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Intracartilaginous Defects in Adult Sheep

Histological, Autoradiographical (^{35}S-sulphate), Ultrastructural, Microradiographical and Fluorochromic Studies*

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Summary. Standardized, intracartilaginous articular cartilage defects were studied in the knee joints of adult sheep at 3, 4 and 6 months. The histologic and autoradiographic examinations of the articular cartilage in the margins of the defects showed superficial fibrillation and retention or increase of sulphate uptake over chondrocytes close to areas without labelled cells. These changes were mainly confined to the defect area. No progress with time could be ascertained. Desintegrated and dead chondrocytes as well as matrix alterations were ultrastructurally found in the margins of the defects. Beneath the defects in the deeper parts of the remaining non-calcified cartilage, there were matrix alterations that were not apparent with the light microscope. Microradiographic and fluorescence microscopic (after Tetracycline) examination showed loss of mineral from calcified cartilage and bone in the immediate vicinity of the defect and increased fluorescence in mineralized structures at 3 and 4 months. Moreover, at 6 months subchondral osteophytes were seen within and outside the defect area.

Thus, in this experimental system superficial articular cartilage defects evoke matrix alterations in the deeper regions of the remaining cartilage and reactive changes in the mineralized structures.

Zusammenfassung. Bei erwachsenen Schafen wurden unter standardisierten Bedingungen intracartilaginäre Defekte des Kniegelenkes über einen Beobachtungszeitraum von 3—6 Monaten untersucht. Die histologischen und autoradiographischen Befunde ergaben, daß der Gelenkknorpel im Randgebiet des Defektes eine oberflächliche Fibrillation aufwies, wo die Chondrocyten eine teilweise erhöhte Aufnahme von markiertem Schwefel zeigten. Ein Fortschreiten dieser Veränderungen über das Defektgebiet hinaus konnte im Versuchsablauf nicht beobachtet werden. Elektronenmikroskopisch fanden sich im Defektrand Nekrosen von Chondrocyten und eine Fibrillation der Matrix. Die Matrixveränderungen im tiefergelegenen, nicht verkalkten Gelenkknorpel unter dem Defekt waren ausgedehnter, als es die lichtmikroskopischen Befunde aufwiesen. Die Mikroradiographie und die Fluorescenzmikroskopie nach vorangegangener Tetracyclingabe ergab einen Mineralisationsverlust im verkalkten Gelenkknorpel und Knochengewebe unmittelbar neben dem Defekt und eine erhöhte Fluorescenz in den Mineralisationszonen nach 3 und 4 Monaten. Nach 6 Monaten wurden subchondrale Osteophyten innerhalb und außerhalb des Defektgebietes gefunden. Aus den Untersuchungen ergibt sich, daß ein oberflächlicher Gelenkknorpeldefekt zu Veränderungen der Matrix in den tieferen Knorpelarealen führt und reaktive Veränderungen in den Mineralisationszonen eingeleitet werden.

Introduction

In a preceding study of intracartilaginous defects in the articular cartilage on the femoral head of adult rabbits, changes in the subchondral bone were

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observed, consisting of initial loss of mineral and locally accelerated new bone formation (Lemperg, 1971 b). These changes were mainly confined to the mineralized tissues beneath the cartilage lesion and no generalized remodelling of the subchondral bone was found at observations up to 12 weeks. In contrast, most previous investigations on intracartilaginous defects, utilizing histological and autoradiographical techniques, have not disclosed any subchondral bone changes (for ref. see Lemperg, 1971 b). The question whether or not intracartilaginous defects may be the cause of accelerated new bone formation is of great importance since the initial event in osteoarthritis probably is a focal articular cartilage lesion (Collins, 1949; Harrison *et al.*, 1953). There is an obvious lack of suitable experimental models for studies on the possible relationship between articular cartilage alterations and reactions in the subchondral structures. The merit of an experimental model for such studies will depend upon the possibility to standardize the conditions and to define the nature of the cartilage lesion with the aim to establish a correlation to eventual subchondral reactions.

The present communication reports observations on standardized intracartilaginous defects in the articular cartilage of the knee joints of adult sheep, 3, 4 and 6 months after creation of the defects. Fluorescence microscopy after Tetracycline administration, autoradiography after ^{35}S-sulphate labelling *in vitro*, microradiography, histology and electron microscopy are used. Histochemical studies were also performed on the same material, but the results of these are not reported here. The main aims were a) to ascertain the occurrence of subchondral changes in an experimental animal other than the rabbit, b) to study the possible progression of changes with time, and c) to complement the previously used methods with an ultrastructural investigation.

Material and Methods

Ten ewes at an age of $2^1/_2$ years and three at an age of about 5 years were used. No animal was pregnant during the experiments.

Operative Procedure

The knee joint was opened laterally. When the joint was extended, the articular surface of the femur was easily exposed after the patella had been lifted up. A 3 mm wide transverse intracartilaginous defect was created in the articular cartilage, corresponding to the femoropatellar joint. The defect reached to about 3 mm from the margins of the femoral condyles, thus being entirely situated within the articular cartilage. A 3 mm wide special stainless steel raspatory with five slender 0.3 mm high jags was used. No attempt at postoperative immobilization was made and no antibiotics were given. The animals were brought to the farm on the first day after the operation.

