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# Further characterization of spontaneous arthritic changes in murine squamo-mandibular joint: histopathological aspects

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**Summary.** The appearance of age-related ulcerative changes in the mouse mandibular condyle were evaluated by light and electron microscopy examinations. Fibrillations appeared along the articular surface and in deeper tissue regions, as early as at six months of age. Such changes were characterized by a marked loss of the tissue's cellularity and by a marked reduction in matrix metachromasia and safranin-0 staining. These microscopical changes were accompanied by a reduced reactivity for both ruthenium red and colloidal iron binding, as noted ultrastructurally. At the same time, increasing numbers of erythrocytes appeared to be adhered to the surface irregularities and were also found in deeper regions within the articular lesions. Using morphological criteria, it became apparent that the degenerative changes of aging articular cartilage started at the more superficial regions of the tissue and only thereafter proceeded toward the chondro-osseous junction. Also, with the advancement of age, the degenerative changes became more severe.

**Key words:** Mouse - Aging - Degeneration - Joint

## Introduction

The articular cartilage in adult and aged humans develops areas of disintegration and erosion (Weiss, 1973; Lee et al., 1974). These pathological areas show signs of fraying and cracking and are known as fibrillations. Morphologically these changes resemble osteoarthritic-like lesions and involve the destruction of the articular surface. Such age-related changes are accompanied by chemical changes in the cartilaginous matrix such as the depolymerization of protein-polysaccharide complexes and the loss of acid mucopolysaccharides in the damaged areas (Meachim, 1972; Ehrlich et al., 1975). Further, with the advancement of age an amorphous proteinaceous layer appears to develop along the articular surface of various joints (Wiltberger and Lust, 1975; Appleton, 1978; Stanescu and Leibovich, 1982). This layer corresponds to the "lamina splendens" that has been previously described at the light microscopy level (Weiss et al., 1968). The latter feature appears to serve as an important functional component of the articular surface and might, therefore, play an essential role in lubrication of articulating joints.

Similar to other studies (Stanescu and Leibovich, 1982) it has been recently shown that, in the mandibular condylar cartilage, this layer is composed of marcromolecules that affix negatively charged moieties, a phenomenon that changes as the animal ages (Laver-Rudich and Silbermann, 1985).

The present study evaluates additional aspects of the age-related changes that characterize the squamo-mandibular joint in the senescent animal. Our observations included new light and electron microscopy findings that might enlighten our understanding of age-related degenerative joint disease.

## Materials and methods

Young (newborn to 2 months), maturing (3 months to 6 months), adults (7 months to 12 months) and old (12 months to 22 months) ICR mice were used. The heads were excised and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 (48 hours, 4° C), followed by

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**Fig. 1.** A section through the condylar cartilage of a 7 month old animal stained with safranin O. Positive staining is seen close to chondrocyte clusters (C) in the lower hypertrophic zone. The rest of the tissue appears nonreactive. Acellular regions (arrow) are seen along the articular surface and throughout the section. A:articular surface; B:subchondral bone. x 240

decalcification in 10% ethylenediaminetetrasodium salt (EDTA) in the same buffer (4-6 weeks at room temperature with constant stirring). Tissue was then dehydrated, embedded in Paraplast, and 6 µm thick sections were stained with hematoxylin and eosin for light microscopy examinations. Other Paraplast-embedded sections were stained with 0.1% toluidine blue in phosphate buffer (pH 1.8) for the demonstration of sulfated proteoglycans. Non-decalcified specimens were embedded in glycol metactylate (JB-4), and 1 µm thick sections were stained with safranin-0 (Rosenberg, 1971). For ultrastructural examinations mandibular condyles were fixed in 5% glutaraldehyde (Ladd Research Labs.) in 0.1 M cacodylate buffer (pH 7.4, 1-5 hours, 4° C), postosmicated (1% $OsO_4$) in the same buffer (1 h. 4° C), dehydrated in graded ethanols and embedded in Epon 812.

Additional specimens of animals aging from newborn to ten months old, were fixed in a mixture of 5% glutaraldehyde and of 0.3% ruthenium red (chroma gesellschaft) in 0.1 M cacodylate buffer (pH 7.4). Specimens were rinsed in the above-mentioned buffer and postfixed in a mixture containing 2% $OsO_4$ and 0.3% ruthenium red in 0.1 M cacodylate buffer (pH 7.4), according to the method described by Luft (1971), and processed for electron microscopy.

Other specimens of similar age groups were immersed (10 min) at room temperature, in a mixture of colloidal iron (undialized, pH 1.5), containing 0.5 M $FeCl_3$ and 12% acetic acid (Gasic et al., 1968). Specimens were consequently rinsed in 12% acetic acid in double distilled water and 0.1 M cacodylate buffer (pH 7.4). The labelled specimens were then processed for electron microscopy as described above. 1 µm thick epon embedded sections were stained with 1% toluidine blue in 1% borax general morphology. Ultrathin (50-70 nm) sections were cut with a diamond knife, mounted on copper grids, stained with uranyl acetate (10 min) and with lead citrate (10 min) and examined with a Jeol 100B and with a Zeiss 10 A electron microscope.

