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Osteoarthrosis

Changes of bone, cartilage and synovial membrane in relation to bone scintigraphy

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This thesis is based upon the following previous publications:

I Christensen SB, Reimann I: Differential histochemical staining of glycosaminoglycans in the matrix of osteoarthritic cartilage. Acta Path Microbiol Scand, Sect A. 88:61-68, 1978.

II Reimann I, Christensen SB: A histochemical study of alkaline and acid phosphatase activity in subchondral bone from osteoarthritic human hips. Clin Orthop 140: 85-91, 1979.

III Christensen SB, Reimann I, Henriksen O, Arnoldi CC: Experimental osteoarthritis in rabbits. A study of ^{133}Xenon washout rates from the synovial cavity. Acta Orthop Scand 53:167-174, 1982.

IV Reimann I, Christensen SB, Diemer NH: Observations of reversibility of glycosaminoglycan depletion in articular cartilage. Clin Orthop 168:256-262, 1982.

V Christensen SB, Krogsgaard OW: Localization of Tc-99m MDP in epiphyseal growth plates of rats. J Nucl Med 22:237-245, 1981.

VI Christensen SB, Arnoldi CC: Distribution of ^{99m}Tc-phosphate compounds in osteoarthritic femoral heads. J Bone Jt Surg 62-A: 90-96, 1980.

VII Christensen SB: Localization of bone-seeking agents in developing experimentally induced osteoarthritis in the knee joint of the rabbit. Scand J Rheum 12:343-349, 1983.

In the text these papers will be referred to by their respective Roman numerals.

Contents

Introduction

Skeletal scintigraphy has come into increasing clinical use during the past decade, after Subramanian and McAfee (1971) introduced the first ^{99m}Tc-phosphate complex. Since that time, the method has been improved, partly by new complexes having a more rapid blood clearance with higher bone-to-soft tissue ratios and partly by better resolution of imaging devices. Owing to the low dose of radiation, benign orthopaedic diseases are increasingly being subjected to bone scintigraphy, and empirically it has been possible to learn interpreting the scintigrams and thus utilize the examination. On the other hand, the physiological basis for the binding of the tracer to skeletal tissue and the cause of increased uptake are still subject to discussion. Moreover, the morphological localization of the bone-seeking agents in various morbid states, e.g. osteoarthritis, has not been clarified.

It is a clinical experience that in arthritic joints the uptake of bone-seeking agents is increased, even at a very early stage. The present investigations were undertaken to elucidate the sites at which the ^{99m}Tc-phosphorous compounds bind to skeletal tissue in general and their topography in the joint at different stages of osteoarthritis in particular, in order thereby to arrive at a better morphological basis for interpreting the scintigrams. It was endeavoured to relate the uptake to abnormal changes in the involved joint to obtain better insight into the pathogenesis of osteoarthritis.

I. Pathogenesis of osteoarthritis

Osteoarthritis is one of the oldest diseases known. Its skeletal changes were demonstrated in the Neanderhtal man (100,000 - 35,000 B.C., Wells 1964). Viewed as a disease entity it by far exceeds all other articular diseases in morbidity rate (Lawrence 1977). Initially in its course, however, there might be different forms of damage to the joint whose final patho-anatomical stage is the same. A comparison with heart failure is justified, as it is assumed that the various "joint noxae" start the same, fairly stereotyped development leading to "joint failure", i.e. osteoarthritis. A distinction, in principle, between primary and secondary osteoarthritis may be used in clinical practice, but it is hardly justified, due presumably to lacking knowledge of the causes eliciting the primary, idiopathic osteoarthritis.

Osteoarthritis is defined as destruction of articular cartilage in diarthrotic joints, giving rise to increased remodelling of the subchondral bone and the formation of marginal osteophytes (Collins 1949). The degeneration of the cartilage, in particular, is pathognomonic. Many authors have aimed at finding the "primary" event, and naturally a large number of them have focussed on early changes in the structure and biochemistry of the cartilage.

CARTILAGINOUS CHANGES

The special shock-absorbing property of cartilage may be explained by the interaction between *collagen, proteoglycans,* and the *extracellular fluid.*

The *proteoglycans* in cartilage matrix are made up of the glycosaminoglycans chondroitin-6 sulphate, chondroitin-4 sulphate, and keratan sulphate which radiate from a central core protein (Rosenberg et al. 1970, Mankin and Lipiello 1971). One end of this core protein is again bound, by special link proteins (Hascall and Heinegård 1974) to hyaluronic acid, forming the so-called PG aggregates (Hardingham and Muir 1974, Ruy et al. 1982). These aggregates bind up to 1000 times their own volume in water in free solution (Comper and Laurent 1976), but are constrained to a much smaller hydrated space in cartilage by the collagen network. This restriction in expansion creates within the proteoglycans' thirst of water a swelling pressure of 2.5 - 5 atmospheres (Maroudas 1976). This pressure is responsible for the compressive stiffness of the cartilage, and indeed it has been demonstrated that the stiffness is correlated to the glycosamine content (Kempson et al. 1970, Harris et al. 1972).

One of the earliest changes demonstrated in osteoarthritis is a reduction in the glycosamine glycan content in the superficial part of the cartilage (Collins 1949, Mankin and Lipiello 1970), fibrillation and loss of orientation of the collagen fibres at the surface (Weiss 1973), and an increase in the water content of the cartilage (McDevitt and Muir 1976, Muir 1977).

These changes may be explained either by traumatic damage to the collagen network which is broken up, and PG leaks out of the cartilage (Freeman 1973, Freeman and Meachim 1973, Maroudas 1976) or by primary loss of PG whereby the collagen fibres lose their supporting matrix, the tissue

grows soft, and the fibres are damaged (Ali and Bayliss 1974). On this question, cartilage students have split up into two schools: A mechanically oriented school, assuming that wear-and-tear causes the primary damage to the collagen fibres, and another school believing that the PG loss takes place primarily, via enzymatic degradation.

The latter hypothesis is based upon the finding that damage to the chondrocytes entails a release of neutral proteases (Sapolsky et al. 1973, Sapolsky and Howell 1982) and cathepsin D (Sapolsky et al. 1973 a, 1974, Ali and Evans 1973), enzymes which degrade the polypeptide chain of PG and which have been found in an increased concentration in osteoarthritic cartilage (Ali and Evans 1973, Sapolsky et al. 1973 b).

Thus, one or two disintegrations in the popypeptide chain will reduce the molecular weight sufficiently for the proteoglycans to diffuse out of the matrix (Morrison et al. 1973). Proteoglycans that can be extracted from osteoarthritic cartilage are smaller (Brandt and Palmosky 1976), a large fraction of them lacks the hyaluronic acid-binding end of the protein core (Inerot et al. 1978), and there is a decrease in the chondroitin sulphate chain length (Bollet and Nance 1966). Whether this is conditioned by a defective production or by enzymatic degradation is not known, but the consequence is the formation of minor aggregates which are more easily extracted from the cartilage (Brandt and Palmosky 1976, Moskowitz et al. 1979). A defect in the link protein has not been found (Ruy et al. 1982).

Compared with the PG metabolism, the changes in the *collagen content* and composition of the cartilage are slight in cases of early osteoarthritic changes. However, there are signs of increased turnover, since there have been reports of increased synthesis (Lipiello et al. 1977) as well as collagenase activity (Ehrlich et al. 1978) in mildly osteoarthritic as compared with normal cartilage. Normal articular hyaline cartilage contains only type II collagen (Miller 1971). In osteoarthritis the formation of type I collagen around chondrocyte clusters has been recorded (Ninni and Deshmukh 1973, Gay et al. 1976, Gay et al. 1983), but several other studies have shown only type II collagen in fibrillated cartilage (Muir 1977, Lipiello et al. 1977).

Nevertheless, the collagen changes have occupied a more central position in the pathogenesis of osteoarthritis after it was demonstrated that the ability of the superficial collagen layer to resist tensile stress decreases with advancing age (Weightman 1976, Kempson 1982).

Present investigations (I)

Although the cartilage loses PG in osteoarthritis, it has high metabolic activity and tries to compensate for the loss by an increased production of glycosaminoglycans. This was first demonstrated by Collins and McElligot (1960) who showed that osteoarthritic cartilage had an increased rate of $^{35}SO_4$ incorporation. Since then, numerous studies have supported the concept that synthetic activity is considerably increased (Bollet 1968, Mankin et al. 1971, Eronen et al. 1978, Palmosky et al. 1980, Mankin et al. 1981), while others deny the existence of an increased PG synthesis (Maroudas 1975, McKenzie et al. 1977).

With advancing age, the production of chondroitin-6 sulphate, chondroitin-4 sulphate, and keratan sulphate alters. Formation of exclusively chondroitin sulphate is characteristic of immature and young cartilage, while keratan sulphate does not form until the cartilage is more ageing (Kaplan and Mayer 1959, Matthews and Glagov 1966).

It was of interest to elucidate whether, apart from the depletion of GAG, osteoarthritic cartilage shows an altered distribution of the various GAGs which could be ascribed to an altered production pattern. This investigation included 6 femoral heads removed from osteoarthritic patients in connection with hip replacement surgery and 6 control heads removed after fracture of the femoral neck or in connection with exarticulation at the hip because of an extremity tumour. After fixation in 4% neutral, buffered formaldehyde, decalcification in formic acid sodium citrate reagent (Morse 1945), and double-embedding in celluidin-paraffin, the following staining methods for GAG were used on the sections: *1. Safranin-O fast green-iron haematoxylin* (Lillie 1965) for histological-histochemical grading of the osteoarthritic cartilage changes according to Mankin et al. (1971). *2. Toluidine blue O (Merck)* at pH 1, 2, 3, 4, 5 (Walpole buffer). The metachromatic staining reaction is dependent upon electrostatic binding of the cationic dye to the acid groups of the glucosaminoglycans in anionic form. Accordingly, the binding depends upon the pK values of the anions and can be affected by altering the pH. This will afford a separation between the reaction from carboxyl groups and sulphate groups. Moreover, the degree of sulphation contributes to determining at which pH the reaction ceases (Krygier and Kasprzyk 1961, Spicer 1962). *3. Alcian blue 8GX (I.C.I.) with critical electrolyte concentration (CEC)* (Scott and Dorling 1964) permits a distinction between highly sulphated (keratan sulphate), sulphated as well as carboxylated (chondroitin sulphate), and only carboxylated glucosaminoglycans (hyaluronic acid).

This investigation revealed an appreciable variation in the osteoarthritic cartilage changes within the same femoral head, the changes increasing towards the weightbearing area. Apart from the superficial GAG depletion, there was no fundamental difference between the osteoarthritic and the control group in the distribution of keratan sulphate, which was found territorially (the matrix zone surrounding the chondrocyte) in the deep part of the cartilage, interterritorially in its other parts. On the other hand, there was a difference in the distribution of chondroitin sulphate in cartilage matrix between the two groups. In addition to the depleted area in osteoarthritic cartilage, there was a relatively increased stainability in the territorial zone, especially around the cell clusters, compared with the stainability in the interterritorial area. This phenomenon was observed throughout the cartilage, also in areas with only mild signs of osteoarthritis. In the cartilage of the osteophytes the GAG content was almost exclusively chondroitin sulphate. Three of the control cartilages showed a distribution of chondroitin sulphate similar to that of the osteoarthritic cartilages, but only in the weightbearing part. These control specimens were from two patients aged 87 and 88 and from one patient 72 years of age with mild, initial osteoarthritic changes of the cartilage.

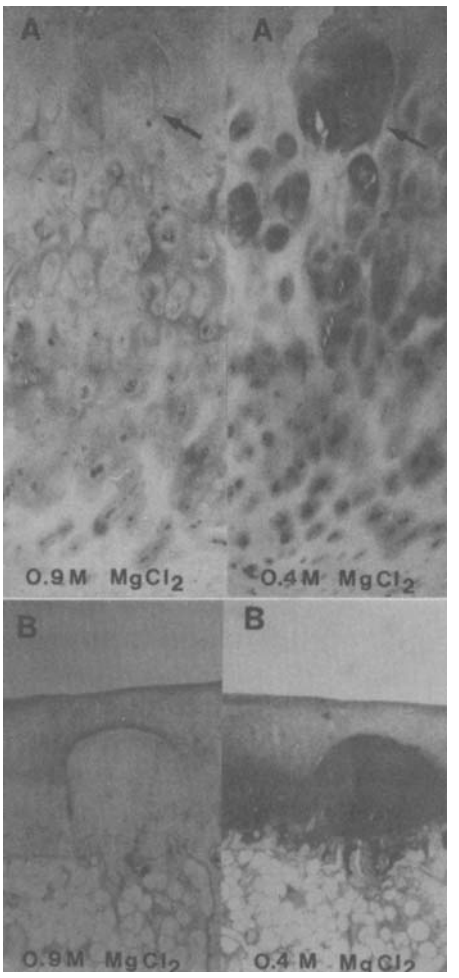


Figure 1. Alcian blue staining by the critical electrolyte concentration (CEC) principle. In cartilage Alcian blue 0.4M $MgCl_2$ will stain both chondroitin 4/6 sulphate and keratan sulphate while at 0.9 M $MgCl_2$ only staining of high molecular weight keratan remains.
A: Osteoarthritic cartilage (grade 8) from the femoral head of a patient aged 50 years. Superficial loss of staining intensity is seen. The increased territorial staining around cell clusters (arrows) is seen to be caused mainly by chondroitin sulphate. Magnification × 67.
B: Osteophyte cartilage from a patient aged 68 years showing that the glycosaminoglycans in the osteophyte cartilage are mainly chondroitin sulphate. Magnification × 21.

