ELECTRON MICROSCOPY OF ARTICULAR CARTILAGE IN THE YOUNG ADULT RABBIT

BY

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Few previous studies have been made of the appearance of articular cartilage under the electron microscope. Cameron and Robinson (1958) examined the matrix of both articular and epiphyseal cartilages in femora of infants who had died in the neonatal period. No observations were made upon the cells as they were unable to assess the extent of *post mortem* change. They described the presence in the articular cartilage of bundles of fibres having the characteristic cross-striations seen in mature collagen; these bundles were arranged parallel with the surface in the superficial 25μ , but irregularly in the deeper parts of the cartilage.

Zelander (1959) examined the appearances of femoral and patellar articular cartilage in adult guinea-pigs, mice, and rats, describing both cells and matrix. He noted the characteristics of one type of chondrocyte, but did not describe any variations in its appearance at different levels, except in the calcified zone, where he described the dying chondrocyte. Each healthy cell was always surrounded by a region almost devoid of fibres, which he termed the capsule. Around this was a zone 2 to 4μ thick in which were fibres of steadily increasing diameter, the innermost fibres being unstriated, but the outer ones showing the characteristic banding of mature collagen. Zelander equated this zone with the "territory" of Bargmann (1956). In the interterritorial regions he found fibres of constant diameter which were uniformly striated with a periodicity of $580 \text{ \AA}$. In the deeper parts of the matrix he described the appearances of early calcified foci.

Martin (1954) studied the appearances of the collagen fibres in cartilage obtained from the developing tibia of the fowl, including the articular surfaces. Zbinden (1953) gave an account of some age changes in articular cartilage from guinea-pigs

and rats. Little and Pimm (1957) and Little, Pimm, and Trueta (1958) described the changes which occur in the collagen fibres of human articular cartilages in the presence of osteo-arthritis.

The remaining studies of the electron microscopic appearance of cartilage have been limited to the epiphyseal region. Robinson and Cameron (1956) studied the distal epiphyseal cartilage in the femora of newborn infants, and described the appearances in the process of ossification. Using material from the proximal humeral epiphysis of young kittens, Scott and Pease (1956) described the appearances seen during ossification, and in particular the degenerative changes seen in the chondrocytes.

Materials and Methods

The material used in the present investigation was obtained from six young adult rabbits of both sexes, varying in age from 62 to 74 days. The rabbits were anaesthetized with chloroform or nembutal and specimens were taken from the distal articular cartilage of the femur. Two shavings were taken from the cartilage so that specimens from the deep and superficial regions were separated: all specimens were cut up in the fixative until the blocks of tissue measured less than a millimetre in at least two axes. The tissue was fixed in ice-cold chrome-osmium fixative (Dalton, 1955) for 2 hours: a shorter period was found to give inadequate fixation. It was then dehydrated following the procedure recommended by Richardson, Jarett, and Finke (1960), using tertiary butyl alcohol throughout. Earlier specimens had been embedded in a methyl-butyl methacrylate mixture, but in the present series araldite was used as the embedding medium; the methods described by Glauert, Rogers, and Glauert (1956) and Sorvall (1959) were tried, but that of Richardson and others (1960) was found to give the most satisfactory results. Thin sections were cut with a Leitz ultramicrotome, using glass knives, and were placed upon copper grids, which had already been covered with a carbon film. The sections were examined in an A.E.I. EM6 microscope.

Some sections were examined without staining, but a variety of staining techniques was tried. The most satisfactory stain for cells and their organelles was found to be uranyl acetate, used according to the method of Sorvall (1959). Lead hydroxide also gave good results for the cells, but it was found difficult to prevent crystallization: the methods described by Watson (1958), Lever (1960), and Dalton and Zeigel (1960) were tried. The fibres of the matrix were found to be stained most effectively by phosphotungstic acid (Barton, 1961). The staining reactions of potassium permanganate, as described by Lawn (1960), were tried, but the results were disappointing.

Results

The articular cartilage covering the lower end of the femur in young adult rabbits ranges from 150 to 450 μ in thickness. Its surface is remarkably smooth (Fig. 1); such irregularities as occur are less than 250 $\text{\AA}$ in depth over a length of 0.5 μ ; there are occasionally somewhat deeper irregularities, up to 0.2 μ in a length of 10 μ . It rests upon the subarticular bone, which may be recognized by the presence of characteristic osteoblasts (Scott and Pease, 1956) and by the absence of uncalcified matrix.

