CHONDROCYTE ULTRASTRUCTURE IN ADULT HUMAN ARTICULAR CARTILAGE

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Age changes in the ultrastructure of articular chondrocytes have been studied in detail in mice (Silberberg, Silberberg, and Feir, 1964) and in rabbits (Davies, Barnett, Cochrane, and Palfrey, 1962; Barnett, Cochrane, and Palfrey, 1963). Less has been published concerning the influence of age on cellular ultrastructure in human articular cartilage. This is due partly to the scarcity of suitable juvenile material for comparison, and partly to the difficulty of studying in adults the effects of ageing alone uncomplicated by those of osteoarthritic fibrillation. The present study is based solely on a series of articular cartilages in adult joints, and the results include observations from both fibrillated areas of cartilage and from areas with an intact articular surface. Fibrillation is characterized by a process of fraying and splitting of the cartilage which can lead to its disintegration and erosion; the process is initially localized, involving only focal areas of an affected joint. Many of the lesions are mild and do not cause clinical symptoms. Collins, however, regarded all grades of fibrillation as forms of “osteoarthritic” cartilage change (Collins, 1949; Collins and McElligott, 1960), and this interpretation has been used in the present investigation.

The observations here reported are an extension of previous electron microscope studies of human articular cartilage from the adult knee (Collins, Ghadially, and Meachim, 1965; Ghadially, Meachim, and Collins, 1965; Meachim and Roy, 1967; Meachim, 1967). Structures normally present in the cartilage cells are described, and the significance of various changes found in the chondrocytes is discussed in relation to ageing and to osteoarthritis.

Material and Methods

Articular cartilage from the human knee was collected at operation before above-knee amputation or during surgical exploration of the joint. Specimens were obtained from eleven patients ranging in age from 37 to 83 years. An average of three tissue blocks was examined from each patient; at least eight serial sections were studied from each block. The exact site or sites sampled in any particular patient varied according to the convenience of the surgeon concerned. In ten patients the tissue was collected from the femoral condyle and in one it came from the patella.

For electron microscopy small pieces of cartilage were fixed in buffered osmium and processed and embedded in araldite (Glaucht, 1961). Sections were stained with lead citrate or occasionally with uranyl acetate, mounted on uncoated copper grids, and examined under an A.E.I. E.M.6 microscope, using an accelerating voltage of 50 or 75 kV.

Results

Since osteoarthritic fibrillation is basically a focal process, the specimens were classified according to the state of the areas sampled rather than according to the state of the joint as a whole. In eight of the eleven patients the tissue taken for study showed evidence of fibrillation; in the other three the articular surface appeared intact in sections examined under the light microscope. It was found that the cells both of the fibrillated and of the non-fibrillated specimens had many ultrastructural features in common, and the following description of adult human articular chondrocytes applies to both groups of specimens except where indicated otherwise.

Cell Membrane.—The chondrocytes are bounded by a limiting plasma membrane. Many of the cells show cytoplasmic processes extending out into the surrounding matrix (Fig. 1); these processes have been noted in the superficial layer of adult human articular cartilage (Ghadially and others, 1965) as well as in its deeper, uncalcified parts. In some cells, irrespective of their zonal distribution, the membrane is rather smooth with only a few short processes (Figs 2 and 3). Small rounded micro-pinocytotic vesicles are often seen in the cytoplasm.
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immediately adjacent to the plasma membrane (Figs 1 and 2), where a few of them can be flask-shaped with an opening to the exterior.

Rough Endoplasmic Reticulum.—The cytoplasm contains a variable amount of rough endoplasmic reticulum. Often this is found as scattered membrane pairs of variable lengths (Fig. 1), but in some cells it shows as a prominent component of the cytoplasm (Figs 2 and 4); occasionally a stack of regularly-aligned membranes is seen (Fig. 5). Cisternal dilatation amongst rough endoplasmic reticulum can occur to some extent in apparently healthy chondrocytes (Fig. 4); however, in one specimen a proportion of the cells near a fibrillated surface showed abnormally dilated and irregular cisternae (Fig. 6).