Observation Times and Preparation of the Material

After 3, 4 and 6 months (five $2^1/_2$ year-old animals each at 3 and 6 months, and three 5 year-old animals at 4 months) the animals were killed. Two days previously they had been given an intramuscular injection of oxytetracycline at a dose of 20 mg/kg body weight. Within 2–3 minutes after death the entire defect area with adjacent normal cartilage was excised. Corresponding samples were taken from the nonoperated side of 5 animals. All the samples were divided into 4 equal parts and treated as follows:

1. Autoradiography and histology. The samples were incubated for 1 hour in 2 ml of Tyrode's solution containing 1 mC ^{35}S-sulphate and were then washed in saturated sodium

sulphate solution (Lemperg, 1967). Then the samples were fixed in 4% aqueous formalin containing 0.5% cetylpyridinium chloride, decalcified in a mixture of equal parts of monosodium citrate (20%) and formic acid (44%), and embedded in paraffin. Serial sections, 5 μ thick, were cut in the sagittal plane. The first 60 sections were used for autoradiography and every following 10th section for histological examination. The entire sample was sectioned. Autoradiography was performed by the dipping technique (Ilford K2 in gel form, diluted with distilled water—2:1). After developing, some specimens were stained with haematoxylin-eosin or 1% toluidine blue in an aqueous solution, while others were left unstained.

Material for histological examination was stained in the same way.

2. Microradiography and Fluorescence Microscopy. The samples were dehydrated in absolute ethanol and embedded in methyl methacrylate. Sections about 1 mm thick were sawn from the plastic block in the sagittal plane and ground down to 80–100 μ. Microradiography was performed with a Siemens tube (AGW 3 ö, with a 1 mm^2 tungsten target and a 1.0 mm thick beryllium window) at 15 kV; the preparations were in direct contact with Kodak spectroscopic plates 649–0.

For fluorescence microscopic examination the specimens were mounted in Permount and examined in a Zeiss fluorescence microscope. The combination of exciter filters BG 38 and UG 1 with barrier filter 41 was used.

3. Electron Microscopy. The sampes were fixed in 2.5% glutaraldehyde in 0.34 M Veronal acetate buffer adjusted to pH 7.4, followed by postfixation in 1% osmium tetroxide in the same buffer. After rinsing and dehydration the specimens were embedded in Epon 812. The sections were cut on an LKB Ultratome III after which they were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiscope 1 A and/or 101.

4. The samples were frozen in liquid nitrogen for histochemical studies (the results are not reported in this paper).

Results

Postoperative Course and Gross Observations

Already on the first day after operation the animals could obviously follow the herd without difficulty, and 3–4 days later they were running without any visible limp. Joint effusion or other complications were not recorded in any of the cases.

The joints showed no changes in the capsule, synovial membrane or synovial fluid. In all cases the defect was clearly visible. At 3 and 4 months the articular cartilage adjacent to the defect was unaffected. At 6 months, 4 of the 5 animals showed small areas with red-gray discoloration of the articular cartilage adjacent to the defect. The changes in the cartilage were mainly localized to the condyles. The defect itself seemed unaffected in all cases. One animal showed a small osteophyte at the periosteal margin of the medial condyle.

General Morphologic Observations

All sections, including those for histochemistry, were examined with respect to the depth of the defect. In all but one of the cases (No. 6) the deepest part of the defect was well above the tidemark (calcified line) and it could be safely excluded that the subchondral bone was primarily engaged in any part of the defect. The features of case 6 will be described under the headings histology and autoradiography.

Histological and Autoradiographical Observations

Articular Cartilage. The bottom of the defect was in most cases about 10–15 cell rows below the articular surface. The deepest part was in three cases nearer

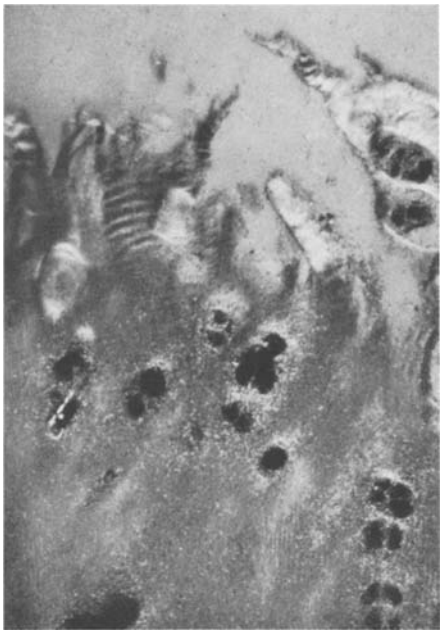

Fig. 1. Articular cartilage defect at 3 months showing superficial fibrillation and ^{35}S-labelling of the chondrocytes at the margins of the fibrillated area. Autoradiogram in polarized light. ×450

to the tidemark but at no point in contact with it. At 6 months one animal (No. 6) showed no uncalcified articular cartilage within a limited part of the defect and fibrous and newly formed cartilaginous tissue were seen in this particular area. Primary lesion of the subchondral bone in this area seemed probable. In the other samples there was only articular cartilage in the defect area.

Superficial fibrillation of the surface in the defect area was present in two cases at 3 and 4 months and in three cases at 6 months (Fig. 1). No definite difference in the extent of fibrillation with progression of time could be ascertained. The articular cartilage outside the defect showed slight superficial fibrillation in two cases. Loss of metachromatic stainability was often seen in

Fig. 2. a Microradiogram of subchondral bone at 3 months (defect area to the left; normal articular cartilage to the right) showing mineral loss from the superficial part of the calcified cartilage in the defect area. Fluorescence microscopy of the same preparation revealed a superficial cartilage lesion. The sharp delimitation between calcified and non-calcified cartilage is demonstrated in the normal region. ×140. b Microradiogram of the same preparation as in c demonstrating loss of mineral around chondrocytes and in vessel canals in the calcified cartilage, as well as on the trabecular bone surfaces delimiting the marrow cavities. ×140. c Fluorescence photomicrograph of the same preparation as in b showing extensive fluorescence in the tidemark and on bone surfaces that in the microradiograms showed loss of mineral. The increased fluorescence is limited to the subchondral structures while the trabecular bone surfaces in the epiphysis exhibit normal fluorescence. ×140

