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Case Reports

Relapsing Polychondritis

An Ultrastructural Study of Elastic and Collagen Fibres Degradation Revealed by Tannic Acid

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Summary. In a case of relapsing polychondritis, ultrastructural study of ear cartilage using tannic acid staining showed patterns of degradation of elastic and collagen fibres. The participation of macrophages and chondrocytes in the resorption of ear cartilage is discussed.

Key words: Relapsing polychondritis – Tannic acid – Elastic fibres – Collagen fibres.

Introduction

Relapsing polychondritis (RP) is a rare connective tissue disease characterized by inflammation of elastic and articular cartilage often associated with other autoimmune diseases.

The clinical picture has been recently reviewed (McAdam et al. 1976).

Electron microscopic studies have been reported in three papers. Mitchell and Shepard (1972) studied articular cartilage, and ear cartilage was examined by Shaul and Schumacher (1975) and by Hashimoto et al. (1977). These ultrastructural studies outlined various alterations of chondrocytes but paid little attention to the degradation of elastic and collagen fibres.

The purpose of this study is to show alterations of the elastic and collagen fibres clearly demonstrated by tannic acid staining, with other ultrastructural lesions of the disease.

Case Report

A 58 year-old white female noted the onset of painful swelling and redness of the pinnae of both ears five months before admission. This episode resolved after several weeks. Two weeks

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before admission the patient developed acute and febrile polyarthritis with recurrence of swelling of the ears.

Physical examination revealed a febrile white female with swelling and tenderness of both wrists and of the second and third metacarpophalangeal joints of the hands. Ten days after admission she developed painful swelling of the bridge of the nose. Sjögren's syndrome with pricking of the eyes was demonstrated by Schirmer's test and biomicroscopy. There was no tracheal tenderness, no hearing loss, no Raynaud's phenomenon or cutaneous vasculitis. Cardiac and thoracic examination was normal and blood pressure was 160/90. X rays of the hands and chest X-rays were normal.

Laboratory examinations: haemotocrit was 36%, haemoglobin 14.5 g/100 ml and WBC count 6,600 with a normal differential. The Westergren sedimentation rate was 140 mm/h. Urinalysis, BUN, serum creatinine, muscular enzymes, hepatic functions and serum immunoglobulins level were within normal limits. Latex fixation test for rheumatoid factor was positive at a titre of 1:2000. Fluorescent antinuclear antibodies were positive at a titre of 1:100 with a speckled pattern not related to RNP or Sm antigens. LE cell preparations were negative and DNA binding capacity was normal. Lupus band test on covered normal skin was negative. Serum total haemolytic complement was elevated (67 u/ml, normal: 42 ± 7 u/ml). Tests for syphilis, cryoglobulins, lupus anticoagulants were negative. Circulating immune complexes were detected with polyethylene glycol precipitation, solid phase Clq fixation test and solid phase conglutinin test. An indirect immunofluorescence test on rat costal cartilage revealed anticartilage antibodies in the patient's serum. A passive haemagglutination test showed antinative collagen type II antibodies in the patient's serum.

Complex HLA haplotype was A9–A32/B8–B27/C1–C7/Dr–Dr4.

Material and Methods

A small piece of ear cartilage was removed under local anesthesia. The specimen, sliced into fragments of 1 mm^2 were immediately fixed in 2% glutaraldehyde solution in 0.1 M cacodylate buffer pH 7.3 at room temperature for 90 min. After rinsing in the same buffer, the specimens were post-fixed in 2% OsO_4 in cacodylate buffer pH 7.3 at 4° C for 90 min and then treated in block according to Simionescu (1975) for 30 min at room temperature with 1% digallic acid (C_{14} H_{10} O_9, tannic acid 1764, Mallinckrodt, chemical works, St Louis, Missouri, USA) in 0.05 M cacodylate buffer pH 7.0 at room temperature for 30 min.

The blocks were subsequently dehydrated in alcohol and embedded in Spurr resin. Ultrathin sections were cut with a Reichert Om U_3 ultramicrotome, stained with lead citrate and examined with a Philips 300 electron microscope at 40 KV.

