EXTRA-CELLULAR LIPID IN THE MATRIX OF HUMAN ARTICULAR CARTILAGE

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The presence of fat and other lipids within the cytoplasm of cartilage cells has often been observed. Collins, Ghadially, and Meachim (1965) in a previous paper have described this phenomenon in some detail. They find that intra-cellular lipid is an almost constant feature of the chondrocytes of adult hyaline cartilage in various anatomical situations in man and rodents, that it is more prominent at ages later than infancy and the early growth period, but that it is not necessarily a manifestation of any form of cellular degeneration, since electron microscopy reveals lipid accumulation in cells whose organelles appear perfectly healthy. In the course of that study, the attention of the authors was drawn to the presence of lipid outside the cells, lying in a granular form within the matrix in some areas of certain cartilages. The occurrence of extra-cellular lipid in cartilage is much less well known than that of the intra-cellular lipids, and only a few previous observations have been recorded. Montagna (1949) speaks of "clouds of very fine sudanophilic particles visible only at high magnification" and of "a faint sudanophilic band in the new matrix just under the perichondrium" in adult tracheal cartilage. Barnett, Cochrane, and Palfrey (1963) observed small clusters of "myelin bodies" together with electron-dense granules interspersed among the fibres of the middle zone of ageing rabbit cartilage; it is possible that this may be a different phenomenon from that noted by Montagna.

Extra-cellular lipid has been observed by the present authors in a small number of costal and bronchial cartilages in man and on occasion in rabbit articular cartilage, but these studies have been concentrated upon human articular cartilage where extra-cellular lipid can quite often be discovered in the superficial zones and where it seemed that it might possibly have pathological significance.

Methods

Articular cartilage was collected at necropsy from the head of the humerus in young subjects and from the central area of the head of the humerus, from near the articular edge of a femoral condyle, and from the patellar groove of the femur in adults. These specimens were prepared for examination under a binocular stereomicroscope and, as frozen sections, under the light microscope. In addition, fresh cartilage was obtained from the femoral condyle in two patients aged 58 and 82 years in the operating theatre prior to mid-thigh amputation of the lower limb; this material was prepared for electron microscopy as well as for sectioning on the freezing microtome.

The ages of the persons whose cartilage was examined ranged from birth to 82 years. In the juvenile group there were four premature babies dying shortly after birth, two full-term babies dying at the age of 4 and 5 months, two girls aged 8 years, and one youth aged 16 years. The adult patients were aged 28 to 82 years. Many of the samples were free from any osteo-arthritic lesion of cartilage, while others showed superficial or deep fibrillation in the area taken for examination.

Stereomicroscopy.—Samples of adult cartilage, either fixed in formol-calcium or unfixed, were prepared as follows. A block of cartilage was divided into thin tangential slices by a series of cuts made at increasing depths parallel to the articular surface. By this means a superficial slice and two or more deeper slices were obtained from the same area of non-fibrillated samples. Deeply fibrillated samples were studied in a single thin tangential slice taken at their roughened surface. The slices were rinsed briefly in 70 per cent. isopropyl alcohol, stained for 10 minutes in a filtered solution of oil red 0, rinsed again in 70 per cent. isopropyl alcohol, and then placed in formol saline until they were examined under a low-power binocular microscope (×12·5 to ×50) with incident light.

Light Microscopy.—Tissue blocks were generally prepared by cutting out a cartilage sample from above the level of its calcified zone. By this method the need for preliminary decalcification is avoided. The blocks were
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fixed in formol-calcium. Frozen sections were then cut, stained in a filtered solution of oil red O for 15 minutes, counterstained with Harris haematoxylin, and mounted in glycerine jelly. The sections were examined for the presence of oil red O-positive material in the intercellular matrix.