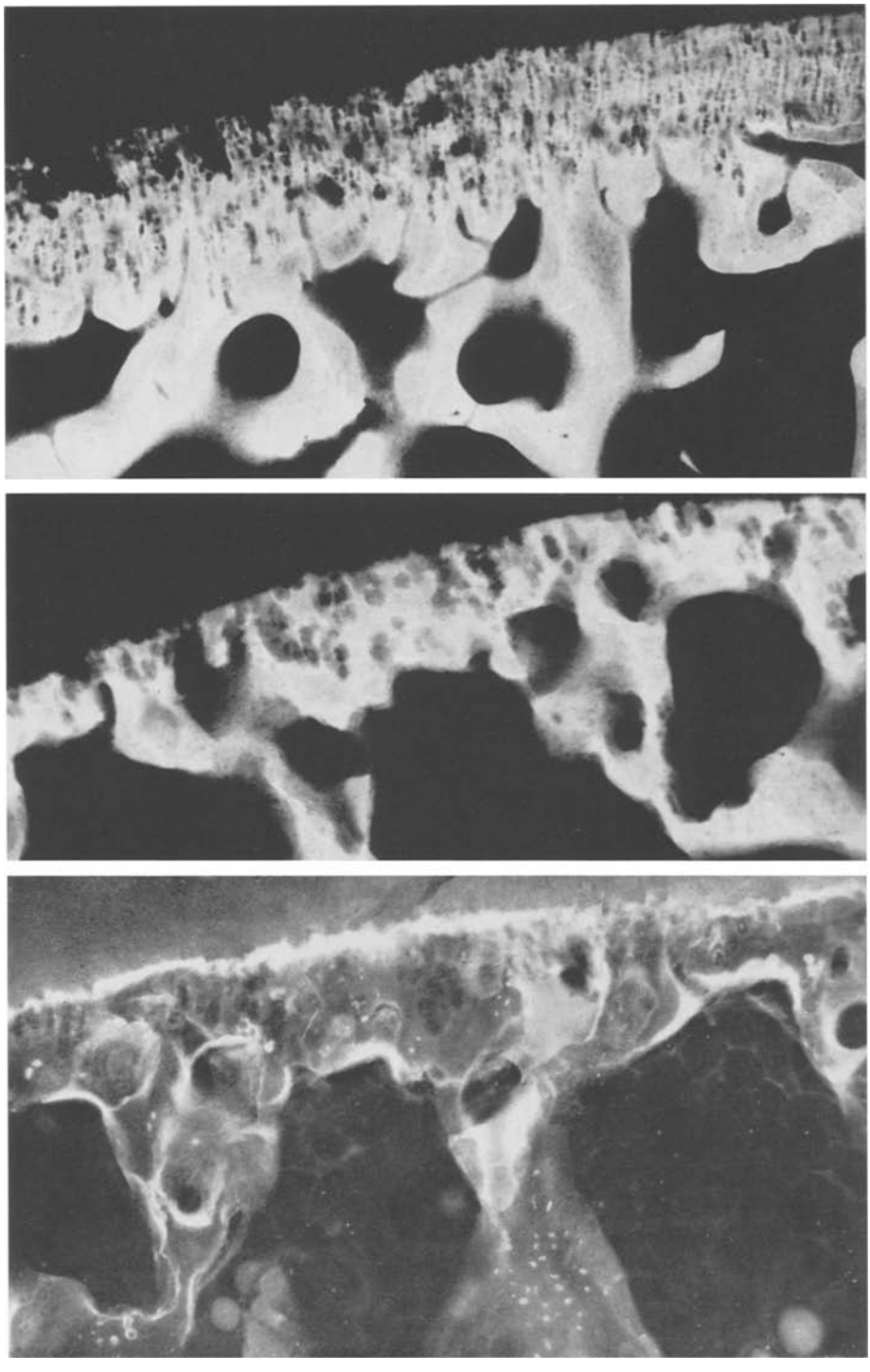

Fig. 2 a–c

Table. *Microradiographic and fluorescence-microscopic observations on the subchondral structures in intracartilaginous defects*

I = Defect area. II = Area adjacent to defect. A = Loss of mineral in calcified cartilage (Fig. 2 a); B = Dilated and perforating vessel canals in calcified cartilage (Fig. 2 b); C = Increased fluorescence in tidemark and/or subchondral bone surfaces (Fig. 2 c); D = Subchondral osteophytes (Fig. 3 a and b). The figures in the columns indicate the number of animals with positive findings.

		A	B	C	D
3 months	I	5	4	5	—
$n=5$	II	2	3	3	—
6 months	I	5	4	4	2
$n=5$	II	4	3	1	2
4 months	I	2	—	3	—
$n=3$	II	—	—	—	—

the margins of the defects and multinuclear chondrones occurred with varying frequency.

The autoradiograms showed areas with normal or high sulphate uptake over chondrocytes in the vicinity of the defects, alternating with areas without such uptake (Fig. 1). There were individual and local differences as to the extension of areas without ^{35}S-sulphate uptake. In some places, minor areas without ^{35}S-labelled chondrocytes were also seen in the deeper part of the non-calcified cartilage. At 4 months, the superficial fibrillation was sparse but the extension of areas without ^{35}S-labelling in the margin of the defect was obviously larger than in the $2^1/_2$ year-old animals.

Subchondral Bone. At 3 months vessels with rather small diameters invading the deeper parts of the calcified cartilage of the defect area were found in all cases. In these intracartilaginous canals there were numerous multinuclear cells of osteoclast type. At 4 and 6 months only a small number of such cells were found in these canals, the number of which was of the same order of magnitude as in the normal joint. Osteoclast counts were performed in an attempt to verify this observation statistically. However, no really good system for counting could be worked out and too few sections were available to obtain data allowing a safe statistical treatment.