Results

Normal Cartilage

In the deep layer of the ear cartilage, characteristics of normal tissue were found. Viable chondrocytes (Fig. 1) were surrounded by a concentric margin of a microfibril-containing matrix. Intercellular spaces were occupied by intermixed collagen and elastic fibres. Collagen fibres (Fig. 2) were of various diameters: thin fibres without a banding pattern were more numerous than thick fibres with typical 64 nm periodicity.

Elastic fibres (Fig. 3), clearly revealed by tannic acid, had an amorphous central portion surrounded by a peripheral margin of microfibrils. Close attachment of banded collagen to elastic fibres was frequently observed (Fig. 4).

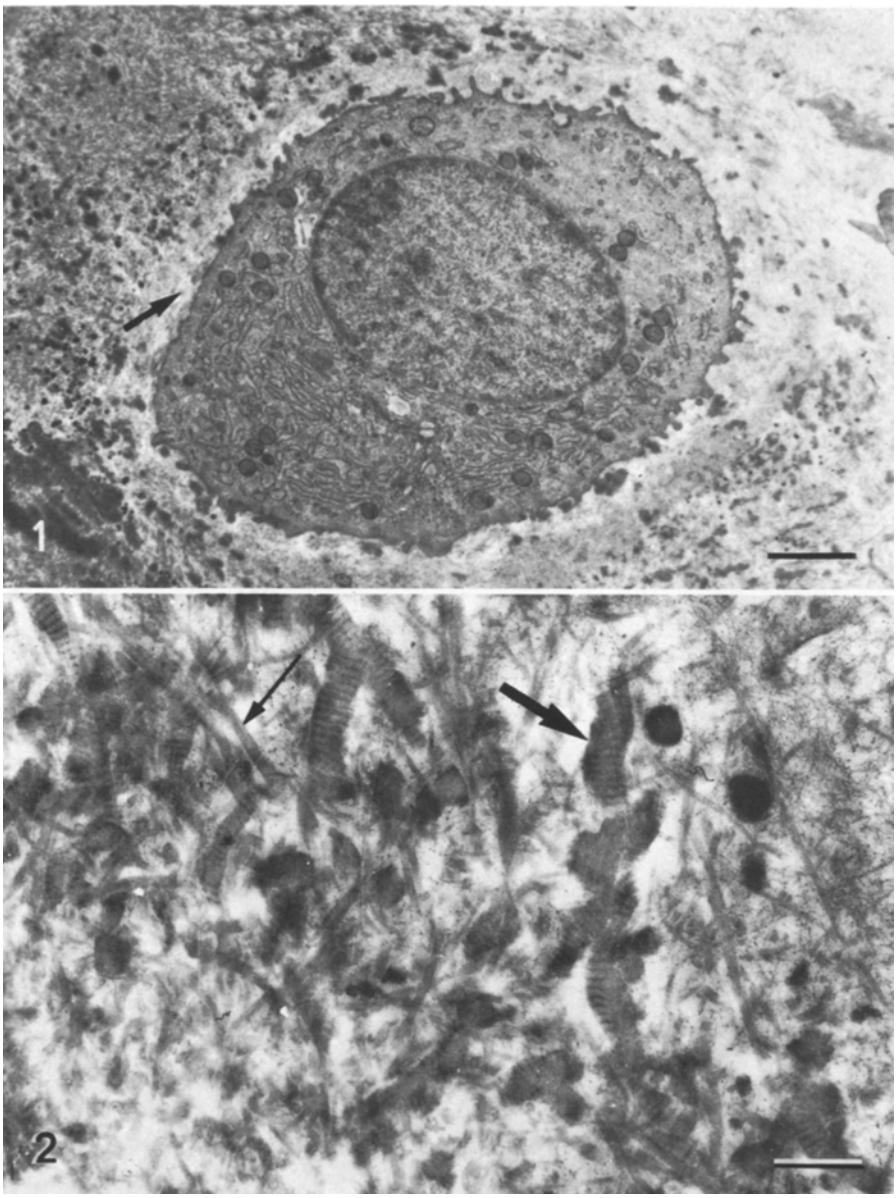
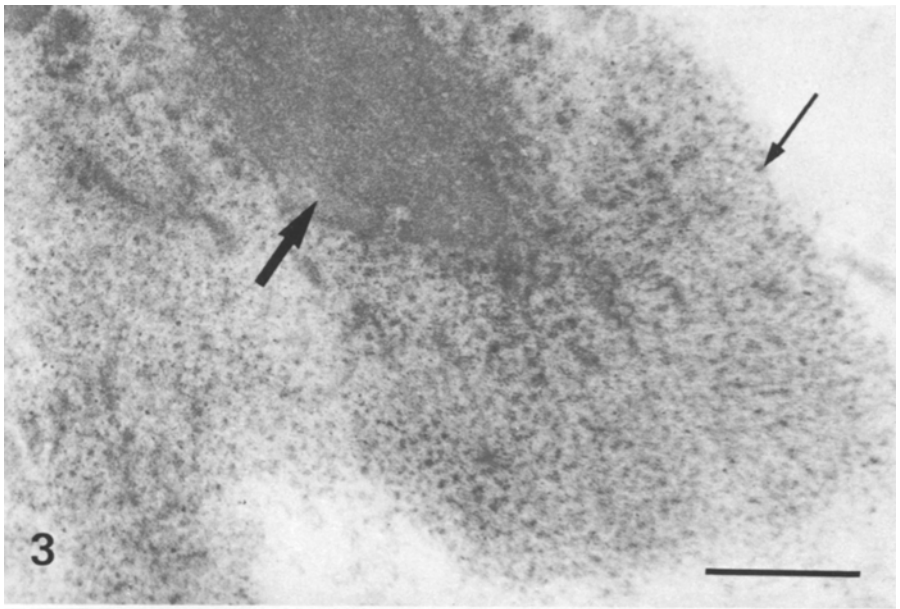


