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Electron microscopic studies on resorption of xenogeneic cartilage implants

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Summary. Xenogeneic implants of devitalized cartilage were used as a model system to study the fine structural details of cartilage resorption. The implants were surrounded by a cellular infiltrate consisting of macrophages, fibroblasts, lymphocytes, and eosinophils. Throughout the observation period (2–12 weeks), macrophages formed a palisade-like layer directly opposed to the surface of the implant. The total size of the infiltrate decreased with time. In the acute phase (2 weeks), lymphocytes and eosinophils were abundant but thereafter decreased in number. Consequently, there was a marked predominance of macrophages and fibroblasts in the chronic phase (4–12 weeks). Large multinucleated cells, believed to represent polykaryons formed by fusion of macrophages, were also observed. These findings indicate that inflammatory cells are capable of resorbing cartilage without the participation of living chondrocytes. Apparently, macrophages have direct responsibility for the resorptive process. The role of the other cell types is less clear. In the early period after implantation, they may interact with the cartilage and release factors that stimulate and direct the function of the macrophages.

Fine structurally, the macrophages were characterized by a large Golgi complex and numerous coated vesicles. In contrast, phagosomes and lysosomes were few. The coated vesicles were located in the Golgi area, where they appeared to form by budding from dilated rims of stacked cisternae, and in the peripheral parts of the cells, opposing the implant. It is suggested that the coated vesicles transport degradative enzymes from the Golgi complex to the plasma membrane for release extracellularly. This conforms to the idea that cartilage resorption is a lytic rather than a phagocytic process. Our findings agree with and partly extend earlier observations on cartilage resorption in connection with inflammatory joint diseases and tumor invasion.

Key words: Cartilage resorption – Macrophages – Coated vesicles

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Resorption of cartilage occurs physiologically during endochondral bone formation. It is also observed in connection with inflammatory and degenerative joint diseases, tumor invasion, and destruction of implants. Vascularized connective tissue (Schenk et al. 1968), inflammatory cells (Vischer 1982), and tumor cells (Kuettner and Pauli 1981) have been implicated in the resorptive process. They are believed to exert their function by secreting lytic enzymes such as collagenase, neutral proteases, and lysosomal hydrolases (Vaes et al. 1981). Another possibility is that the chondrocytes themselves produce and release lytic enzymes under influence of catabolic factors of mononuclear cell origin (Dingle 1981).

In the present study we have used transmission electron microscopy to shed further light on the mechanism of cartilage resorption and particularly the role of inflammatory cells. Xenogeneic implants of calcified and uncalcified cartilage were used for this purpose. In order to exclude participation of the chondrocytes, the cartilage was devitalized by repeated freezing and thawing before the implantation. This could lead to release of enzymes from the cells and partial degradation of the pericellular matrix. Nevertheless, recent experiments have shown that prior devitalization does not per se lead to implant resorption (Pawlowski et al. 1982). We also made experiments in which the cartilage was treated with hydrochloric acid and/or sodium chloride before implantation. These treatments have earlier been found to modify the susceptibility of cartilage to resorption (Kuettner and Pauli 1981; Pawlowski et al. 1982).

Materials and methods

Animals. Adult female rabbits (4.0–4.5 kg) were used as donors and adult (6-month-old) female Sprague-Dawley rats as recipients of cartilage implants.

Preparation of material for grafting. Whole xiphoid processes and calcified parts of cartilaginous ribs were dissected out and carefully cleaned from surrounding tissues including perichondrium. The tissue was then frozen and thawed three times for devitalization. Ribs with a diameter of 2 mm were chosen and cut into 1.5 mm cylinders. Peripheral, uncalcified parts of the xiphisternal cartilage were cut into 2×2 mm pieces. The collected material was divided into four groups and treated as follows:

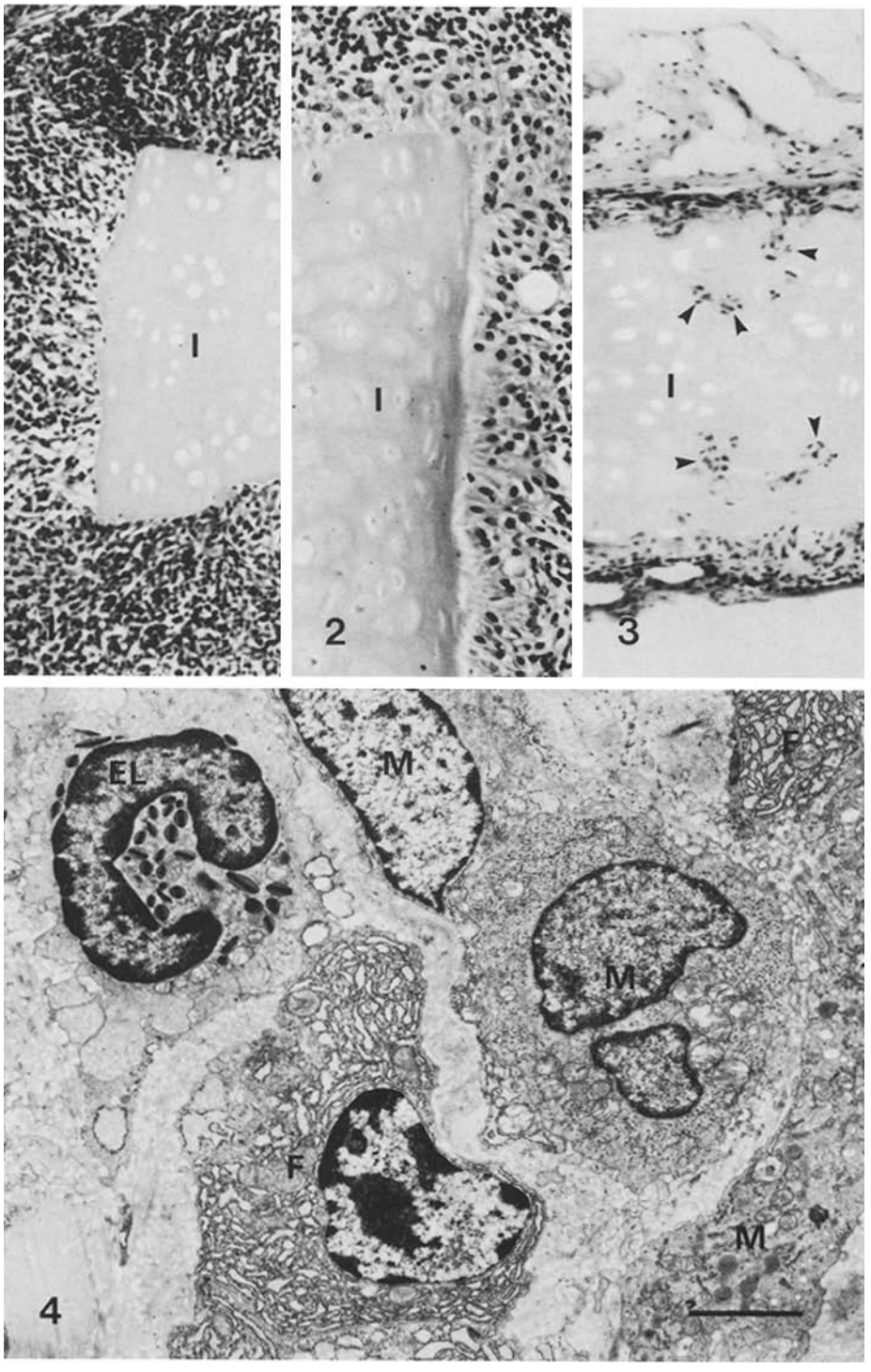
1. untreated (control)
2. decalcification with 0.6 N HCl for 1.5 h at 4° C
3. extraction with 1.0 M NaCl, pH 5.8, for 48 h at 4° C
4. decalcification with 0.6 N HCl followed by extraction with 1.0 M NaCl

The tissue was then rinsed with phosphate-buffered saline (PBS) and stored at −20° C before use. All steps were done under sterile conditions.

Implantation procedure. Animals were anesthetized with ether. After incision of the skin the abdominal muscles were exposed and cartilage fragments were inserted into small intramuscular pockets after rinsing in PBS.