Fig. 3. a Microradiogram of the same preparation as in b showing an osteophyte in the defect area and vessel canals reaching the tidemark. Mineral loss is less prominent than in Fig. 2 b. ×140. b Fluorescence photomicrograph showing cartilage lesion and multinuclear chondrones. Tidemark fluorescence is limited to some parts of the defect area. ×140. c Fluorescence photomicrograph at 6 months from the same animal as in a and b (the margin of the defect that was present in the outermost left part of this preparation, is not seen) showing a subchondral osteophyte in the right part. Note the low fluorescence on the epiphyseal bone surfaces. ×50

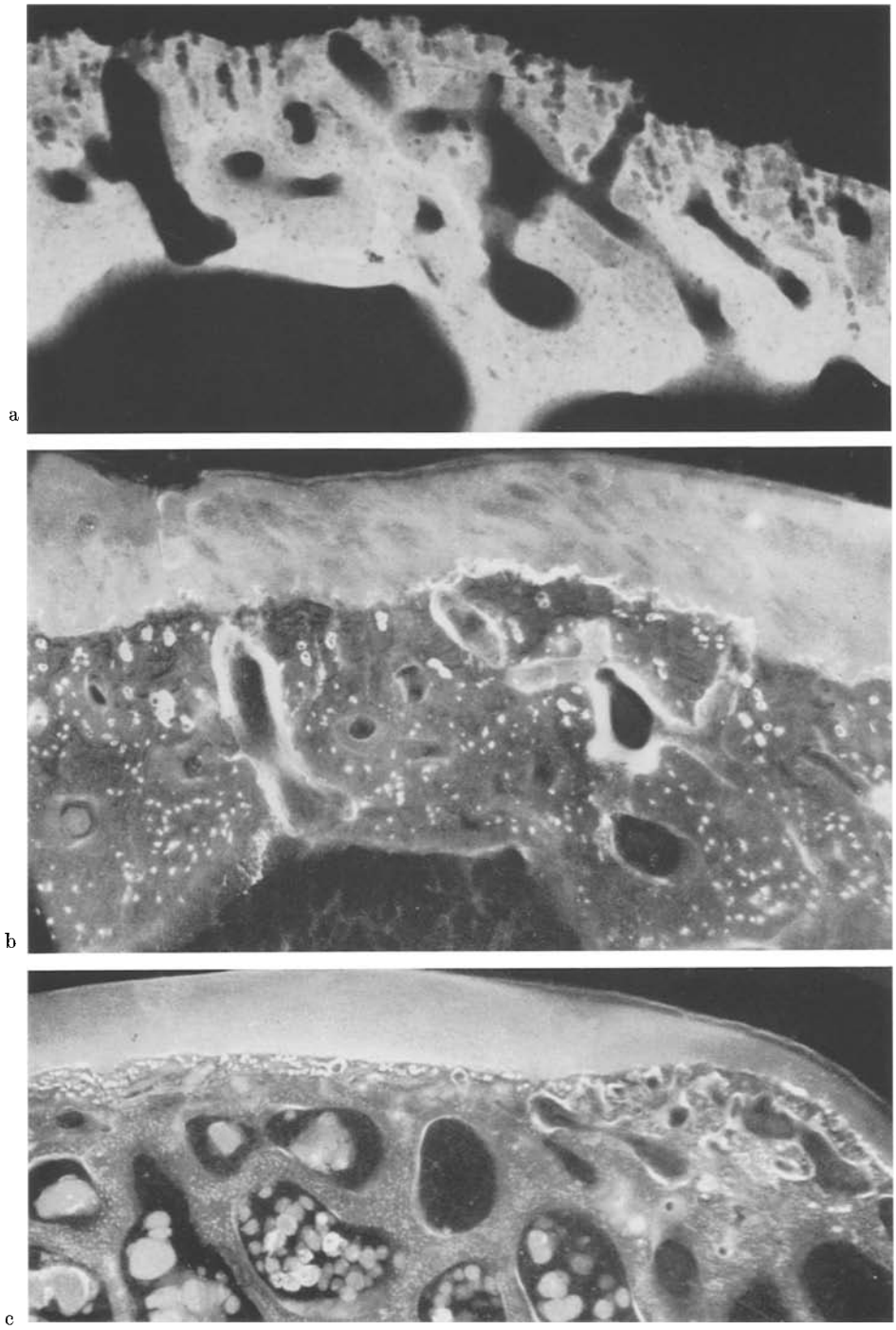

Fig. 3 a–c

Ingrowth of vessels was accompanied by new bone formation and resorption of calcified cartilage. The basophile lines of the tidemark had disappeared in these areas. Immediately outside the defect similar changes were occasionally seen.

Microradiographic and Fluorescence Microscopic Observations

The changes described below were sometimes focal. The borderlines of the subchondral structures corresponding to the cartilage defect could not be delimited. The microradiograms were compared with the corresponding fluorescence microscopic specimens in order to identify the defect area and to exclude artifacts due to loss of marginal tissue in the grounding procedure.

At 3 months, the defect area showed loss of mineral from the superficial part of the calcified cartilage (Fig. 2a) and enlarged chondrocyte lacunae (Fig. 2b). Dilated vessel canals perforated the tidemark. Loss of mineral was seen in osteons and on lamellar bone surfaces beneath the defect area (Fig. 2b). Extensive fluorescence was observed in the tidemark and on bone surfaces showing loss of mineral (Fig. 2c). These changes were mainly confined to the defect area but were occasionally also found adjacent to it (Table).

