An experimental model of osteoarthrosis in the temporomandibular joint of the rabbit

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Degenerative changes in the temporomandibular joint were induced in 24 rabbits by surgical perforation of the disk. The incongruence obtained between the joint surfaces caused a gradual increase in macroscopic and microscopic changes, including gross remodeling, loss of tissue volume, and altered cell morphology within a 16-week observation period. These changes occurred concurrently with major alterations in the composition of the matrix, as demonstrated by increase in the glycosaminoglycan content of both condylar cartilage and disk and by loss of hydroxyproline in the disk. The lesions in the disk tissue were clearly discernible, whereas those in the condylar cartilage were less extensive. The described method is concluded to give alterations in the temporomandibular tissues, as seen in degenerative joint disease of an early stage. □ *Biochemistry; fibrocartilage; temperomandibular joint disk*

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The disk of the temporomandibular joint (TMJ) develops from dense fibrous tissue, with an abundant matrix consisting mostly of collagen and proteoglycans (PGs) (1). The collagen fibers give tensile strength and rigidity to the tissue, whereas the PGs, owing to their content of glycosaminoglycans (GAGs), provide a high charge density and a high osmotic pressure. Together these substances form an elastic tissue that reduces friction and distributes the load during articulation.

Osteoarthrosis (OA) is primarily a non-inflammatory degenerative disease of synovial joints. Studies of both natural and experimental OA have mostly comprised synovial joints with hyaline articular cartilage, such as the knee. The TMJ may also be affected (2–4) by changes occurring in the disk tissue and the articular cartilage. A typical feature of OA is fibrillation of the joint surface resulting from disintegration of the collagen network (5), and this has been associated with degradation and loss of PGs, mainly resulting from proteolytic activity (6). When the collagen starts to disintegrate and the matrix loses its content of polyanionic constituents, the elasticity diminishes. A vicious circle may thus be established in that the continued load on the less elastic tissues causes further cell damage, the release of more proteolytic enzymes, and more degradation of the matrix.

Several experimental animal models have been developed to study the onset and progression of OA (for references, see Ref. 7). Degenerative changes have been observed in the TMJ after discectomy (8). Meniscectomy creates an incongruence of the articular surfaces in the knee and has therefore been used to initiate OA-like lesions (7). Similar changes were also experimentally induced in rats after removal of molar teeth (9). A distinct incongruence of the TMJ surface may be induced by perforation of the disk, as the incongruence will result in an uneven distribution of the load. Late OA-related changes, such as denudation of the soft tissue articular surfaces and osteophytes on the condyle, have been reported to follow experimental perforation of the TMJ disk in monkeys (10). However, the mechanisms underlying such TMJ destruction have not been studied in detail.

To create an experimental animal model for the study of earlier events in the development of OA, we have analyzed the effects of a perforation in the TMJ disk in rabbits. The induced changes were monitored by the histologic findings, using a light microscope, and by the gross biochemical composition of the matrix.

Materials and Methods

Thirty male adult New Zealand White rabbits, 2.5–3.5 kg, were utilized in the study. With the guidance of an operation microscope, the disks of the right TMJ of 24 rabbits were perforated with rabbits under general anesthesia (first, intracutaneous injection of an anticholinergic drug, 0.1 ml/kg body weight, then an intramuscular injection of a neuroleptic analgesic such as fentanyl–fluamizone, 0.3 ml/kg body weight, followed by intravenous injection of diazepam, 0.3 ml/kg body weight). Through a lateral incision, a subcutaneous dissection was performed up to the lateral wall of the joint capsule, which was penetrated. The posterolateral part of the disk was isolated, and a laterocentral circular perforation with a diameter of approximately 1.5 mm was made by means of a small myringo-punch. The joint was then thoroughly irrigated with saline solution and closed. No antibiotics or drugs were administered postoperatively. All rabbits were maintained on a standard diet of pellets and water ad libitum. The rabbits were killed at intervals of 4, 8, 16, and 32 weeks after surgery.