Figs. 1–3. Light micrographs of two- (**Fig. 1**) and twelve-week-old (**Fig. 3**) implants of decalcified rib cartilage and four-week-old implant of untreated sternal cartilage (**Fig. 2**). The implants (*I*) are surrounded by a cellular infiltrate that decreases in size with time (cf. **Figs. 1 and 3**). **Figure 2** demonstrates how the cells next to the cartilage arrange themselves in a palisade-like pattern. In **Fig. 3**, the infiltrating cells have penetrated deep into the cartilage (*arrowheads*). **Fig. 1**, ×120; **Fig. 2**, ×260; **Fig. 3**, ×160

Fig. 4. Part of the cellular infiltrate surrounding a two-week-old implant of untreated sternal cartilage. Macrophages (*M*), fibroblasts (*F*), and an eosinophilic leukocyte (*EL*) are shown. Bar 2.0 μm. ×8,000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Morphological examination. After 2, 4, 8, and 12 weeks the animals were killed and the implants were excised together with surrounding tissues and immediately fixed in 95% ethanol/35% formaldehyde (2/1) or glutaraldehyde. For light microscopy, the specimens were prepared according to standard procedures and stained with haematoxylin and eosin. Calcified implants were demineralized in 1 N HNO_3 overnight. For electron microscopy, the specimens were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer, pH 7.3, with 0.05 M sucrose. They were then postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, stained with uranyl acetate, dehydrated in ethanol, and embedded in low-viscosity epoxy resin. For decalcification, the tissue was immersed in glutaraldehyde fixative with 0.1 M EDTA, pH 7.3, for at least 4–5 days. Thin sections were cut on an LKB Ultrotome IV, stained with lead citrate at alkaline pH, and examined in a Philips EM 300 electron microscope operated at 80 kV.

Results

Light microscopic observations

Two-week-old implants were surrounded by large inflammatory infiltrations consisting of mononuclear cells (Fig. 1). Numerous eosinophils were also noted. At the subsequent intervals, a successive decrease in the size of the cellular infiltrate was evident (Figs. 2 and 3). Macrophages arranged in a palisade-like pattern directly opposed to the surface of the implant (Fig. 2). Fibroblasts together with some lymphocytes and eosinophils were found outside this layer. In eight- and twelve-week-old implants, large multinucleated cells also appeared along the border of the cartilage. They were found in contact with calcified and uncalcified regions.

The above description applies to all experimental groups. Thus, no distinct effect of decalcification and/or salt extraction on the reaction of the host tissue against the implant was detected.

Electron microscopic observations

Fine structural examination was concentrated on the inner part of the infiltrate, opposed to the implant. In two- and four-week-old implants, this layer consisted of macrophages, fibroblasts, lymphocytes and eosinophils (Fig. 4). As soon as two weeks, the macrophages showed a tendency to arrange themselves in a palisade-like pattern along the surface of the implant. They were columnar in shape with the nucleus located away from and the cytoplasm towards the cartilage (Fig. 5). A large Golgi complex was located in the juxtanuclear region of the cells (Fig. 6). Numerous small vesicles, often of the coated type, were found in this region and appeared to be formed by budding from stacked Golgi cisternae (Figs. 6 and 7). Abundant coated vesicles were also present close to the plasma membrane and were seen to open extracellularly towards the adjoining cartilage (Figs. 8 and 9). In contrast, only few typical phagosomes and lysosomes were observed. In eight- and twelve-week-old implants large multinucleated cells appeared with cytoplasmic processes penetrating into the cartilage matrix (Figs. 10 and 11). The central cytoplasm of these cells contained a large Golgi complex, many small vesicles, and numerous mitochondria.