After further incubation with testicular hyaluronidase and subsequent staining, it was confirmed that the territorially relative increase in GAG consisted of chondroitin sulphate, as testicular hyaluronidase digests chondroitin sulphate, but not keratan sulphate (Mayer et al. 1956, Leppi and Stoward 1965). The results may be taken to indicate that osteoarthritic cartilage has acquired an altered production pattern of GAG towards a young cartilage. The finding in some of the control cartilages may suggest that increased territorial staining of chondroitin sulphate is a very early manifestation of initial osteoarthritis or a sign of stress actions upon the joint.

CHANGES OF THE SUBCHONDRAL BONE IN OSTEOARTHRITIS

The skeletal changes in the juxta-articular bone present a mixed appearance of bone formation, resorption, cystic degeneration, and the development of marginal osteophytes (Collins 1949, Sokoloff 1969, Jeffrey 1973).

It has been emphasized by Radin and co-workers (1973, 1976, 1982) that the changes in the subchondral bone may constitute the primary pathogene-

sis and cause the cartilaginous degeneration. Their hypothesis is that an increased impulsive loading upon the joint causes subchondral, trabecular microfractures which, in the course of healing, lead to increased subchondral sclerosis and thereby a stiffer basis for the cartilage. In that case, the cartilage itself has to absorb a greater part of the loading energy, and this increases the risk of cartilaginous degeneration.

Vascular changes have been demonstrated in the juxta-articular bone, in the form of arterial hyperplasia (Harrison et al. 1953), intraosseous hypertension (Arnoldi et al. 1972), and venous engorgement (Hulth 1959, Arnoldi et al. 1972). The intraosseous hypertension can be related to the occurrence of rest pain (engorgement pain syndrome) (Arnoldi et al. 1971, 1975). However, the pathogenetic explanation of the intraosseous hypertension has not been clear until a correlation was found experimentally between joint effusion with increased intraarticular pressure and increased intraosseous pressure in the juxta-articular bone (Arnoldi et al. 1979, Bünger et al. 1981). These findings support the increasing interest in synovial changes in osteoarthritis (Arnoldi and Reimann 1979).

Present investigation (II)

The juxta-articular bone is the focus of increased uptake of bone-seeking isotopes in osteoarthritis. Consequently, it is of interest to evaluate the remodelling activity in various sites of bone and to correlate it to the degree of the osteoarthritic changes in overlying cartilage. For this purpose, histochemical alkaline and acid phosphatase activity tests were used, although their significance in skeletal tissue has not yet been fully elucidated.

Alkaline phosphatase is bound to the plasma membrane in osteoblasts, preosteoblasts, hypertrophic chondrocytes, fibroblasts and to the membrane of the matrix vesicles (Andersen 1970, Göhlin and Erichson 1973) in which calcium accumulation prior to mineralization is observed (Anderson 1973). In these sites the enzyme is imagined to influence the membrane transport of phosphate by transphosphorylation (Arsenis et al. 1976). Others believe that the enzyme in skeletal tissue influences bone formation through its pyrophosphatase activity, whereby the pyrophosphate inhibition of apatite crystal formation is removed (Fleich and Neumann 1961, Anderson and Reynolds 1973). The transport of calcium across cell membranes against a concentration gradient requires energy, provided by ATP hydrolysis as a part of an ATP-driven calcium pump, and alkaline phosphatase might play a role in the Ca accumulaton during its ATPase activity (Fortune et al. 1980). Although the mechanism of the enzymatic effect still remains unelucidated, it is generally assumed to be involved in bone formation. Increased activity is found in growth zones (Jeffree 1969, Andersen 1970), in healing fractures (Timmer 1968), in Paget's disease (Woodard 1959), and in certain bone tumours (Jeffree and Price 1965).

Osteoclasts, and their presumed precursors, mononuclear macrophages, contain large quantities of acid hydrolytic enzymes, including acid phosphatase in the lysosomes (Burstone 1959, Doty and Schofield 1976) and in the ruffled border channels (Lucht 1971). The lysosomal hydrolases of the

osteoclast are believed to be active in the breakdown of bone substances. This is supported by the finding that parathyroid extract-stimulated osteolysis in tissue cultures has shown an increase of enzyme, both in the medium and in the cellular fraction (Vaes 1968).

In the present study non-specific alkaline phosphatase activity was demonstrated by the simultaneous coupling method of Burstone (1962), using naphthol-AS-BI-phosphate as substrate and Fast Red Violet LB as indicator. Acid phosphatase activity was demonstrated by the method of Barka and Andersen (1963) using hexazotized pararosaniline as indicator.

The activity was measured semiquantitatively on frozen sections of whole osteoarthritic joints by the *initial time*, i.e. the reaction time from incubation with medium until the first colour reaction (Hopsy and Glenner 1965).

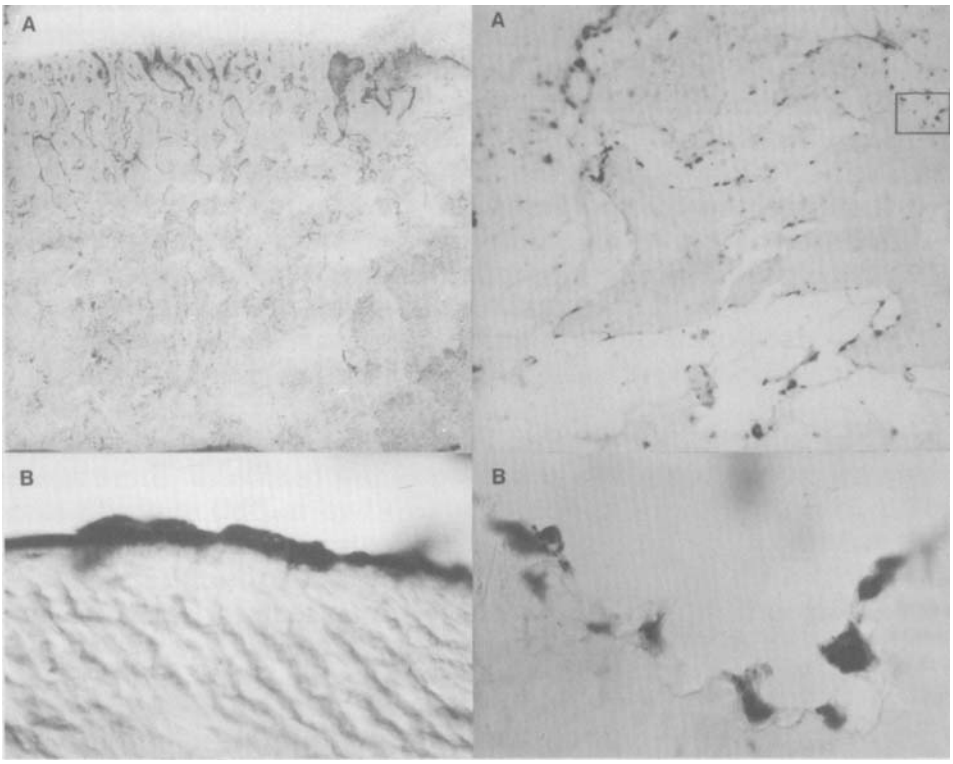


Figure 2. (A) Photomicrograph of frozen unfixed section 16 micra thick from femoral head with osteoarthritis stained for alkaline phosphatase. The reaction (black colour) is seen to be most pronounced at the surface. (Magnification × 12). (B) Same, interference contrast, magnification × 250. The alkaline phosphatase activity (black colour) is seen at the surface of the trabecular bone in the osteoblasts upon a thin layer of osteoid.

Figure 3. (A) Photomicrograph of frozen unfixed section 16 micra thick from osteoarthritic femoral head, weight-bearing area, stained for acid phosphatase. The reaction is seen in cells at the surface of the trabecular bone (magnification × 32). (B) Same (magnification × 125) illustrating osteoclasts in Haversian lacunae with punctate reaction (lysosomes containing acid phosphatase).

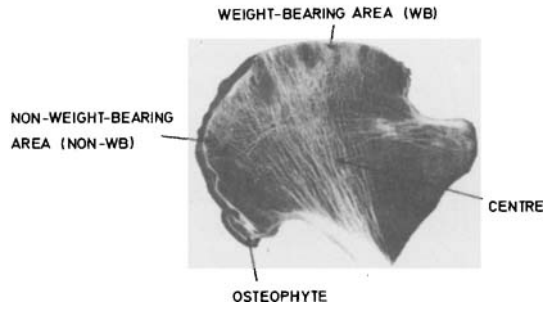
Twenty-four femoral heads from patients with grade 2-3 osteoarthritis (Collins 1949) were removed at replacement surgery, freeze-cut on a hard-tissue cryostat (Ullberg 1956), and unfixed sections of the entire head were incubated with the incubation medium in the following areas: subchondrally in the weightbearing and non-weightbearing area respectively, centrally, and in osteophytes. Adjacent sections were stained with safranin-0 iron haematoxylin with a view to grading (Mankin et al. 1971) the osteoarthritic changes over the areas measured.

This demonstrated no uniformly distributed enzyme activity in the femoral head, but there was a considerable variation between the individual areas in the same head. The distribution of enzyme activity between acid and alkaline phosphatase activity proved parallel. The activity was at a maximum in the subchondral weightbearing area and osteophytes, and at a minimum centrally. When excluding the activity in the osteophytes, subchondral enzyme activity was found to increase with increasing severity of cartilaginous degeneration.

Lerim et al. (1975), in biochemical studies of the alkaline phosphatase activity in bone homogenate from human osteoarthritic knee joints, did not find any difference from the activity in normal bony tissue. However, in biochemical analysis of enzyme activity, the more detailed *in situ* information about activity is lost, and in their study it was not possible to take samples from the adjacent subchondral bone where the enzyme activity is highest.

The increased remodelling after increasing cartilaginous degeneration found in the present study can be explained partly on the basis of a greater

Fig. 4.



ALKALINE PHOSPHATASE:
WB ---------- 22.1 ± 8.0 sec
NON-WB ------ 39.8 ± 13.7 sec
OSTEOPHYTE - 26.6 ± 4.7 sec
CENTRE ------ 50.7 ± 20.6 sec

ACID PHOSPHATASE.
WB ---------- 41.2 ± 13.8 sec
NON-WB ---- 94.5 ± 57.4 sec
OSTEOPHYTE - 69.7 ± 29.0 sec
CENTRE ---- 126.9 ± 56.6 sec

Fig. 5.

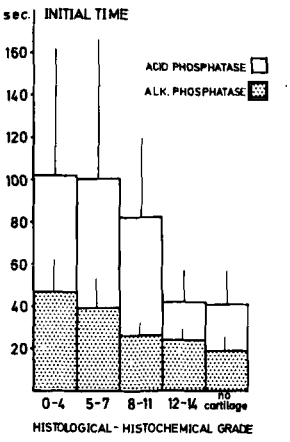


Figure 4. Radiographs of femoral head with osteoarthritis showing the different areas estimated. The lists below illustrate the variations in the initial time according to the site studied (mean ± SD).

Figure 5. Diagram showing the correlation between alkaline and acid phosphatase activity expressed by initial time (mean ± SD) compared with the histological-histochemical grade of the cartilage lesions.

mechanical action upon subchondral bone resulting from the gradually decreasing biomechanical ability of the cartilage as a shock absorber, and partly it is likely that the synovial fluid in areas denuded of cartilage has a toxic effect upon the bone and bone marrow (Fassbinder 1975). The high enzyme activity in the osteophytes reflects their growth.

CHANGES IN THE SYNOVIAL MEMBRANE AND FLUID

In manifest osteoarthritis there is fibroblastic synovitis with hypertrophy of the lining cells and subsynovial fibrosis (Lloyd-Roberts 1953). These changes have proved to correspond to histochemically demonstrated increased lysosomal activity in synoviocytes and an increased fibroblastic activity (Reimann and Christensen 1979).

The content in the synovial fluid of enzymes, such as plasmin (Lack and Ali 1967), hyaluronidase (Bollet 1967), elastase (Barrett 1978), and other neutral proteinases (Peltonen et al. 1981) has been considered to contribute to the cartilaginous destruction. But in view of the great content of enzyme inhibitors, including in particular α_2 macroglobulin, cartilage destruction by synovial fluid proteinases has been called in question (Barret and Starkey 1973). However, a condition of imbalance between leakage of enzymes from phagocytic lining cells (A-type) and production of inhibitors from B-type lining cells is believed to exist (Glynn 1977), and this may contribute to the development of osteoarthritis.