In order to evaluate the occurrence and the degree of the degenerative changes in the condylar tissue, animals were divided into five major age groups: newborn to two months old, three to five months old, six to eleven months old, and twelve to 22 months old. In each age group the sections were evaluated for: (1) the appearance of surface irregularities; (2) adherence of erythrocytes to the articular surface; (3) the appearance of deep fibrillations and (4) association of RBC with these fibrillations. At least five determinations were made for each interval and the values were expressed as a percentage of the total determinations.

## Results

A portion of the overall appearance of the mandibular condyle in a fully matured (seven months old) animal is shown in Figure 1. In the above case the articular surface appears intact, yet the underlying cartilage is composed of matrix that is only vaguely reactive for safranin-0, while "clones" of chondrocytes are scattered throughout. The cartilage-bone interface is as yet very distinctive. However, at the same age group there are already animals that reveal ulcerative lesions at the articular surface underlined by a thick "reparative" cartilaginous tissue (Fig. 2). Moreover, focal regions of ankylosis, between the condyle and the articular disc, are often seen. In more severe cases the articular lesions extend down to the subchondral bone (Fig. 3). In one year old animals, the pathological

processes are more protracted along the articular surface as well as within the cartilage itself (Fig. 4). Both the superficial clefts and the deeper defects were found to be filled with erythrocytes (Figs. 4, 5). When the articular deformations are followed by serial sectioning, it seems that the articular fibrillations serve as the source for the more profound cracks that extend deep into the subchondral bone regions. In the damaged areas an intense metachromatic staining is associated with chondrocyte clusters (Fig. 6). Ultrastructural examination of the condylar cartilage of one year old animals reveals that in non-fibrillated areas an electron-dense amorphous layer covers the articular surface (Fig. 7). A similar layer has not been noticed in younger animals. The region immediately underlying the above layer contains collagen fibers as well as vesicular structures. The chondrocytes in this region are surrounded by a lacuna filled with fibrillated matter organized in a parallel fashion to the longitudinal axis of the cell.

Using the ruthenium red fixation method, the structure of the collagen fibers along the articular surface can be easily demonstrated, as shown in Figures 8 and 9. Furthermore, it is seen that the collagen mass is split by areas that contain electron lucent material, thus separating between the thick bundles of collagen. For the most part, ruthenium red-positive material is noticed merely within the lacunae surrounding a chondrocyte (Fig. 8). Within deeper regions of the condylar articular cartilage, electron opaque granules indicative of proteoglycans are aligning collagen fibers within the intercellular matrix (Fig. 9).

As already noted above, the condylar cartilage of maturing animals was undergoing clear signs of age-related degeneration. By transmission electron microscopy it was shown that the amorphous layer along the articular surface