Fig. 1. Normal chondrocyte in the deep layer of ear cartilage. Arrow: concentric margin of a microfibril containing matrix. Magnification: 5,850; Bar: 2 μm

Fig. 2. Normal collagen fibres in the deep layer of ear cartilage. Thick arrow: thick fibre with 64 nm periodicity. Thin arrow: thin fibre without banding pattern. Magnification: 24,000; Bar: 0.5 μm



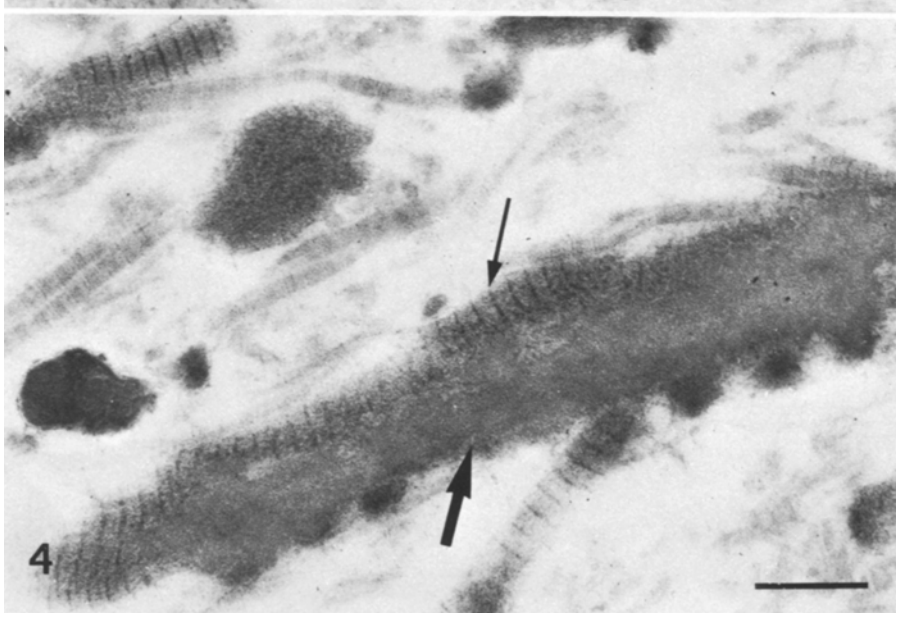


Fig. 3. Normal elastic fibre in the deep layer of ear cartilage. Thick arrow: amorphous central portion. Thin arrow: peripheral microfibrils. Magnification: 81,000; Bar: 0.25 μm

Fig. 4. Banded collagen fibre (thin arrow) closely attached to elastic fibre (thick arrow) in normal ear cartilage. Magnification: 60,000; Bar: 0.25 μm