It is of interest that Dingle et al. (1979) have isolated a non-enzyme, catabolin, produced by the synovial membrane and which stimulates the catabolism of the cartilaginous matrix by the chondrocyte itself.

It has been generally assumed that the synovitis is secondary to the cartilaginous destruction and that it can be explained by phagocytosis of detritus (Lloyd-Roberts 1953).

However, a primary inflammatory factor is thought to be present in osteoarthritis, associated with deposition of crystals of calcium pyrophosphate dihydrate (McCarthy 1975) or hydroxyapatite (Dieppe et al. 1976). So-called inflammatory osteoarthritis is probably more common than previously assumed (Huskisson 1979), and even primary osteoarthritis could be the end result of a series of attacks of synovitis, sometimes extending over a long period of years (Arnoldi and Reimann 1979).

II. The experimental osteoarthritis model

Morphological studies on human osteoarthritis are restricted to tissue removed from patients with clinically manifest osteoarthritis. As most pathogenetic interest attaches to the early changes in the joint, animal experimental models have gained wide usage.

A number of different models have been described, and in the case of all models it is a matter of discussion, more or less, whether the induced changes simulate the pathogenesis in human osteoarthritis which *per se* must be assumed to be multifactorial.

Rabbit models have been based upon scarification of cartilage (Meachim 1963), intraarticular injections of the proteolytic enzyme papain (Bentley 1971), induced alteration of weightbearing by valgus osteotomy on the tibia (Reimann 1973), intermittent impact loading of the joint (Radin et al. 1973), immobilization of joints in compression (Salter and Field 1960) or in knee extension (Langenskiöld et al. 1979), partial meniscectomy (Moskowitz et al. 1973), and *in vivo* freezing of the cartilage (Simon et al. 1981).

Today the most used models are, however, those which induce articular instability, either by cutting the anterior cruciate ligaments in dogs (Pond and Nuki 1973, Gilbertson 1975, McDevitt and Muir 1976) or by cutting of the cruciate ligaments, excision of the medial collateral ligament, and extirpation of the medial meniscus (Hulth et al. 1970, Telhag and Lindberg 1972, Bohr 1976).

As the intention was to study the uptake of bone-seeking agents in experimental osteoarthritis, models with primary damage to bone had to be excluded. For this purpose, the rabbit model based on joint instability was used, and the pathogenetic changes in this model were further elucidated by investigating changes in synovial perfusion and by assessing changes in the GAG content of the articular cartilage histochemically.

PERFUSION CHANGES IN THE SYNOVIAL MEMBRANE (III)

Like most other models, that of Hulth and Telhag (1970, 1971) is created by an initial surgical trauma, and although arthrotomy has been done on the control knee, differences in the synovial reaction cannot be disregarded. This has not previously been elucidated; nor has the time relation of the synovial reaction to the development of osteoarthritis.

An attempt has been made to evaluate these factors by calculating the ratio between the washout rates of ^{133}xenon injected intraarticularly into both knees. Radioxenon is an inert, lipophilic gas which easily crosses cell membranes and whose recirculation is negligible as practically all of it is expired after one circulation through the lungs (Lassen 1964).

Phelps et al. (1972) found a biexponential washout curve following intraarticular injection af ^{133}xenon. When analysing the tissue localization of ^{133}xenon in the joint during the fast and slow phases of the clearance curve,

they observed that the initial, rapid washout was explicable by washout to the blood vessels across the synovial membrane, whereas the slower, subsequent washout represented washout of xenon from articular fat.

In 6 rabbits, followed for a minimum of 1 year, biexponential washout curves were found, and the initial washout proved faster on the osteoarthritic side throughout the period. The ratio between the initial washout rate constants in the osteoarthritic and the control knee was particularly high during the first 16 weeks. At that time it was significantly higher than the ratios of the washout rate constants at 6 months and later ($p < 0.001$, Mann-Whitney).

Fig. 6.

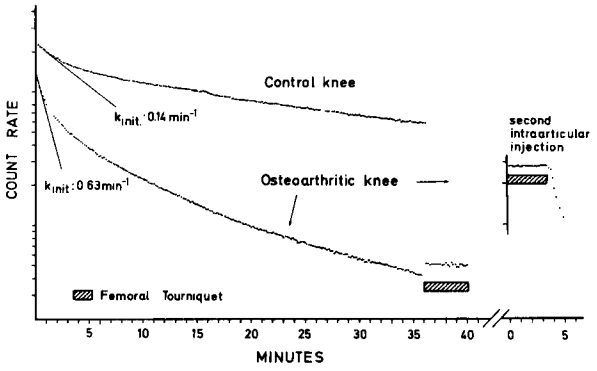


Figure 6. ^{133}Xe washout curves from the knee joints 23 weeks postoperatively. The biexponential curve and the constant count rate after circulatory arrest is seen both in the late slow phase and the initial fast phase of the washout curve.

Fig. 7.

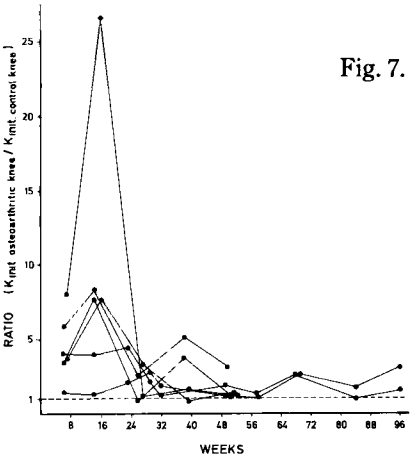


Figure 7. The ratio between the washout rates of the osteoarthritic and the control knee of 6 rabbits at different periods postoperatively. Ratios above 1 indicate increased clearance from the osteoarthritic knee. The highest ratios are seen within the first half year.

Methodological sources of error have to be considered. Changes in articular fat influence the washout rate of the lipophilic gas xenon. Loss of fat would cause a more rapid washout, mostly in the latter slope of the clearance curve, but it would to some degree also influence the initial washout. It is not known whether changes in the fat content of the synovial membrane in the osteoarthritic knee occur. A conceivable, but unrealistic loss of all fat would entail an increase in the initial washout rate of 40%, if the calculations are performed with a partition coefficient of 0·67 ml.g^{-1}, a 4% fat content of the synovial membrane, and a haematocrit value of 50% (Phelps et al. 1972). This can by no means explain the 500% increase in washout rate found in the early stages. Scintigraphy of the knee region of rabbits given ^{99m}Tc microspheres intracardially showed a marked increase in the flow to the knee region on the osteoarthritic side, in support of the xenon measurements.

The study thus showed a marked increase in the synovial flow during the early stages of experimental osteoarthritis, but owing to the methodological

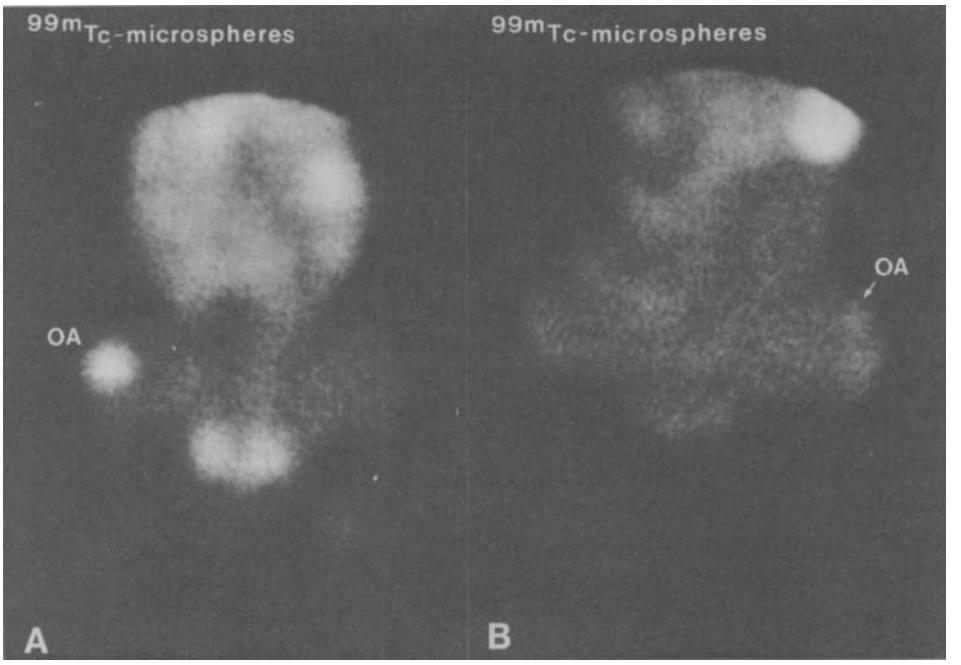


Figure 8. Flow distribution of ^{99m}Tc-microspheres to the lower part of the body of rabbits 3½ months (A) and 16 months (B) postoperatively. Accumulation of microspheres in the osteoarthritic joint region is seen particularly at the early stage of the disease.

considerations, it is difficult to draw any conclusions as to the less increased washout ratios during the later stages.

The flow increase reflects partly a major surgical trauma, but probably more so the traumatic synovitis caused by the great instability of the knee. In other words, an appreciable synovial reaction for several months is incorporated in the model during the early stages of osteoarthritic development. However, this also applies to other models, such as cutting of the anterior cruciate ligament in dogs after which there is effusion in the joint for up to 12 weeks after the operation (Gilbertson 1975). Even in a non-traumatic model, like Langenskiöld's, in which rabbit knees are immobilized, there will be joint effusion and histologically evident traumatic synovitis (Finsterbusch and Friedman 1973).

It is possible that the posttraumatic synovitis contributes to the development of osteoarthritis and is responsible for some structural changes in the joint which are otherwise considered typical of osteoarthritis.

GAG DEPLETION AND REGENERATION (IV)

In the present experimental model it was intended to elucidate the time of GAG depletion during the development af osteoarthritis and possibly find signs of GAG regeneration.

The GAG content of the cartilage was evaluated for exclusively sulphated glycosaminoglycans by staining with 0.01% toluidine blue 0 at pH 3 (Kry-

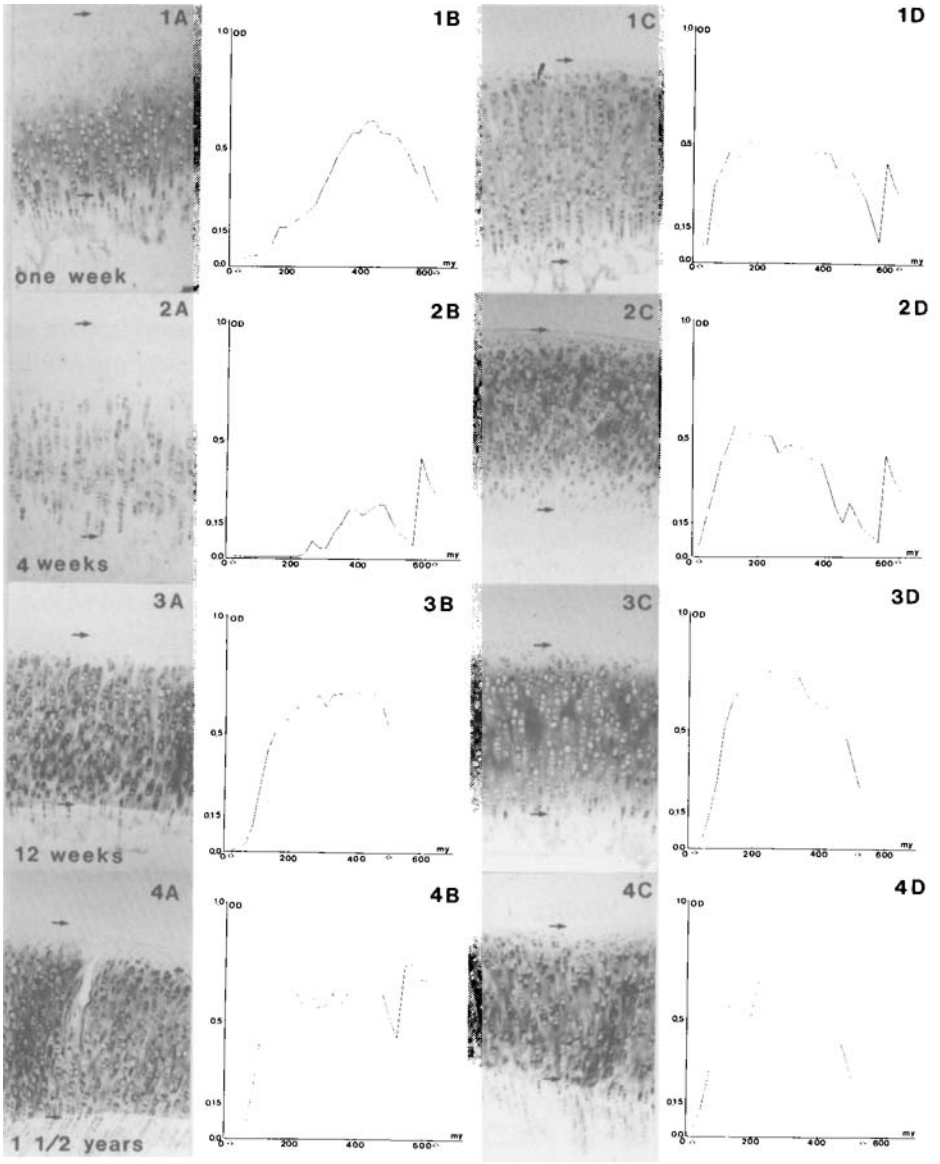


Figure 9. Illustrations of visual and optical measurements from sections of articular cartilage prepared at various postoperative intervals. (A) Histological sections for visual measurements of surface depletion of GAGs from unstable joints. Toluidine blue pH 3, original magnification × 25. (B) Diagrams showing the results from scanning measurements of optical density from surface of articular cartilage to tide mark (arrows) in micra (my). These measurements are from the same section as in A. (C) Histological sections from contralateral control joints (toluidine blue pH 3, original magnification × 25). (D) Diagrams from measurements of optical density from contralateral joints.
1A-1D: one week. 2A-2D: four weeks. 3A-3D: twelve weeks. 4A-4D: one and a half years.