Electron Microscopy.—A narrow superficial strip of articular cartilage was excised at operation from near the edge of the femoral condyle before amputation, and small pieces were treated rapidly with osmic acid, embedded in Araldite, sectioned on the ultra-microtome, mounted on copper grids, stained with lead citrate or uranyl acetate, and examined under the A.E.I. EM6, using an accelerating voltage of 50 or 75 Kv. A parallel block of cartilage was prepared for light microscopy by frozen section.

Results

Macroscopy and Low-power Stereomicroscopy

To the naked eye the articular cartilage at birth is semi-translucent and colourless. During childhood and adolescence it becomes opaque. Adult articular cartilage is either white or slightly yellow.

When tangential slices of adult articular cartilage were prepared and stained as described above, it was found in some of the non-fibrillated cartilages, and particularly in those from the head of the humerus, that the superficial layer stained sufficiently intensely with oil red O for it to acquire a definite pink or red colour visible to the naked eye (Fig. 1).

Light Microscopy of Frozen Sections

Juvenile.—All the articular cartilages examined in this group were taken from the head of the humerus and none showed osteo-arthritic fibrillation. No extra-cellular lipid was demonstrated in any of the premature babies or infants, and none was seen in a 16-year-old boy. However, two specimens from 8-year-old girls both showed a weakly-positive reaction in the superficial layer of the cartilage matrix which was seen under high magnification to be due to the presence of a fine cloud or peppering of lipid particles near some of the chondrocytes in the first and second rows of cells beneath the articular surface.

Adult.—Extra-cellular lipid was much more frequently noted in the articular cartilage from adult subjects (28 to 82 years of age). It was found in the superficial zone of the articular cartilage in sixteen out of nineteen non-fibrillated cartilages and in three out of four specimens in which early osteo-arthritic fibrillation was present. Only in a single specimen was stainable lipid discovered in the deeper zones of the cartilage where the chondrocytes were arranged in more or less radial columns. In six sections in which the superficial cartilage layers were substantially eroded as the result of osteo-arthritis, no extra-cellular lipid was demonstrable in the cartilage which remained.

Under the high-power objective of the light microscope, the oil red O-positive material is seen in the form of multiple, mainly fine, red granules. The intensity of the colour reaction as seen under low magnification seems to be related to the concentration of the particles, being deep red in areas where they appear more tightly packed and paler in areas where they appear more widely spread.

Fig. 1.—Two tangential slices of human articular cartilage from the head of humerus of a man aged 74 years. The slices, cut by hand, are approximately 0.5 mm. thick. The dark one, on the left, is the superficial slice and contains diffuse lipid which has stained positively with oil red O; the right-hand specimen is the next slice, containing the middle zone of the articular cartilage, and shows no stainable lipid. × 8.

Superficial slices showing this phenomenon were then examined further under the stereomicroscope (up to × 50). Stereomicroscopy of the surface and of the cut edges of these slices shows that the red-stained material lies just beneath the articular surface and extends into the cartilage for only a short distance; as seen through the surface of the slice, the staining reaction is not uniform but tends to show areas coloured to varying intensity. Stereomicroscopy fails to demonstrate any distinctly positive staining in the deeper slices of non-fibrillated cartilage. It also fails to show any lipid staining within cartilage slices from samples in which the superficial zone has been substantially eroded away by osteo-arthritic change, although these specimens do often show stained globules of synovial fat entrapped between the fibrillated processes of the cartilage.

The possibility that lipid accumulation is responsible for the yellowish tinge of some adult cartilages was investigated, but it was found that abundant lipid was present both in some of the white and also in some of the pale yellow cartilages.
In the present investigation the distribution of lipid material in the matrix could usually be classified into one of two main patterns:

(a) Irregular collections of lipid particles, generally peri-cellular, in the matrix at the level of the first or second row of chondrocytes, extending sometimes to the third or fourth row of cells below the articular surface;

(b) A more or less continuous and intensely-stained band of lipid particles in the matrix just below the articular surface (Fig. 2).