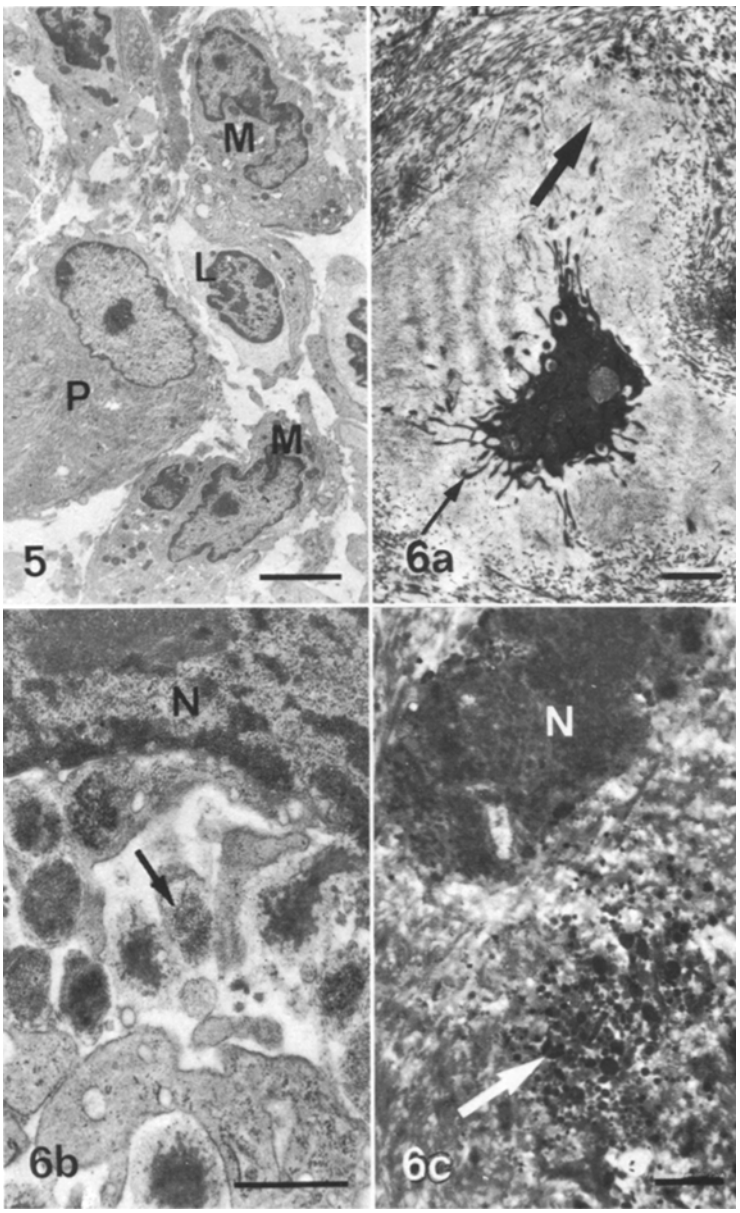


Fig. 5. Cellular infiltrate in the perichondrium. *M* macrophages *P* plasma cell *L* lymphocyte. Magnification: 5,400, Bar: 2 μm

Fig. 6 a–c. Altered chondrocytes in superficial layer of ear cartilage. **a** Retraction of the cytoplasm. Thin arrow: cytoplasmic digitation Thick arrow: broad pericellular matrix. Magnification: 6,750; Bar: 1 μm. **b** Release of cytoplasmic inclusions Arrow: myelin figures. *N* nucleus Magnification: 15,000; Bar: 1 μm. **c** Complete necrosis of the chondrocyte Arrow: cytoplasmic dense inclusions *N* nucleus Magnification: 8,400; Bar: 1 μm

