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The Subchondral Bone Plate of the Femoral Head in Adult Rabbits

II. Changes Induced by Intracartilaginous Defects Studied by Microradiography and Tetracycline Labelling

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Summary. Standardized, intracartilaginous articular cartilage defects on the femoral head in adult rabbits were studied histologically and autoradiographically after ^{35}S-sulphate labelling at 7 observation times between 5 days and 12 weeks. Undecalcified sections from the same defects were studied microradiographically and fluorescence microscopically after administration of Tetracycline. Interest was devoted mainly to the mineralized structures beneath the articular cartilage defect. The following histological and autoradiographical observations were made:

The articular cartilage in the defect area showed loss of matrical metachromasia, retention or increase of sulphate uptake over chondrocytes close to areas of chondrocytes with no sulphate uptake, multinuclear chondrones and in isolated cases fibrillation of the matrix at longer observation times.

The microradiographic and fluorescence microscopic examinations showed the following:

1) There was a rapid loss of mineral in the calcified articular cartilage, around vascular canals in the cortical part of the subchondral bone, from osteones and from adjacent lamellar bone surfaces after 5 days. This progressed up to 2–3 weeks.

2) The number of fluorescent bone surfaces increased markedly up to 3 weeks, after which there was some decrease, with the exception of the lamellar bone surfaces which still showed increased fluorescence after 12 weeks.

3) Massive fluorescence was seen in the tidemark from 2 weeks onwards.

4) The changes were limited to tissues beneath the defect area.

Introduction

Numerous experimental procedures have been used in an attempt to shed light on the aetiology of the clinical picture designated "primary osteoarthritis" (for references, see Key, 1931; Gardner, 1960; Meachim, 1963). Experimental methods which lead to rapid destruction of the articular cartilage causing secondary changes in the bone, apparently resembling human osteoarthritis, distort its true picture, however (for references, see Thaxter *et al.*, 1965). The beginning of osteoarthritis probably consists of a local disintegration of the cartilaginous matrix, with a slow tendency to progression (for references, see Harrison *et al.*, 1953), while the chondrocytes have been found to have a normal or even increased uptake of ^{35}S-sulphate, indicating retention of cellular function (Collins and McElligott, 1960). Meachim (1963) took these observations into consideration in his animal experiments with "scarification" of the articular cartilage in the knee joint of adult rabbits. Changes of the articular cartilage resembling those in osteoarthritis were

observed, but the subchondral bone was described as normal up to 44 weeks postoperatively. Other investigators have also found, on histological examination, that the subchondral bone has remained unchanged in experimental studies of superficial articular cartilage defects, for example Bennet and Bauer (1935) in the dog, Bucher (1955) in the guinea pig, Imerlishvili (1957) in the cat and DePalma *et al* (1966) also in the dog.

The question of whether intracartilaginous lesions of the articular cartilage can lead to changes in the subcartilaginous mineralized structures and to accelerated remodelling of the subchondral bone is of topical interest, however, since the latter process is an integrating component of the pathological picture in osteoarthritis (for references, see Harrison *et al.*, 1953; Johnson, 1959; Stecher, 1961).

In a previous investigation the structure and spontaneous remodelling of the subchondral bone plate in the femoral head of adult rabbits was studied by means of microradiography and fluorescence microscopy after administration of Tetracycline (Lemperg, 1971). The subchondral bone tissue was found normally to have a low metabolism in rabbits over 1 year of age.

The principal aim of the present investigation was to study the early effect of limited intracartilaginous articular cartilage defects on the calcified articular cartilage and the subchondral bone tissue in the area of the defect. Further, studies were made to determine whether such articular cartilage defects have any general stimulatory influence on the remodelling of the subchondral bone tissue outside the defect area in the femoral head. Microradiography and fluorescence microscopy after administration of Tetracycline, as well as histological methods and autoradiography after *in vitro* labelling with ^{35}S-sulphate, were used.