Various controls were used to study the effects of surgery. To evaluate whether the contralateral joint of the operated rabbits could serve as a control, these tissues were compared with joints from three untreated rabbits. Moreover, a sham operation following the above guidelines was performed on the right TMJ of three rabbits. Here the operation was interrupted when the disk had been identified, the wound was closed without damage to the disk, and the tissues were examined at 4, 8, and 16 weeks, respectively, after surgery.

Fifteen of the operated animals were used to monitor the light microscopy changes after perforation of the disk. After the animals had been killed, the left and right joints were removed en bloc with the joint capsules preserved. The joint components were further dissected with the aid of a stereomicroscope. The macroscopic form and size of disks and mandibular condyles were recorded and photographically documented with a Nikon Single Lens Reflex camera and Ektachrome 64 ASA film (Figs. 1 and 2), whereafter the disks were carefully oriented and pinned by the margins to a cork mat before fixation in 4% buffered formalin. The condylar heads were dissected free from the capsular tissues and similarly fixed in formalin. After 2 days of fixation the bone tissue was decalcified with a formic acid/citrate buffer at a pH of 1.2 (22% v/v formic acid and 10% w/v monosodium citrate). The tissues were then embedded in methacrylate, and 2-μm-thick sagittal sections were cut from the condyles and the disks by means of a Leica Historange Microtome. Sections were made at 0.2-mm intervals to cover the perforation and 2 mm on each side. The sections obtained were stained with hematoxylin–eosin and with the toluidine blue–van Kossa method. These stainings enabled

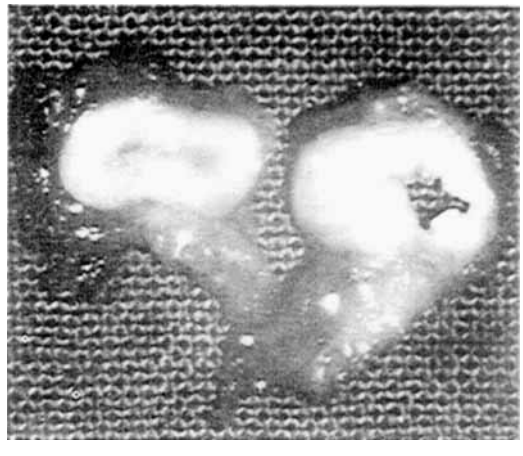

Fig. 1. Experimental (right) and control (left) disks 16 weeks after perforation. The experimental disk has an altered form, and the diameter of the defect is more than twice as large as that of the myringo-punch instrument used.

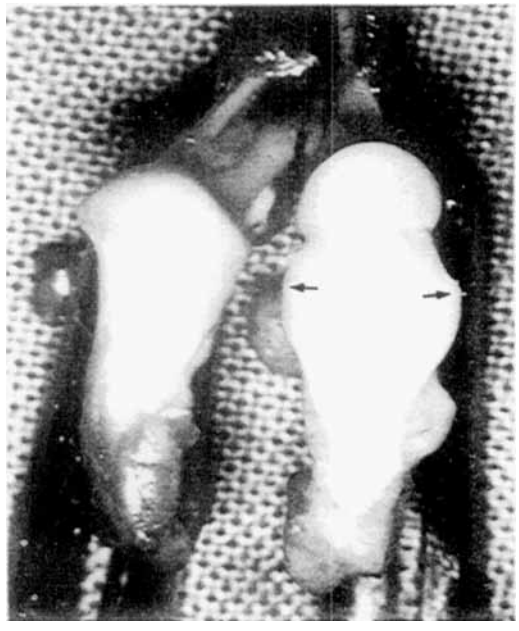

Fig. 2. Experimental (right) and control (left) condyles seen from above 16 weeks after disk perforation, showing differences in shape due to remodeling (arrows).

us to study gross tissue morphology, including matrix organization and structure, cell morphology, inflammatory cell reaction, and mineralization.