At 6 months, the principal alterations were similar to those at 3 months. However, the loss of mineral appeared to be less pronounced in the subchondral structures. Additionally, two cases showed subchondral osteophytes within the defect area (Figs. 3a and b). In two cases subchondral osteophytes were also found 2–3 mm outside the defect area without direct connection with the defect (Fig. 3c). The animal (No. 6), that in histological sections showed a suspect primary engagement of the subchondral bone, had a superficial cartilage defect in the plastic embedded sections. The other changes in this animal did not differ from those observed in the other animals.

The animals observed at 4 months showed only limited superficial resorption of the calcified cartilage and increased fluorescence in the tidemark. An increased number of fluorescent subchondral bone structures was also observed in one animal. It should be observed that these animals were older than those studied at 3 and 6 months.

The trabecular surfaces in the deeper parts of the epiphyseal bone adjacent to the defect area exhibited the same extension of fluorescence as on the non-operated side.

Correlation between Changes in Articular Cartilage and Subchondral Bone

Attempts were made to establish a correlation between certain changes in the articular cartilage such as fibrillation, depth of the defect, more extensive areas of chondrocytes without ^{35}S-labelling, and alterations in the calcified structures. One correlation which could possibly be established was a negative one. There was no correlation between more extensive areas with non-labelled chondrocytes and subchondral reactions. The other correlation was lack of, or only weak reaction subchondrally in areas with superficial defects where only the outermost 3–6 cell rows had been removed.

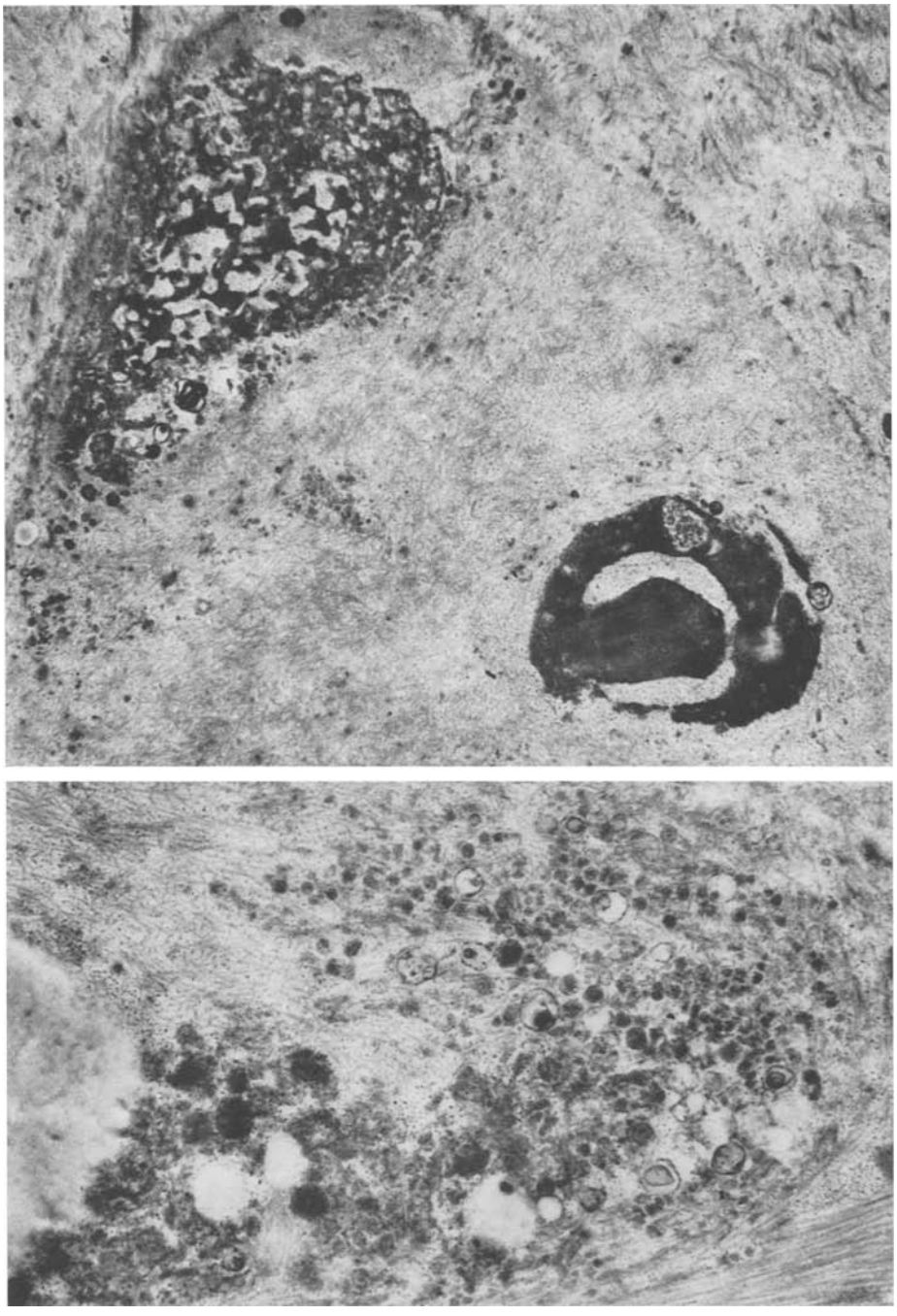

Fig. 4. a This and the following pictures represent electron micrographs from the non-calcified articular cartilage beneath the defect. This figure shows degenerating and dead chondrocytes. ×8000. b Area close to a degenerating chondrocyte showing vesicles and small bodies of somewhat varying electron density. ×22000