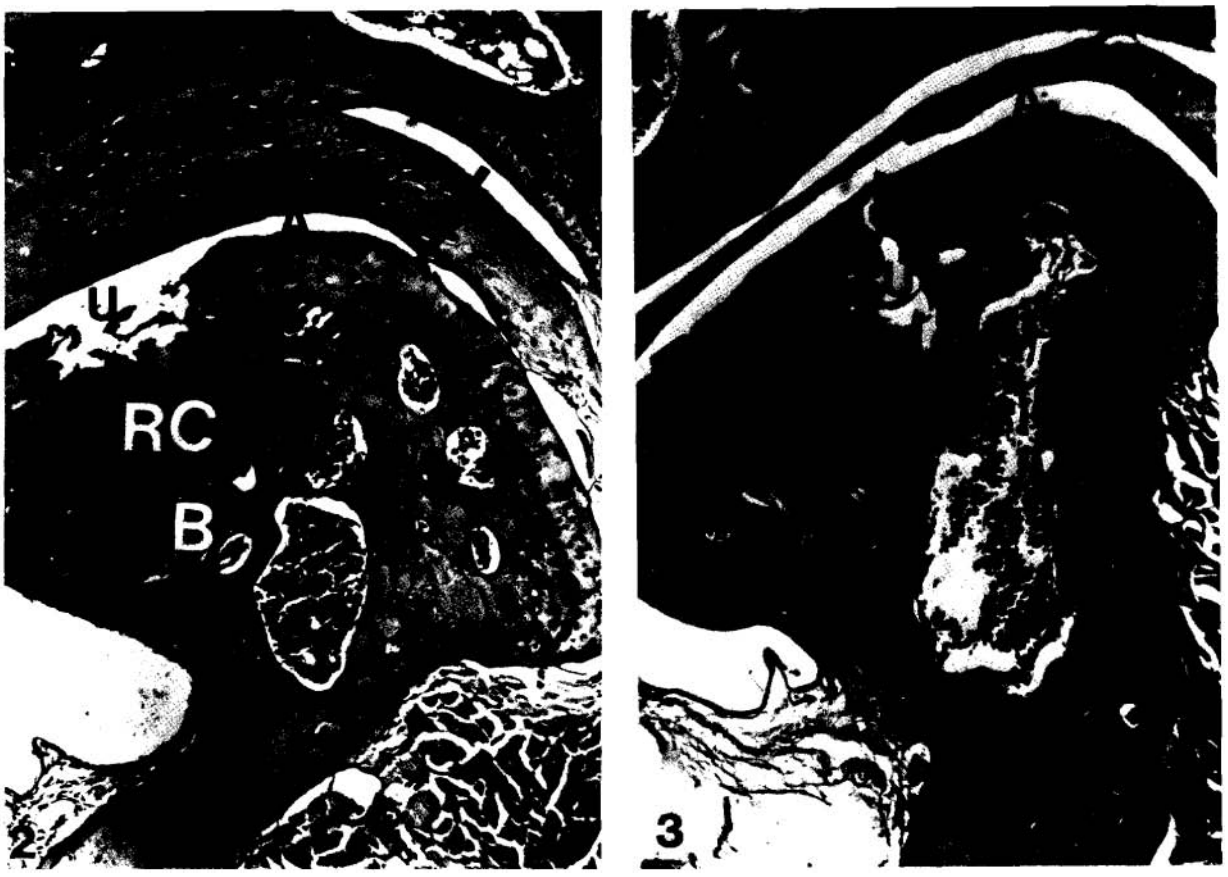



Fig. 2. The appearance of the mandibular condylar cartilage as seen in a seven month old animal. Note an ulcerative lesion (U) over the articular surface (A) along with focal ankylosis (arrow) between the condylar surface and the articular disc (D). The squamosal bone S forms a continuous ankylosis (K) with the disc. Underneath the ulcerative lesion an area of "reparative cartilage" (RC) is observed. This area forms a direct contact with the mandibular ramal bone (B). *x* 96

Fig. 3. A similar section to that shown in Figure 2 demonstrating a deeper ulcerative lesion (U) which extends to the subchondral bone (B). Articular surface (A), articular disc (D), squamosal bone (S). *x* 96

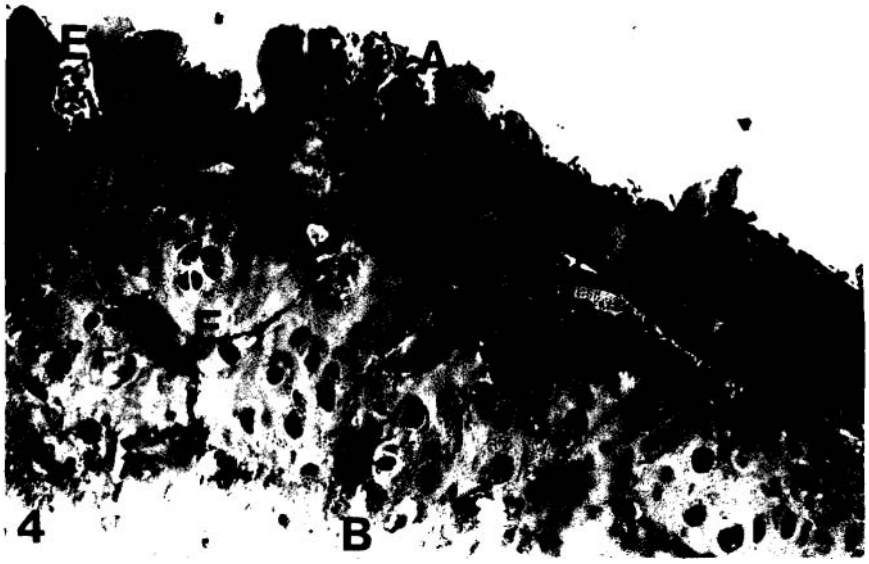



Fig. 4. A frontal section of a condylar cartilage obtained from a oneyearold animals, embedded in epon and stained with basic toluidine blue. The articular surface (A) appears highly irregular. Erythrocytes (E) have accumulated within these fibrillated regions as well as with in deeper tissue lesions. (B) bone. × 240

Fig. 5. .A higher magnification of a simila specimen to that shown in Figure 4. Note the densely packed erythrocytes (E) located within the deep ulcerations in the condylar cartilage (C Also, erythrocytes are seen along and within the surface irregularities. A:articular surface. , 240