Mitochondria.—A number of mitochondria are usually identified in any one section through a chondrocyte (Figs 2 and 5); they show a double lining membrane and contain cristae which are sometimes irregularly orientated or indistinct in outline. In one specimen cells with an apparent increase in the

Fig. 1.—Ultrastructure of a chondrocyte from cartilage with deep fibrillation, showing many cytoplasmic processes (M), numerous micropinocytotic vesicles (V), scattered rough endoplasmic reticulum (R), fine filamentous material (F), and a lipid droplet (L). Note the presence of nuclear pores (P) on the nuclear membrane. Nucleus (N). × 18,600. Inset. Nuclear pores at higher magnification. × 37,000.
number of mitochondria were encountered (Fig. 3). In another specimen chondrocytes near a fibrillated surface showed swollen mitochondria with disorientated cristae (Fig. 7), structures with multilayered whorled membranes, and other membrane-bound inclusions containing disintegrating cell organelles (Fig. 8). The cytoplasmic inclusions seen in these cells can be classified as "residual bodies", or as "cytolysomes" (autophagic vacuoles), as they sometimes contain morphologically recognizable cytoplasmic components (De Duve and Wattiaux, 1966). Many of these structures show a double membrane and appear to have derived from the abnormal mitochondria.

Golgi Complex.—Golgi apparatus, when present, is identified as one or more areas of closely-spaced agranular lamellae with dilatations and formation of vesicles of variable sizes (Fig. 2). Often the vesicular component is poorly developed, but sometimes the apparatus shows as a prominent complex in which vesicles are large and numerous (Fig. 9).

Other Vesicles.—In addition to the vesicles of Golgi complex and to those of pinocytosis at the cell membrane, smooth-walled vesicles can also occur elsewhere in the cytoplasm. They vary a good deal in their pattern. The larger ones often show a similar morphology to that of large vesicles found in the Golgi area containing mainly electron lucent or a small amount of electron dense material. Small vesicles with a mainly clear electron lucent content appear to be either of pinocytotic or of Golgi origin. Another type is occasionally seen, which contains indistinct small rounded structures suggestive of inner vesicles (Fig. 2), and may thus represent a "multivesicular body" (De Duve and...
Wattiaux, 1966; Smith and Farquhar, 1966); but in a few instances it also shows a double lining membrane or contains cristae-like structures, suggesting an altered mitochondrion. Other types of vesicle present a variety of forms, each of which is found occasionally in chondrocytes in the present material.

Lipid Drops.—In the electron micrographs one or more intracytoplasmic lipid globules is often demonstrated in the plane of section through a chondrocyte (Collins and others, 1965). The globules are rounded but sometimes irregular in outline (Figs 1, 2, and 3) and in adult human articular cartilage their content varies in electron density from pale grey to black. A complete or incomplete rim of electron dense granules is often seen at the edge of the globule, and the content itself can be partly granular. At times one or more mitochondria are seen in close association with the lipid globules.

Glycogen.—This is often seen. It occurs as scattered particles, or as a small or large aggregate in which the particles are often closely packed (Figs 4, 5, and 9).

Intracytoplasmic Filaments.—Many of the chondrocytes contain one or more finely filamentous areas in their cytoplasm (Figs 1 and 2), as described in a previous report (Meachim and Roy, 1967). The filaments lie directly within the cytoplasm and are not enclosed in membrane-bound vacuoles. They are typically perinuclear in situation; less commonly they are seen in a more peripheral part of the cell. Chondrocytes with filaments are a frequent finding both in the fibrillated and in the non-fibrillated specimens.
Fig. 4.—Chondrocyte from cartilage with superficial fibrillation, showing dilatation of cisternae of most of the rough endoplasmic reticulum (R). Note prominent Golgi apparatus (A) and aggregates of glycogen (G). Nucleus (N). × 20,000.

Fig. 5.—Chondrocyte from non-fibrillated cartilage with rough endoplasmic reticulum (R), showing parallel alignment of the membrane pairs. Note the presence of many mitochondria and aggregates of glycogen (G). × 35,000.
**Nucleus.**—In adult human articular cartilage the ultrastructure of the cell nucleus (Figs 1 and 2) is essentially similar to that in many other tissues. Thus it is bounded by a double membrane and contains electron dense granular nucleoplasm; a nucleolus is occasionally present (Fig. 2) and in tangential sections nuclear pores approximately 800 Å in diameter often with a central dot (Fig. 1) are apparent. There is, however, much variation in the shape of the nuclei. They can be spherical, oval, or elongated, and nuclear indentation is sometimes seen as described in adult rabbit articular cartilage (Barnett and others, 1963).

**Centriole.**—In an occasional cell centrioles are seen near the nucleus (Fig. 2).
Fig. 7.—Part of a degenerating chondrocyte from deeply fibrillated cartilage containing swollen mitochondria (M) with deformed cristae, marked dilatation of cisternae (C) of rough endoplasmic reticulum, and a dense body (D) containing membrane structure. × 40,000.