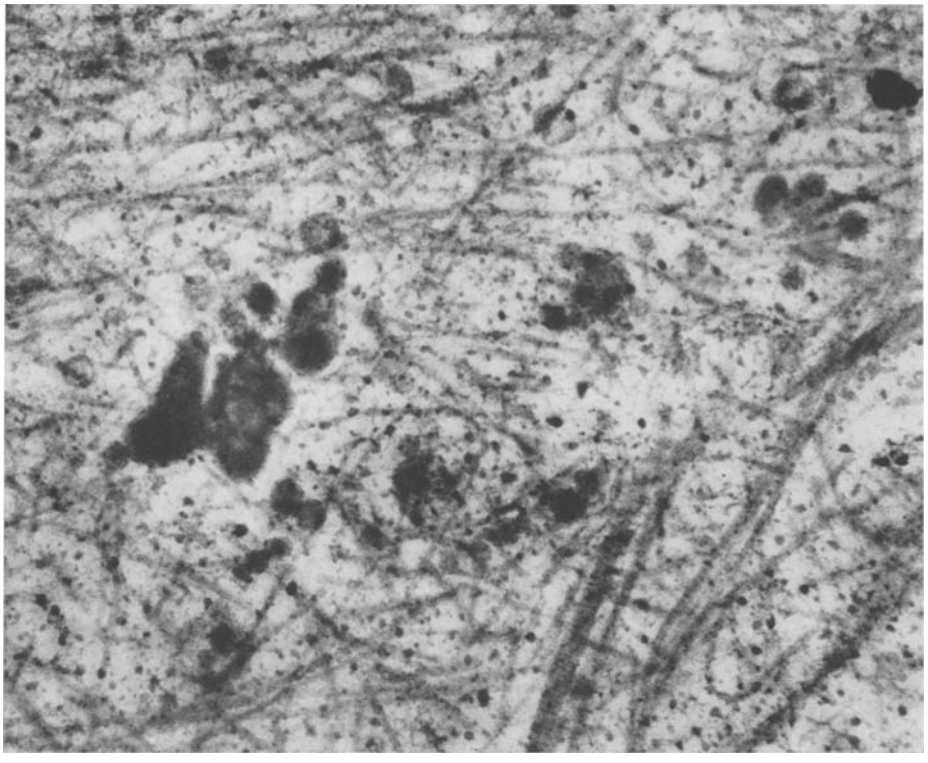

Fig. 5. Matrix exhibiting irregularly arranged fibres of varying thickness, occasionally with periodic banding. Electron dense bodies of varying size and shape are also seen. ×58000

Anatomical Remarks

In the central parts of the femoropatellar joint of sheep, $2^1/_2$ and 5 years of age, the borderline between calcified and non-calcified articular cartilage is distinct (Fig. 2a). A narrow faintly fluorescent band is often, though not constantly, present in the tidemark. Numerous vessel canals occur ni the calcified cartilage. They do not penetrate the tidemark. The subchondral bone shows low spontaneous remodelling, indicated by few bone surfaces showing Tetracycline-induced fluorescence. The marrow cavity is occasionally in direct contact with the calcified cartilage. In the 5-year-old animals the calcified cartilage appears thicker, and fluorescence in the calcified structures is more sparse than in the $2^1/_2$ year-old animals.

Electron Microscopic Findings

The unaffected articular cartilage of the sheep showed no peculiarities as compared with that of other species. At all observation times the non-calcified cartilage beneath the defect showed degenerative alteraitons fo varying severity. Slight degenerative changes were also seen in the cartilage close to the sides of the defect, as well as in the control material.