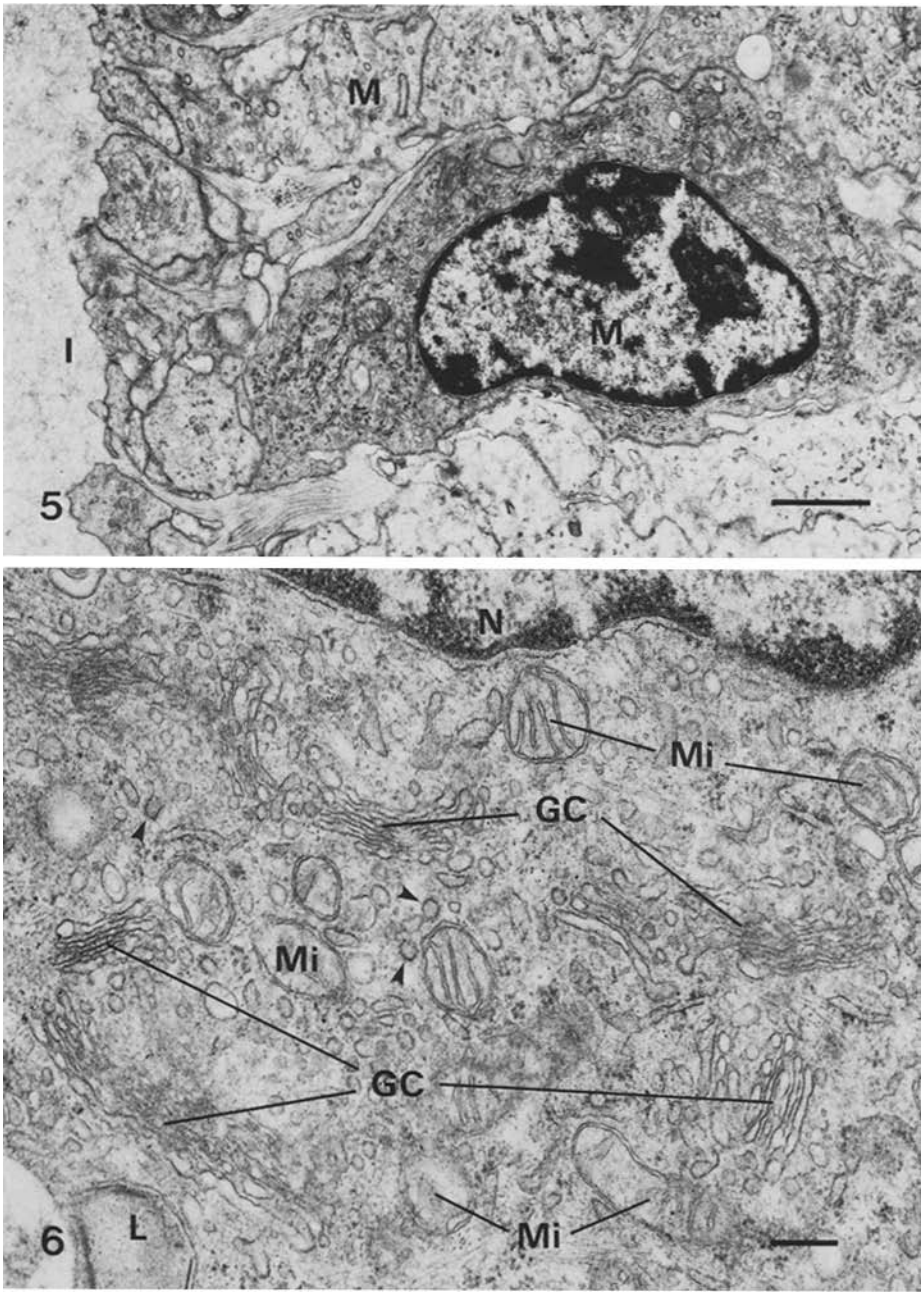
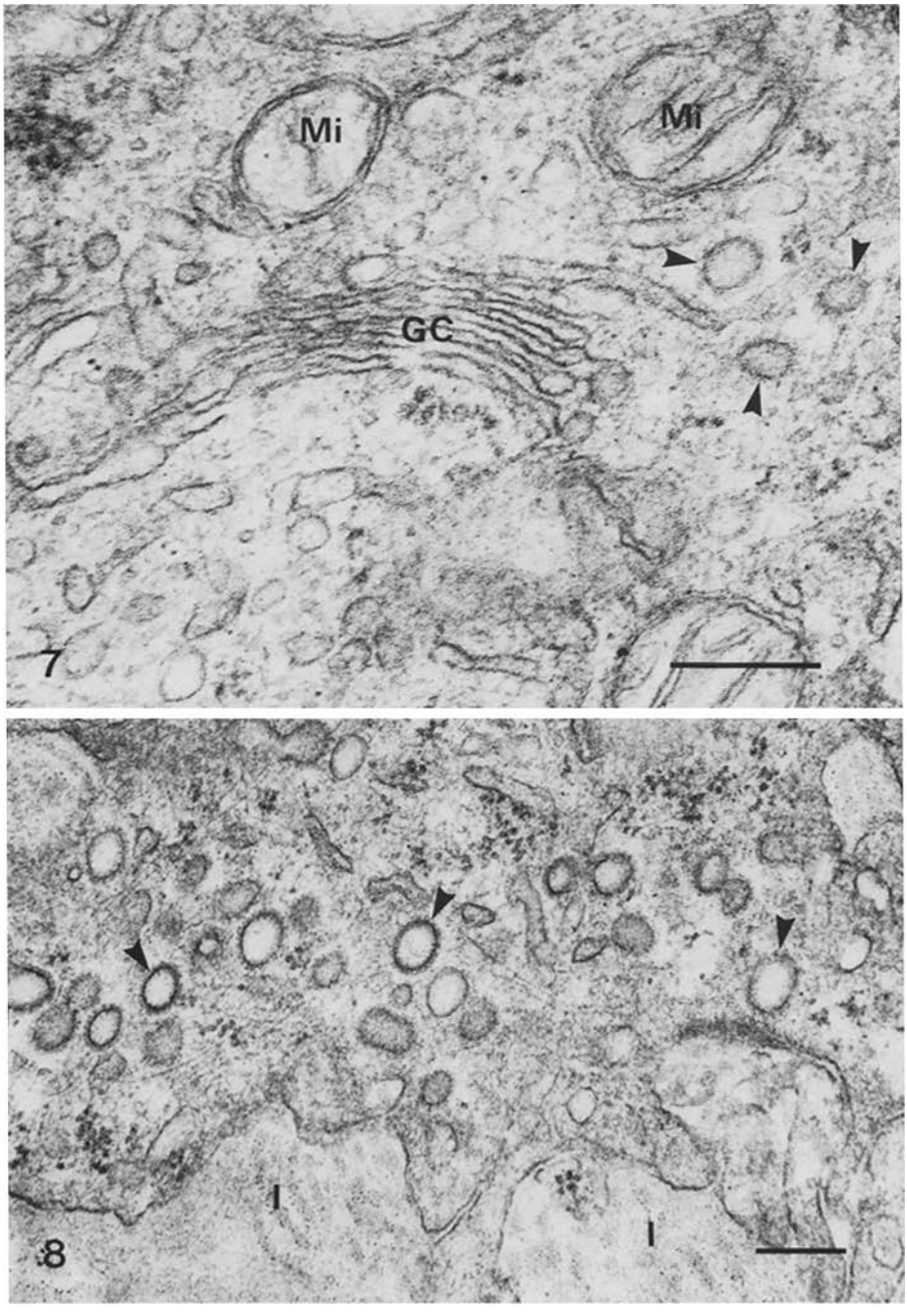