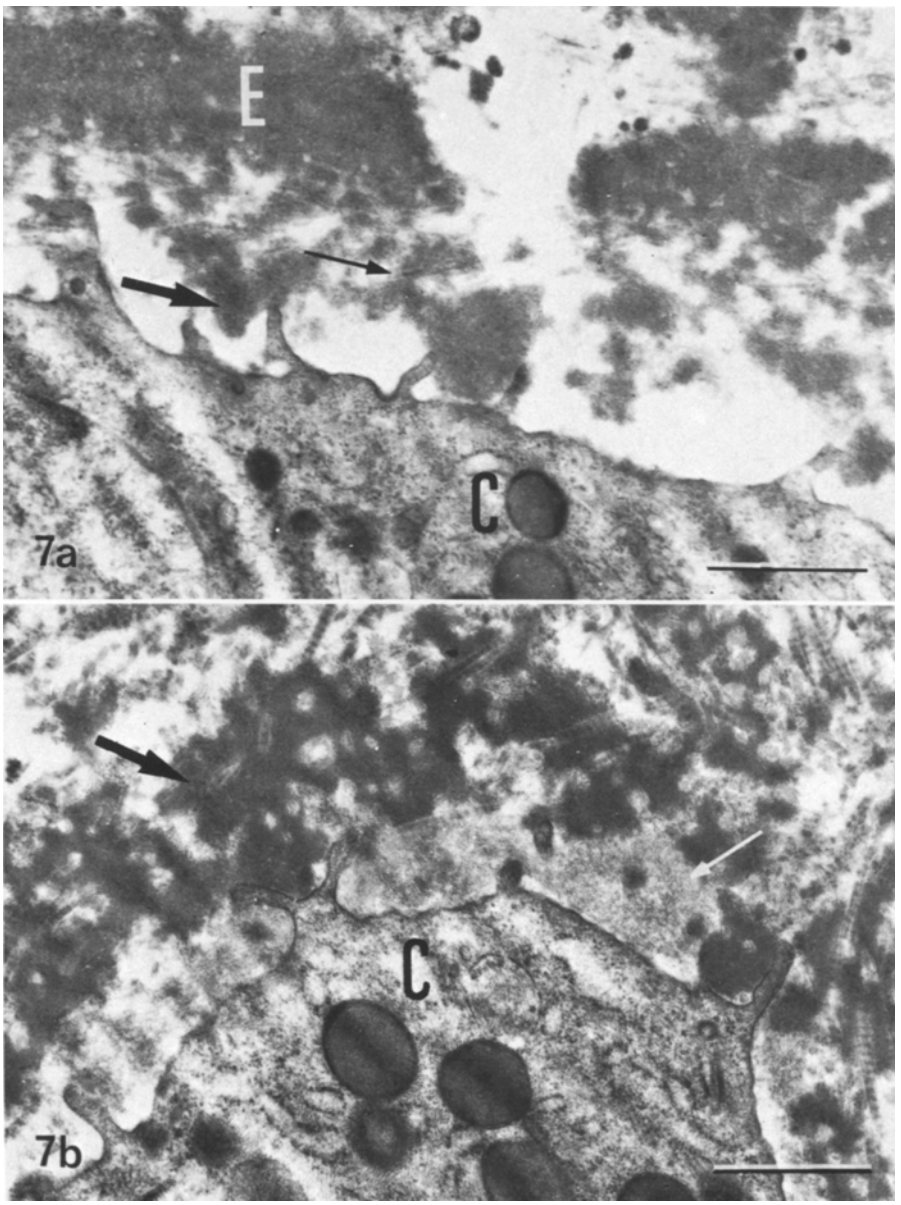


Fig. 7 a and b. Fragmentation of elastic fibres in superficial layer of ear cartilage. **a** Peripheral release of amorphous *(thick arrow)* and fibrillar (*thin arrow*) components. *E* elastic fibre *C* chondrocyte Magnification: 20,400; Bar: 1 μm. **b** Virtually complete disintegration of amorphous portion (*thick arrow*) Thin arrow: fibrillar component. *C* chondrocyte. Magnification: 20,400; Bar: 1 μm