Fig. 8.—Structures (S) containing whorled membranes and much rough endoplasmic reticulum are present in this chondrocyte from the same specimen as in Fig. 7. Other double membrane-bound bodies (B) containing remnants of cell organelles are also seen. Nucleus (N). × 20,000.
Fig. 9.—Chondrocyte from non-fibrillated cartilage with prominent Golgi complex, composed of smooth parallel lamellae and many smooth vesicles of variable size. Note aggregates of glycogen particles. × 29,400.

Fig. 10.—Complex bodies containing lipid droplets (L). Chondrocyte from non-fibrillated cartilage. × 40,000.
Complex Bodies.—A variety of large complex bodies with components of contrasting morphology have been encountered (Figs 10 and 11). Typically they contain one or more lipid drops; sometimes they also include electron dense particulate or fine fibrillary material. They are not a common finding, but have been noted in one of the non-fibrillated and in two of the fibrillated specimens. Small complex bodies of similar morphology are occasionally seen.

Electron Dense Bodies.—Membrane-lined rounded and ovoid bodies with a copious content of moderately or intensely electron dense material are common in the deeply fibrillated specimens, where certain of the cells contain an increased number of these structures (Fig. 12). Some of them appear to have an incomplete double membrane and attenuated cristae. They are infrequent in the non-fibrillated cartilages and in those showing only a mild degree of osteoarthritic fibrillation.

Disintegrated Cells amongst the Matrix.—One pattern of chondrocyte necrosis seen in man is characterized by a complete disintegration of the cell with the formation of organelle and cytomembrane remnants, rounded membranous bodies, and granular particles (Fig. 13). Occasional instances of this have been noted amongst the matrix both in fibrillated cartilages and in the superficial layer below an intact articular surface in non-fibrillated specimens.

Cell Shedding into the Joint Cavity.—No evidence of cell shedding into the joint cavity was seen on electron microscopy of the non-fibrillated cartilages. Exposure of cells at the joint surface was, however, noted occasionally in a few of the specimens from fibrillated areas.

Discussion
Normal Cytoplasmic Organelles

The ultrastructure of mammalian articular chondrocytes has been described in the rabbit by Davies, Barnett, and their colleagues (Davies and others, 1962; Barnett and others, 1963; Palfrey and Davies, 1966) and in the mouse by Silberberg and others (1964). Many of the cytoplasmic structures seen in the present study have their counterpart in the rabbit and mouse, and can be regarded as normal constituents of adult human chondrocytes. In man they have been observed both in cartilage with an intact articular surface and in specimens showing osteoarthritic fibrillation.

The mitochondria of chondrocytes no doubt have a similar function to that in cells of other tissues,
being concerned in the production of energy-rich substances for use in biochemical processes. Similarly the rough endoplasmic reticulum with its ribosomes is presumably the site for synthesis of enzymes and other proteins. Autoradiographic studies in the larval salamander (Revel and Hay, 1963) and the embryonic rat (Godman and Lane, 1964) indicate that vesicles of the Golgi apparatus are concerned in the segregation and transport of material manufactured by the chondrocyte and secreted into the matrix. This conclusion is consistent with the known secretory function of the apparatus in fibroblasts (Curran, Lovell, and Clark, 1965), synovial cells (Roy and Ghadially, 1967), and intestinal epithelium (Peterson and Leblond, 1964). However, it cannot be assumed that extracellular secretion is the only function of the Golgi complex in chondrocytes, since studies of anterior pituitary cells suggest that the complex may also contribute small vesicles to cytoplasmic bodies concerned in intracellular digestive processes (Smith and Farquhar, 1966).

The small rounded vesicles seen in the cytoplasm of chondrocytes immediately adjacent to the plasma membrane are termed micro-pinocytotic vesicles (Silberberg and others, 1964; Palfrey and Davies, 1966), implying that they transport material into the cell from the exterior. In addition to these micro-pinocytotic vesicles at the plasma membrane and to vesicles in the Golgi area, smooth-walled
Fig. 13.—Area containing remnants of a disintegrated chondrocyte. Non-fibrillated cartilage. × 31,000.
vesicles can also occur elsewhere in the cytoplasm of adult human articular chondrocytes. Some of them are considered to be of pinocytotic and others of Golgi origin, but the nature and origin of the others is often uncertain. Glycogen deposits in chondrocytes probably serve as a readily available source of raw material for biochemical processes (Stockwell, 1967a), and intracellular lipid drops may also serve this purpose (Collins and others, 1965; Stockwell, 1967a). Microtubules were not identified in the present study, which was made on tissue fixed directly in buffered osmium without use of glutaraldehyde. They have, however, been demonstrated in rabbit articular chondrocytes fixed with glutaraldehyde before being treated with osmium (Palfrey and Davies, 1966).

