Changes in Permeability of Rabbit Articular Cartilage Caused by Joint Contracture as Revealed by the Peroxidase Method

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Summary. Changes in permeability of adult rabbit articular cartilage caused by joint contracture were studied by light and transmission electron microscopy, employing horseradish peroxidase (HRP) as an indicator. The knee joint was plaster-immobilized for 0, 2, 4, 6, or 8 weeks in the flexion position. One ml of 4% HRP was administered in the articular cavity of the knee joint and allowed to diffuse and permeate into the articular cartilage. Distribution of the permeated HRP was visualized in the cartilage taken from the lateral condyle of the femur, utilizing the DAB-H2O2 reaction. In the normal and the non-immobilized joints, the permeated HRP reached to the matrix and chondrocytes situated in the deep layer of the articular cartilage. HRP was heavily deposited in the intercellular matrices, particularly around the chondrocytes, and was actively endocytosed by these cells. In the plaster-immobilized joints, especially after 4 weeks or longer of immobilization, the administered HRP had not permeated well and was restricted to the surface (lamina splendens) and the superficial layer of the cartilage. These results show that administered HRP diffuses into the deep layer of the articular cartilage and is actively endocytosed by chondrocytes and that the permeability of articular cartilage is remarkably reduced by joint contracture.

In the articular cartilage of the rabbit femur, considerable morphological changes in chondrocytes and intercellular matrices are induced when the knee joint is subjected to experimental contracture (OHTA et al., 1981). In parallel with the structural changes, alkaline phosphatase (ALPase) activity also changes (OHTA et al., 1983), suggesting that functional aspects of the articular cartilage might be affected by the plaster-immobilization as well.

Disturbances in permeability of nutrients, in particular, are strongly expected to occur in contractured articular cartilage. Since the adult joint cartilage lacks blood capillaries, nutrients for the chondrocytes and intercellular matrices in the cartilage are supplied from the synovial fluid by diffusion through the articular cartilage surface (HODGE and MCKIBBIN, 1969; HONNER and THOMPSON 1971; OGATA et al., 1978).

In the present study, therefore, attempts were made to examine the permeability

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in the articular cartilage and its changes related to joint contracture by light and electron microscopy, employing HRP as an indicator. The uptake of the administered HRP within chondrocytes was also studied.

**MATERIALS AND METHODS**

The articular cartilage at the lateral condyle of the femur of adult rabbits (approximately 3.5 kg in average weight) was used. The knee joint of the right hind limb of each rabbit was plaster-immobilized with maximal flexion for either 0, 2, 4, 6, or 8 weeks, five animals being immobilized for each specified period. As a control, the corresponding portion of the cartilage of the untreated left hind limb was used.

The animals were anesthetized with Ketalar. The knee joint articular cavities were washed two times with sterile physiological saline by subcutaneous injection through the articular capsule. After being washed, 1 ml of 4% HRP (Sigma, type VI, U.S.A.) in physiological saline was injected into the cavity. The knee joints were then kept in the flexion position for 30 min. The central area of the articular cartilage was quickly taken out from the lateral condyle of the femur using a sharpened scalpel. The specimens were fixed with a mixture of 2% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C for 1 hr. They were then washed two times with chilled phosphate-buffered saline for 30 min and washed again with 0.05 M Tris-HCl buffer (pH 7.4) for 30 min. Frozen sections of about a 40 μm thickness were made, immersed in 3, 3'-diaminobenzidine·HCl (DAB, 0.2 mg/ml)-H2O2 (0.005%) for 15 min, and rinsed with distilled water. After being washed, some of the sections from each specimen were examined by light microscopy. Others were osmicated for 2 hr, dehydrated through a series of graded ethanols, embedded in Epon 812, and processed for transmission electron microscopy.

As a cytochemical control experiment, the same volume of physiological saline instead of HRP was injected into the knee joint cavities. After 30 min, the corresponding area of the articular cartilage was removed, processed for the DAB reaction as above, and examined.

**RESULTS**

1. **Light microscopic findings**

In the *normal* and the *untreated* joints, DAB reaction product showing the presence of the diffused HRP was distributed from the surface amorphous layer (lamina splendens) to the deep layers of the cartilage (Fig. 1). HRP activity, in particular, was strongly positive in the surface amorphous and superficial layers. Both the matrix and the chondrocytes in all the layers of the cartilage were positive for the DAB reaction.