Material and Methods

The studies were made on 54 rabbits of both sexes with a nominal age of at least 8 months and weighing 3.5–4.4 kg.

Operative Procedure

Under Nembutal anaesthesia and with sterile conditions a defect measuring about 3×3 mm was created on the dorsal articular surface of the femoral head about 2 mm medial to the borderline between the head and neck of the femur (zone III)[1]. The joint was not luxated in this procedure. A small curved raspatory was used and an attempt was made to avoid penetration of the defect beyond the borderline between calcified and non-calcified articular cartilage. The joint capsule and muscles were sutured with catgut. No attempt at postoperative immobilization was made and no antibiotics were given.

In a control group of 5 animals only the joint capsule was opened and the articular cartilage was left intact.

Observation Times and Preparation of Examination Material

After 5 days or 1, 2, 3, 5, 8 or 12 weeks (7 animals with a defect at each observation time and in the control group 1 animal at 1 week and 2 each at 5 and 12 weeks) the animals were killed. Two to four days before death they were given an intravenous injection of oxytetracycline in a dose of 25 mg/kg body weight. The femoral head and neck and adjacent trochanteric area from the operation side were excised after macroscopic inspection of the hip joint. With a fine bandsaw the femoral head was divided in the coronal plane through

1 The division of the articular surface of the femoral head into 7 zones is shown in Fig. 1 in the preceding article in this volume (Lemperg). Zone III is the defect area.

the middle of the defect area. One half of the head was fixed, dehydrated in absolute alcohol and embedded in methyl methacrylate. The other half was incubated in 2 ml of Tyrode's solution containing 1 mC ^{35}S-sulphate [2] for 1 h and washed in saturated sodium sulphate solution as described previously (Lemperg, 1967). The examination material of the other side was treated in the same way.

Microradiography and Fluorescence Microscopy

Sections about 1 mm thick were sawn from the plastic-embedded block, starting from the divisional surface in the coronal plane. The sections were ground down to a thickness of 80–100 μ, and some further to 50 and 30 μ. Microradiography was performed in a Philips PW 1010/30 apparatus at 15 kV; the preparations lay in direct contact with Kodak spectroscopic plates 649–0. For fluorescence microscopy the preparations were mounted in Permount and examined in a Zeiss fluorescence microscope. The filter combinations of exciter filters BG 38 and UG 1 with barrier filter 41 and exciter filters BG 38 and BG 12 with barrier filters 50 and 65 were used. The fluorescence preparations were examined with a linear magnification of 80 and 200 times.

Histology and Autoradiography

The preparations were fixed in a 4% aqueous formalin solution containing 0.5% cetylpyridinium chloride, decalcified in a mixture of equal parts of monosodium citrate (20%) and formic acid (44%) and embedded in paraffin. Beginning from the divisional surface in the coronal plane, serial sections 5 μ thick were cut. The first 80 sections were used for autoradiography and every following 5th section for histological examination. The entire defect area and adjacent intact parts of the joint were sectioned. Autoradiography was performed by the dipping technique (Ilford K 2 in gel form, diluted with distilled water 2:1). After developing, some preparations were stained with haematoxylin and eosin or 1% toluidine blue in an aqueous solution, while others remained unstained. The histological preparations were stained in the same way.

Results

Macroscopic Observation

All animals were able to move about unhindered during the period of observation. In 2 animals, one observed at 5 weeks and the other at 8 weeks, an increased amount of turbid fluid was found in the joint and macroscopically the articular cartilage appeared eroded. These two animals were excluded from further study. In the other animals nothing noteworthy was observed apart from the expected postoperative changes. The mobility of the joint was completely normal. The defect on the femoral head was well visible in all cases.

Histological and Autoradiographic Observations

The preparations from all animals were examined with respect to the depth of the articular cartilage defect. The following observations were regarded as a sign that the subchondral bone was primarily involved: opened vascular canals in the calcified articular cartilage with a visible defect in the bone tissue, small cartilaginous islets or granulation tissue in the bone tissue beneath the defect, and the presence of mesenchymal cells at different stages of differentiation and fibrous tissue in the articular cartilage defect. These animals were excluded from the presentation of the results.