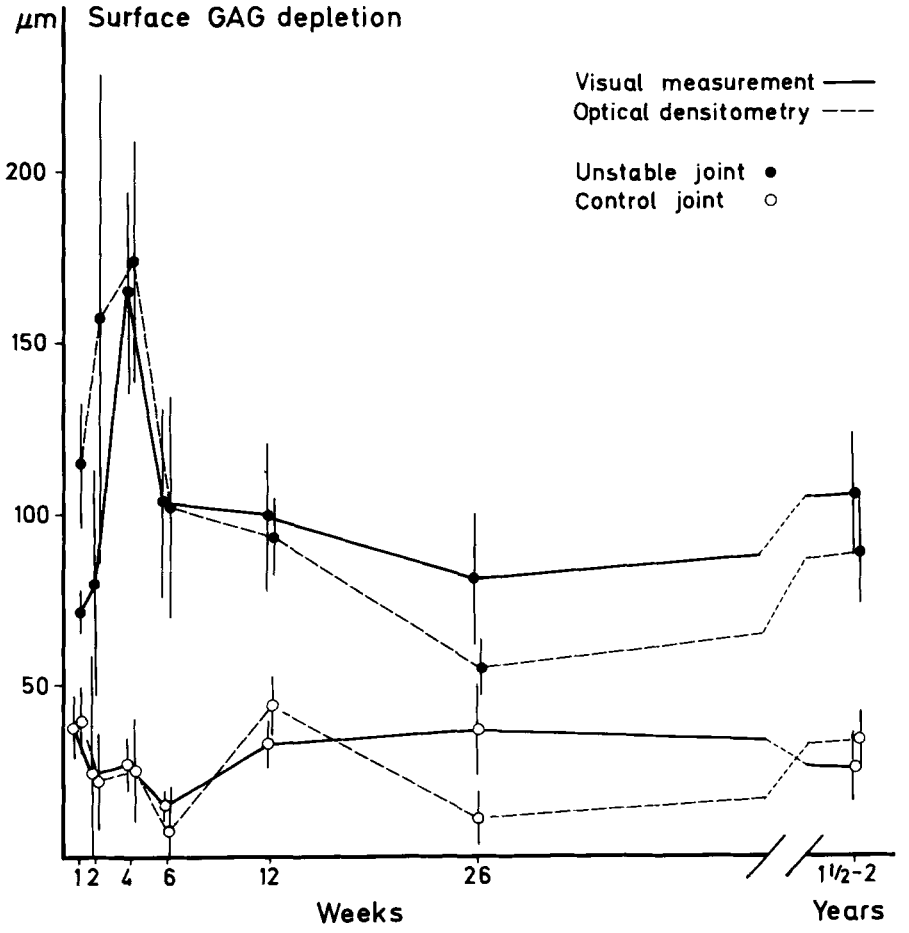


Figure 10. Visual and optical densitometric measurements of the surface depletion of GAGs expressed as mean ± SEM.

gier and Kasprzyk 1961, Spicer 1962). Patellar cartilage from osteoarthritic and control knees of 42 rabbits was examined 1, 2, 4, 6, 12, and 26 weeks as well as 18-24 months postoperatively, 6 rabbits in each group. The thickness of the non-stainable, GAG-depleted superficial zone was measured, in part visually and in part by optical densitometry (Leitz Texture Analysis System) in which the cartilage was scanned from its surface to the "tide mark".
The densitometric measurements were performed in monochromatic light of a wavelength corresponding to the γ-band of toluidine blue (540 μm) on which it was demonstrated that the reaction in mounted tissue sections with only bound dye may follow the Lambert-Beer law (Kramer and Windrum 1955, Kelly 1958, Kelly 1966) by which a relative quantitation of sulphated GAG is obtained.

The main results of the investigations, however, are based upon the size of a superficial, non-stainable zone, defined by an optic densitometry of less

than 0.15, and in this respect there was agreement between the visual measurements and the optic densitometric scanning.

As early as one week postoperatively, there was increased surface depletion of GAG. The heights of the unstained superficial zone rapidly increased during the subsequent weeks, reaching maximum GAG depletion at 4 weeks. Thereafter, the depleted areas decreased, and at 26 weeks postoperatively their height was significantly lower than at 4 weeks ($p < 0.05$, Mann-Whitney), indicating regeneration of proteoglycans in the matrix.

This experimental model affords primarily cartilaginous degeneration in the medial femorotibial joint, so that a loss of GAG on the patellar cartilage can be interpreted as a sign of initial, generalized action upon all articular cartilage, not as a local reaction. It is reasonable to relate the very early generalized GAG depletion to the simultaneous, severe posttraumatic synovitis demonstrated by the xenon whashout (I). In this phase, the cartilage is soft and vulnerable, but if the mechanical forces are not too great and the synovial reaction subsides, the findings indicate a possibility of healing through filling of the demasked collagen network with proteoglycans. If, on the other hand, the arcaded construction of the collagen fibres is destroyed, the possibility of regeneration at some time or other passes a point of no return, and development of osteoarthritis is inevitable. The cartilaginous degeneration will manifest itself on the most heavily loaded or stressed areas, in this model the medial joint chamber. However, from the very start there has been a question, in this model, of a non-specific, generalized weakening of the biomechanical properties of the cartilage which need not necessarily represent osteoarthritis.

III. Bone scintigraphy

HISTORY

The first labelling of bone tissue by radiotracer was performed by Chiewitz and Hevesy (1935) in the Niels Bohr Institute, Copenhagen, where adult rats were labelled with ^{32}P-phosphate. This altered the conception that skeletal tissue was metabolically inactive.

The increased supply of radioisotopes, which became available after 1945, and the development of the rectilinear scanner in the early 1950's formed the basis of incipient bone scintigraphy. In the role of tracers the components of hydroxyapatite itself, radioactive Ca and P, did not prove wellsuited. ^{45}Ca and ^{32}P are pure beta emitters, unsuited for scintigraphy, and the gamma radiation of ^{47}Ca was too high (1.3 MeV) for ordinary collimators, although this isotope has been used (Bauer and Wendelberg 1959). ^{131}Barium and ^{140}Barium (Bauer et al. 1957, Spencer et al. 1970), ^{47}Scandium (Basse-Cathalinat et al. 1968), and more rare nuclides, such as ^{177}Lutetium, ^{176m}Lutetium, ^{153}Samarium, and ^{171}Erbium (O'Mara, McAfee and Subramanian 1969) have been used as bone-seeking tracers, but have not come into wide use.

On the other hand, Ca analogues, such as the alkali metals ^{85}Strontium and ^{87m}Strontium, turned out to be wellsuited (Bauer 1969) and were for a long time the isotopes of choice in many laboratories. It has been demonstrated that the distribution of tracer doses was like that of Ca (Bauer and Ray 1958). However, the physical (65 days) and biological (800 days) half-life of ^{85}Strontium limits the dose that can be administered, and this results in a poor count rate. Its use is broadly speaking restricted to patients with proven malignancy, but good information has been obtained by regional scintimetry in benign orthopaedic diseases (Bauer 1978). ^{87m}Strontium has a shorter half-life (2.8 hours) and can be administered in higher doses, but it does not give the high focus background ratio of scanning by ^{85}Strontium at the end of 7-10 days, and the generator cost was high.

^{18}Fluorine is an excellent tracer for bone scintigraphy, having a high affinity for skeletal tissue and a rapid blood clearance (Blau et al. 1962, Heerfordt, Vistisen and Bohr 1976). Its energy is too high for "low resolution collimators", and its half-life is short (1.8 hours). This limits its use to laboratories close to a cyclotron.

The introduction of ^{99m}technetium-labelled phosphate complexes (Subramanian and McAfee 1971) marked a great advance in bone scintiscanning which had so far been reserved for only a few laboratories and had not yet become a procedure that could be used as a routine in daily clinical practice. The radionuclide ^{99m}Tc is ideal for diagnostic purposes. The emitted gamma radiation has an energy of 140 keV, which affords good utilization, particularly of gamma cameras with thin crystals (1/2 inch) in which up to 90% of the radiation is absorbed. Furthermore, thin-walled collimators with a high solubility can be used. The radionuclide is eluted as pertechnetate (TcO_4^-) from a ^{99}Molybdenum generator in which neutron-irradiated molybdenoxide

is loaded on a column of aluminium oxide, where it decays into ^{99m}Tc. The bone-seeking agents are prepared by mixing the eluted sodium pertechnetate with a phosphorous compound as carrier substance and Sn^{2+} as reducing agent. Before technetium can be labelled on any of the phosphorous compounds, it must be reduced to $Tc^{(4+)}$ (Subramanian and Mc Afee 1971, Cox 1974).

Labelled tripolyphosphate was the first phosphate complex to be introduced (Subramanian and McAfee 1971). It was soon succeeded by polyphosphate (Subramanian et al. 1972 a) and pyrophosphate (PP) (Perez et al. 1972). Now, these organic phosphate complexes have been superseded by the stable, non-hydrolysable disphosphonates with P-C-P bonds. Thereamongst ethylene-1 hydroxy-1,1 disphosponate (EHDP) (Castronovo and Callahan 1972, Subramanian et al. 1972 b) and methylene diphosphonate (Subramanian et al. 1973, 1975) have been most used.

In general, it may be said that the percentage quantity of the administered bone-seeking isotope that labels skeletal tissue does not differ much for the various agents, while the phosphonate complexes, in particular, have a rapid plasma clearance and thus, within the same time interval, a lower blood background (Krishnamurthy et al. 1974, 1975). Besides, these agents are not taken up by soft tissues, e.g. the liver, as are the condensed phosphate complexes.

FACTORS INVOLVED IN SKELETAL UPTAKE

The mechanism underlying the binding of the bone-seeking isotopes to skeletal tissue has long been a subject of discussion, and several, fundamentally different hypotheses concerning the determining factors have been adduced:

Flow: Perfusion through bone is essential for the uptake of the bone-seeking isotopes. Indeed, it is a well-known experience that in the absence of perfusion, as in aseptic necrosis of bone, there wil be a cold area (Bohr and Heerfordt 1977, Greiff et al. 1980). But it is a question whether an increased uptake is explicable mainly by increased vascularity (Siegel et al. 1976). It seems to have been established that an increase in blood flow can explain a somewhat increased uptake. In hypo/hyperthermia studies on rat limbs, Genant et al. (1979) observed that a flow ratio of 0.42 (^{99m}Tc-microspheres) between a cold and warm limb had a corresponding influence upon the ratio of the bone scintigraphic uptake of 0.70 for ^{99m}Tc-EHDP and 0.43 for ^{18}Fluorine. In the same way, sympathectomy entails an increased uptake, presumably by opening closed arterioles (recruitment), so that the tracer gains access to new bony surfaces (Charkes 1979, 1980).

On the other hand, experimental studies have demonstrated that an increase in perfusion of up to 4 times normal has entailed a maximum increase in tracer uptake of 70% (Sagar 1978). Reversely, it has been shown by Lavender et al. (1979) that a skeletal trauma (osteotomy on canine tibiae) entailed a rise in the flow of 100% and a far greater increase in the uptake of the bone-seeking isotopes (800%).

Charkes et al. (1978, 1980) have set up a 5-compartment model of the tracer kinetics of bone-seeking isotopes: blood, non-bone ECF (extracellular fluid), tubular urine, bone ECF, and bone surface. It was again deduced that after a significant increase in blood flow, there was only relatively little increase in skeletal uptake of the bone-seeking agent.

Hughes et al. (1977) and Kelly et al. (1977) correlated capillary extraction of various tracers, such as fluorine and Tc-phosphate complexes, to their diffusion constants. They found the transport to be conditioned by passive diffusion through clefts in the capillary wall. However, this transport is fast and non-limiting for scintigraphic uptake (McCarthy et al. 1980) for several hours after the administration. The ECF of the bone is assumed to have been "saturated" with tracer (Charkes 1979), and apart from the recruitment phenomenon opening new capillaries, which can explain the perfusion-conditioned increase in skeletal uptake, there must be local differences in the extraction from ECF to bone, whereby a focus arises.