In the joints *immobilized for 2 weeks*, HRP was also distributed in the matrix and the chondrocytes in all the layers of the cartilage as seen in the normal and the non-immobilized joints (Fig. 2).

In the joints *immobilized for 4 weeks*, the positive reaction became much weaker both in the matrix and in the chondrocytes in the middle and deep layers of the cartilage (Fig. 3). In the superficial layer, on the other hand, the DAB reaction was still strongly positive.

In the joints *immobilized for 6 weeks*, only the surface amorphous layer was positive.
Fig. 1-6. Light micrographs of the articular cartilage from untreated or plaster-immobilized joints.
S cartilage surface. × 800

Fig. 1. Untreated joint. Reaction product of HRP activity, showing the extent of HRP permeation, is demonstrated in all the layers. HRP activity, in particular, is strongly positive in the surface amorphous and the superficial layers.

Fig. 2. Joint immobilized for 2 weeks. The reaction product can here also be traced far into the deep layer. Its distribution pattern is nearly the same as that seen in Figure 1.

Fig. 3. Joint immobilized for 4 weeks. The reaction product is localized in the surface amorphous and the superficial layers.

Fig. 4. Joint immobilized for 6 weeks. The deposition of the reaction product is restricted exclusively to the surface amorphous layer.

Fig. 5. Joint immobilized for 8 weeks. The reaction product is present merely in the surface amorphous layer as seen in Figure 4.

Fig. 6. A cytochemical control. No positive reaction is seen in any layers of the articular cartilage from the untreated joint.
Furthermore, the positive reaction detected on the superficial layer of the cartilage was remarkably reduced in intensity (Fig. 4), as compared to that seen in the non-immobilized and 2- or 4-week-immobilized joints.

In the joints immobilized for 8 weeks, practically no positive reaction was seen in any layers of the articular cartilage (Fig. 5), except in the surface amorphous layer where merely a faint reaction was detected.

In cytochemical control studies, endogenous peroxidase activity was not observed in the cartilage of the untreated or immobilized knee joints (Fig. 6).

2. Electron microscopic findings

In the normal and the non-immobilized, joints the reaction product showing the permeated HRP activity was seen in the chondrocytes and matrix through the superficial layer.

Fig. 7 and 8. Electron micrographs of the articular cartilage from the untreated joint. S cartilage surface.

Fig. 7. Superficial layer of the articular cartilage. The reaction product is present in the amorphous matrix (arrow) and collagen fibrils (arrowheads), which are arrayed in parallel with the cartilage surface. × 10,000

Fig. 8. Middle zone of the articular cartilage. A strong DAB reaction is present in the pericellular matrix. In the chondrocytes, the reaction product is localized mostly in the plasma membrane, pinocytic pits (arrowheads), and vesicles (arrows). × 26,000. Inset shows a magnification of the matrix of the superficial layer. Note that the DAB reaction product is present in the collagen fibrils as well as in the amorphous matrix. × 24,000

for HRP. Furthermore, the positive reaction detected on the superficial layer of the cartilage was remarkably reduced in intensity (Fig. 4), as compared to that seen in the non-immobilized and 2- or 4-week-immobilized joints.

In the joints immobilized for 8 weeks, practically no positive reaction was seen in any layers of the articular cartilage (Fig. 5), except in the surface amorphous layer where merely a faint reaction was detected.

In cytochemical control studies, endogenous peroxidase activity was not observed in the cartilage of the untreated or immobilized knee joints (Fig. 6).
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... to the deep layers of the cartilage. In the matrix, in particular, HRP activity was strongly positive around the perichondrocytic area (Fig. 7, 8). Under higher magnification, the reaction product was seen to be heavily deposited on and between the collagen fibrils in the pericellular matrix (inset in Fig. 8). The permeated HRP was actively endocytosed by chondrocytes, in which DAB-stained pinocytic pits and vesicles were frequently observed. Intracellularly, the DAB reaction was also positive in the vesicles of the Golgi region, but not in the mitochondria or in the rough endoplasmic reticulum (Fig. 8).

During the course of the immobilization, the reaction product progressively decreased in amount in the matrix, and DAB-positive cells were encountered less frequently—especially in the middle and deep layers of the cartilage. The longer the joints were immobilized, the less intense was the DAB staining reaction found in the matrix and chondrocytes in all the layers of the articular cartilage. This tendency was most evident in the joints immobilized for 8 weeks.