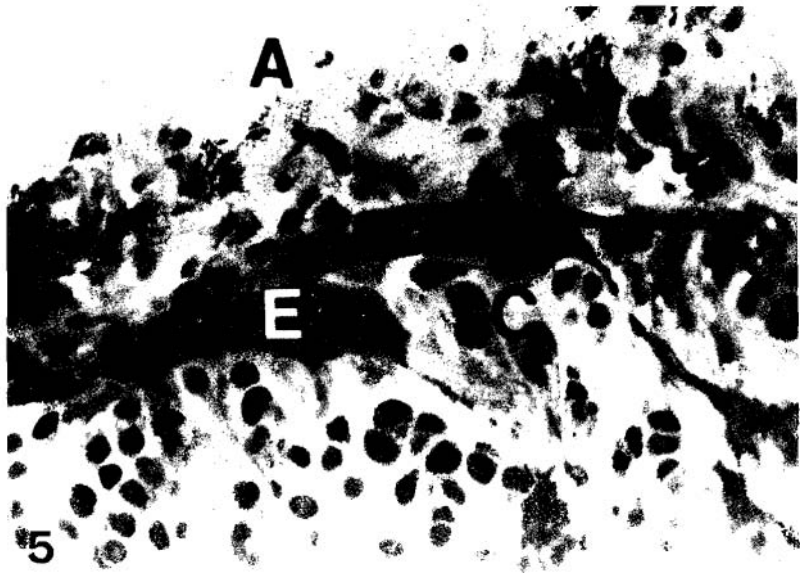




Fig. 6. .A typical appearance of a deep crack in the subchondral region as seen in a oneyearold animal. Metachromatic staining is associate with cell clusters. T subchondral tissue. B: A articular surface. stained with acidic toluidine blue.

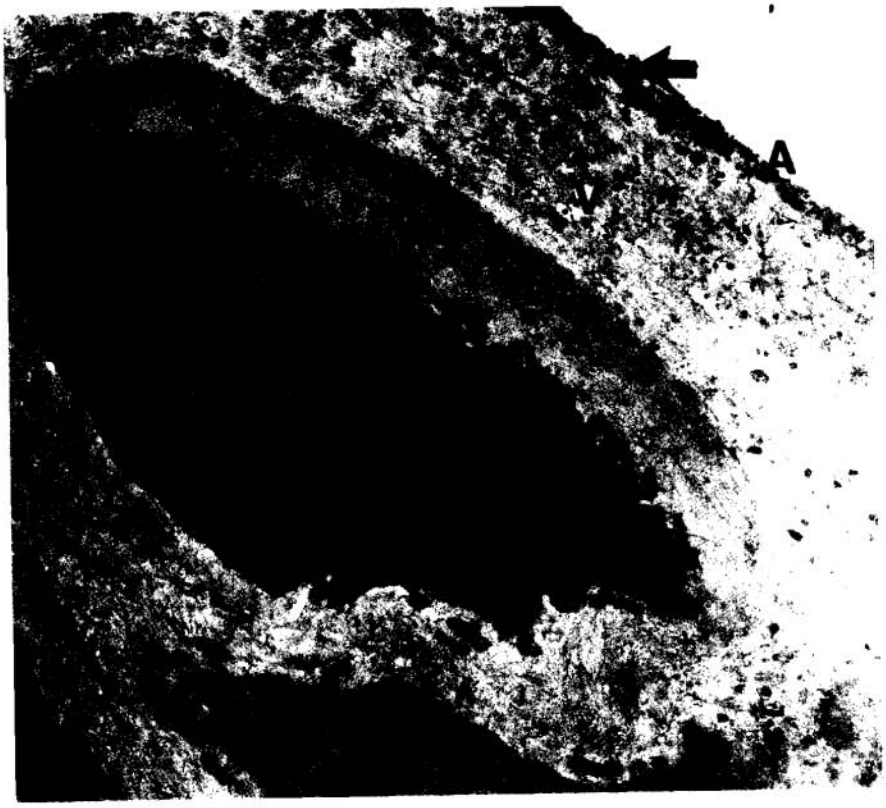



**Fig. 7.** .An intact articular surface (A) of a oneyearold animal. Note the amorphous layer (arrow) covering it. The matrix beneath the above layer contains vesicular structures (V). A chondrocyte (C) is surrounded by a lacuna (L) which is filled with fine collagen fibrils. × 20,000

**Fig. 8.** .Electron micrograph of a one year old animal, fixed with ruthenium red prior to the processing of the tissue. Positive proteoglycan aggregates (open arrow) are seen clustered in a lacunar region (L) in the lower portion of this micrograph. Aggregates of collagen fibers (long arrows). The collagen fibers appear to run in a parallel orientation to the articular surface (A). × 40,800