Fig. 5. Palisade-like arrangement of macrophages (*M*) in the cellular infiltrate surrounding a two-week-old implant (*I*) of sternal cartilage treated with 0.6 N HCl. Bar 1.0 μm. ×13,000

Figs. 6–8. Details of macrophages in the cellular infiltrate surrounding a four-week-old implant of sternal cartilage extracted with 1.0 M NaCl.



Figures 6 and 7 show large juxtanuclear Golgi complexes consisting of stacks of cisternae (*GC*). Numerous small vesicles are also present throughout the Golgi area. Many of these are of the coated type (*arrowheads*). **Figure 8** shows abundant coated vesicles in the peripheral part of a macrophage, directly opposed to the matrix of the implant (*I*). *L*, lysosome; *Mi*, mitochondria; *N*, nucleus. Bars 0.2 μm. **Fig. 6**, ×44,000; **Fig. 7**, ×101,000; **Fig. 8**, ×61,000

Abundant fibroblasts were found outside the 'palisade' of macrophages (Fig. 4). Their number, as a proportion of the cells in the infiltrate, increased with time, in parallel with a decrease in the number of eosinophils and lymphocytes. The fibroblasts had an extensive rough endoplasmic reticulum, often with dilated cisternae, and a large juxtanuclear Golgi complex. They were not located at the very borderline of the implant but showed cytoplasmic extensions that extended in between the macrophages and approached the cartilage.

The implant itself consisted of dead chondrocytes and an extensive extracellular matrix. Thin collagen fibrils were the main structural component of the matrix. With increasing time after implantation, these fibrils showed a tendency to become wider and more clearly cross-striated (Figs. 12 and 13). In contrast to the situation in living cartilage, proteoglycan granules (Thyberg 1977) were difficult to distinguish. Although the specimens were decalcified before sectioning, the borderlines between calcified and uncalcified matrix could be recognized due to the existence of laminae limitantes (Scherft 1972). In a narrow zone next to the macrophages and the large multinucleated cells, the matrix was poorly stained and appeared partly digested (Figs. 10 and 11).