Bone mineral: Factors concerning the surface of the hydroxyapatite crystal are of essential importance to its kinetics and its binding of substances. According to Neumann and Neumann (1958), the superficial ions are assumed to be hydrated. Thus, there are three zones between which ions can be exchanged: The crystal interior, crystal surface, and a bound hydration shell. Transport to and from the crystal lattice itself is slow, whereas exchange on the surface and on the bound hydration shell is rapid (Neumann and Neumann 1958). It has been demonstrated that pyrophosphate and diphosponates are chemisorbed by electrocovalent or covalent bindings to the crystal surface (Francis 1969) where they inhibit the growth and dissolution of the crystals (Francis et al. 1969, Fleisch et al. 1969).

It is assumed that the labelled phosphate complexes are absorbed undissociated onto the crystal surface, but mention has been made of the possibility that technetium may split off on the crystal surface (van Langevelde et al. 1977), whereupon the labelled tracer is said to be deposited as technetium oxide (TcO_2).

Immature collagen: Rosenthall and co-workers, in their *in vitro* studies on bony tissue which had, in addition to decalcification, been through various chemical modifications, found increased uptake in the organic phase of the bone compared with untreated bone. They concluded that the bone-seeking isotopes are bound predominantly to immature collagen (osteoid) (Kay et al. 1975, Rosenthall and Kay 1975, 1976).

Enzyme systems: On the basis of experimental findings of an inhibition of phosphatases incubated with diphosphonates, Zimmer et al. (1975) and Schmith et al. (1974) assume that the bone-seeking technetium compounds complex especially with alkaline phosphatase. This was supposed to explain an increased uptake in areas showing osteogenesis.

In view of the uncertainty which still attaches to the mechanism underlying the binding of bone-seeking isotopes to skeletal tissue, it was attempted in the present study to elucidate it by means of its autoradiographic localization, comparison of the latter with the distribution of alkaline phosphatase activity, collagen formation, tetracycline labelling, and evaluation of the affinity of the bone-seeking isotopes for the inorganic phase of the skeletal tissue by studying the decalcification of sections labelled *in vivo*.

LOCALIZATION OF ^{99m}Tc-MDP IN SKELETAL TISSUE (V)

An attempt was made to elucidate the localization of ^{99m}Tc-MDP in rat epiphyses (V) in which there is good morphological distinction between the various bone-forming stages of enchondral ossification: provisional calcification, ossification, and resorption.

^{99m}Tc-MDP was administered intravenously to 4-5-week-old rats 3 hours before they were killed. The knee region was freeze-embedded and cut on a hard-tissue cryostat by Ullberg's technique (1954). Contact autoradiography of the sections was performed within the cryostat using nuclear plates coated with a G5 emulsion. Thereupon, they were postfixed in 4% Baker's formol calcium (4% formaldehyde) and stained. Microautoradiography was carried out on fixed sections (formol calcium) by the dip method (Kopriwa and LeBlond 1962), viz. dipping the tapes suspended on metal frames.

The following staining methods were used: *Poststaining* by Mayer's haematoxylin-eosin. *Prestaining* for alkaline phosphatase activity (Burstone 1962) and acid phosphatase activity (Barka and Anderson 1963). This was done partly to elucidate the hypotheses on the binding of Tc-phosphate complexes to the hydrolytic enzymes, in particular alkaline phosphatase, and partly to compare radionuclide uptake with osteoblastic activity (alkaline phosphatase) and osteoclastic activity (acid phosphatase).

Vital staining. In order to compare in another way the radionuclide uptake with the remodelling processes in the skeletal tissue, rats were *in vivo* labelled with oxytetracycline, administered, with a short labelling interval (3 hours), after administration of ^{99m}Tc-MDP. This labels both formative and resorptive bone surfaces (Harris et al. 1962, Olerud and Lorenzi 1970). The sections were either photographed under incident fluorescence microscopy (Leitz Ploem Opak fluorescence microscope, type G filter) prior to the autoradiographic procedure or else coated with 0.5% celloidin which somewhat diminishes the fading of the fluorescence conditioned by the autoradiographic procedure.

To investigate the hypothesis on the binding of bone-seeking isotopes to immature collagen, the uptake of bone-seeking agents was compared with the *collagen formation,* visualized autoradiographically by labelling with ^{3}H-proline (Weinstock and LeBlond 1974). After fixation in neutral buffered formaldehyde 4%, the tissue was decalcified in EDTA 4%, double-embedded in celloidin paraffin, and microautoradiography of the sections was performed by the dipping method.

To guard against chemography, sections from unlabelled rats were run

through the procedure, both for the frozen material and the decalcified, paraffin-embedded material.

The radionuclide uptake of ^{99m}Tc-MDP was compared with the *flow distribution in the epiphyseal region,* the latter being visualized autoradiographically by using a diffusible radioactive isotope administered intravenously (Sapirstein 1958). ^{99m}Tc-pertechnetate was administered 15 sec. and 5 min. respectively prior to flow stop by tourniquet on the thigh. Thereafter, contact autoradiography on the freeze-cut block was done.

With the object of elucidating *the affinity of ^{99m}Tc-MDP for the mineral phase of the bony tissue,* labelled sections were mounted on tape, counted in a gamma spectrometer before and after incubation for 10 min. in water or in 4% EDTA which decalcifies the sections. The activity released from the sections was examined chromatographically in 4 chromatographic systems, and the R_F values were compared with those for ^{99m}Tc-MDP, ^{99m}Tc-pertechnetate, ^{99m}Tc-Sn colloid, ^{99m}Tc-albumin, and ^{99m}Tc-EDTA in the corresponding systems.

Contact autoradiography afforded a good survey of the distribution of Tc-MDP which exhibited a narrow belt of particularly high activity at the

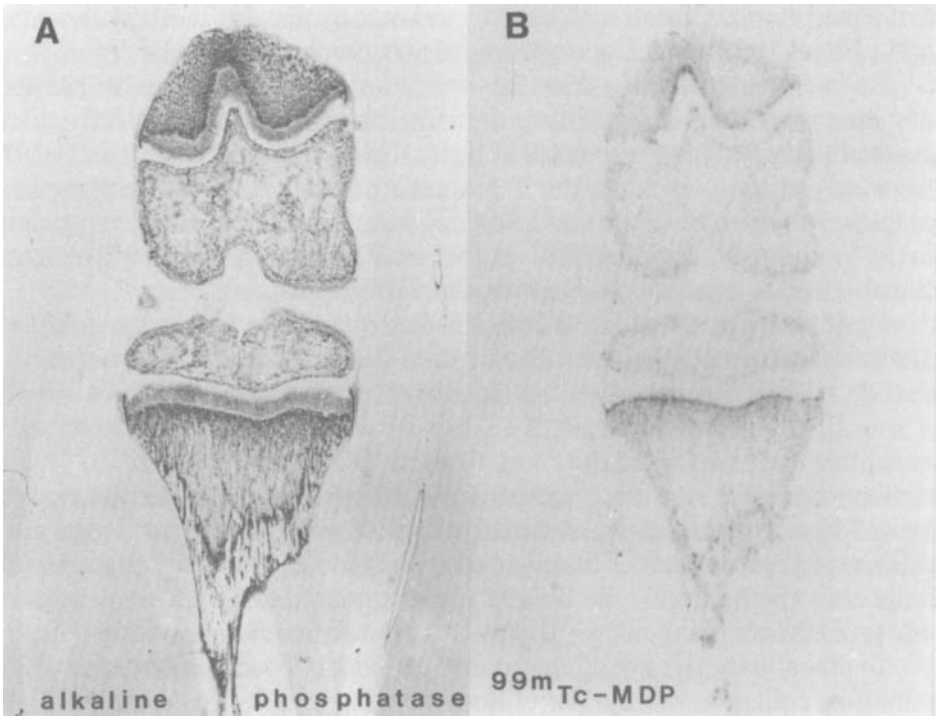


Figure 11. Macroautoradiographic distribution of Tc-MDP in epiphyseal growth plate and its relation to alkaline phosphatase activity. (A) Section of knee region stained for alkaline phosphatase activity. (B) Corresponding contact autoradiograph. Particularly high uptake of Tc-MDP is seen at osteochondral junction. At low magnification, gross distribution of radionuclide roughly corresponds with alkaline phosphatase activity. However, no uptake is seen in the alkaline-phosphatase-active cells of the hypertrophic zone of the epiphyseal cartilage, and in areas with more widespread activity, such as the periosteum, radioactive accumulation is seen in the innermost mineralized layer and not in relation to the area of enzyme activity (A and B × 4,7).

junction of epiphysis and metaphysis. This uptake was located microradiographically to that part of the provisional calcification in the cartilage matrix which was situated at the end of the vascular invasion into the cartilage. In low magnification there was quite good agreement between the radionuclide distribution and the distribution of alkaline phosphatase activity, at least in the bony tissue. On the other hand, there was no uptake of ^{99m}Tc-MDP in the alkaline phosphatase-active hypertrophic chondrocytes, and the provisional calcification with high radionuclide uptake did not show a high enzyme activity. This militates against the enzyme itself being the target of the Tc-phosphate complexes. There was a patchy increase in uptake in the periosteal area below the metaphysis, where a particularly marked osteoclastic activity was found, visualized by their acid phosphatase activity. At this site, the uptake was localized predominantly to the walls of Howship's lacunae, but there was some uptake also in the clasts themselves.

The tetracycline labelling with short labelling interval showed fluorescence of both formative areas, in particular the provisional calcifications and of the resorptive surfaces in Howship's lacunae. There was good agreement between the fluorescence of tetracycline and the autoradiographic uptake of ^{99m}Tc-MDP within the same section.

In contrast, there was poor correlation between the distribution of bone-seeking isotopes and collagen formation, assessed by ^{3}H-proline labelling. In

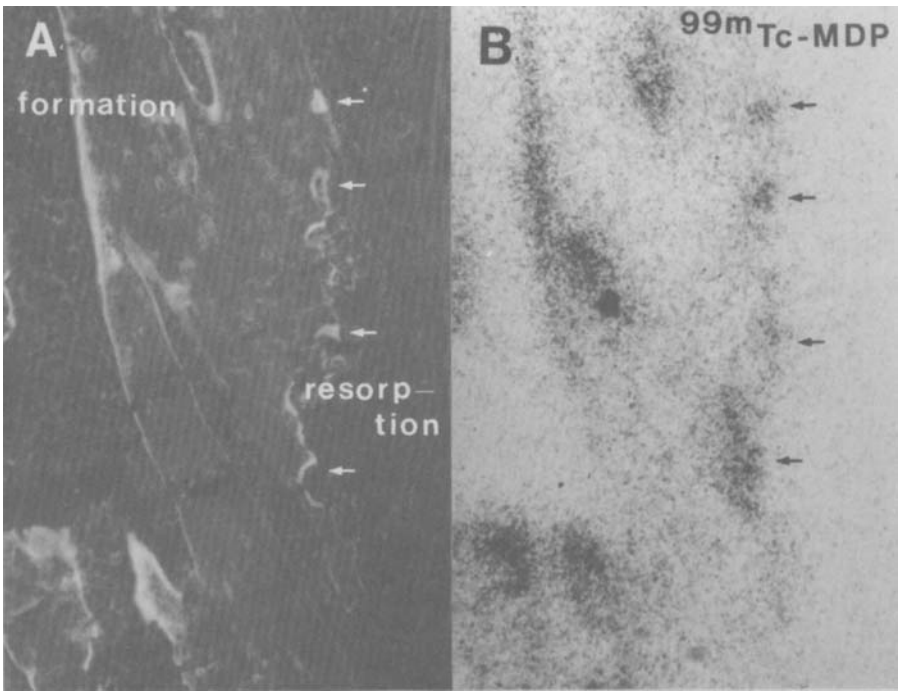


Figure 12. Uptake of Tc-MDP in periosteal osteoclastic area compared with tetracycline labelling. (A) Tetracycline fluorescence micrograph. Bone trabecula is seen at center with periosteal area to its right and marrow to its left. (B) Autoradiograph showing corresponding uptake of Tc-MDP. Radionuclide accumulation coincides with tetracycline fluorescence with short labelling period, since both label areas of resorption as well as of formation (× 108).