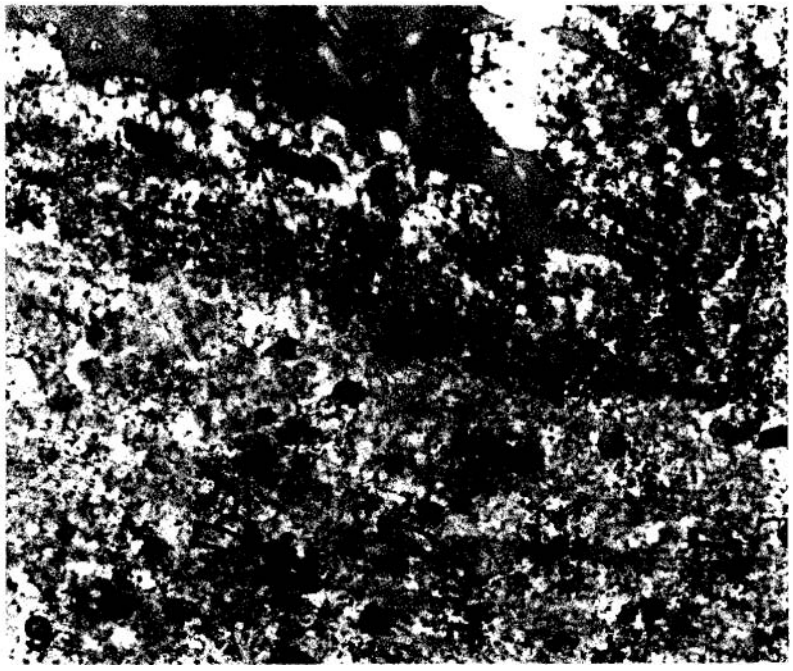
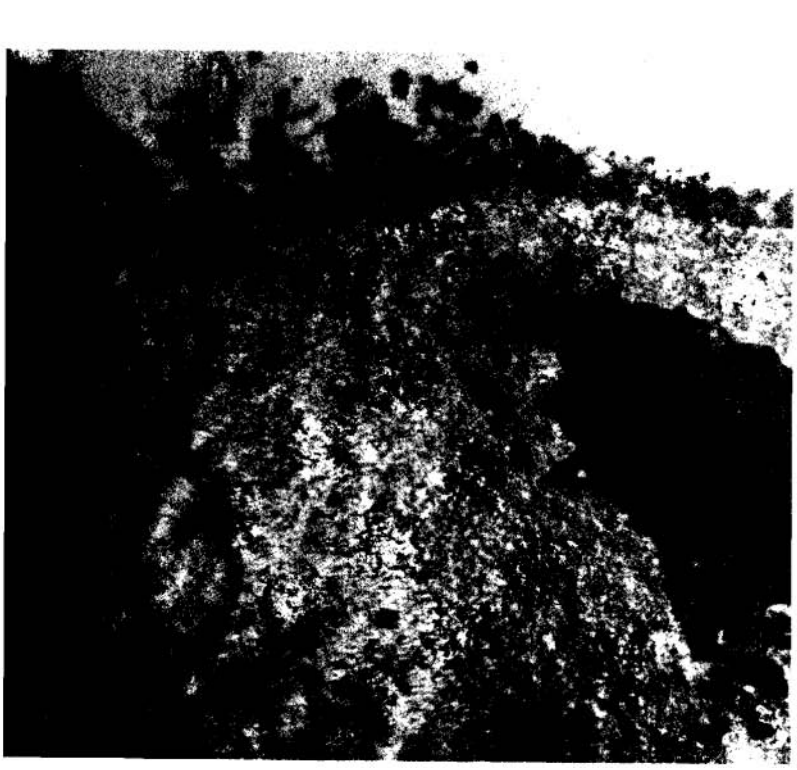


Fig. **9.** .A section through a deeper region in a similar specimen to that shown in figure 8 (stained with RR). In this region the proteoglycan aggregates are clearly seen in the lacunar region (L) of a chondrocyte (C). RR positive structures are also seen in the extracellular matrix closely associated with collagen fibrils (open arrow) Electron dense matrix vesicles (dark arrow) can be identified within the intercellular matrix. *x* 40,000

Fig. **10.** .Signs of an early stage of articular clefting (arrow). It can be seen that the amorphous layer is following the invagination into the underlying matrix. A portion of a chondrocyte (C) and matrix vesicles (V) are seen underneath the articular surface (A). × 40,800

Fig. **11.** .Electron micrograph of a tissue treated with colloidal iron prior to processing for ultrastructural observation. The amorphous layer along the articular surface (A) binds colloidal iron particles (arrows). U: early stage of ulceration. *x* 30,000