The following report refers only to the 25 animals in which the base of the defect lay within the non-calcified articular cartilage or on the borderline between this and the calcified articular cartilage. The distribution of the animals according to the different observation times is given in the Table.

2 Carrier-free ^{35}S-sulphate, SJSI. The Radiochemical Centre, Amersham, England.

Table. *Observations on Tetracycline labelled structures at different observation times*

Observation times	Number of animals	Zone III				Zone II + IV			
		a	b	c	d	a	b	c	d
5 days	3	—	—	1	2	—	—	1	1
1 week	3	1	1	1	2	—	—	—	—
2 weeks	5	2	4	5	5	—	1	1	1
3 weeks	4	4	2	4	3	—	—	1	2
5 weeks	2	—	2	2	1	—	—	—	—
8 weeks	3	1	—	2	2	—	—	—	—
12 weeks	5	2	1	1	4	—	—	—	—

The figures in columns a–d indicate the number of animals showing a greater number of labelled structures on the treated side than on the untreated side. Zone III: defect area; zones II and IV: areas laterally and medially adjacent to zone III. a = tidemark, b = osteones, c = intracartilaginous canals, d = lamellar bone surfaces delimiting medullary cavities (for details, see text).

A typical picture of the *defect area* is shown in Fig. 1. Small areas containing chondrocytes with no sulphate uptake alternated with areas of chondrocytes showing a high sulphate uptake. Multinuclear chondrones were often observed in these areas at longer observation times. The staining with toluidine blue of the cartilaginous matrix in the defect area and the articular cartilage at the margin of the defect was usually reduced. No appreciable difference was observed with regard to these changes at the different observation times up to 12 weeks. Fibrillation of the cartilage was seen in some cases at observation times over 5 weeks but was not a regular finding.

The *subchondral bone* beneath the defect area showed no changes at observation times up to 2 weeks. In 2 cases, at 3 and 5 weeks, occasional enlarged intracartilaginous vascular canals were seen, and the zone of calcified cartilage appeared to be rather narrower than in the adjacent articular cartilage. At observation times of 8 and 12 weeks, in 2 further animals, similar changes were seen. In one case at 12 weeks the tidemark (calcified line) lay at a somewhat higher level in parts of the defect area (Fig. 2), while in other parts it was not visible. In this case some blood vessels had invaded the deepest layer of the articular cartilage (Fig. 2).

The articular surface of the femoral head outside the defect area showed no changes wether in the articular cartilage or in the subchondral bone.

In the animals in the *control group* neither the articular cartilage nor the subchondral bone showed any changes.

Microradiographic and Fluorescence Microscopic Observations

The right and left femoral heads were placed together and the zones I–VII were marked as identically as possible on the preparations with a fine mapping pen. The occurrence of tetracyclineinduced fluorescence was recorded in the following structures: a) tidemark, b) osteones, c) intracartilaginous vascular canals and d) lamellar bone tissue bordering against the medullary cavity lying nearest to