For the analysis of GAGs and hydroxyproline contents the joints of the remaining nine operated rabbits were excised 16 weeks after the perforation of the disk. Approximately 2 × 2 mm of full-thickness condylar surface cartilage adjacent to the perforation and all disk tissue from a 1-mm margin outlining the perforation were dissected for this purpose. Control tissues were obtained from corresponding areas of the left TMJ. The tissues were dried and defatted in cold acetone for 2 days, whereafter the pooled materials were weighed on a Cahn electrobalance. After digestion of the tissue fractions with papain (Sigma, St. Louis, Mo., USA) (11), the acid polysaccharides and protein remains were separated by ion-exchange chromatography (diethylaminoethyl), and the GAGs were then isolated by subsequent precipitation reactions (12). The uronic acid content of the GAG fraction was determined by a high-performance liquid chromatography (HPLC) procedure, as described by Karamanos et al. (13). The hydroxyproline analysis was carried out after hydrolysis, by means of the modified method of Stegemann & Stalders (14).

Results

The left untreated joints of the experimental rabbits, the sham-operated joints, and the joints from completely untreated rabbits were all similar macroscopically. The condylar and temporal articular surfaces were covered with cartilage; that is, no exposure of bone was seen, and the joint surfaces seemed smooth and even. The disks were biconcave, with smooth and unruffled surfaces. Nor did light microscopy show any differences (Figs. 3a and 4a).

Four and 8 weeks after the perforation the morphology of the disks was similar. Macroscopically detectable vascularization or signs of regeneration could not be seen at the perforation borders. Microscopic evaluation showed that these borders were slightly thinner, and in some disks we found increased cellularity. The fibroblast-like cells in the perforation margins were mainly oriented parallel to the surface of the defect—that is, perpendicular to the plane of the disk and thus smoothing the rough borders of the perforation. In some areas the cells had a rounded form and were surrounded by a more basophilic matrix. These chondrocyte-like cells were more abundant posterior to the lesion, whereas in the normal disks the flattened fibroblast-like cells were evenly distributed throughout the fibrous tissue. There were no signs of inflammatory cell response in the load-bearing area of the disk. The condylar and temporal components of the operated right joints showed no significant change during the first 8 weeks after perforation.

Sixteen weeks after the operation the changes observed in joint morphology were more marked. The findings were similar to those seen after 32 weeks. The altered zone at the perforation margin and the diameter of the tissue defect had increased considerably