Intracytoplasmic Filaments

Healthy chondrocytes often contain finely filamentous areas as a normal feature in their cytoplasm (Meachim and Roy, 1967). There is, however, ultrastructural evidence that human articular chondrocytes with an unusually large amount of filaments are in many instances undergoing a form of involution or "degeneration". In such cells there is a reduction in the total number of organelles because of their loss from areas replaced by filaments, and the organelles which remain often tend to be poorly preserved (Meachim and Roy, 1967). Excessive accumulation of intracytoplasmic fine filaments in articular chondrocytes also occurs in experimentally produced chronic haemarthrosis (Roy, 1968). In this condition, too, other evidence of cell degeneration is seen. The fact that in the normal rabbit intracytoplasmic filaments in articular cartilage are seen more commonly in older than in young animals (Barnett and others, 1963) is in keeping with their occurrence in adult human articular chondrocytes. Both in man and in the rabbit intracellular filament accumulation has been observed beneath an intact articular surface; it can therefore be regarded as a feature of age change rather than as a specific consequence of osteoarthritis.

Cell Necrosis and Cell Shedding

A change in the cell density of articular cartilage could in theory come about in one or more of the following ways: by death of chondrocytes in situ; by shedding of cells into the joint cavity from the articular surface; by proliferation of surviving chondrocytes; and by alteration in the amount of intercellular matrix independent of any change in the total cell population. Electron microscopy has shown no evidence of cell shedding from a non-fibrillated articular surface either in mice (Silberberg and others, 1964) or in rabbits (Davies and others, 1962; Barnett and others, 1963) or in man in the present study. Ultrastructural findings indicate instead that the age-correlated decline in cellularity which occurs in rabbit femoral articular cartilage is due mainly to death of chondrocytes in situ, since the remains of necrotic cells can be observed in the cartilage matrix of mature adult rabbits (Barnett and others, 1963). In adult man light microscopy has shown that loss of cellularity can occur in the superficial layer of non-fibrillated areas of femoral articular cartilage from the knee (Meachim, Ghadially, and Collins, 1965; Stockwell, 1967b). The present study with the electron microscope suggests that here again the phenomenon is due to death of chondrocytes in situ. The non-fibrillated cartilage specimens showed occasional instances of disintegrated necrotic cells (Fig. 13) amongst the matrix of the superficial layer, but no evidence of cell shedding into the joint cavity. Exposure of chondrocytes at the articular surface was, however, noted occasionally in a few of the fibrillated specimens. Thus in fibrillated cartilage cell shedding can probably occur as well as death of cells in situ. In areas of deep fibrillation the influence of these processes on cartilage cellularity is often counteracted by a proliferation of surviving deeper chondrocytes to form abnormal multi-cellular clusters (Meachim and Collins, 1962).

Chondrocyte Degeneration

Unless a chondrocyte is frankly necrotic, caution is necessary before ultrastructural features are interpreted as evidence that it is degenerate. For example, the present study indicates that cisternal dilatation amongst rough endoplasmic reticulum can occur in healthy chondrocytes (Fig. 4). Again, in non-cartilaginous tissues, the cytoplasm can show focal alterations which represent attempts to dispose of superfluous, effete, or damaged organelles, or attempts to remove secretory products which are no longer required (Smith and Farquhar, 1966); it is reasonable to assume that a similar phenomenon can occur in chondrocytes without the cell as a whole being degenerate. However, in one of the specimens in the present study, adult human articular chondrocytes near a fibrillated surface showed excessive destruction of mitochondria, as evidenced by mitochondrial swelling with disorganisation of cristae, cytolysome formation, and the presence of whorled membranous residual bodies (Figs 6, 7, and 8); a
proportion of the affected cells also showed abnormally dilated and irregular cisternae (Fig. 6). The ultrastructural features strongly suggest that these chondrocytes were undergoing a form of degenerative change. A similar type of degeneration has been described by Palfrey and Davies (1966) in normal articular cartilage from rabbits, and by Roy (1968) in articular cartilage of rabbits in experimental haemarthrosis.

**Complex Bodies**

The nature and significance of the large complex bodies with components of contrasting morphology (Figs 10 and 11) is uncertain. Apparently they have not been encountered in articular cartilage from mice (Silberberg and others, 1964) or from rabbits (Davies and others, 1962; Barnett and others, 1963; Palfrey and Davies, 1966). In the present study of adult human articular cartilage they were noted in one of the non-fibrillated and in two of the fibrillated specimens; small complex bodies of similar morphology were occasionally seen. It is tempting to speculate that the large and small complex bodies are an unusual form of heterophagic or autophagic digestive vacuole in which material such as lipid has proved relatively resistant to attack by lysosomal enzymes (Smith and Farquhar, 1966).