The articular cartilage may be divided into four main zones on the basis of its electron microscopic appearance: a superficial zone ranging from 10 to 20 μ in thickness; an upper middle zone ranging from 30 to 50 μ in thickness; a lower middle zone, 50 to 250 μ in thickness; and a deep zone, 50 to 100 μ in thickness. Variations in the total thickness of the articular cartilage are largely accounted for by variations in thickness of the lower middle zone.

Superficial Zone.—This is relatively cellular, the chondrocytes being arranged in two or three layers parallel to the articular surface. The cells occupy about 35 per cent. of the area of a section; this is equivalent to about 20 per cent. of the volume of the cartilage. At the surface there is an acellular zone which is generally 2 to 3 μ thick.

The cells are lenticular in shape (Fig. 1); the diameter of their profiles is from 10 to 20 μ and their thickness from 3 to 5 μ . Their surface (particularly that facing the joint cavity) is generally smooth, but does exhibit occasional short stumpy processes which only rarely branch (Fig. 3). These are more evident at the margin of the cell. The nucleus is ovoid in shape and measures 5 to 10 μ in length and 2 to 3 μ in width. It possesses a well-defined smooth double membrane (Fig. 2), which exhibits nuclear pores ranging from diameters of 500 $\text{\AA}$ to 750 $\text{\AA}$, at intervals of 0.5 to 0.75 μ . It possesses a typical nucleolus. Within the cyto-

plasm short lengths of cytomembranes are seen in small numbers, bearing Palade granules on their outer surfaces (Fig. 3). The interval between the members of a pair of cytomembranes, measuring 420 $\text{\AA}$ in width, is no more electron dense than the surrounding cytoplasm. There are abundant mitochondria with obvious cristae; these are predominantly transversely orientated and appear branched. Fat droplets have not been observed. There are many small oval vesicles in the cytoplasm; these are concentrated close to the cell membrane. They possess an outer electron dense membrane surrounding a central area of low density, and sometimes open to the surface of the cell by narrow necks. The major diameter of these vesicles ranges from 550 to 850 $\text{\AA}$ and the minor from 400 to 600 $\text{\AA}$ (Fig. 4).

The matrix in the superficial zone contains many fine fibres of 80 to 250 $\text{\AA}$ diameter, those of the smaller diameter being nearer the surface (Fig. 5). When seen in longitudinal section the fibres show striations of periodicity $100 \pm 10 \text{\AA}$. They tend to run in bundles of four to six, more or less parallel to the articular surface. The matrix forming the surface, to a depth of about 0.2 μ , has few fibres and those that do penetrate into this superficial region have very indistinct outlines. Among the fibres are occasional rounded or cylindrical bodies that resemble the blunt processes of the cells; they possess a somewhat indistinct outer membrane and finely granular contents. Much of the interfibrillar matrix is clear, but shows scattered electron densities in some preparations. There is no increase in the electron density of the interfibrillar matrix around the cells.

Fig. 1.—Section of superficial zone. The articular surface is on the right. Stained with uranyl acetate. $\times 5,800$.

Fig. 2.—Part of a cell in superficial zone, showing a nuclear pore (np) and part of a mitochondrion (m). Stained with lead hydroxide. $\times 29,500$.

Fig. 3.—Part of a cell in superficial zone, showing short, stumpy processes at the surface (p), a mitochondrion (m), and short lengths of cytomembranes carrying Palade granules (cy). Stained with uranyl acetate. $\times 29,500$.

Fig. 4.—Part of a cell in superficial zone which has been cut obliquely and shows the concentration of pinocytotic vesicles (above and to left of letter V) near the surface of the cell. Part of the nucleus is visible at the lower right (n). Stained with lead hydroxide. $\times 20,500$.

Fig. 5.—Matrix of cartilage at surface, showing a superficial region in which there are few or no fibres (c), with small bundles of fine fibres (fb) in the deeper part of the section. Many of the fibres (e.g. where indicated by arrow) show cross-striations in the original print. Stained with uranyl acetate. $\times 29,500$.

Fig. 6.—Pair of cells from upper middle zone with Golgi vesicles (gg), mitochondria (m), and cytomembranes (cy). Stained with uranyl acetate. $\times 6,000$.