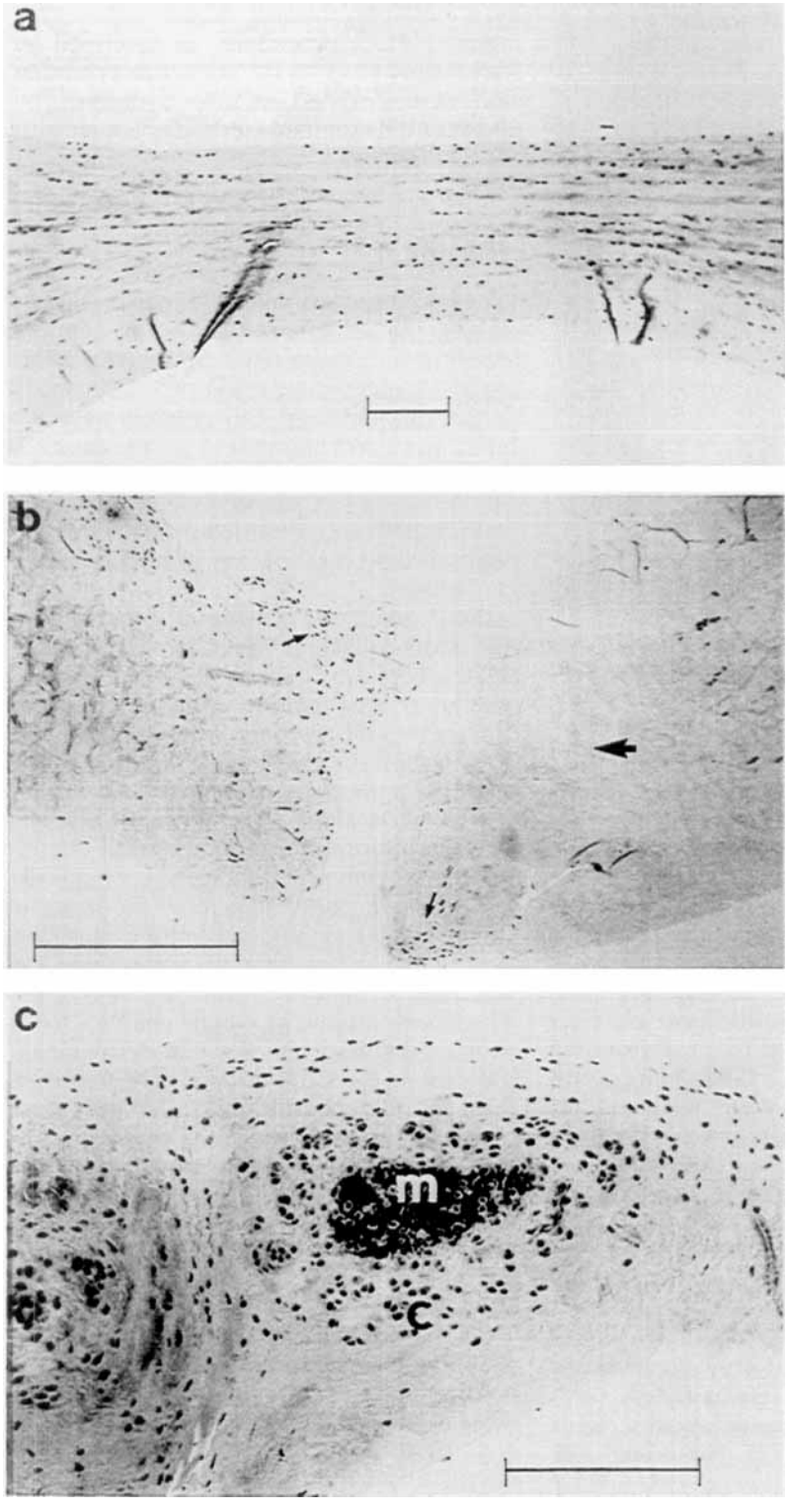

Fig. 3. Disks from the temporomandibular joint. Control disk (Fig. 3a), and 16 weeks after perforation of the disk (Figs. 3b and 3c). The celluarity is increased close to the tissue defect (→), whereas somewhat deeper the tissue is hypocellular (➔) (Fig. 3b). The remaining cells are often rounded (c), and the tissue contains foci of mineralization (m) (3c). Bar = 1 mm. (Hematoxylin and eosin stain).