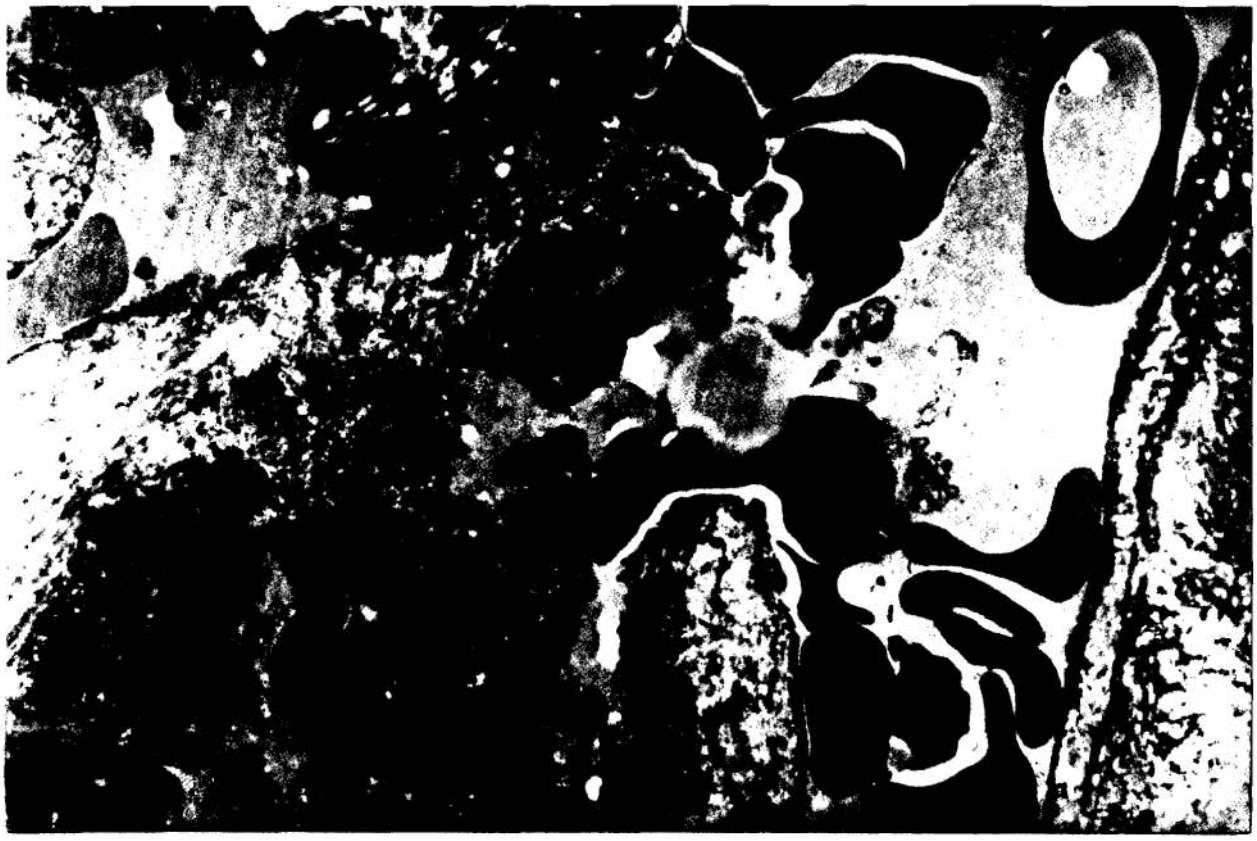
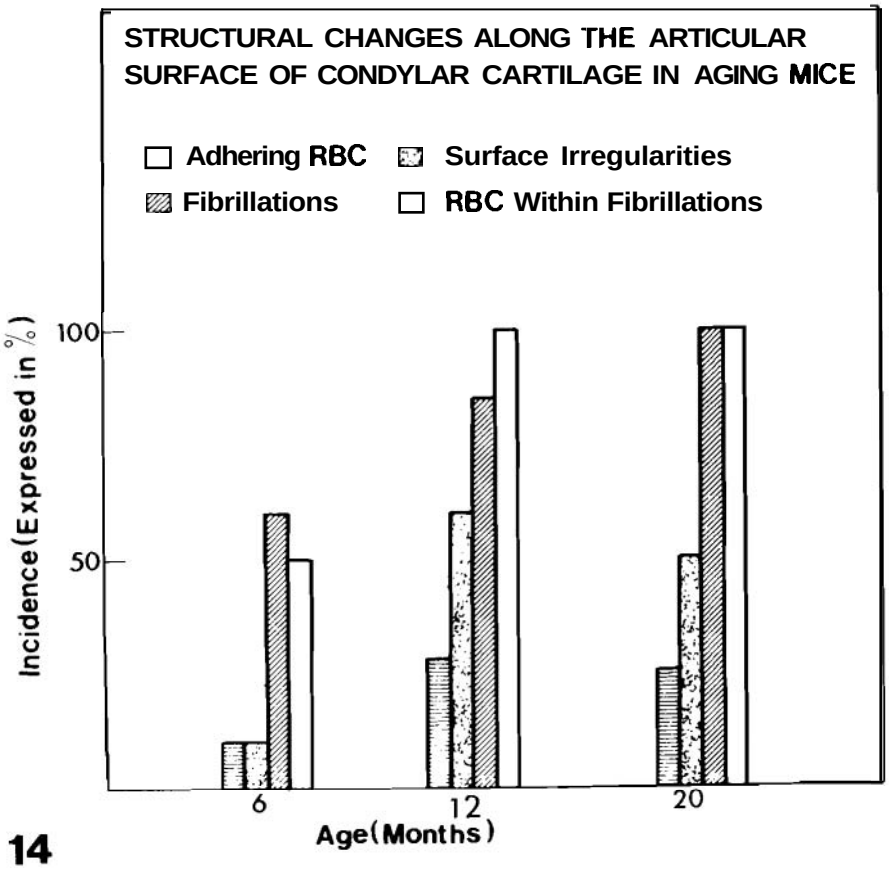