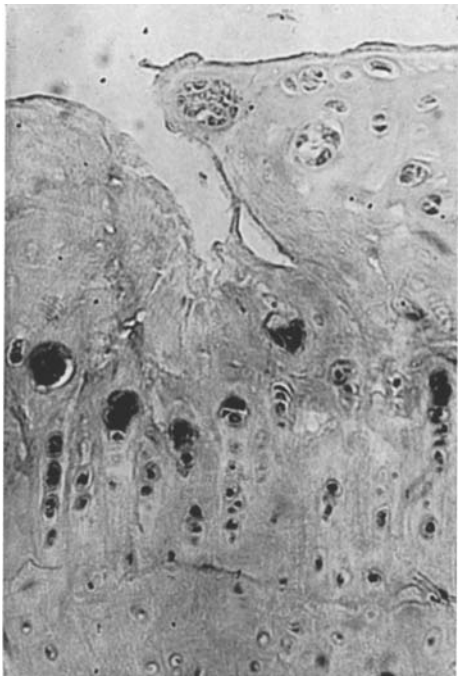


Fig. 1

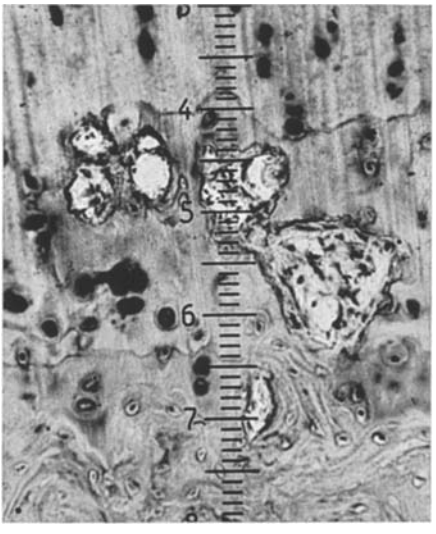


Fig. 2

Fig. 1. The defect area at an observation time of 8 weeks. Multinuclear chondrones are lying at the margin of a fissure in the articular cartilage. The matrix shows disintegration and a number of chondrocytes show low or no uptake of ^{35}S-sulphate. Intensive ^{35}S-sulphate uptake over the condrocytes in the deepest part of the articular cartilage. Autoradiogram after labelling with ^{35}S-sulphate, stained with haematoxylin and eosin. ×360

Fig. 2. Borderline area between articular cartilage and bone beneath the defect at an observation time of 12 weeks. The tidemark (calcified line) is broken and part of it lies at a higher level. Some osteocytes are seen close to invading blood vessels. The chondrocytes of the articular cartilage show a high uptake of ^{35}S-sulphate. Autoradiogram after ^{35}S-sulphate labelling, stained with haematoxylin and eosin. ×360. Calibration of the micrometer scale: 1 scale divison = 8 μ

the articular cartilage; only that surface facing the articular surface was evaluated. In the tidemark fluorescence was recorded as present or absent. In the subchondral bone and the calcified articular cartilage the number of labelled structures b–d were counted, and identical areas from the treated and untreated femoral heads were compared.

Observations on the Untreated Side

In order to check that the untrated side was "normal", the total number of labelled structures from the whole group was compared with the findings in the previously studied normal material (Lemperg, 1971). No difference was found for structures a, b and d. For structure c somewhat higher values were obtained (see below). Further, the values from animals with observation times of 5 days–3 weeks were compared with those from animals with observation times of 5–12 weeks; no differences were found. On comparison of the values for structure c in zone III with those for the same structure in the other zones, slightly higher