Fig. 7.—Fine fibres from matrix between a pair of cells, showing cross-striations (arrows). Stained with uranyl acetate. $\times 100,000$.

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Upper Middle Zone.—The transition from the superficial to the upper middle zone is relatively sharp. Here pairs of cells are first seen (Fig. 6). One cell of a pair is commonly sectioned near its periphery or through its processes alone. The cells are shorter and more rounded, the length of their profiles being about 11μ and their width about 6μ . Their occasional processes are longer, more irregular and sometimes branched; they arise with equal frequency from all aspects of the cell. The nucleus often has an undulating surface so that parts of the membrane appear less distinct. In the cytoplasm, cytomembranes are more frequently seen and the Palade granules are more obvious. The cytomembrane interval, measuring $420 \text{ \AA}$, is somewhat more electron dense than in surface cells. Small fat globules are occasionally present. The mitochondria resemble those in the surface cells. The small oval vesicles noted in the surface cells are present, but they are less numerous and are uniformly distributed throughout the cytoplasm. In addition, Golgi vacuoles are now visible. These lie at one side of the nucleus and contain apparently particulate matter, most of which is concentrated near the centre of the vacuoles. At high magnifications the contents appear as a fine reticulum. Although most of the Golgi vacuoles are only about 0.5μ in diameter, some are enlarged to a diameter of nearly 2.5μ ; these large Golgi vacuoles often impinge upon the cell wall (Fig. 6).

The matrix around the cells and between pairs of cells contains fine fibres of 150 to $350 \text{ \AA}$ in diameter; the fibres exhibit cross-striations with a periodicity of about $100 \text{ \AA}$ (Fig. 7). Further from the cells these give way to the coarse fibres of the general matrix. These have a diameter of 400 to $550 \text{ \AA}$ and have indistinct cross-striations of about $400 \text{ \AA}$ periodicity. The coarse fibres are generally cut transversely or obliquely, but occasionally appear in longitudinal section in lengths of up to about a micron. There is a gradual transition from the coarse fibres of the general matrix of the upper middle zone to the fine fibres of the superficial zone.

Lower Middle Zone.—This is usually the thickest zone of the articular cartilage. The cell density is much lower than in the superficial zone; they occupy about 8 per cent. of the area of a section. This is equivalent to about 2 per cent. of the volume of the cartilage. The cells are commonly arranged in pairs or groups of three or four. They are much more variable in shape and their profile is highly irregular. The cells exhibit two types of irregularity. First, the surface is often scalloped through the

presence of rounded bays, which contain finely granular or reticular material; the appearance of this material is similar to that of the contents of the Golgi vacuoles (Fig. 8). These bays measure from 0.5 to 2μ across, dimensions which are similar to the diameter of the Golgi vacuoles. The second type of irregularity of the cell outline is the presence between the bays of large numbers of irregular branching processes, which extend into the matrix and often make direct contact with its fibres. The nucleus has a well-defined membrane, but shows many shallow undulations. The nucleoplasm in most preparations is more electron dense than the surrounding cytoplasm.

The cytomembranes are numerous, extensive, often concentrically arranged around the nucleus and bear many Palade granules (Fig. 10). The interval between cytomembrane pairs, which measures $400 \text{ \AA}$, is of much greater electron density than that of the remainder of the cytoplasm, which is pale, so that the cell as a whole often has a characteristic banded appearance. This is in contrast to the uniform density of these parts in the superficial and upper middle zones. Occasionally the paired cytomembranes are more widely separated over distances of about 0.2μ to 0.5μ , with an interval ranging up to $1,000 \text{ \AA}$; the intervening material in these regions is homogeneous and electron dense. Fat globules, homogeneous dense spherical bodies from 1μ to 2μ in size, are visible in many cells (Fig. 9). At the surface of these fat globules pairs of cytomembranes are often arranged concentrically. Mitochondria are again clearly visible, but most appear to be smaller, more electron dense and with less evident cristae. Large Golgi vacuoles are again in evidence, often clustered together at one end of the cell. These large vacuoles are frequently seen to open at the surface of the cell.

Fig. 8.—Part of a cell from lower middle zone, showing the similarity of the material in the bays (b) and in the Golgi vesicles (gg). Stained with uranyl acetate. $\times 20,500$.