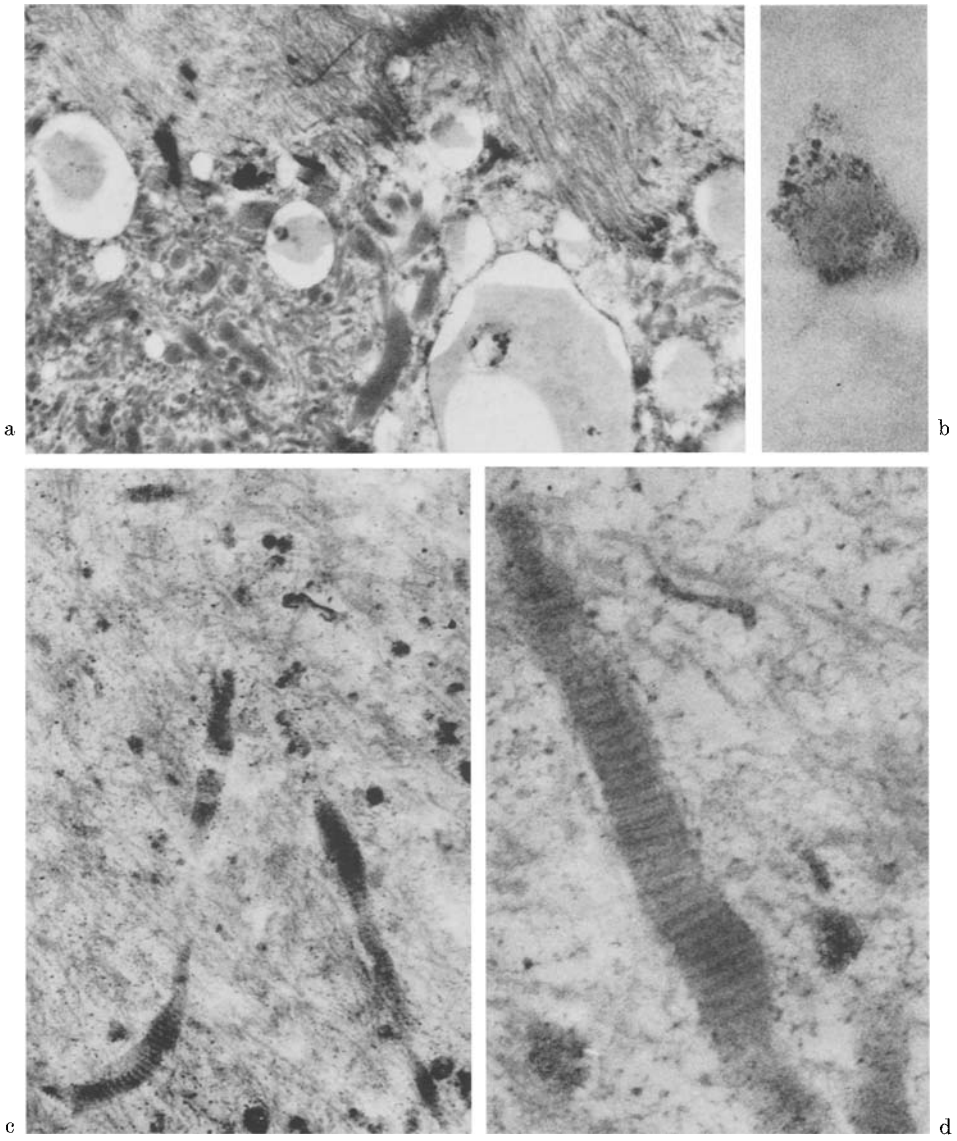

Fig. 6. a Matrix showing delicate fibres, coarse fibres without obvious banding, and lipoid droplets. 45000. b Portion of matrix showing a moderately dense body with associated small dense particles. ×94000. c Matrix showing coarse fibres with periodic banding, tapered ends, and varying thickness and electron density. Delicate fibres and electron dense particles are also seen. ×24000. d Higher magnification of another coarse fiber to demonstrate the periodic banding and variation in thickness and electron density. ×67000

Degenerating and dead chondrocytes occurred mainly in the deep and intermediate portions of the non-calcified cartilage beneath the defect (Fig. 4a). The degenerating chondrocytes were characterized by irregular, condensed, electron

dense nuclei and an irregularly dilated endoplasmic reticulum that was partly disorganized and disintegrated. The mitochondria were swollen and their cristae disintegrated. Other chondrocytes were completely necrotic and transformed into irregular, electron dense shrunken masses, occasionally lying in large lacunae. Close to degenerating or dead chondrocytes there were electron dense bodies, vesicles and membranes of varying size and shape (Fig. 4b). Many of these seemed to represent remnants of disintegrated chondrocytes. In the superficial portion of the non-calcified cartilage beneath the defect some chondrocytes showed electron dense cytoplasm and vesicular endoplasmic reticulum. In all regions of the cartilage in the defect area there were also more or less unaffected chondrocytes.

The matrix in the deep and intermediate parts of the non-calcified cartilage beneath the defect consisted of an irregular network of fibers of varying thickness, with or without a periodic banding (Fig. 5). Typical collagen fibers with characteristic banding occurred intermingled with more delicate fibers. Diffusely scattered among these fibers there were electron dense bodies of varying size ans shape. Some of these were small, rounded and rather homogenous in appearance, whereas others were larger, more irregular and possessed varying electron density. Other bodies showed an electron dense periphery and an electron lucent interior.

Other kinds of bodies consisted of groups of small dense particles, occasionally arranged in an annular fashion. Larger bodies with low or moderate electron opacity were also observed, probably representing lipoid material (Fig. 6a). Membranous structures were sometimes seen in the matrix. Small electron dense, sometimes crystalloid particles were found in association with small, membrane-bound, moderately dense bodies (Fig. 6b). Occasionally, coarse, often tapered collagen fibers were seen, showing prominent main periodic banding (Figs. 6c and d). The interfibrillary matrix (ground substance) possessed low electron density. In the deep portions of the non-calcified cartilage but clearly above the tidemark, areas of calcification occurred. The matrix in the superficial portion of the non-calcified cartilage in the defect area showed only sparse bodies and particles of the types described above.

Discussion

The results of the present study on standardized intracartilaginous articular cartilage defects in skeletary mature sheep agreed in principle with observations made on similar defects in adult rabbits (Lemperg, 1971b). Thus degenerative changes in the articular cartilage and mainly localized subchondral reactions were found at 3 months. Additionally, at six months subchondral osteophytes within the defect area but also cartilage erosion and osteophytes outside of it were identified. The last mentioned phenomenon may safely be interpreted as a progress of the joint alteration, leading to a thinning of the articular cartilage, also in areas primarily not engaged in the experimentally induced lesion. However, in the articular cartilage of the defect area progress of alterations could not be ascertained with the methods used.

The design of a experimental model is crucial for the validity of conclusions which may be drawn concerning the relationship between primary articular

cartilage lesion and subsequent changes in the calcified structures. In our model the cartilage defect is strictly local and the operative procedure does not interfere with joint function or stability. Alterations in the cartilage outside the primary defect can thus reasonably be interpreted as secondary to the defect and localized changes in the calcified tissues beneath and adjacent to the defect as a response of the subchondral structures to the cartilage alterations. The limited extension of the defect permitted the use of subchondral areas adjacent to the defect as a control tissue, in addition to the non-operated joint. This point is important since the structure and the spontaneous remodelling of the subchondral bone show local and individual variations (Lemperg, 1971a). Inasmuch as loss of mineral from the calcified cartilage and penetration of vessel canals through the tidemark, as well as accelerated new bone formation were integrating parts of the subchondral bone response, it seems to be an advantage to use fully skeletally mature animals. In not fully mature animals there is still an intimate contact between subchondral vessels and the non-calcified cartilage and also a marked spontaneous bone formation (Holmdahl and Ingelmark, 1950; Mankin, 1963; Lemperg, 1971a). These facts interfere with the evaluation of minor alterations in the borderline regions.