**Electron Dense Bodies**

An increased number of electron dense bodies was noted in some of the cells of the cartilage specimens showing deep fibrillation of osteoarthritic type. This observation is of interest in view of the biochemical findings in osteoarthritis. In fibrillated areas of human articular cartilage there is a localized chemical degradation of the protein-polysaccharide complexes of the ground substance in the intercellular matrix (Matthews, 1953; Collins and McEl- liggott, 1960; Bollet, Handy, and Sturgill, 1963; Bollet and Nance, 1966; Barland, Janis, and Sandson, 1966). Enzymatic degradation of the matrix is believed to be the major mechanism responsible for this localized change, and various suggestions have been made concerning the nature and origin of the enzymes concerned (Chrisman, 1964; Bollet and Nance, 1966). Barland and others (1966) suggest that the enzymes are derived from lysosomes in the cartilage cells. The morphology of the dense bodies sometimes observed in increased numbers in the chondrocytes of fibrillated human cartilage would be consistent with a lysosomal nature (Fig. 12). Some such bodies appear to have incomplete double membrane containing attenuated cristae and may be of mitochondrial origin. One cannot, however, assign a matrix-degrading role to these bodies solely on the basis of their morphology. They may well be merely a secondary feature of osteoarthritis, and play no major part in its pathogenesis.

**Summary**

Ultrastructure of chondrocytes in articular cartilage from eleven adult individuals have been studied. The specimens include eight cartilages showing varying degrees of fibrillation and three with an intact surface. Apart from normal cells containing varying amounts of cytoplasmic organelles seen in cells of other tissues, many degenerative changes are also noted. These include marked dilatation of cisternae of rough endoplasmic reticulum, swelling of mitochondria with deformed cristae, cyto- somes with variable morphological appearance, and lysosomal bodies. Some of these abnormalities, increase in lysosomal bodies for example, are more common in chondrocytes from cartilage showing fibrillation. Remnants of organelles indicating necrosis of chondrocytes in situ has also been observed in both fibrillated and non-fibrillated cartilage.

The function of normal articular chondrocytes, the possible mechanism of age-correlated reduction of chondrocytes, and the significance of the observed abnormalities in relation to osteoarthritis are discussed.

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**REFERENCES**


L'ultrastructure du chondrocyte dans le cartilage articulaire de l'homme adulte

RéSUMÉ
On étudia l'ultrastructure des chondrocytes du cartilage articulaire provenant d'onze hommes adultes. Les prélèvements comprenaient huit cartilages présentant une fibrillation plus ou moins intense et trois cartilages avec surface intacte. En dehors de cellules normales contenant de différentes quantités d'organelles cyto-

Ultraestructura del condrocito en el cartílago articular del hombre adulto

Sumario
Se estudió la ultraestructura de los condrocitos del cartílago articular en once personas adultas. Ocho espécimens acusaron una fibrilación más o menos intensa y tres cartílagos tuvieron la superficie intacta. Además de células normales con varios números de organitas citoplásmicas observadas también en otros
plasmiques observées dans d'autres tissus, on nota beaucoup d'altérations dégénératives, telles que dilatation des cisternae du réseau endoplasmique grossier, enflentement des mitochondries avec cristae déformées, cytolysomes d'apparence morphologique variable et inclusions lysosomiques. Certaines de ces anomalies, telle que le nombre augmenté des inclusions lysosomiques, étaient plus fréquentes dans des chondrocytes provenant des cartilages avec fibrillation. Des vestiges des organelles indiquant une nécrose des chondrocytes in situ on été observés tant dans le cartilage fibrillé que celui non fibrillé.

On discute la fonction normale des chondrocytes articulaires, le mécanisme possible de la réduction des chondrocytes par rapport à l'âge et la portée des anomalies observées à l'égard de l'ostéoarthrose.

tejidos, se notaron muchas alteraciones degenerativas: dilatación de las cisternae del retículo endoplásmico grosor, hinchazón de las mitocondrias con crista deformadas, citolíosomas de apariencia morfológica variable e inclusiones lisosómicas. Algunas de estas anomalías, tales como un número aumentado de inclusiones lisosómicas, se vieron más a menudo en condrocitos de los cartilagios con fibrillación. Vestigios de organitas, indicando una necrosis in situ, se observaron tanto en el cartílago fibrillado como en el no fibrillado.

Se discute la función normal de los condrocitos articulares, el mecanismo posible de la reducción de condrocitos en relación con la edad y la importancia de las anomalías observadas respecto a la osteoartrosis.
Chondrocyte ultrastructure in adult human articular cartilage.

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