Fig. 9.—Part of a cell from lower middle zone showing a fat globule (f) in relation to cytomembranes (cy). Stained with uranyl acetate. $\times 15,500$.

Fig. 10.—Part of a cell from lower middle zone, showing the concentric arrangement of the cytomembranes (cy) around the nucleus (nu) and the transition from fine fibres near the cell (ff) to the coarse fibres of the general matrix (cf). Stained with uranyl acetate. $\times 15,500$.

Fig. 11.—Section from lower middle zone, showing the network of fine fibres around and between the cells and the dark interfibrillar matrix between a pair of cells. Stained with uranyl acetate. $\times 6,000$.

Fig. 12.—Cell from deep zone in which the nucleus (nu) is ill defined. Stained with uranyl acetate. $\times 7,500$.

Fig. 13.—Cell from deep zone, showing short lengths of cytomembranes (cy) with irregularly scattered granules (gr) in the cytoplasm. Stained with uranyl acetate. $\times 15,500$.

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Occasional degenerate cells are seen, but comparable cells are commoner in the deep zone, with which they are described.

In the matrix of the deep middle zone there is a thin layer of fine fibres surrounding many of the cells and predominantly arranged parallel to the cell surface. This layer is continuous with a loose, interlacing network lying between groups or pairs of cells (Fig. 11). The fibres are similar in calibre to those lying around the groups of cells in the upper middle zone, but the cross-striations seen on these fine fibres now have a periodicity of about 400 Å. The remainder of the matrix contains coarser fibres, ranging in diameter from 400 to 700 Å, with cross-striations having a period of about 600 Å; these fibres are usually cut transversely or obliquely (Fig. 10). The inter-fibrillar matrix is somewhat electron dense between pairs or groups of cells, but elsewhere it remains relatively pale.

Deep Zone.—This is distinguished by the presence of degenerate cells, which occur in increasing proportion as the bone is approached, and by the appearance of calcification in the deeper portions of the matrix. Healthy cells similar in appearance to those in the middle zone are interspersed among the degenerate cells. The cells are generally rounded in form, ranging from 8 to 16 μ in diameter. Sometimes they appear in irregular columns; closely apposed pairs are rarely seen.

Two types of degenerate cells are found. In the first, the nucleus is characteristically seen lying near one edge of the cell and is nearly always of lower electron density than in the more superficial zones of the cartilage. It possesses an irregular surface and an indistinct nuclear membrane, so that it is not easily distinguished from the surrounding cytoplasm (Fig. 12). The cytomembranes are seen in short lengths and in places the pairs are separated by small masses of granules similar to Palade granules (Fig. 13). There is an increase in the relative amount and a decrease in the electron density of the cytoplasm between the pairs of cytomembranes. The cell membrane is often indistinct and in places disintegrated (Fig. 14), and is surrounded by a fine granular material (Fig. 15). Typical mitochondria are not seen, but electron dense bodies of comparable dimensions and frequency are found.

The second type of degenerate cell consists of an elongated central body from which radiate a small number of long narrow branching processes. Their overall extent ($\sim 10\mu$) is similar to the dimensions of the cells in the upper middle zone, the body

forming a third or less of the overall diameter. Many sections pass through the processes only (Fig. 16). The processes vary from 500 to 1,000 Å in width and partly enclose bays of up to nearly 1 μ across. These bays contain granular material similar to that already described within the Golgi vacuoles. When present the nucleus exhibits the same appearances as in the first type of degenerate cell. Typical organelles are identifiable only in cells showing less advanced degeneration. A few cells cannot with certainty be assigned to one or other of the two degenerative types described above.

The intercellular substance has three distinct regions. Immediately around the degenerate cells there is a shell made up of a fine granular reticulum, extending outward from the bays (Fig. 17). Surrounding this there is a network of fine fibres which are about 80 to 150 Å in diameter and have cross-striations with a periodicity of 400 Å; the fibres are more numerous than in the middle zones, and are usually arranged irregularly, though a few run parallel to the surface of the cell. Outside the latter are the coarse fibres of the general matrix. These are from 400 to 600 Å in diameter. Along the length of the fibre the dark and light cross-striations alternate at intervals of 650 Å. Within each light band are three faint lines and there are two denser lines within each dark band.