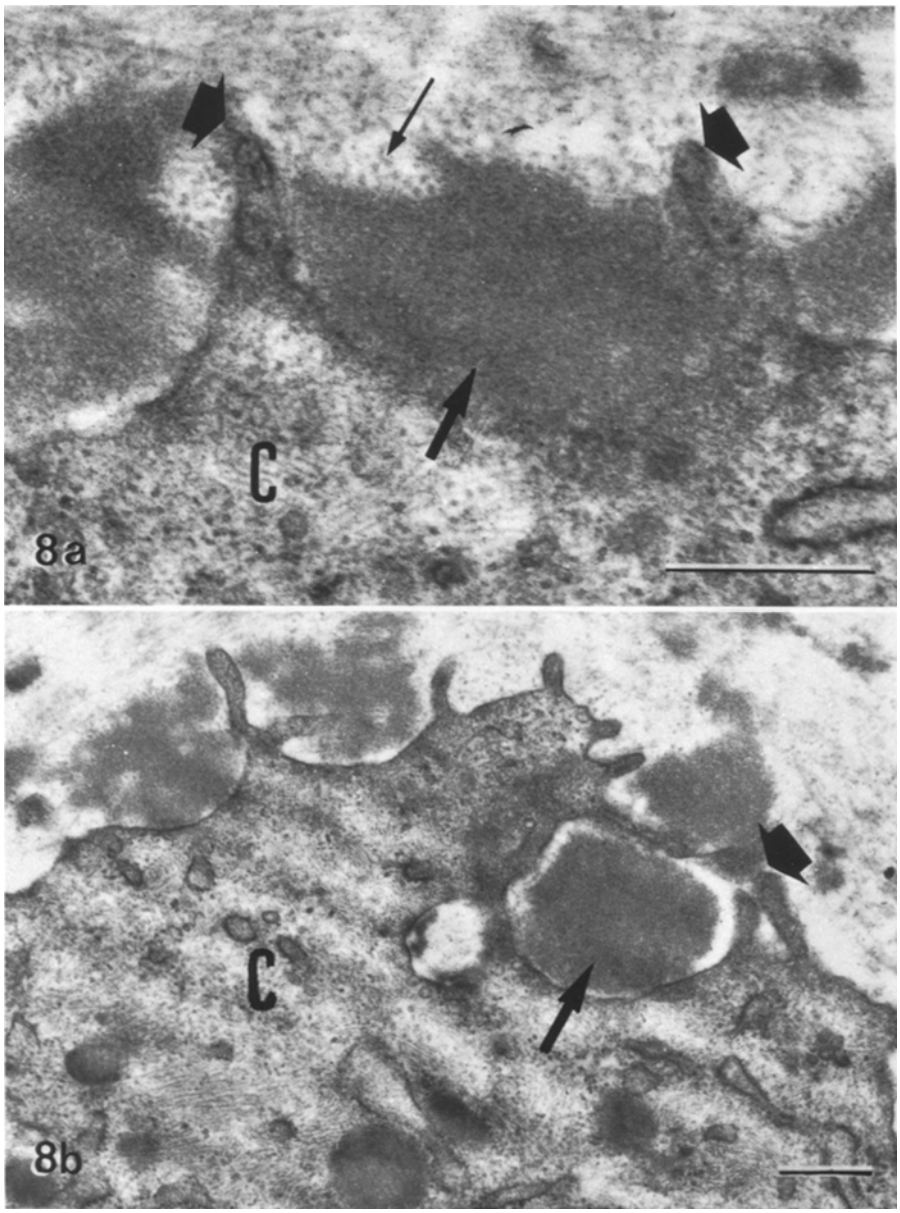


Fig. 8 a and b. Phagocytosis of disintegrated elastic fibre by chondrocyte in superficial layer of ear cartilage. **a** Amorphous (*thick arrow*) and fibrillar (*thin arrow*) components are partially surrounded by cell processes (*short arrows*) *C* chondrocyte. Magnification: 55,000; Bar: 0.5 μm. **b** Amorphous component (*thick arrow*) is completely surrounded by cell processes (*short arrow*). *C* chondrocyte. Magnification: 24,600; Bar: 0.5 μm