The first pattern of distribution of lipid was seen in cartilage form near the edges of the femoral condyles and in a few specimens from the central area of the head of the humerus. It was found both in intact cartilage and in cartilages which showed early fibrillation. Sometimes only a very small amount of intercellular lipid was seen in the area sectioned.

The second, intense band-like pattern of lipid distribution was seen almost exclusively in cartilages which showed no osteo-arthritis erosion. In fact, this distribution was found only in the articular cartilage of the humerus. The upper border of the band is fairly sharply defined and is separated from the articular surface by a narrow zone of clear matrix. The lower border of the lipid band is less sharp, and the matrix immediately beneath often shows peri-cellular collections of lipid particles which are more dense and extend further on the side of the tangentially-disposed cells which lie towards the cartilage surface (Fig. 2). It is exceptional to find any lipid lying in the matrix around the deeper, radially-disposed chondrocytes.

**Electron Microscopy**

Electron micrographs of both samples of cartilage examined show granular material distributed in the matrix in an irregular manner near some of the chondrocytes (Fig. 3, opposite, and Fig. 4, p. 140). When it lies near a cell, the material tends to be concentrated on one side of the cell (Fig. 3, opposite), as is also seen by light microscopy.

The granular particles, most if not all of which are believed to be lipid in nature, usually lie around cells which appear to have been alive and healthy, with a well-defined cell wall and intact nucleus (Figs 3 and 4), but sometimes, in contrast, similar material is encountered in clefts within the matrix in which also lie recognizable remnants of dead and disintegrating chondrocytes (Fig. 5, overleaf, p. 141). At high magnification, the granular matter in the matrix is seen to comprise rounded bodies varying in size and appearance. Some of these bodies are uniformly electron dense, while others contain an eccentric or central region which is less dense, or even clear, in appearance (Fig. 6, overleaf, p. 142). The more prominent bodies are seen against a background of smaller, less electron-dense particles of indistinct outline. Occasional particles which seem to show membranous formations are also identified (Figs 3, 4, 5, and 6). There is a resemblance between the particles seen in the matrix of the two human cartilages and the lipid particles found in adult rabbit articular cartilage (Fig. 7, overleaf, p. 143) which had earlier been reported by Barnett, Cochrane, and Palfrey (1963). In the human tissue, however, bodies showing clearly-defined membranes are much less apparent than in the rabbit cartilage.

![Fig. 2.—Frozen section of articular cartilage of humerus (from a man aged 52) stained by oil red O, showing a dark continuous band of particulate lipid just below the surface and a concentration of lipid adjacent to the cells in the first and second superficial rows of chondrocytes. × 310.](http://ard.bmj.com/)
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Fig. 3.—Two chondrocytes, each containing an intra-cytoplasmic lipid drop, and a dense cloud of fine lipid particles in the extra-cellular matrix. Adult human femoral cartilage. Electron micrograph. × 7,500.
Fig. 4.—Electron-dense lipid particles in matrix adjacent to a chondrocyte of adult human femoral articular cartilage. Note the cytoplasmic processes of the cartilage cell. There is an intra-cellular lipid droplet near the nucleus. × 18,400.
Fig. 5.—A cleft in the cartilage matrix which is the site of a disintegrated chondrocyte, showing lipid droplets and whorled phospho-lipid membranes as well as other cellular remnants. Adult human femoral cartilage. Electron micrograph. ×18,000.
Fig. 6.—Electron-dense granular particles in extra-cellular matrix, showing their varying size and density and the probable presence of membrane formations in some. Adult human femoral cartilage. × 48,000.
Fig. 7.—Extra-cellular lipid particles in rabbit femoral articular cartilage. For comparison with Fig. 6. × 48,000.
It is impossible to be sure that some of the electron-dense granules in the cartilage matrix do not represent deposits of calcium salts or of other mineral matter, as depicted by Silberberg, Silberberg, and Feir (1964) in mouse articular cartilage, but the morphology of most of the material is consistent with its being lipid, and its peri-cellular distribution corresponds with that of the extra-cellular lipid identified in stained frozen sections by light microscopy.