Calcification is first seen among the coarse fibres of the general matrix and at some distance from any cell surface. This takes the form of fine needle-like electron dense spicules scattered among the fibres, which are clumped together to form rosettes (Fig. 18). These merge to give rise to continuous masses of electron dense material. Diffraction patterns obtained from early calcified masses show rather broad rings, indicating the presence of randomly orientated apatite crystals of small size. The patterns from the adjacent sub-articular bone show sharper rings, with the (002) ring broken into two arcs. This indicates larger crystals and partial orientation of the crystallographic c-axes.

Fig. 14.—Cell from deep zone in which the cell membrane has disintegrated. Calcified matrix (cm) is also visible. Stained with lead hydroxide. $\times 7,500$.

Fig. 15.—Disintegrated cell from deep zone around which fine granular material can be seen (fg). Stained with lead hydroxide. $\times 15,000$.

Fig. 16.—Section through branching processes of a degenerate cell lying among fine granular material. The electron-dense material is calcified. Stained with lead hydroxide. $\times 4,000$.

Fig. 17.—Degenerate cell from deep zone around which the fine granular (fg), fine fibrous (fb), and coarse fibrous (cf) parts of the matrix can be seen. Stained with phospho-tungstic acid. $\times 9,500$.

Fig. 18.—Part of matrix from deep zone, showing calcified masses in relation to the coarse fibres. Stained with uranyl acetate. $\times 29,500$.

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Discussion

Matrix.—It is convenient to discuss in detail the structure of the matrix from the articular cartilage of the young adult rabbit before going on to consider the form and functions of the cells.

The narrow surface lamina, 0.2μ in thickness, relatively devoid of fibres, probably corresponds to the *lamina splendens* described by MacConaill (1951), using light microscopy. The surface of the matrix is remarkably smooth. The irregularities seen under the electron microscope are of the same order of magnitude as those present on highly polished metal or glass (Knauer and Stern, 1929). Despite occasional irregularities of greater size, it is clear that, in conjunction with various lubrication mechanisms within synovial joints (Barnett and Cobbold, 1962), this smoothness contributes materially to their ease of movement.

Throughout the matrix there are large numbers of fibres, varying in size and arrangement. In the superficial zone there are fine fibres only; in the other zones such fibres are found around and between the cells, and these give place quite abruptly to the coarser fibres in the general matrix. Two problems require consideration: the source of the fine fibres and the inter-relationship of the fine and the coarse fibres.

The superficial fine fibres could be formed in three ways. They may originate in association with the long, branching processes of cells in the upper middle zone, especially in the interval between pairs of cells, whence they subsequently move towards the surface of the cartilage. A second possibility is that the surface cells are responsible for fine fibre production, but at a very slow rate, so that a newly-formed fibre would be observed only occasionally in relation to the cell surface. Finally, it is feasible that the surface fibres have been produced by surface cells at an earlier stage of development. This suggestion could be confirmed only by reference to articular cartilage from young and foetal rabbits; if it proved correct, there would seem to be no mechanism for replacing any matrix that may be rubbed off the mature articular surface.

In young adult rabbits no predominance of fibre bundles perpendicular to the surface has been demonstrated by the electron microscope, though these have been described by Benninghoff (1925) and others.

The course fibres of the middle and deep zones almost certainly originate from fine fibres produced locally, but the rather abrupt transition from one type to another is difficult to interpret in terms of simple maturation. The sudden increase in diameter might be attributed to the conglomeration of several

fine fibres or to the growth of the fibres (Jackson, 1956). The comparative absence of coarse fibres cut in longitudinal section in the middle zones probably indicates the assumption of a tight helicoidal form. Electron microscope studies do not allow an analysis of the arrangement of the coarse matrix fibres because the section thickness is much less than the diameter of the suggested helix. The fibres in the region of calcification are commonly cut in longitudinal section, indicating that many are in straight lengths: it also suggests that the helicoidal form found more superficially is not the general arrangement here.