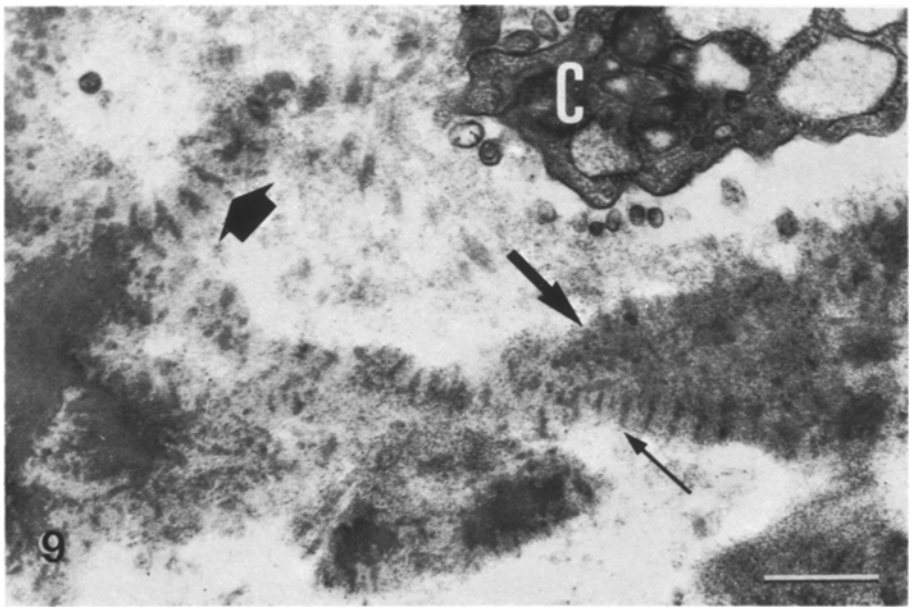


Fig. 9. Fragmentation of collagen fibre (*thin arrow*) closely attached to altered elastic fibre (*thick arrow*) in superficial layer of ear cartilage. Short arrow: banded structure with periodicity of about 90 nm. *C* chondrocyte. Magnification: 30,000; Bar: 0.5 μm

Perichondrium

In the perichondrium, pericapillary infiltrates consisted of macrophages, lymphocytes and plasma cells (Fig. 5).

Cartilage Alterations

In the vicinity of the perichondrium, the superficial layer of the ear cartilage exhibited diffuse lesions of the chondrocytes and of the intercellular matrix.

Alterations of the chondrocytes were of variable degree and included: (a) retraction of the cytoplasm (Fig. 6a) with prominent cytoplasmic digitations and broad pericellular matrix, (b) disintegration of the cytoplasm (Fig. 6b) with release of cytoplasmic inclusions, some having the pattern of myelin figures, (c) complete necrosis of the chondrocyte (Fig. 6c) with diffusion of various dense inclusions into the intercellular space.

Alterations in the intercellular matrix concerned both elastic and collagen fibres. Elastic fibres were more often affected by disintegrating lesions than collagen fibres. In the vicinity of viable chondrocytes, elastic fibres underwent variable degrees of fragmentation, from peripheral release of fibrillar and amorphous components (Fig. 7a) to virtually complete fragmentation of the amorphous central portion (Fig. 7b). Some chondrocytes phagocytosed disintegrated elastic fibres (Fig. 8). Collagen fibres were more rarely altered than elastic fibres.

Loose aggregates of banded material with a periodicity of about 90 nm, corresponded to resorption of collagen attached to elastic fibres (Fig. 9).

Discussion

Normal Cartilage

Previous ultrastructural studies (Shaul and Schumacher 1975; Hashimoto et al. 1977) described normal appearing tissue in the deep layer of ear cartilage with viable chondrocytes surrounded by a thin fibril containing area and collagen fibres of various diameters, including thin fibres without a distinct banding pattern and thick fibres with a typical 64 mn periodicity. Elastic fibres were poorly revealed with conventional staining. The amorphous central component did not stain and the peripheral envelope of microfibrils appeared more distinctly at the cut edge of the fibre. Hashimoto et al. (1977) mentioned the close attachment of collagen fibres to elastic fibres that we have observed. With the use of tannic acid, the amorphous central component stains intensely and peripheral microfibrils can be seen around the fibre (Cotta-Pereira et al. 1976).

Perichondrium

In previous light microscopic studies (Pearson et al. 1960; Verity et al. 1963; Shaul and Schumacher 1975; McAdam et al. 1976), hypervascularisation of the perichondrium and cellular infiltration by mononuclear round cells have been regularly reported. The presence of macrophages in the perichondrium was clearly demonstrated in our ultrastructural study, and we will discuss later the participation of their lysosomal content in matrix degradation.