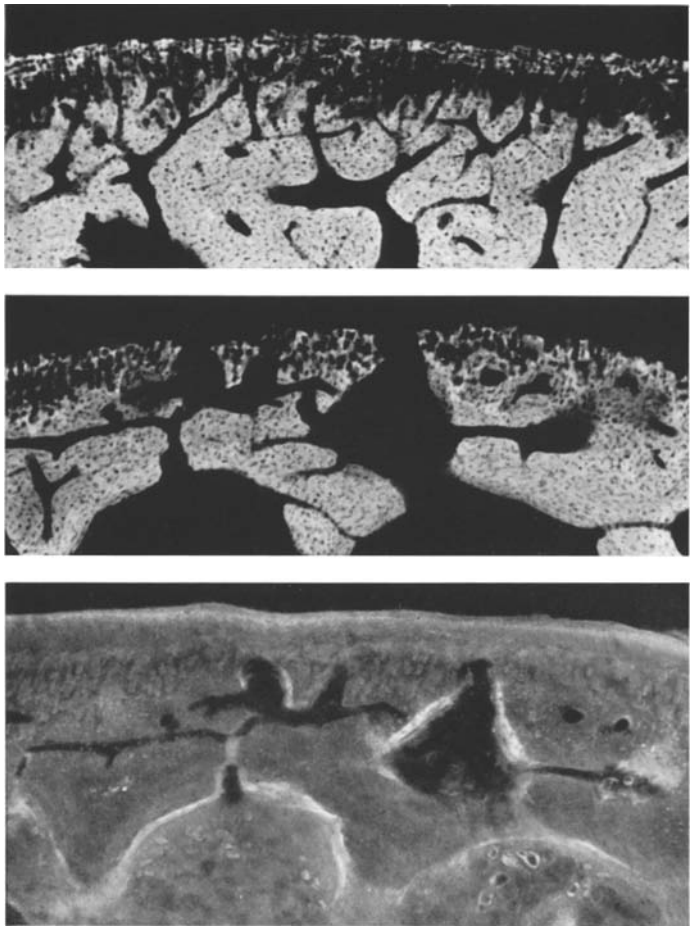


Fig. 3. a Microradiogram of the subchondral bone on the untreated side. b Identical area to (a) on the treated side. Mineral loss in the calcified articular cartilage and around intracartilaginous vascular canals. Signs of mineral loss are also seen in osteones. c Fluorescence photomicrograph of the same preparation as in (b). Note that the defect has not penetrated to the tidemark. Tetracycline-induced fluorescence on some bone surfaces around vascular canals showing mineral loss. Observation time 1 week. ×100

values were found in zone III. This difference may be due to the fact that on examination of this zone, which corresponds to the defect area, higher magnifications were often used, which would systematically give these higher values. This would also explain the fact that for structure c higher values were obtained for the whole group. Individual differences in the labelling frequency were found in different animals for all structures.

Observations in the Defect Area (Zone III) at Different Observation Times

The Table gives the number of animals at the different observation times in which no signs of primary involvement of the subchondral bone were found either on histological or fluorescence microscopic examination. In most cases only one preparation including the defect area was obtained.

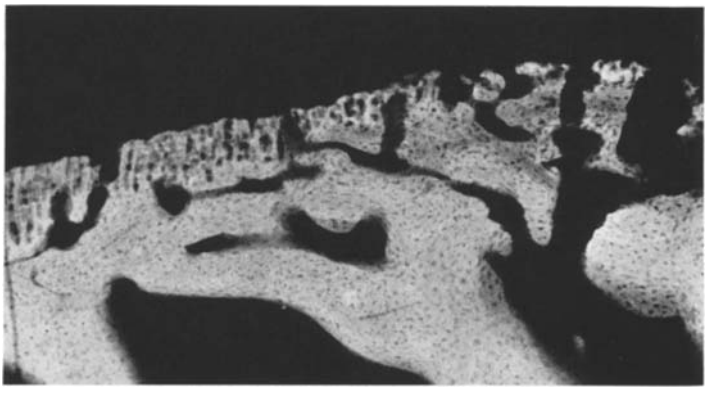


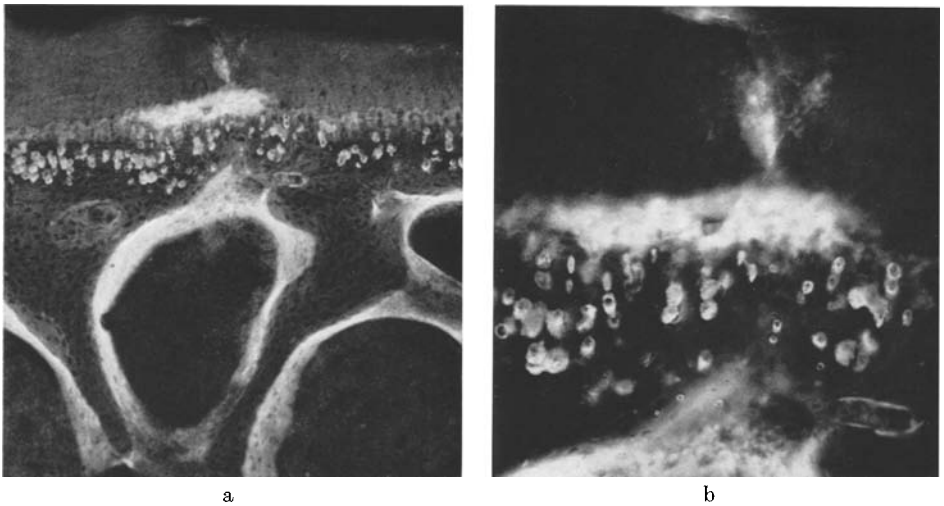
Fig. 4. Microradiogram of the defect area after an observation time of 2 weeks. In several places there is loss of mineral from the calcified articular cartilage and around vascular canals in the cortical part of the subchondral bone. Mineral loss from adjacent lamellar bone surfaces is also observed. ×100