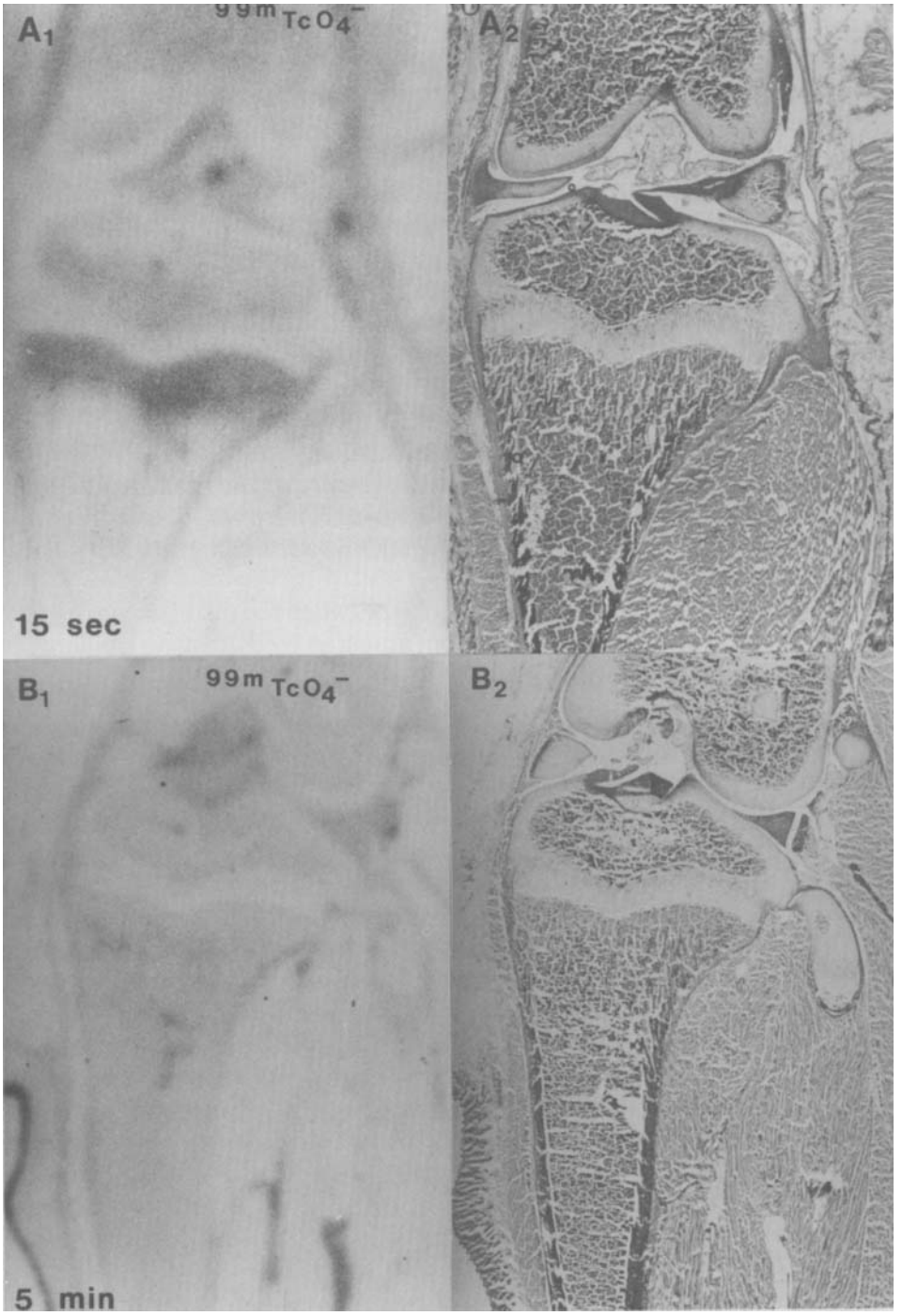


Figure 13. Blood flow of knee region illustrated by the distribution of pertechnetate given intravenously 15 sec. and 5 min. before arrest of flow. (A_1, B_1) Macroautoradiographs. (A_2, B_2) H & E staining of neighbouring sections (A:×13, B:×9). The whole metaphyseal area appears highly vascularized, but with low extraction rates as compared with other tissues, such as skin (B: 5 min). Blood flow alone does not seem to account for the high uptake of bone-seeking agents at provisional calcification (Fig. 11).

this labelling the uptake did not show a maximum at the provisional calcification, but osteoid formation increased just below the vascular coil at the vascular invasion into the epiphyseal cartilage. This militates against collagen formation as the factor determining the uptakes of bone-seeking isotopes.

Visualization of the flow distribution at a flow stop 15 sec. after administration of pertechnetate exhibited a marked flow in the metaphyseal bone, but not a distribution which could afford the sole explanation of the high uptake of ^{99m}Tc-MDP in the provisional calcification. Thus, it must be assumed that factors other than the flow influence the uptake of the bone-seeking isotopes.

In the decalcification experiments on *in vivo* labelled sections only 1.6% of the radioniclide uptake remained on the sections after 10 minutes' incubation in EDTA, while 98.8% of the activity remained on the sections after incubation in water.

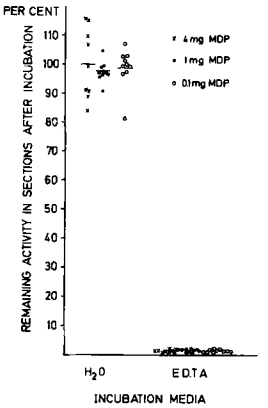


Figure 14. Decalcification experiments on sections labelled in vivo with Tc-MDP and incubated in either water or 4% EDTA. Almost complete loss of activity from EDTA-incubated sections is seen, and released radioactive compound could not be distinguished from Tc-MDP by chromatography.

The released activity could not, in the 4 stystems, be chromatographically distinguished from ^{99m}Tc-MDP, indicating that ^{99m}Tc-MDP labels the mineral phase of the bony tissue, and that no dissociation occurs on the crystal surface as suggested by Langenvelde et al. (1977).

In autoradiographic studies on the uptake of bone-seeking agents, Tilden et al. (1973) labelled human osteoarthritic femora. They found a linear uptake at the transition between bony tissue in different stages of maturity. In his able study on the uptake of bone-seeking agents in fracture healing, Greiff (1978, 1983) observed a more diffuse uptake, restricted to mineralized bone in callus tissue and epiphyseal growth plates.

In a microautoradiographic investigation Guillemart et al. (1978, 1980) used ^{95m}Tc and ^{96}Tc. Their longer physical half-lives (61 days and 4.3 days) allowed paraffin embedding and autoradiographs with good resolution, but the uptake of bone-seeking agents could not be related to enzyme activity. They observed, as we did, an increased uptake in relation to the osteoclasts.

By macroautoradiography Rohlin (1977) and Rohlin et al. (1978) found, following incubation of sections in a ^{99m}Tc-pyrophosphate-containing medium, an increased uptake on growing bone surfaces. This is compatible with the present findings.

IV. Localization of bone-seeking isotopes in osteoarthritis

LOCALIZATION IN ADVANCED HUMAN OSTEOARTHRITIS (IV)

Using ^{47}Ca and ^{85}Sr, Danielsson et al. (1963) performed scintimetric studies on 109 patients with osteoarthritis of the hip. They found increased uptakes in the osteoarthritic hip. This uptake was proportional to the severity of the disease, painful osteoarthritis of recent origin, however, showing a greater uptake than osteoarthritis of similar severity, but which had developed slowly.

With increasing use of ^{99}Tc phosphate complexes, bone scintigraphy has come into use in the diagnosis of articular diseases. In osteoarthritis, rheumatoid arthritis, sacroiliitis, infective arthritis as well as in posttraumatic synovitis, for example, there is an increased uptake (Hoffer and Genant 1976). Thus, the examination must be called non-specific, but extremely sensitive, unlike radiography.

In bone scintigraphy, even when using a gamma camera with a high resolution collimator, it is difficult to locate the bone-seeking isotopes accurately in the osteoarthritic joint and its surroundings. Therefore, it is of pathogenetic interest to elucidate the distribution by macroautoradiography.

Patients with osteoarthritis of the hip were injected intravenously, 2 hours before arthroplasty, with 10 mCi of either ^{99m}Tc-MDP or ^{99m}Tc-polyphosphate. The osteoarthritic femoral head was freeze-embedded and freeze-cut into halves on a hard-tissue cryostat. The cut surface of the remaining part of the head formed the background of the macroautoradiography, and the sections cut last were used for comparison after staining with heamatoxylin-eosin, for alkaline phosphatase activity, and with von Kossa's stain for calcium phosphate (Pearse 1968). The macroautoradio-

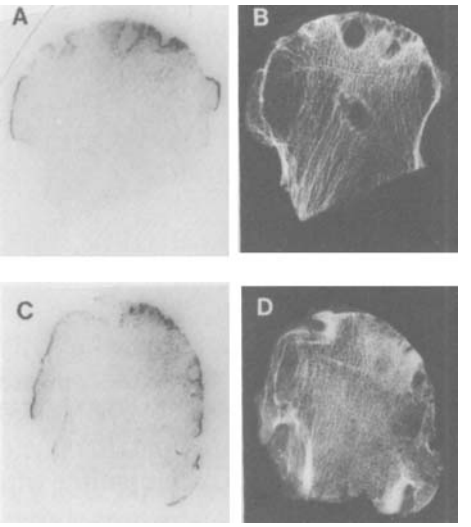


Figure 15. Autoradiographs of hemisections and roentgenographs of thin slices of osteoarthritic femoral heads from two patients, one given ^{99m}Tc-polyphosphate (Fig. 15, A) and the other ^{99m}Tc-methylene diphosphonate (Fig. 15, C) preoperatively.
On the right (Figs. 15, B and 15, D) are the roentgenographs of thin slices of the femoral heads that included the surface, on which each film had been placed to make the autoradiographs. The radionuclide has predominantly accumulated in the weight-bearing areas, particularly in the cyst walls and in the osteophytes at the osteochondral junctions.

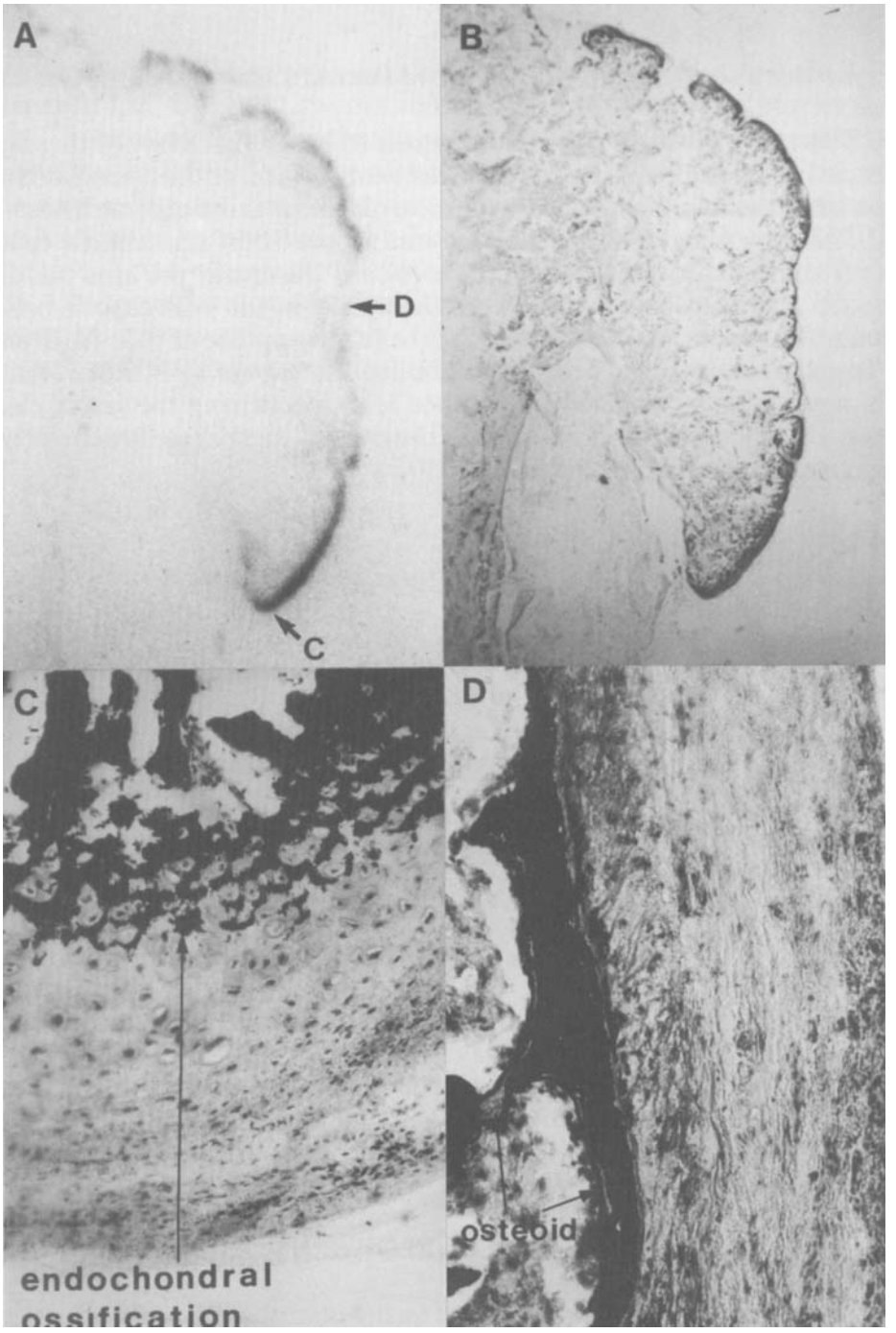


Figure 16. Radionuclide and alkaline phosphatase distributions in an osteophyte from a patient who was given ^{99m}Tc-methylene diphosphonate preoperatively.
Fig. 16, A: Autoradiograph (× 9.3). Fig. 16, B: Section adjacent to 16, A stained for alkaline phosphatase activity (× 9.3). Figs. 16, C and 16, D: Sections stained for calcium phosphate (von Kossa stain, × 1.16). Figs. 16, C and 16, D correspond to areas C and D marked on Fig. 15, A.
In Figs. 16, A and 16, B the distributions of radionuclide and alkaline phosphatase seem to coincide. Areas showing high uptake of the radionuclide at the osteochondral junction correspond to the areas of pronounced enchondral ossification in the section stained for calcium phosphate (Fig. 16, C), whereas areas with no enchondral ossification but containing osteoid (Fig. 16 D) show less uptake.

graphy was also compared with X-rays of the remaining half of the femoral head.