Cartilage Alterations

As in the present report, variable degrees of alteration of the chondrocytes were found in articular cartilage by Mitchell and Shepard (1972) and in the superficial layer of ear cartilage by Shaul and Schumacher (1975) and by Hashimoto et al. (1977). Release of cytoplasmic organelles by severely damaged chondrocytes was described in detail by Hashimoto et al. (1977). These authors are of the opinion that the dense granules diffusing into the intercellular space are probably of lysosomal nature, and contribute to the alteration of the matrix. Multivesicular bodies observed in articular cartilage by Mitchell and Shepard (1972) have to date not be found in ear cartilage.

Destruction of the ear cartilage matrix is an obvious feature of RP. However, previous ultrastructural studies make little mention of fibre alterations. Shaul et Schumacher (1975) described deposits of a finely granular electron-dense material between collagen fibres and at the surface of elastic fibres. Elastic and collagen fibres are normal in appearance according to Hashimoto et al. (1977).

In the present study, alterations of the elastic fibres were clearly demonstrated with the use of tannic acid. Disruption of elastic fibres by proteolytic enzymes has been extensively studied by Ross and Bornstein (1969). Whereas the peripheral mantle of microfibrils is removed by chymotrypsin, the amorphous central portion is selectively digested by elastase, with complete disorganisation of the fibre. In our study, the fragmented pattern of the amorphous central component with persistence of the microfibrils suggests the action of elastase on elastic fibres. Chondrocytes are closely associated with the degradation of the elastic fibres. Disrupted elastic fibres are always found around viable chondrocytes, phagocytosing this degraded material. In RP this pattern has not been observed to date in ear cartilage. However, in articular cartilage Mitchell and Shepard (1972) reported a clear-cut pattern of phagocytosis by chondrocytes of a striated extracellular material.

Patterns of collagen fibre degradation were infrequent in our patient and consisted of striated material with atypical periodicity of about 90 nm. Ultrastructural patterns of collagen resorption have been extensively studied by Perez-Tamayo (1970). In the experimental model of carrageenin granuloma, this author described progressive fragmentation then separation and finally disintegration of extracellular collagen fibres. At the stage of fragmentation of the fibres, the banding pattern was still apparent, with a measurable periodicity of about 90 nm. Nemetschek-Gansler et al. (1977) have described banded fibrous structures in the extracellular spaces of connective tissue from human endometrium, in the Ehler-Danlos syndrome and in cases of tendon ruptures. These authors interpreted the banded structures as states of an enzymatically induced degradation of collagen. In RP, previous ultrastructural studies make no mention of collagen resorption in ear cartilage. However in articular cartilage, Mitchell and Shepard (1972) showed micrographs of chondrocytes phagocytosing striated material that could be a product of collagen degradation.

Mechanisms of Cartilage Lesions in Relapsing Polychondritis

Pathophysiological mechanisms remain unclear in RP. In the present study, the presence of rheumatoid factor, antinuclear antibodies, circulating immune complexes and specific antinative collagen type II antibodies in the patient's serum, support the hypothesis of an autoimmune disease with an humoral response specifically directed against collagen of articular and elastic cartilages. Nevertheless, the role of cellular events in cartilage lesions cannot be ruled out. The presence of numerous macrophages in the perichondrium, near the altered cartilage, strongly suggests the participation of these cells in tissue lesions. Among the lysosomal enzymes of the macrophages, elastase and collagenase (Baggiolini et al. (1979) represent an important source for enzymatic breakdown of the cartilage. Release of elastase might also explain the vascular lesions of RP and the aneurysms of large arteries (McAdam et al. 1976).

The role of the chondrocytes in cartilage degradation also seems to be important. Phagocytosis of matrix degradation products was clearly demonstrated in articular cartilage by Mitchell and Shepard (1972) and in ear cartilage

in the present study. Release of lysosomes by altered chondrocytes was observed by ourselves and by Hashimoto et al. (1977). These authors postulated that lysosomal contents released by chondrocytes may produce inflammation and reduce the proteoglycan content of cartilage. On the other hand, collagenase and elastase have not been demonstrated in chondrocyte lysosomes (Stockwell and Meachim 1973).

Thus, whereas immunological events may initiate cartilage lesions in RP, macrophages and chondrocytes seem to be the active and non-specific agents of elastic and collagen fibre destruction observed in thise disease.

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