At 5 days and 1 week the changes were most marked in the microradiograms. In all animals loss of mineral was seen, to varying extents, in the calcified articular cartilage, around intracartilaginous vascular canals and around vascular canals in the layer of cortical bone, as well as signs of incipient resorption in osteones (Fig. 3b). In no case were any corresponding changes found on the untreated side (Fig. 3a). On fluorescence microscopic examination of the same preparation it was seen that the defect had not penetrated to areas showing loss of mineral (Fig. 3c). In 2 animals there was an increased number of fluorescent surfaces in the defect area—close to structures showing mineral loss (Fig. 3c) but also in intracartilaginous "blindly" terminating vascular canals. Changes of the mineral content on the lamellar bone surfaces (Structure d) could not be evaluated with certainty. In 2 cases there was no fluorescence of the tidemark in zone III, while this was seen in zones II and IV.

At 2 and 3 weeks the changes seen on microradiography were similar to those described above, but the reduction of the mineral content seemed to be more general in the cortical bone layer and in many cases was also clearly evident on isolated lamellar surfaces (d) (Fig. 4). The fluorescence was distinctly higher in all structures, a–d, of the defect area. In some cases the tidemark showed intensive, broad fluorescence extending into adjacent, non-calcified articular cartilage (Fig. 5a and b). A decreased mineral content was observed in corresponding areas on the microradiograms (Fig. 6).

At *5–12 weeks* the microradiograms showed somewhat varying pictures. The findings were similar to those made earlier but the mineral content seemed to be somewhat higher and more even at the longer observation times. At the site of the tidemark fluorescence, mineral could be seen on the microradiograms (Fig. 7a and b). A tendency to a decrease of the number of structures exhibiting fluorescence was found with increasing observation times.

The Table summarizes the changes observed on fluorescence microscopy. The table gives the number of animals which at each observation time showed a greater



a b

Fig. 5. a Fluorescence photomicrograph of the defect area after an observation time of 2 weeks. There is intensive fluorescence in part of the tidemark where a fissure is seen in the articular cartilage. The lamellar bone surfaces beneath the defect show intensive fluorescence. ×150. b Detail from the same area, ×350. Fluorescence is also visible in a fissure in the articular cartilage. The photographs are taken with the filter combination of BG 38 and BG 12, with barrier filters 50 and 65; the fluorescence in the chondrocytes is autofluorescence. Examination of the same preparation with a filter combination of BG 38 and UG 1 and barrier filter 41 showed that the fluorescence in the tidemark and articular cartilage was induced by Tetracycline

number of labelled structures in zone III (the defect area) than in zone III on the untreated side. The criteria laid down for the existence of a difference were that in structure a fluorescence should be found in zone III on the treated side which was not present or was considerably less in zones II and IV and which was not present either on the untreated side, and that for structures b, d and c, respectively, the number of fluorescent structures in zone III should exceed the number of fluorescent structures in zone III on the untreated side by at least two. In absolute figures this meant in most cases that, for example, no or one osteone showed fluorescence on the untreated side, whereas 2–3 were fluorescent in the defect area. Three cases formed exceptions. In these one of each structure showed fluorescence in the defect area while no fluorescence was found in any of the remaining subchondral bone of the whole femoral head, or on the untreated side. It can be seen in the table that an increase of the number of fluorescent structures was observed up to 2 and 3 weeks, after which there was a slight tendency to a decrease. The changes were most marked in the tidemark and in the intracartilaginous vascular canals. Further, it can be seen that in most cases these changes were limited to zone III, while zones II and IV showed such changes in only 3 cases.