Fig. 12. .A more advanced stage of cartilage splitting (arrow) as seen in the condylar cartilage of an older animal. Note the funneling process (F) amidst an amorphous matrix (M) containing multiple vesicles (V). A:articular surface; E:erythrocyte. x 5,000

Fig. 13. .The appearance of an osteoarthritic area in the condylar cartilage of an old mouse. Note the entrapment of erythrocytes (E) within masses of degenerated cartilage matrix (M). x 6,000

Fig. 14. .Schematic representation of age-related changes in the articular cartilage of the mandibular condyle in ICR mice. The appearance of fibrillated cartilage is already apparent at 6 months of age and involves over 50% of all animals.




was in some way involved in the initial stage of tissue degeneration (Fig. 10). The latter articular layer contained negatively charged particles, as it was found that it had bound colloidal iron. The labelled particles followed the invaginating process of the surface (Fig. 11). With advancing age, the surface originated invaginations further penetrated the deeper regions within the articular condylar cartilage, and such clefts often contained blood bearing cells (Fig. 12). In general, the matrix adjacent to the invaginating cleft was acellular and disorganized and was composed, for the most part, of an amorphous material containing collagen and vesicular structures. Further, cartilage involved with such lesions often contained fragments of necrotic tissue, erythrocytes and exudate-like material (Fig. 13). The entrapped erythrocytes often underwent marked changes in their shape and form (Fig. 13). Figure 14 represents a semiquantitative scheme of age-related changes in maturing and aging mice. The initial signs of degeneration are already apparent at six months and are most pronounced at one year. Of interest is the finding that in twenty month old animals there is no real difference in comparison to that noticed in one year old ones.

## Discussion

The susceptibility of the condylar cartilage to degenerative changes is related to forces that are exerted upon it due to its specialized function in the craniofacial complex. Whereas most of the joints in the body are of the load-bearing type and their articular cartilage is hyaline, in the squamo-mandibular joint the cartilaginous tissue experiences muscular forces of the stretch type and consequently the articular cartilage is of a fibrocartilage type (Durkin et al., 1973; Appleton, 1975, 1978).

In the present study we have demonstrated that this specialized joint is prone to the development of histopathological alterations at a relatively early stage in the animal life span. Similar changes are known to be associated with osteoarthritis of aging articular cartilage (Weiss, 1973; Wiltberger and Lust, 1975). Also, we were able to show a decrease in toluidine blue and safranin-0 staining, a feature that was most pronounced in the articular surface. These findings are consistent with reported histological and biochemical findings indicative of a decrease in the glycosaminoglycan content of articular cartilages (Mankin et al., 1971; Muir, 1977;

Sweet al., 1977; Bayliss and Ali, 1978; Inerot et al., 1978; Christensen and Reinman, 1980; Ryu et al., 1984). Our ultrastructural studies, using the ruthenium red method, have shown that positive ruthenium red particles, representing proteoglycans, (Luft, 1971; Myers et al., 1973; Shepard and Mitchell, 1977) were, for the most part, located in deeper regions of the tissue. While the most reactive sites for ruthenium red were the lacunar areas, such reactivity was almost absent from regions along the articular surface.

One of the noteworthy features observed in this study was the appearance of a so-called reparative cartilage underneath and surrounding the ulcerative lesions along the articular surface. Our results appear consistent with those of Mankin et al. (1977) which showed an inverse correlation between the severity of the ulcerative process and the proteoglycan content in the tissue. It has been suggested that the depletion of the glycosaminoglycan could be due to a diffusive leakage of glycosaminoglycan via the fibrillated areas (Maroudas et al., 1973).

Recently, we have reported on the possible role of proteolytic enzymes in the degradation of cartilage matrix in mature and aged animals (Livne et al., 1985). As the early lesions usually appear along the articular surface, they might have resulted from age-related changes within the cartilaginous tissue proper as well as from factors originating in the synovial tissue. It has indeed been shown that the articular cartilage can be stimulated for self destruction under the influence of catabolic messengers such as catabolin, a macromolecule isolated from synovial tissue (Dingle, 1983). A characteristic feature of the articular surface of condylar cartilage was a layer of fine granular electron-dense material. This layer normally contains a proteinaceous component with negatively charged moieties. One of the observed age-related features was the reduced ability to bind electron dense tracers. Other changes include the appearance of fibronectin type I collagen along the articular surfaces of osteoarthritic cartilages (Wurster and Lust, 1982) and aging condylar cartilage (Livne et al., 1985).

In an in vitro study chondrocytes were found to synthesize fibronectin only in the absence of extracellular matrix. Further, fibronectin is normally present in tissue rich in type I collagen but is usually absent in intact cartilage (Dessau et al., 1978). Hence, it could be suggested that the presence of fibronectin in old cartilage may be indicative of changes in basic metabolic pathways that are age-related. Such changes may lead to the collapse of the articular amorphous layer. As has been shown here, this phenomenon appears to be associated with the adhesion of erythrocytes to the damaged matrix.