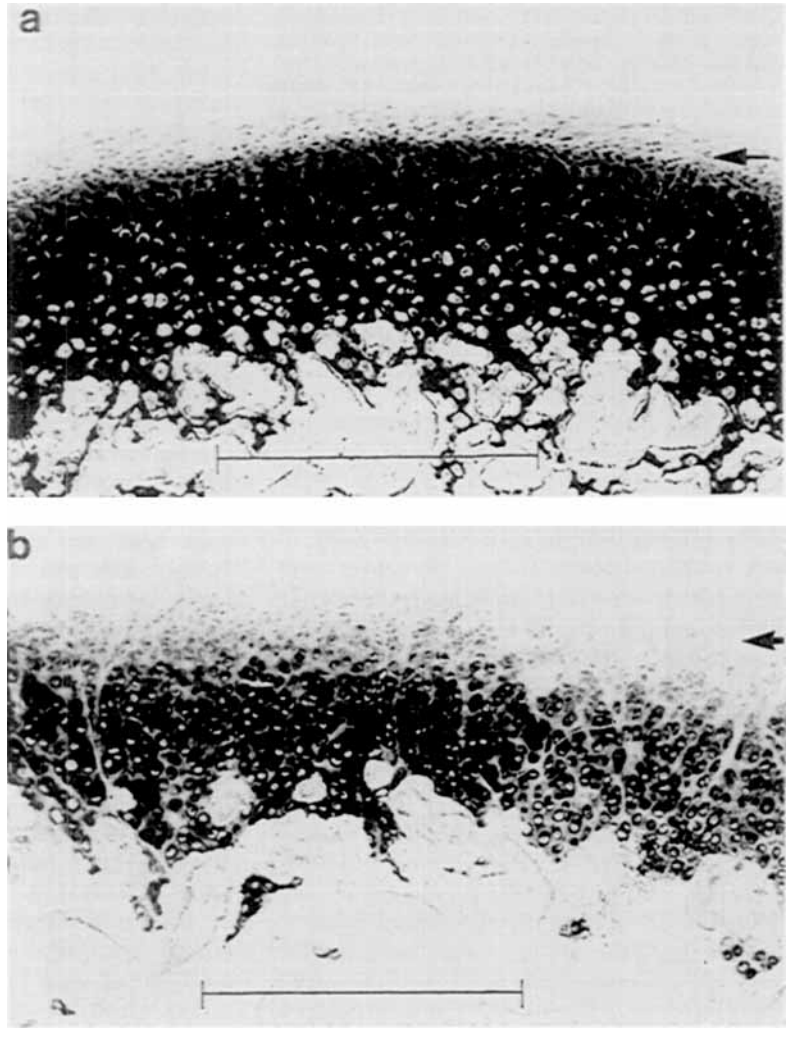

Fig. 4. Articular cartilage from mandibular condyles. The unaffected tissue (Fig. 4a) shows a fibrous lining (➔) with cells mainly oriented parallel to the surface, whereas 16 weeks after disk perforation (Fig. 4b) this layer is less organized and uneven (➔). Bar = 1 mm. (Hematoxylin and eosin stain.)

(Fig. 1). These more pronounced degenerative features of the disk were also seen histologically (Fig. 3b, c). The perforation borders showed areas with fragmentation. The normal arrangement of the collagen fibers parallel to the disk surface was lost, the direction of the fibers being less organized. The increased cellularity seen close to the tissue defect as early as after 4 weeks was still more pronounced, whereas the tissue distant to the defect had become almost acellular. The chondrocyte-like differentiation was commoner, and in some areas clusters of such cells had a hypertrophic appearance. The basic reactions of the surrounding matrix were also more marked, and calcified areas were seen in association with these hypertrophic cells. Simultaneously with the development of a cartilage-like matrix, the contents of GAGs increased considerably, whereas there was a parallel decrease in the hydroxyproline content (Table 1).

Macroscopically, the condyles showed extensive remodeling 16 weeks after surgery (Fig. 2), whereas only minor changes were observed 4 and 8 weeks after disk perforation. When examined histologically,

Table 1. Total contents of glycosaminoglycans and collagen in rabbit temporomandibular joint disks and condylar cartilage after unilateral experimental perforation of the disk. The contralateral side was used as control (n = 9 rabbits)

	Total GAGs*	Total hydroxyproline†
Disks		
Experimental	4.07	61.2
Control	2.57	97.8
Cartilage		
Experimental	14.1	68.1
Control	7.51	64.0

* In μg UA/mg dry tissue weight.
† In μg hydroxyproline/mg dry tissue weight.

changes were not so consistent and apparently progressive as in the disks. Some of the condylar heads 16 and 32 weeks postoperatively showed areas with thickening and thinning of the fibrous lining (Fig. 4b). The collagen arrangement of this lining was disturbed, and the orientation in bundles parallel to the surface was less obvious under the microscope. The normally even distribution of flattened fibrocyte-like cells, with their long axes parallel to the joint surface, was disordered and showed both hypo- and hyper-cellular areas. Scattered rounded chondrocyte-like cells were also found in this layer of the experimental tissue. The bone tissue at the osteochondral junction seemed somewhat coarser in the experimental tissue, but we detected no signs of altered bone formation, and the osteoid seams were similar on the operated and control sides. The changes in the articular cartilage were accompanied by an increased amount of GAGs, whereas the collagen content seemed unaffected (Table 1). No certain light-microscopic changes in condyles were found 4 and 8 weeks after surgery.