Other Observations

In zones V and VI no changes were observed, except in 1 animal at an observation time of 12 weeks, where a larger number of labelled lamellar surfaces (d) was found in zone VI, not present on the untreated side.

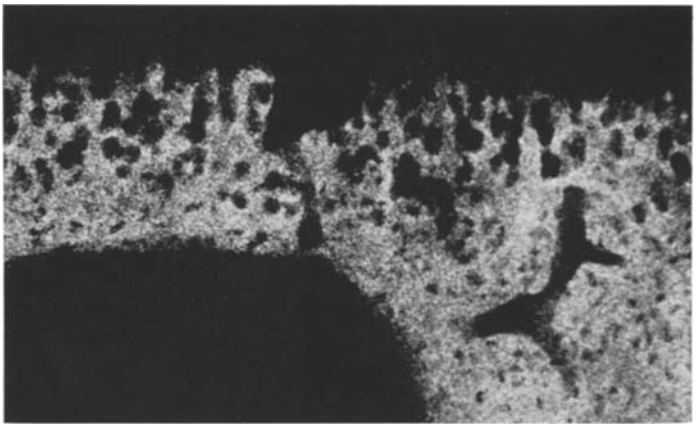


Fig. 6. Microradiogram of same preparation as in Fig. 5b. A reduced mineral content is seen below the fluorescent area. ×350

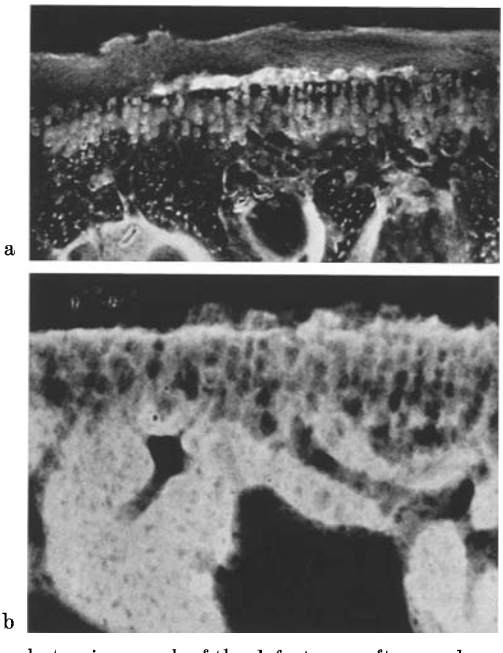


Fig. 7. a Fluorescence photomicrograph of the defect area after an observation time of 8 weeks. Intensive fluorescence is seen in the tidemark and on lamellar surfaces. ×100. b Microradiogram from part of the same preparation as in Fig. 9. At the site of the fluorescence mineral is seen to lie on the articular side of the margin of the original calcified cartilage. ×350

In zones I and VII subperiosteal new bone formation was observed to a very small extent at 2–8 weeks; no such formation was found at the other observation times. No definite difference was found in the labelling of the bone surfaces in the cancellous bone of the metaphysis and epiphysis between the treated and untreated side, apart from the fact that the lamellar surfaces adjacent to structure d in zone III usually showed fluorescence when structure d showed fluorescence (Fig. 5a).

Observations on the Control Animals

No changes were observed in zones II to VI in any of the animals. In both animals observed at 5 weeks subperiosteal new bone formation was seen in zone I.