Numerous procedures have been used for the production of "experimental osteoarthritis". The older literature has been reviewed by Gardner (1960) and Meachim *et al.* (1963). Thompson and Bassett (1970), performing compression—immobilization and condylar—resection experiments in skeletally not fully mature rabbits, have discussed these models. They pointed out that various reactions occur in the subchondral bone after immobilization and resection, respectively. The former was accompanied by hypertrophy of the subchondral bone and the latter by resorption of calcified cartilage and vessel invasion, although similar articular cartilage changes were observed in both experiments. These observations would suggest that factors other than cartilage alterations may be operative upon the subchondral bone obscuring a possible direct effect of cartilage degeneration on subchondral bone reaction. Moreover, Thaxter *et al.* (1965) in an autoradiographic (^{35}S-sulphate) study on rat knee joints emphasized the fact that the cartilage alterations in immobilization experiments have other characteristics than those in human osteoarthritis that have been described by Collins and McElligott (1960). Recently, Hulth *et al.* (1970) published a preliminary report on experimental osteoarthritis produced by resection of menisc and ligaments in the knee joints of adult rabbits. Degenerative cartilage changes of the "osteoarthritis type" were found. Alterations of the subchondral bone were not mentioned. Although some of these experimental models produce articular cartilage changes histologicaly resembling those in osteoarthritis, they give poor prerequisites for establishing a clear correlation between cartilage changes and subchondral bone alterations.

The changes in the articular cartilage as observed in this study with light microscopic and autoradiographic examinations were confined to the defect area. They were of degenerative type and showed increased ^{35}S-sulphate uptake in the immediate vicinity of the defect (*cf.* Collins and McElligott, 1960; Lemperg, 1971b). The changes were interpreted as belonging to grade I and in a few areas possibly to grade II according to Collin's (1949) classification. No change in the

alterations with progression of time could be ascertained. There was no correlation between the extension of areas without sulphate-labelled chondrocytes and subchondral bone reaction.

The ultrastructural features of the articular cartilage outside the defect area and in the non-operated joints seemed to have the same characteristics as adult rabbit and human articular cartilage (Barnett *et al.*, 1963; Meachim *et al.*, 1965; Palfrey and Davies, 1966; Ghadially and Roy, 1969). In the margins of the defect disintegrated and necrotic chondrocytes were rather frequent, mainly within areas showing matrix fibrillation. This conforms to the light microscopic findings. Surprisingly it was found that in the deepest parts of the remaining non-calcified cartilage beneath the defect, disintegrated chondrocytes and chondrocyte remnants appeared to be more numerous than closer to the defect. In these deeper parts of the remaining non-calcified cartilage, but well above the calcified cartilage, there were numerous small particles showing some resemblance to those described as calcification centres in the epiphyseal plate by Bonucci (1970). The matrix in the deepest part of the defect area contained occasional abnormally coarse and irregular fibres with prominent periodic banding. These were similar to those reported to occur in osteoarthritic human cartilage (Ghadially and Roy, 1969). Electron dense bodies of presumably lysosomal type were found in chondrocytes both within and outside the defect area.

Thus the ultrastructural findings suggested that mainly the matrix alterations were more extensive in the deeper part of the defect area than could be disclosed by light microscopic and autoradiographic examinations. In a recently performed microchemical study on rabbits, articular cartilage with only slight superficial fibrillation showed significant alterations of the glycosaminoglycan pattern (Hjertquist and Lemperg, in press). This also strongly indicates that matrix alterations may occur without conspicuous microscopical changes.

Two types of response could be seen in the calcified structures beneath the non-calcified articular cartilage of the defect. One consisted of loss of mineral, sometimes restricted to the superficial part of the calcified cartilage. This change was the predominating phenomenon at 3 months and seemed to be a continuation or remnant after the primary loss of mineral demonstrated in rabbits already at 1 and 2 weeks (Lemperg, 1971 b). The other response was a sometimes very marked new bone formation and mineralization in areas showing loss of mineral, occasionally creating subchondral osteophytes in the defect area at 6 months. This accelerated new bone formation in the subchondral bone showed a tendency to spread also outside the defect area, sometimes resulting in subchondral osteophytes in the vicinity of the defect. Unfortunately no material from cartilage corresponding to such areas was investigated ultrastructurally. Thus the essential question of possible matrix alterations prior to ossification in such areas remains unanswered.

The following partly speculative, sequence of processes in our experimental system seems probable: In the primary cartilage lesion death of chondrocytes occurs as a direct consequence of the operative trauma. The matrix around these chondrocytes disintegrates and shows varying degrees of fibrillation. Inasmuch as similar features are known to occur in normal articular cartilage also (Meachim *et al.*, 1965; Ghadially and Roy, 1969) this process is probably not of specific

nature but seems to be more pronounced in association with the defects than in normal cartilage. These superficial matrix alterations may alter the diffusion of solutes to and from the deeper part of the cartilage. This step postulates that articular cartilage receives its nutrition mainly from the synovial fluid (*cf.* Maroudas *et al.*, 1968). The metabolically disturbed chondrocytes will produce a matrix with abnormal composition. Inasmuch as the deepest part of the articular cartilage probably exchanges solutes with the subchondral vessels (*cf.* Greenwald and Haynes, 1969) it would not be surprising to get a response from the cells in these vessels. The essential point in this hypothesis is to demonstrate whether or not there is a "metabolic disturbance" in the chondrocytes in the deep part of the cartilage, and if there is such a disturbance it is important to elucidate of what type it may be.

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