In these joints immobilized for 8 weeks, the cartilage surface was uneven and irregular, and the filamentous and amorphous surface layer was thick. The reaction product was sparsely distributed in the surface amorphous layer of the degenerated cartilage, and the cartilage matrix immediately beneath the articular surface was also slightly positive (Fig. 9). In the middle and deep layers, the reaction product was not observed either in the intercellular matrix or within cells (Fig. 10).

DISCUSSION

In the present study, the permeability of nutrients from the articular surface into the...
cartilage matrix and chondrocytes as well as the changes in permeability related to joint contracture were clarified by light and electron microscopy using HRP as an indicator. The permeability of articular cartilage has repeatedly been studied using various tracers such as dyes (Brower et al., 1962; Maroudas et al., 1968), radioisotopes (Hodge and McKibbin, 1969; Honner and Thompson, 1971; Mankin, 1963), and hydrogen gases (Ogata et al., 1978). Although these tracers do permeate through the cartilage surface and reach into the deep zone of the articular cartilage as well, their application is primarily at the light microscopic level. Furthermore, they offer rather poor information regarding permeability, owing to their low quality of resolution. However, HRP is a substance whose location can be easily visualized and identified both by light and electron microscopy.

When administered into the non-immobilized knee joint cavity, HRP permeated by diffusion and could be traced into the deep layer of the articular cartilage. Both chondrocytes and intercellular matrices were strongly positive for the DAB reaction. By electron microscopy, the permeated HRP was found to be heavily deposited in the intercellular matrices, especially surrounding chondrocytes. Also pinocytic pits and vesicles of chondrocytes were filled with HRP. These observations show that the permeated HRP is actively endocytosed by chondrocytes.

The uptake of HRP itself is fairly common among various cells and tissues. For instance, exogenous HRP is incorporated in the Golgi vesicles and saccules in adeno-hypophyseal cells when incubated for 4 hr (Pelletier, 1973). In macrophages, HRP uptake occurs more rapidly. Pinocytic vesicles are filled with HRP within 5 min, while secondary lysosomes are saturated after a 45-60 min incubation (Steinman et al., 1976). The HRP uptake observed in the present study may be regarded as a process of endocytosis, which has been extensively described for various cells and tissues. It is quite likely that nutrients for the cartilage, at least some of them, may be taken up by chondrocytes in a way similar to that by which HRP permeates into the area and is finally endocytosed.

Plaster-immobilization of the joint causes remarkable changes in the distribution of the permeated HRP. HRP was restricted to the superficial region of the articular cartilage, despite the fact that the same volume and concentration of HRP was administered as was used for the normal and the non-immobilized joints, in which HRP reached far into the deep layer. During the course of the immobilization period, the deposited HRP decreased markedly in amount, especially in the middle and the deep layers of the cartilaginous matrix. Also chondrocytes, which actively endocytosed HRP, were gradually reduced in number and finally became sparse after long-term immobilization. Thus, joint contracture apparently induces changes in permeability and uptake of HRP in articular cartilage. It is accompanied by morphological changes such as the thickening of amorphous substances on the articular surface, undulation and coarseness of the matrix, deposition of necrotized substances in the matrix, decrease in cell density, and irregular cell arrangement in the articular cartilage (Ohta et al., 1983). A felt-like amorphous layer on the surface of the cartilage thickened during the course of the plaster immobilization. This layer could be a barrier to the permeation of various substances including HRP. In addition, the articular fluid decreases in volume as well, depending on the length of the immobilization period (Ohta et al., 1981).

In the immobilized joints, changes in permeability occur probably owing to structural disturbances of cartilaginous cells and the matrix. Chondrocytes reveal remarkable changes in structure (Ohta et al., 1981), but still retain some function as they are
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positive for ALPase activity (OHTA et al., 1983). Permeability of HRP seemed to be prevented by the disorder of the matrix, so that HRP could not reach these cells, even if they did retain the capacity to take up HRP. In the immobilized joints, therefore, disorders in the intercellular matrix and the subsequent decrease in permeability seem to be responsible for the decrease in uptake of nutrients from the articular surface into the cartilage. It is, thus, strongly suggested that the joint functioning in a mobile manner plays an important role in maintaining the permeability of the articular cartilage and subsequently allowing of adequate nutrient supply to it as well as prevention of its structural disturbances.

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