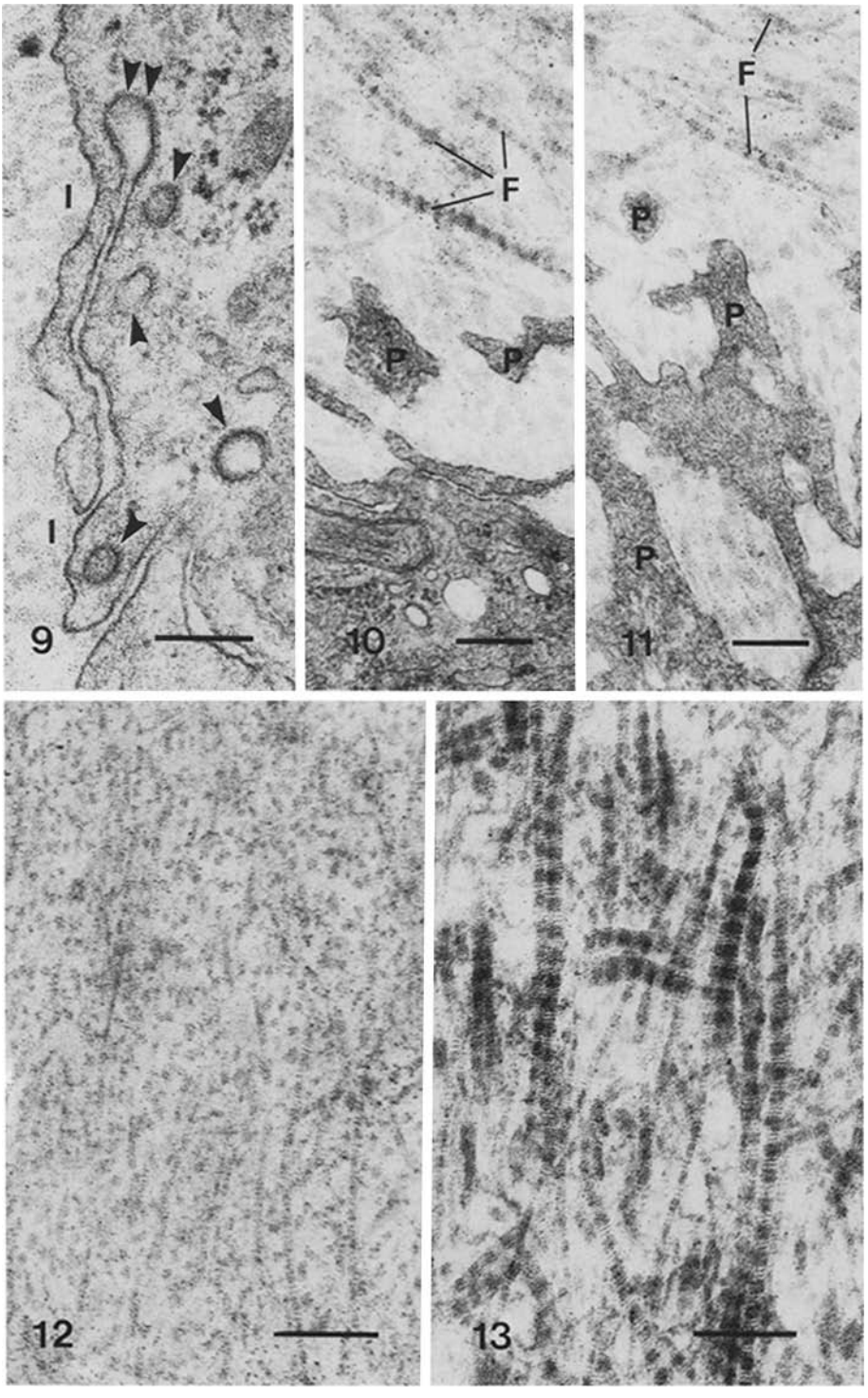
Decalcification and/or salt extraction of the cartilage before implantation did not clearly affect the fine structure of the organic matrix nor the composition and morphology of the cellular infiltrate surrounding the implant.

Discussion

In the present study, xenogeneic implants of devitalized cartilage were used as a model system to study cartilage resorption. Our findings indicate that inflammatory cells are capable of resorbing cartilage and that the participation of living chondrocytes is not required. This does not exclude a role for the latter in other experimental systems and in the destruction of cartilage that occurs in connection with degenerative joint diseases (Ali and Evans 1973; Dingle 1981; Vischer 1982).