This study revealed a markedly increased uptake in the weightbearing area, in cystic walls beneath the denuded joint surface, at the osteochondral junction of the osteophytes, and in fibrocartilaginous areas of some cysts.

The autoradiographic distribution was supported by scintimetric counting of activity in various areas of the sections. The uptake per area was expressed as a focus-background ratio, the uptake in the joint capsule being used as the background. Comparison of the relative uptake of ^{99m}Tc-MDP and ^{99m}Tc-polyphosphate gave higher values for the former ($p < 0.01$, Mann-Whitney, Fisher's combined significance test), confirming the faster clearance of diphosphonates from the blood and thereby a lower focus-background ratio (Subramanian et al. 1975).

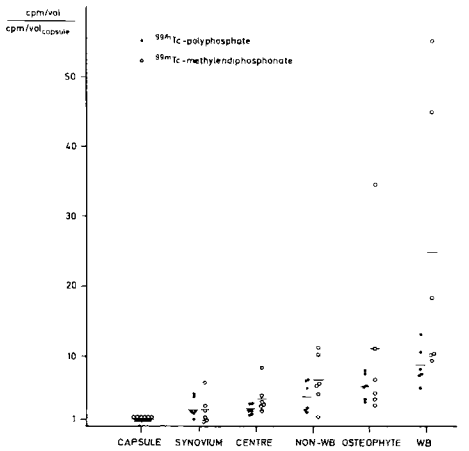


Figure 17. Target-to-background uptake ratios for different areas in the twelve osteoarthritic femoral heads studied, using the uptake of radionuclide in the fibrous capsule as background and the denominator of the ratios. Non-WB = non-weight-bearing area and WB = weight-bearing area.

In other words, the increased uptake by the osteoarthritic femoral head was not evenly distributed, and the distribution of radionuclide uptake was analogous with that of alkaline and acid phosphatase activity (II).

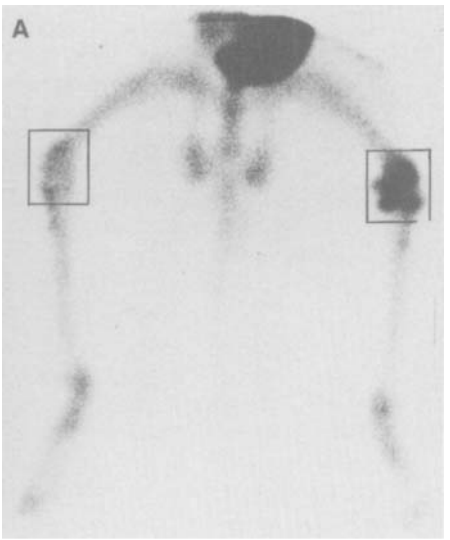
Thus, the increased bone scintigraphic uptake in osteoarthritic joints reflects the remodelling processes subchondrally, primarily in the weightbearing, denuded area, and also the growth of osteophytes.

LOCALIZATION DURING THE DEVELOPMENT OF EXPERIMENTAL OSTEOARTHRITIS (VII)

In osteoarthritis an increased uptake of technetium phosphorous compounds has been noted even at very early stages of the disease (Hoffer and Genant 1976). This has been found also when using calcium isotopes or their analogues (Danielsson et al. 1963).

Accordingly, it was of pathogenetic interest to elucidate where an increase, if any, in the uptake is situated morphologically in the pre-osteoarthritic joint, while it is still radiographically normal or shows only very mild signs of incipient osteoarthritic changes. To this end, we used Hulth and Telhag's experimental rabbit model, based upon articular instabi-

lity. A total of 42 rabbits were included in the experiments, groups of 6 being examined 1, 2, 4, 6, and 12 weeks and also 6 and 18 - 24 months postoperatively. The unstable as well as the sham-operated control knee were subjected to scintimetry 3 hours after i.v. administration of 5 mCi ^{99m}Tc-MDP, the rabbits were killed, and the knees were examined by macroautoradiography, partly as in the human studies by X-ray films, partly by nuclear plates (G5 emulsion, Agfa Gevaert), upon which tape with sections attached was fastened. After separation and fixation, the sections were stained with HE, for glycosaminoglycans with safranin-O, for calcium phosphate by the von Kossa method, and for alkaline phosphatase activity by the method of Burstone.



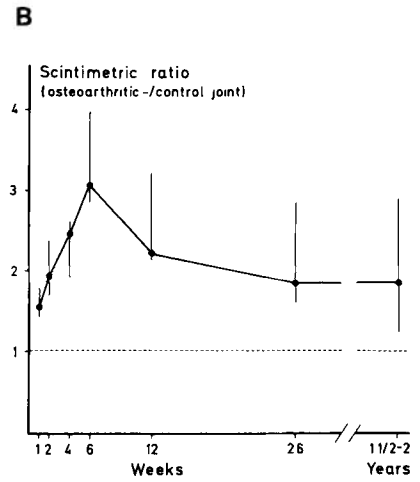


Figure 18. Bone scintigraphy of rabbit with OA of the left knee 6 months post-operatively showing the increased uptake on the osteoarthritic side and the region of interest used in scintimetry (A). B: Scintimetric ratios between the uptake in the unstable and the control joint at different intervals postoperatively (median and quartiles). Highest ratios were recorded in the early stages of the osteoarthritic development.

Scintimetric measurements over the knee regions revealed a relatively increased uptake in the unstable joint at all postoperative intervals as compared with the uptake in the control knees. The ratio was significantly

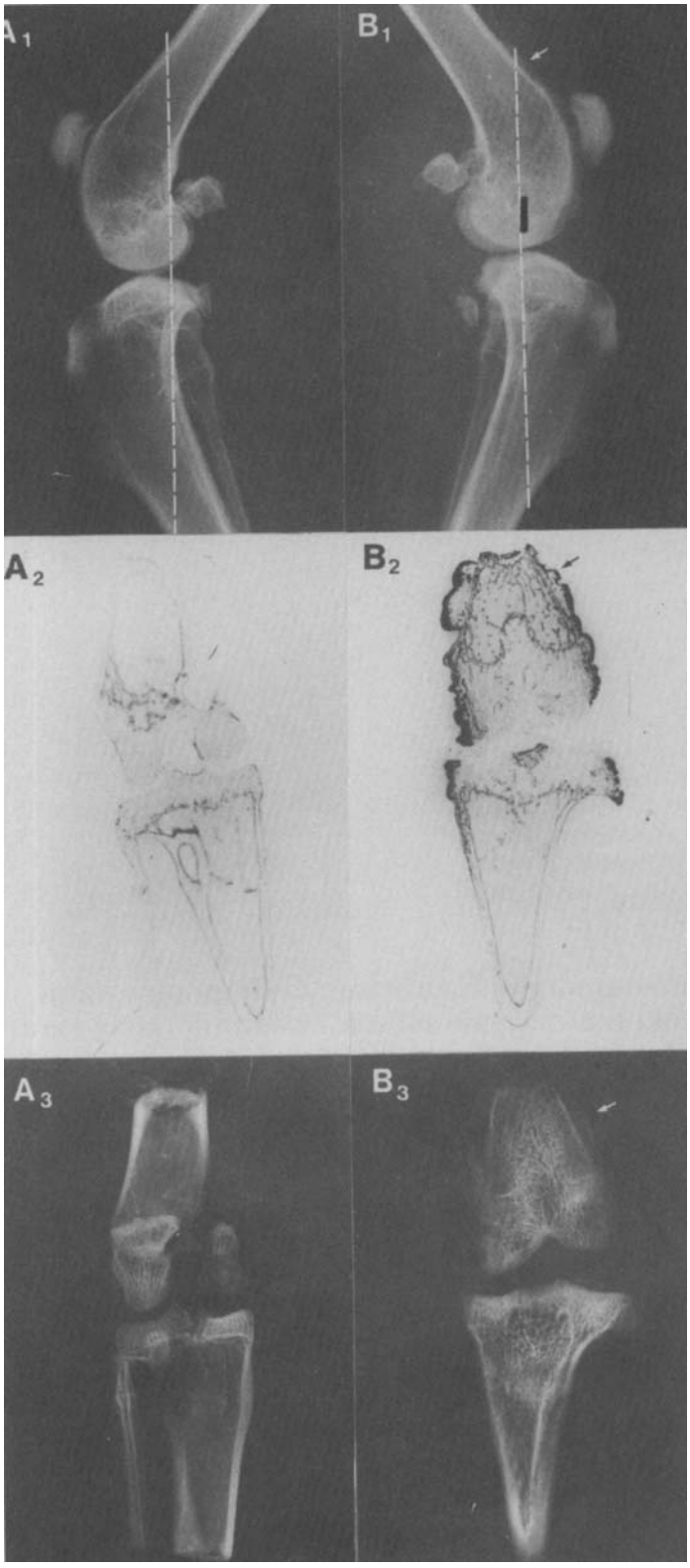


Figure 19. Distribution of ^{99m}Tc-MDP in the unstable (B) and the control joint (A) 6 weeks postoperatively. Radiographs of the control (A_1) and the unstable knee (B_1), showing osteophytic formation in the unstable joints (arrow) and the approximate planes used for autoradiography after sectioning. A_2B_2: Contact autoradiography on X-ray films compared with radiographs of the embedded specimens (A_3B_3). Highly increased osteophytic uptake (arrows) is seen in the unstable joint (B_2), but no obvious increase in uptake is visible in the subchondral bone.

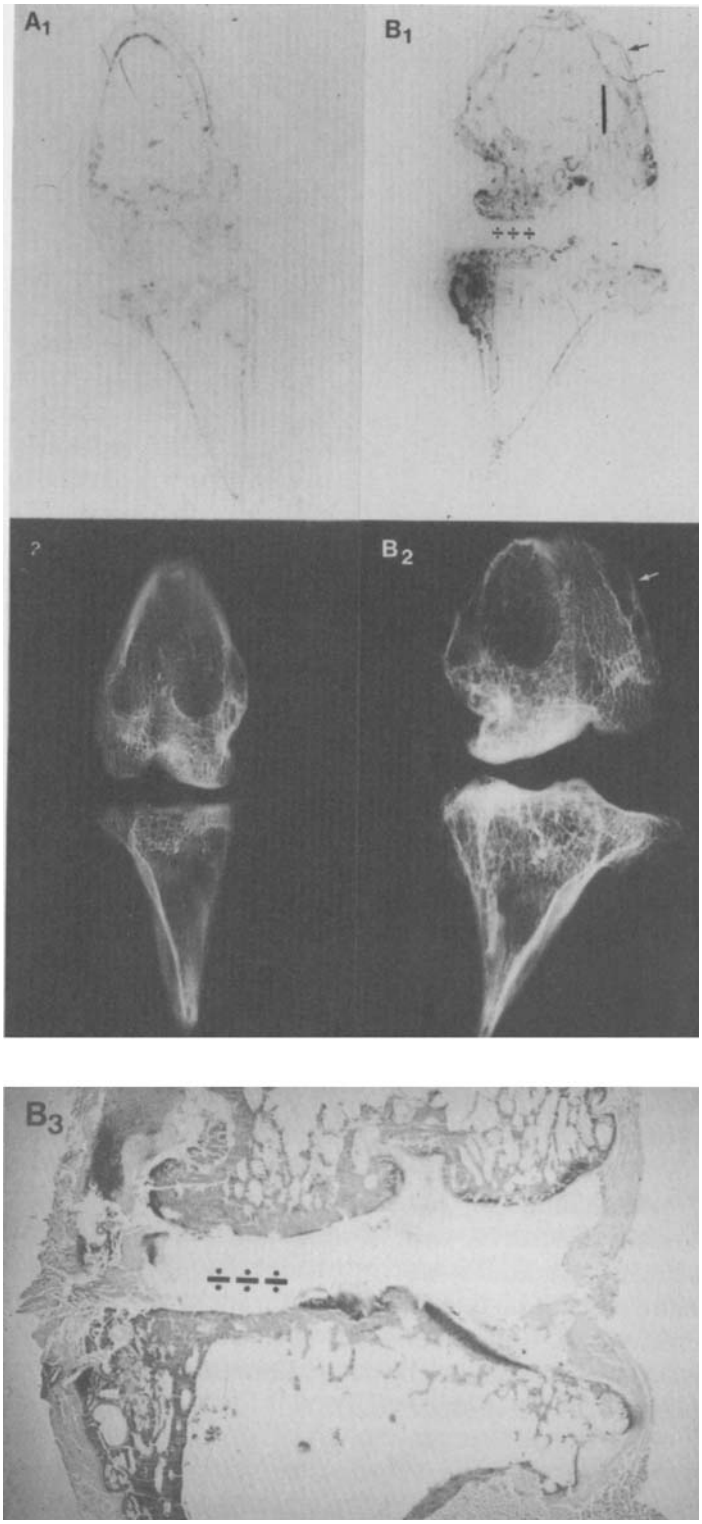


Figure 20. Uptake of bone-seeking agents in advanced experimental osteoarthritis 2 years postoperatively. A_1B_1: Contact autoradiographs from the control (A) and the osteoarthritic knee (B). A_2B_2: Corresponding radiographs of the embedded specimens. Autoradiographs were based on the activity in sectioned surfaces of the specimens. B_3: Adjacent section of the osteoarthritic joint, stained for glycosaminoglycans with Safranin-O, showing eburnization with lack of cartilage in the medial compartment of the joint (× 7). +++ indicate area lacking cartilage. In advanced OA, increased uptake of bone-seeking agents is seen in the subchondral bone in the denuded area and around marginal cysts (synovial cysts). A majority of the osteophytes have at this stage of the experimental model no increased uptake (arrows).