There is some doubt as to the nature of the fibres within the matrix. The coarse fibres in the deep and lower middle zones have a diameter and a periodicity of cross-striation indicating that they are collagen fibres. On the other hand, the fine fibres do not show the typical $640\text{ \AA}$ banding of mature collagen. Evidently the macromolecules in these fine fibres are not aggregated together in the same way as in adult collagen: the conditions under which this type of aggregation takes place apparently do not hold in the superficial zone nor in the immediate neighbourhood of chondrocytes. Even in the coarse fibres the cross-striations are not clear-cut except in the straight fibres of the deep zone. These straight fibres show sub-banding and are similar to those described in rat tail tendon by Randall (1953). It has been shown by Schmitt (1959) that collagen fibres with $640\text{ \AA}$ periodicity and with sub-banding have a specificity for calcification. The presence of such fibres induces the formation of apatite nuclei in solutions which would not otherwise crystallize. Fibres of different periodicity, formed by reconstituting in other ways, do not have this effect. It would seem, therefore, that this special variety of fibre which is found only in the deep zone plays a specific part in calcification.

The electron density of the interfibrillar matrix varies from one region to another. It usually has a dense appearance in the immediate vicinity of the cells, especially between pairs. Elsewhere, the matrix has a lower electron density. However, it is not possible to detect a region of electron dense interfibrillar matrix corresponding in dimensions to the capsule of light microscopists around individual cells or cell groups. There is a change in the diameter of the fibres in the matrix around the cells as described by Zelander (1958), but the position at which this change occurs does not accurately correspond with the position of the capsule as seen under the light microscope. A change in the chemical properties of the matrix may also contribute to the appearance of the capsule.

A clear interval filled with a homogeneous material between the matrix and the cell wall has been described (Zelander, 1958). Comparable intervals in the present material have been interpreted as a shrinkage artefact, the interval being filled by the embedding medium. These artefacts are particularly common when cartilage is embedded in methacrylate or when fixation in osmic acid is limited to half an hour. These shrinkage artefacts are to be distinguished from the regions found around many of the cells in the deep zone, described as "moats" by Cameron and Robinson (1958). These are filled by material which has a fine granular appearance but contains no distinct fibres. They are interpreted as fluid-filled intervals around partially degenerate cells.

Cells.—The general form and arrangement of the cells in articular cartilage are well known from light microscope studies (Barnett, Davies, and MacConaill, 1961). Branching processes projecting from all parts of the cell surface have been seen in epiphyseal and articular cartilage by electron microscopy (Scott and Pease, 1956; Sheldon and Robinson, 1958; Zelander, 1958). Similar processes are seen in all articular cells in the rabbit except those immediately beneath the articular surface.

The nuclei of the cells in the superficial and middle zones show all the characteristics of nuclei elsewhere, including a fine granular content with a more electron dense nucleolus, a double layered nuclear membrane, and nuclear pores. In places the membranes are less distinct around nuclei in the upper and lower middle zones than around superficial nuclei, but this may be accounted for by their undulating form. It should be stressed that no evidence has been found that the nuclei of cells at the surface are in any way degenerate, since no pyknosis, no breaking down of nuclear membrane, nor any displacement of the nucleus from the centre of the cell has been found. In the deep zone the nuclei commonly show one or more of these features, providing evidence that the cells in these regions are degenerating.

The well-developed cytomembrane system within the cytoplasm is characteristic of cells responsible for the discharge of protein secretions (Birbeck and Mercer, 1961). In cartilage cells the cytomembranes are of the granular type except in the vicinity of Golgi vacuoles where smooth cytomembranes are sometimes seen.

The cytomembrane system is more extensive and attached Palade granules are more distinct

in the cells of the lower middle zone than in those nearer the surface. This suggests a greater secretory activity in these middle zone cells, a suggestion that might be correlated with the formation of new fibres or with the formation of the electron-dense inter-fibrillary substance, or with both processes. The scanty cytomembrane systems of the superficial zone cells indicates that little protein secretion occurs here. In the deep zone, well-developed cytomembranes are clearly visible in the occasional healthy cell, but in the majority the break-up of organized membrane systems and the appearance of Palade granules in the cytoplasm may be regarded as evidence of degenerative change.

The density of the interval between cytomembrane pairs in the cells of the middle and deep zones is usually high, even in unstained material. It is in contrast to that seen in bone cells in the sub-articular region, where the material between pairs of membranes is commonly of the same electron density as the remainder of the cytoplasm. In cartilage cells the interval is enlarged in the middle and deep zones and the contained substance is then homogeneous and non-granular; this may indicate storage or transport of a secretion. Its electron density is similar to that of much of the inter-fibrillary matrix around the cells.