Discussion

A widely accepted theory about the development of OA is that the functional forces exceed the adaptive ability of the joint surface and lead to deterioration of the tissue (for references, see Refs. 15, 16). It has been suggested that the pathogenesis of OA in this joint is similar to that in other synovial joints (17). One way in which OA may develop in the TMJ is by excessive grinding or clenching of the teeth, which may also overload the TMJ (18). It has further been suggested that a loss in molar support and a change in the condylar position in the fossa may set off degenerative changes in the joint tissues (19). Moreover, discectomy is associated with a diminished adaptation of the joint surfaces, and also here clinical radiographic examinations have shown an increased risk of degenerative joint disease (8). The condylar TMJ cartilage has been thought to have a considerable regeneration capacity due to the presence of the undifferentiated mesenchymal cell layer immediately beneath the fibrous surface lining (20). Despite this capacity to regenerate, an autopsy study showed macroscopic lesions in about 20% of the subjects more than 40 years old (21).

Signs of cell proliferation in the perforation margins of the disk were noted only 4 weeks after surgical perforation, and this change became more marked, especially in the posterior part. The loss of disk tissue adjacent to the edge of the perforation seen after 16 weeks indicated extensive degeneration of the disk. This was also reflected by the rapid decrease in the collagen content of the disk, whereas in the condylar cartilage no such changes were found.

The condylar cartilage also reacts with proliferation, as demonstrated by macroscopically obvious remodeling. Since the thickness of soft cartilage tissue is unaltered, such a process must also be affected by changes in the osteochondral junction, although they could not be followed by light microscopy. These lesions, observed 16 weeks after the experimental damage to the joint surfaces, include concurrent degeneration and proliferation and thereby fulfill the criteria for OA in the TMJ. The finding of deteriorated articular surfaces and disk tissue in association with remodeling of joint components characterizes the diagnosis of OA in the TMJ (3, 17, 22, 23). The use of the contralateral joint as a control in the operated animals seemed appropriate, since

the findings did not differ from those of joints with sham operation or joints from untreated rabbits. The microscopic structure of the normal TMJ disk in rabbits seems not to differ significantly from the histologic appearance of the human TMJ disks (24–26). Its function as a load-distributing, shear-resistant, and shock-absorbing tissue has been proposed by several authors, and its composition is well suited to meet such demands (24, 26, 27). Previous studies have shown that experimental perforation or extirpation of the disk resulted in arthrotic changes after 40–61 weeks and 12 weeks, respectively (10, 28). This does not contradict the findings in our study, in which 16 weeks were necessary to produce equivalent lesions. The exact overloading mechanism by which this degeneration develops, however, may vary in the different experimental models. Thus, no vascularization of the disks (10) could be demonstrated in the present material.

The finding of altered staining qualities in the disk matrix and simultaneous chondrocyte-like differentiation of the normally flattened fibroblast-like cells is similar to what has recently been described in sheep (29). In fact, the tissue contains foci that look like fully developed hyaline cartilage with hypertrophy of chondrocytes and mineralization of the surrounding matrix. These foci bear some resemblance to the epiphyseal growth zones or cartilagenous callus, although there are no signs of subsequent bone formation.

The change in morphology after perforation of the disk may reflect an adaptation with chondral metaplasia, which also accords well with the finding of a considerably increased content of GAGs together with a lowered content of collagen. The increase in GAG contents differs from our previous findings in arthrotic human TMJ disks (30). However, all of these human disk tissues presented extensive lesions, and this discrepancy may well reflect differences between early and late stages of the disease. The results indicate that the biochemical composition of the tissue is influenced at an early stage and probably of great importance for the further development of this condition. Studies aiming at the characterization of these biochemical events have already been initiated in our laboratory.

Conclusion

The perforation of TMJ disks in rabbits will lead to progressive morphologic and biochemical alterations in adjacent cartilage tissues. The thickness of disk tissue was here considerably decreased, and the tissue structure was affected with altered collagen organization and signs of mineralization. The form of the condylar head was also changed, and its cartilage showed structural aberrations mainly in the superficial layers. It seems appropriate to view the lesions obtained as an experimental equivalent to the naturally occurring OA in the human TMJ.

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