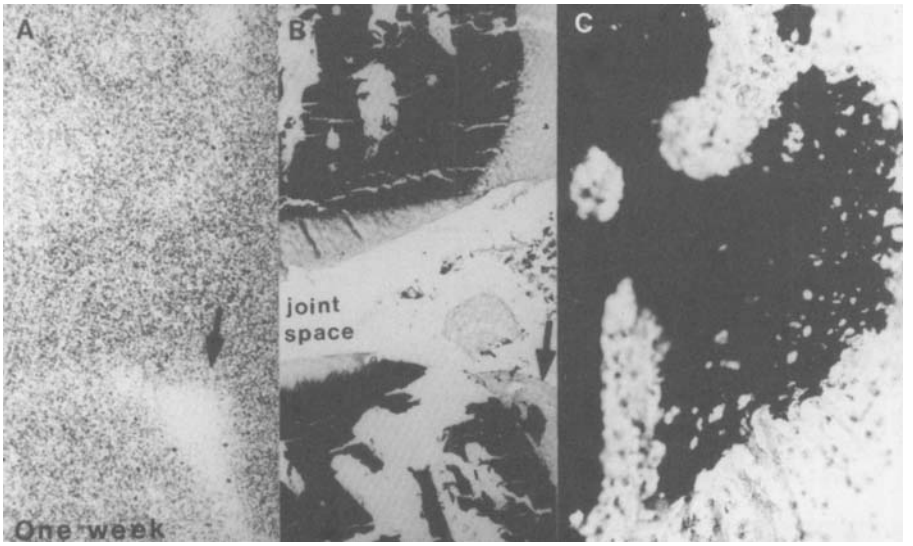


Figure 21. Osteophytic uptake as early as one week postoperatively. A: Contact autoradiography on nuclear plate (dark-field microscopy showing silver grains white) from section shown in B. B: von Kossa stain for calcium deposits in section showing the joint space with marginally localized calcifications in the tibial periosteal layer. Increased uptake corresponds to the area of periosteal calcifications (arrows) (AB: × 12,5). C: Osteophytic area at higher magnification (× 125) showing the first sign of an osteophyte one week postoperatively, viz. deposition of calcium (black) in the periosteum.

higher in the early stages (4-12 weeks) than in the later ones, 18 - 24 months ($p < 0.01$, Mann-Whitney). According to the autoradiography, uptake by the osteophytes was responsible for the high, early uptake, whereas subchondral uptake in the medial joint chamber was not found until the osteoarthritis was advanced.

Incipient osteophyte formation, with an increased uptake of bone-seeking isotopes, was seen on histological and autoradiographic examination, but not on radiography, as early as one week postoperatively. In late stages many osteophytes in this model had lost the marked uptake at the osteochondral junction.

The above-mentioned findings in this model do not support Radin's theory of subchondral microfractures, with newformation of bone, preceding the cartilaginous degeneration. It is worthy of note that a few arthrotomized control knees showed faint histological and autoradiographic signs of incipient osteophyte formation. Consequently, it is reasonable to imagine that the osteophyte formation was due to the posttraumatic synovitis incorporated in this model during the early stages (III).

Final considerations

The present studies have demonstrated an increased uptake of the bone-seeking agents, on formative as well as resorptive surfaces, including in particular areas showing provisional calcification in the enchondral ossification. Even though the perfusion has some influence upon the quantitative uptake, local factors on the bony surface must be responsible for a differing extraction of the bone-seeking isotopes.

The ^{99m}Tc-phosphate complexes bind to the inorganic phase. It is most reasonable to assume that it is the area of free crystal surface, available for adsorption, which differs. In mature skeletal tissue the crystals are of an inter-as well as intra-fibrillar location, surrounded by the collagen fibrils (Glimcher 1976, Lees 1979). Indeed, it has been demonstrated that polar groups in collagen block adsorptive crystal surfaces (Holmes et al. 1971). The absence of collagen block may explain the increased uptake in the formative areas as well as in the resorptive surfaces in Howship's lacunae.

By electron microscopy, Lucht (1972 a, b) has demonstrated free HAP crystals in and beneath the ruffled border of the osteoclast, and these free crystals may cause the increased uptake in the resorptive areas. The hypothesis of a collagen block is supported by the fact that even in solution partially soluble collagen polypeptide can inhibit the adsorption of MDP onto HAP crystals *in vitro* (Christoffersen et al. 1983).

In the present study on experimental osteoarthritis the extremely early growth of osteophytes, demonstrated by ^{99m}Tc-MDP labelling, coincided with the early, marked GAG depletion diffusely in the joint which later proved reversible. It is reasonable to assume, therefore, that these changes result from the early posttraumatic synovitis demonstrated by the ^{133}xenon washout. This is indicated also by the fact that the control-arthrotomized knees also showed faint changes during the very early postoperative period. Accordingly, the osteophyte formation, and even the GAG depletion, may be non-specific reactions to a joint damage which need not necessarily lead to osteoarthritis. If the irritative condition of the joint subsides sufficiently soon, the capacity for regeneration must be assumed to be enough for the injury to heal, which it presumably very often does. This is compatible with the findings of Danielsson (1964) who followed patients with accidentally diagnosed osteophytes in the hip joint for 11 years, and only one out of 86 developed manifest osteoarthritis. Thus, the scintigraphic finding of osteophyte uptake is a non-specific early reaction, whereas the later subchondral uptake is more related to osteoarthritis and reflects the remodelling processes in the subchondral bone, usually beneath the joint surface, showing the most widespread cartilaginous degeneration in the weightbearing area. The image resolution obtainable by bone scintigraphy does not yet allow a distinction between the two fundamentally different forms of uptake. Therefore, it still indicates only an active, non-specific joint affection.

Summary

The present study was undertaken to investigate the sites at which the ^{99m}Tc phosphorous compounds bind to skeletal tissue in general, and their localization at different stages of osteoarthritis in particular, in order thereby to arrive at a better morphological basis for interpreting the scintigrams. It was endeavoured also to relate the uptake of bone-seeking agents to abnormal changes in cartilage, synovium, and subchondral bone to obtain better insight into the pathogenesis of osteoarthritis.

1. The bone remodelling acitivties in subchondral bone in osteoarthritic human femoral heads were elucidated by the alkaline phosphatase activity of osteoblasts and the acid phosphatase activity of osteoclasts. The enzyme activity was measured semiquantitatively by the initial time for the histochemical reaction. The distribution of the activity of the two enzymes in different areas proved parallel, and considerable variation in enzyme activity was seen between different areas within the same femoral head. Increased osteoarthritic cartilaginous changes were associated with increased subchondral enzyme activity, highest in denuded weightbearing areas and in the osteophytes and lowest in non-weightbearing subchondral bone and centrally in the femoral head.

2. Studies on different histochemical staining of glycosaminoglycans in the matrix of human osteoarhritic cartilage and of normal cartilage revealed a heterogeneous distribution of the different glycosaminoglycans through the cartilage. Except for superficial loss of glycosaminoglycans, no difference was found in the distribution of keratan sulphate between osteoarthritic cartilage and control cartilage. In osteoarthritis, however, a relative increase in stainability for chondroitin sulphate was found in the territorial area, especially around the cell clusters, and only chondroitin sulphate was present in the cartilage of osteophytes. These findings were interpreted as an increased GAG metabolism, its mode of production being like that of very young cartilage.

3. In the experimental rabbit model used in studying the uptake of bone-seeking agents this GAG regeneration was able to refill demasked collagen network with glycosaminoglycans in certain areas of the joint. The height of the depleted superficial area was estimated on patellar cartilage stained for sulphated GAG with toluidine blue-0 at pH 3, visually and by optical densitometry using the wavelength corresponding to the γ-band of toluidine blue. The time relation of the surface depletion was elucidated. A marked depletion of GAG, seen one week postoperatively, reached a maximum at 4 weeks. Thereafter, the depleted area diminished as a result of GAG regeneration. The initial, severe depletion was regarded as a non-specific consequence of the simultaneous synovitis demonstrated in the ^{133}xenon washout studies, and not necessarily as a typical morphological sign of osteoarthritis.

4. Studies of ^{133}xenon washout rates from the synovial cavities of rabbit knees with experimentally induced osteoarthritis showed that an appreciable synovial reaction was incorporated in this model based on joint instability for up to six months postoperatively.

5. In skeletal tissue an increased uptake of ^{99m}Tc-MDP was found in mineralization areas, especially in areas of calcification, such as the provisional calcification of the epiphyseal growth plate. In addition to areas of mineralization, resorption surfaces showed an increased uptake in relation to osteoclasts. The autoradiographic uptake corresponded to the fluorescence of tetracycline given with a short labelling period.

Neither alkaline phosphatase nor newly formed collagen, the latter judging by ^{3}H-proline labelling, was the target of the bone-seeking agents. Decalcification experiments on sections labelled *in vivo* confirmed that the mineral phase of bone was labelled with the bone-seeking agents.

6. In advanced human osteoarthritis of the hip the macroautoradiographic distribution of the bone-seeking agents within femoral heads removed at replacement surgery was not evenly distributed. Their distribution was similar to that of alkaline and acid phosphatase activity. The highest uptake was seen in weightbearing subchondral bone, cyst walls, and at the osteochondral junction of the osteophytes. The autoradiographic findings were confirmed by impulse counting in the different zones.

Thus, the increased bone scintigraphic uptake in advanced ostearthritic joints reflects the remodelling processes of the subchondral bone and the growth of osteophytes.

7. In osteoarthritis of rabbit knees experimentally induced by joint instability, the very early increase in uptake from one week postoperatively was found in growing osteophytes even before they could be visualized radiographically. They caused the highest scintimetric ratios between the osteoarthritic and the control knees obtained after 6 weeks. Autoradiographically increased subchondral uptake was not seen until at advanced osteoarthritis of the medial joint chamber.

The studies of the experimental osteoarthritic model have intensified interest in the synovial reaction in osteoarthritis, as typical osteoarthritic changes, such as osteophyte formation and an early superficial loss of glycosaminoglycans from the cartilage, may be caused by, and did indeed conicide with, the posttraumatic synovitis incorporated in this model.

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The studies underlying the present paper were carried out in the Calcified Tissue Research Laboratory of the Department of Orthopaedic Surgery U, Rigshospitalet, Copenhagen, where I was serving as research assistant.

From the outset, I was involved in morphological studies, but as research assistant it was my duty to be in charge of the bone scintigraphic investigations of orthopaedic-surgical patients on our rectilinear scanner. This task was soon taken over by the Department of Nuclear Medicine, and we were left with an insight into the clinically applicable scintigraphic method - and also with a distinct feeling of ignorance concerning the underlying physiological mechanisms and the localization of the uptake of bone-seeking isotopes. As the Calcified Tissue Research Laboratory possessed a heavy-duty cryomicrotome, I saw a chance of being able to elucidate, at least morphologically, the localization of the administered tracer in the skeletal tissue. From the very beginning I was greatly encouraged in this work by Professor Carl Arnoldi from whom I have at all times received support and competent guidance. With Inge Reimann, M.D., who introduced me to bone morphology, I have ever since enjoyed a long-lasting, close and pleasant collaboration. By her enthusiasm and constructive criticism she has created the research atmosphere in the Laboratory.

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Copenhagen, June 1985
Steen Bach Christensen

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