Fat globules are not uncommon within the cytoplasm of cartilage cells of the lower middle zone, but are rarely seen elsewhere in the articular cartilage. Although the present work does not allow a dogmatic statement, it is likely that the fat is enclosed between a pair of cytomembranes. Because of their occurrence predominantly in those cells thought to be highly active and healthy, it seems unlikely that the fat is a sign of degeneration, and more probable that it is fat which will be used in metabolic activity.

Zelander (1958) reported a very small number of mitochondria in the chondrocytes of guinea-pigs, mice, and rats, and he associated the apparent paucity of mitochondria in his material with a low metabolic activity. In the chondrocytes of the rabbit, typical mitochondria are present in considerable numbers within the cytoplasm of the superficial and middle-zone cells, a difference that can perhaps be accounted for in terms of species variation. In cells of the deep zone, numerous electron dense bodies of comparable dimensions to the mitochondria in more superficial cells almost certainly denote mitochondria undergoing a degenerative change.

It cannot be stated that rabbit chondrocytes have few mitochondria or that they are poorly equipped for glycolytic processes. Indeed, biochemical studies

of articular cartilage indicate that *per cell* chondrocytes exhibit well-marked glycolysis (Bywaters, 1937).

The small vesicles concentrated close to the cell wall and occasionally opening upon it are present only in the cells of the superficial zone and upper middle zone. They resemble pinocytotic vesicles (Bennett, 1956) in dimensions and appearance. Such vesicles are found also in mesothelial cells lining the peritoneal cavity, which absorb fluid and particulate matter from the cavity (Odor, 1956). It is thought that the superficial third of articular cartilage is nourished exclusively from the synovial fluid percolating within its substance (Ekholm, 1955), and it is tempting to associate these small vesicles with the uptake of fluid that comes into contact with the superficial cells.

The larger vacuoles are of the Golgi type, although typical Golgi membranes are uncommon. In some cells these vacuoles are quite small and confined to one side of the nucleus. However, in the majority of the cells of the lower middle and deep zones, one or more vacuoles is considerably enlarged and is near to the surface of the cell. The Golgi apparatus is a prominent feature in one type of degenerating chondrocyte. In many of the deepest cells the cytoplasm contains large numbers of vacuoles, up to 1μ in diameter, some of which have apparently burst through to the outside of the cell and discharge their contents. Ultimately little of the cytoplasm is left; the remnants of the cell remain as an irregular, spidery structure. Thus the cavity within the matrix, formerly filled by a healthy cartilage cell, now contains a shrivelled cell and a large quantity of fluid. The fine fibres previously enveloping the healthy cell remain bordering the cavity, and are surrounded by the larger fibres of the general matrix.

Scott and Pease (1956) have described a different form of degeneration in the calcifying epiphysal plate of the cat. In this process the cell enlarges, the cytoplasm appears watery, its contained organelles are widely dispersed, and "finally the plasma membrane seemingly ruptures, but for a while at least traces of endoplasmic reticulum, mitochondria, and granules persist *in situ*". This corresponds with the first type of degeneration described in this article. Both types of degeneration may occur simultaneously in different parts of the same cell.

Zelander (1958) has described "structureless domains" in the cytoplasm of chondrocytes and suggests that they represent glycogen. No such areas have been seen in the present study, nor has this study given any indication of the location of the glycogen in the cells. It should be noted that

methods of preparation used in the present study may be unsuitable for the demonstration of glycogen.

Cell Replacement.—It is often thought that articular cartilage is similar in its behaviour to skin, the new cells and matrix of the deep layers passing slowly to the surface where they are worn away. No evidence in support of this belief has come from the present study. On the contrary, although the surface cells appear to be relatively inactive so far as fibre and protein formation are concerned, they show no evidence of degeneration and none has been observed at the surface without a covering of matrix. Moreover, in all the specimens examined, no depression has been seen on the articular surface which might have accommodated a surface cell. It may be that in young adult animals the smooth articular surface, lubricated by synovial fluid, permits movement of the joint without any wear and tear of the surface. No evidence of cell division—whether by mitosis or amitosis—has been found. However, the large number of degenerate cells in the deep zone of the young animal indicates that during the growth period, unless new cells are formed, there must be a steady decline either in the cellularity or in the thickness of the articular cartilage. Both processes have been shown to occur in cartilage by light microscopy and other studies; it is possible, therefore, that cells which degenerate and die are not replaced.