Discussion

The histological and autoradiographic findings in the articular cartilage of the defect area after ^{35}S-sulphate incubation *in vitro* were similar to those described by Carlsson (1957), Meachim (1963) and Lemperg (1967) concerning loss of matrical metachromasia, the occurrence of multinuclear chondrones and retention or increase of sulphate uptake of chondrocytes. Fibrillation of the cartilaginous matrix was a relatively rarely observed phenomenon, however, and chondrocytic necrosis appeared to have been rather more extensive in the present experimental model than with the scarification of the articular cartilage described by Meachim. The histological findings concerning changes in the subchondral bone beneath the articular cartilage defect deviated in some respects, however, from those in previous comparable studies (Bennett and Bauer, 1935; Bucher, 1955; Imerlishvili, 1957; Meachim, 1963; DePalma *et al.*, 1966). In 4 out of 14 cases at observation times of 3–12 weeks some widened vascular canals were seen in the calcified articular cartilage and in 1 of 5 cases at 12 weeks the tidemark lay at a high level and there was invasion of the deepest part of the articular cartilage by some blood vessels. These changes were indistinct and limited.

The microradiographic and fluorescence microscopic examinations showed a sequence of changes which were not detected on the histological preparations. Initially, after 5 days and 1 week, loss of mineral was observed around intracartilaginous vascular canals and in the upper layer of the calcified cartilage. Resorption in osteones and on the immediately adjacent lamellar bone surfaces followed rapidly. This initial loss of mineral did not show any tendency to progression after 2 weeks and was limited to the mineralized tissue beneath the articular cartilage defect. In each individual preparation it could be established by fluorescence microscopic examination that the defect in the area in question had not penetrated to the mineralized structures (Figs. 3, 5 and 6). The loss of mineral was followed by remineralization and new bone formation, as indicated by Tetracycline-induced fluorescence on bone surface (for references, see Ibsen and Urist, 1964). This reached its highest activity after 2 and 3 weeks and then showed a tendency to decrease. Mainly the lamellar bone surfaces close to the cancellous bone of the epiphysis showed continued new bone formation for as long as up to 12 weeks.

Massive fluorescence in the tidemark and also in adjacent, non-calcified articular cartilage was observed in places where microradiograms showed a decreased mineral content or no mineral at shorter observation times. This indicates changes of the matrix in the defect area, leading to increased incorporation of Tetracycline. At longer observation times additional mineral was observed in places where fluorescence was seen, indicating that the fluorescence in the tidemark and adjacent, non-calcified cartilage meant active mineralization. Of some interest in this connection is the hypothesis presented by Otte (1958) that damage to the tangential layer of the articular cartilage leads to disturbance of an ossification-inhibiting

mechanism, which is claimed to be controlled by enzyme systems in the tangential zone (see also Urist, 1964). All these changes were localized to the defect area, and generalized accelerated remodelling of the subchondral bone in the remaining parts of the femoral head was observed in only one case.

Observations in the present investigation thus differ from those made in the studies cited above with regard to changes in the subchondral bone after creation of superficial articular cartilage defects. Disturbance of the joint function can hardly have played any role in the development of these changes, since the animals were able to move completely unhindered and showed normal articular mobility. Factors which might have led to the subchondral bone changes observed on compression of the knee joint in the rat (Evans *et al.*, 1960) and rabbit (Trias, 1961) were lacking in the present investigation.

The changes in the articular cartilage in the defect area resembled to some extent those observed by Collins and McElligott (1960) in human osteoarthritis. No fibrillation of the articular cartilage was seen, however, in most cases at observation times up to 12 weeks. The articular cartilage changes were followed rapidly by loss of mineral around vascular canals and thereafter by accelerated new bone formation and mineralization of the deepest layer of the articular cartilage. Up to 12 weeks these changes were limited to the defect area and did not lead to generalized remodelling of the subchondral bone. This experimental model may be of value for further studies of changes in the matrix and of cellular function which may possibly be the cause of the locally disturbed mineralization process.

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