A cellular infiltrate dominated by macrophages surrounded the implants. They formed a palisade-like layer that encircled the cartilage. Fibroblasts were located outside this layer and admixed with lymphocytes and eosinophils. In the first few weeks after implantation, the two latter cell types were quite abundant but thereafter decreased in number. Moreover, with increasing time large multinucleated cells appeared which probably represented polykaryons formed by fusion of macrophages. They were not restricted to implants of mineralized tissue and are regarded as chronic inflammatory cells rather than cells specialized for resorption of calcified matrix (osteo- or chondroclasts).

These findings suggest that the macrophages had direct responsibility for the resorption of the cartilage. Phagosomes or lysosomes containing chondrocyte remnants were occasionally noted within them. Otherwise, no signs of high phagocytic activity were detected. This is in agreement with previous observations on cartilage resorption by pannus cells in rheumatoid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arthritis (Kobayashi and Ziff 1975) and by tumor cells (Kuettner and Pauli 1981) and supports the idea that the process is lytic rather than phagocytic (Vaes et al. 1981).

The macrophages were characterized by the abundance of coated vesicles. They were concentrated in the Golgi area, where they appeared to arise by budding from dilated rims of stacked cisternae, and to the peripheral parts of the cells, directly opposed to the implant. Coated vesicles have been implicated in receptor-mediated endocytosis (Goldstein et al. 1979; Pastan and Willingham 1981) and transport of lysosomal enzymes from the Golgi complex to lysosomes (Bainton 1981; Farquhar and Palade 1981). The scarcity of lysosomes in the macrophages studied here suggest that their coated vesicles fulfilled the function of transporting lysosomal and/or other enzymes from the Golgi complex to the plasma membrane for release extracellularly. It is known that macrophages secrete not only lysosomal enzymes but also neutral proteases and collagenase (Page et al. 1978), i.e. enzymes capable of degrading the organic matrix of cartilage and other connective tissues. However, the organelles responsible for this secretory activity have not been identified.

The role of the other cell types in the infiltrate surrounding the implants is less clear and no definite conclusions can be drawn from the present study. Previous investigations have suggested that fibroblasts secrete collagenase and other matrix-degrading enzymes (Vaes et al. 1981). This could possibly be the case in our system also. However, the general impression from our observations is that the importance of the fibroblasts in the resorptive process is much less significant than that of the macrophages. Lymphocytes were most abundant during the first few weeks after implantation and may have influenced the function of both the macrophages and the fibroblasts by release of lymphokines and/or other factors (Vischer 1982).

The structure of the cellular infiltrate and the mechanism of resorption appeared unaffected of whether calcified or uncalcified cartilage was used and whether or not the tissue was treated with hydrochloric acid and/or

Fig. 9. Detail of a macrophage in the cellular infiltrate surrounding a four-week-old implant of decalcified rib cartilage. Coated vesicles (*arrowheads*) are shown in the peripheral part of the cell, opposed to the matrix of the implant (*I*). One of them (*double arrowhead*) is continuous with the plasma membrane and opens toward the extracellular space. Bar 0.2 μm. × 69,000

Figs. 10 and 11. Eight-week-old implant of decalcified rib cartilage. Peripheral parts of two large, multinucleated cells with cytoplasmic processes (*P*) penetrating into the matrix of the implant are demonstrated. The matrix next to the cells is poorly stained and appears partly dissolved when compared with that further out. In the latter, distinct collagen fibrils (*F*) are seen. Bars 0.2 μm. × 52,000

Figs. 12 and 13. Extracellular matrix in four- and eight-week-old implants of decalcified rib cartilage. In the younger implant (**Fig. 12**), the matrix consists of thin collagen fibrils with only a faint cross striation. In the older implant (**Fig. 13**), the collagen fibrils are much wider and distinctly cross-striated. Proteoglycans are not evident in either of the specimens. Bars 0.2 μm. × 72,000

sodium chloride before the implantation. This contrasts to recent findings on tumor invasion (Kuettner and Pauli 1981) and resorption of syngeneic and allogeneic implants (Pawlowski et al. 1982) and indicates that the immunogenicity of xenogeneic implants is too strong to make it possible to modify the reactivity of the host tissue by extraction procedures of this type.

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