Summary

The appearance under the electron microscope of articular cartilage from young adult rabbits is described. In the superficial zone the cells are flattened, but show no evidence of degeneration; they possess mitochondria, cytomembrane systems, and many small cytoplasmic vesicles of the pinocytotic type. These surface cells are surrounded by hyaline matrix containing many fine fibres; the articular surface is remarkably smooth. At a deeper level the cells are more rounded, exhibiting well-developed cytomembranes, mitochondria, Golgi vacuoles, occasional fat droplets, and branching processes closely associated with fine fibres of the surface type. These fibres merge peripherally with larger fibres, helicoidally arranged, that show the characters of adult collagen. At a deeper level, degenerating chondrocytes are seen. Many show breaking up of the cytomembranes, together with enlargement of the Golgi vacuoles, which release their fine granular contents at the cell surface. Some cells show a different type of degeneration, the wall breaking down and the organelles being dispersed without the production of large vacuoles. In the

deepest part of the cartilage, calcification is seen, commonly as rosettes of apatite crystals which are randomly scattered among large collagen fibres with complex striations.

There is nothing to suggest that surface cells are degenerate or that they are worn away by friction. No evidence of cell multiplication has been observed.

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Microscopie électronique du cartilage articulaire de lapin adulte jeune

RÉSUMÉ

On décrit l'aspect du cartilage articulaire de jeune lapin adulte sous le microscope électronique. Dans la zone superficielle les cellules sont aplaties, mais ne montrent pas de signes de dégénérescence; elles ont des mitochondries, des systèmes cytomembraneux et beaucoup de petites vésicules du type pinocytotique. Ces cellules superficielles sont entourées d'une matrice hyaline contenant beaucoup de fines fibres; la surface articulaire est remarquablement lisse. A un niveau plus profond les cellules sont plus arrondies, montrant des cytomembranes bien développées, des mitochondries, des vacuoles de Golgi, quelquefois des gouttelettes de graisse et des procès ramifiants associés étroitement aux fibres fines du type superficiel. Ces fibres s'unissent vers la périphérie à des fibres plus grandes, disposées hélicoïdalement et portant le caractère du collagène adulte. Plus en profondeur, on voit des chondrocytes en dégénérescence. Beaucoup d'entre eux montrent des signes de dissolution des cytomembranes et l'augmentation des vacuoles de Golgi, qui libèrent leur fin contenu granulaire à la surface cellulaire. Certaines cellules accusent un type différent de dégénérescence, avec la rupture de la paroi et la dispersion des organelles sans production de grandes vacuoles. Dans la partie la plus profonde du cartilage la calcification prend habituellement une forme de rosettes de cristaux d'apatite, dispersés au hasard parmi de grandes fibres de collagène avec des stries complexes.

Rien n'indique que les cellules superficielles soient dégénérées ou effacées par la friction. On n'a pas trouvé de signes de multiplication cellulaire.

Microscopia electrónica del cartilago articular de conejo adulto joven

SUMARIO

Se describe el aspecto del cartilago articular de conejo adulto joven bajo el microscopio electrónico. En la zona superficial las células se ven aplastadas pero sin evidencia de degeneración; tienen mitocondrias, sistemas citomembranosos y muchas pequeñas vesículas del tipo pinocitótico. Estas células superficiales se ven rodeadas de una matriz hialina conteniendo muchas fibras finas; la superficie articular es destacadamente lisa. A un nivel más profundo las células presentan una forma más redonda, con citomembranas bien desarrolladas, mitocondrias, vacuolas de Golgi, a veces gotitas de grasa y procesos de ramificación estrechamente asociados con fibras finas del tipo superficial. Estas fibras se unen

hacia la periferia con fibras mayores, dispuestas helicoidalmente que presentan el caracter del colágeno adulto. Más profundamente se observan condrocitos en degeneración. Muchos de estos acusan signos de disolución de las citomembranas y de aumento de las vacuolas de Golgi, que liberan su contenido granular fino a la superficie celular. Ciertas células muestran un tipo diferente de degeneración, con rotura de la pared y

dispersión de los organitos sin producción de grandes vacuolas. En la más profunda parte del cartilago la calcificación toma generalmente una forma de roseta de cristales de apatita, dispersos casualmente entre las grandes fibras de colágeno con estrías complejas.

No hay indicación de que las células superficiales sean degeneradas o gastadas por la fricción. No se encontraron indicios de multiplicación celular.