## References


Appleton J. (1975). The ultrastructure of the articular tissue of the mandibular condyle in the rat. Archs. Oral Biol. 20, 823-826.

Appleton J. (1978). The fine structure of a surface layer over the fibrous articular tissue of the rat mandibular condyle. Archs. Oral Biol. 23. 719-723.

Bayliss M.T. and Ali S.Y. (1978). Isolation of proteoglycans from human articular cartilage. Biochem. J. 169, 123-132.

Christensen S.B. and Reinman I. (1980). Differential histochemical staining of glycosaminoglycans in the matrix of osteoarthritic cartilage. Acta Path. Microbiol. Scand. Sect. A, 88, 61-68.

Dessau W., Sasse J., Timple R., Jilek F. and von der Mark K. (1978). Synthesis and extracellular deposition of fibronectin in chondrocyte cultures. Response to the removal of extracellular cartilage matrix. J. Cell Biol. 79, 342-355.

Dingle J.T. (1983). The role of catabolin in the control of cartilage matrix integrity. J. Rheumatology, suppl. 11, 38-42.

Durkin J.F., Heeley J.D. and Irving J.T. (1973). The cartilage of the mandibular cartilage. Oral Sci. Rev. 2: 29-99. Original article series (A.H. Melcher and G.A. Zarb, eds.).

Ehrlich M.G., Mankin J.J., Jones H., Grossman A., Crispen, C. and Aucona D. (1975). Biochemical confirmation of an experimental osteoarthritis model. J. Bone Jt. Surg. 571, 392-396.

Gasic G.J., Berwick L. and Sorrentino M. (1968). Positive and negative colloidal iron as cell surface elctron stains. Lab. Invest. 18, 63-71.

Inerot S., Heinegard D., Audell L. and Olsson S.E. (1978). Articular cartilage proteoglycans in aging and osteoarthritis. Biochem. J. 169, 143-156.

Laver-Rudich Z. and Silbermann M. (1985). Cartilage surface charge. A possible determinant in aging and osteoarthritic processes. Arth. Rheum. 28, 660-670.

Lee P., Rooney P.J., Sturrock R.D. and Kennedy A.C. (1974). The etiology and pathogenesis of osteoarthrosis, a review. Semin. Arthritis Rheum. 3, 189-218.

Livne E., von der Mark K. and Silbermann M. (1985). Morphologic and cytochemical changes in maturing and osteoarthritic articular cartilage in the temporomandibular joint of mice. Arth. Rheum. 28, 1027-1038.

Luft J.H. (1971). Ruthenium red and violet: chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat. Rec. 171, 347-368.

Mankin H.H., Dorfman H., Lippiello L. and Zarins A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J. Bone Jt. Surg. 53-A, 525-537.

Maroudas A., Evans H. and Almeida L. (1973). Cartilage of the hip joint: topographical variation of glycosaminoglycan content in normal and fibrillated tissue. Ann. Rheum. Dis. 32, 1-9.

Meachin G. (1972). Articular cartilage lessions in osteoarthritis of femoral heads. J. Pathol. 107, 199-210.

Muir H. (1977). Molecular approach to the understanding of osteoarthrosis. Herbeden oration, 1976. Ann. Rheum. Dis. 36, 199-208.

Myers D.B., Highton J.C. and Rayns D.G. (1973). Ruthenium red-positive filaments interconnecting collagen fibrils. J. Ultrast. Res. 42, 87-92.

Rosenberg L. (1971). Chemical basis for the histological use of safranin-0 in the study of articular cartilage. J. Bone Jt. Surg. 53-A, 69-82.

Ryu J., Treadwell B.V. and Mankin H.J. (1984). Biochemical and metabolic abnormalities in normal and osteoarthritic human articular cartilage. Arth. Rheum. 27, 49-57.

Shepard N. and Mitchel N. (1977). The use of ruthenium red and P. phenylenediamine to stain cartilage simultaneously for light and electron microscopy. J. Histochem. Cytochem. 25, 1163-1168.

Stanescu R. and Leibovich J.S. (1982). The negative change of articular cartilage surfaces. An electron microscopic study using cationized ferritin. J. Bone Joint Surg. 641, 388-398.

Sweet M.B.E., Thonar E.J., Immelman A.R. and Solomon L. (1977). Biochemical change in progressive osteoarthrosis. Ann. Rheum. Dis. 36, 387-398.

Weiss C. (1973). Ultrastructural characteristics of osteoarthritis. Fed. Proc. 32, 1459-1466.

Weiss C., Rosenberg L. and Heflet A.J. (1968). An ultrastructural study of normal young adult human articular cartilage. J. Bone joint Surg. 50A, 663-674.

Wiltberger A. and Lust G. (1975). Ultrastructure of canine articular cartilage: comparison of normal and degenerative (osteoarthritic) hip joints. Am. J Vet. Res. 36, 727-740.

Wurster N.B. and Lust G. (1982). Fibronectin in osteoarthritic anine articular cartilage. Biochem. Biophys. Res. Commun. 109, 1094-1101.

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