Discussion

These investigations prove that the extra-cellular matrix of hyaline cartilage may contain lipids in addition to those which can more generally be found within the cytoplasm of the cartilage cells. The lipid observed is detected by its reception of the fat-soluble dye oil red O. No attempt has been made to identify it specifically as neutral fat, fatty acid, or phospholipid, though it is probable that those particles which under the electron microscope show some membranous formations are composed partly of phospholipids.

Extra-cellular lipid is distributed mostly in the matrix of the superficial layers of articular cartilage, where it tends to be more concentrated around the tangentially-orientated chondrocytes of the upper one or two cell layers.

The origin of the lipid in the matrix is a matter for discussion. It may arrive in the superficial zone of articular cartilage by absorption from the synovial fluid; its incorporation in the matrix may be analogous to the incorporation in cartilage of urates in gout and of pigment in ochronosis.

The second possibility is that the lipid is formed in the chondrocytes and passed out into the matrix. This appears to be the more attractive hypothesis. The lipid often shows a juxta-cellular distribution, and membranous structures, presumably lipoprotein in nature, are sometimes to be identified by electron microscopy among the granular particles.

A striking characteristic of the chondrocyte revealed by the electron microscope is its irregular spiky outline which is due to the presence of cytoplasmic processes of varying length and shape.

These processes seem to be more in the nature of pseudopodia, or ephemeral transitory temporary protrusions, than of pedicles or foot processes attached to a basement membrane or some other structure. Occasionally electron microscopy reveals long processes, or pseudopodia, extending from a chondrocyte well out into the matrix (Fig. 8, opposite).

Such processes may contain lipid droplets and lipoprotein membranes (see Fig. 12, Collins and others, 1965). Should they become detached from their parent cell and disintegrate, free extra-cellular lipid may be released into the cartilage matrix.

While this theory may account for most of the extra-cellular lipid, some may be derived from the disintegration of whole cartilage cells in the manner depicted in Fig. 5. But lipid aggregates derived in this way are seen to lie in electron-lucent clefts in the matrix in association with cellular remnants.

If the theory that matrix lipid is derived from the cells is correct, then its concentration in the superficial zone of articular cartilage must be explained by assuming that the chondrocytes of this zone rather than those of the deeper zones are the more active in throwing out cytoplasmic processes and depositing lipid material into the matrix.

There is no evidence that the accumulation of lipid-containing material in cartilage matrix is a consequence of osteo-arthritis fibrillation. The material has frequently been seen in non-fibrillated samples, and in fact seems to be absent in samples in which the superficial layer has been substantially eroded by osteo-arthritis change. Moreover, sudanophilic material also occurs in the matrix of non-articular cartilage. In cartilage matrix at the upper end of the humerus, lipid accumulation often commences at an early age, yet this site is frequently free from fibrillation even in the elderly. It would thus seem that lipid accumulation in the matrix does not in itself predispose to osteo-arthritis fibrillation.

Conclusions

Human articular cartilage and some other cartilages contain, in addition to intra-cellular lipid, lipid which is extra-cellular and lying in particulate form in the matrix.

In human cartilage this is most prominent just below the surface, and the lipid particles are often concentrated near the chondrocytes of the superficial cell layers. The formation is seen best in intact cartilages, such as those covering the head of the humerus in adult patients.

The nature of this lipid has not been identified further than by its acceptance of fat-soluble oil red O and by its electron microscopic appearance. Occasional particles show membranous structure and presumably consist of lipoprotein of cellular origin.

Though some free lipid undoubtedly originates from disintegrating cartilage cells, most of the extra-cellular lipid lies in the neighbourhood of healthy intact cells, and the theory is advanced that it derives from their extruded cytoplasmic processes.
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Fig. 8.—Chondrocyte of human articular cartilage with a long cytoplasmic process extending into the surrounding matrix which is studded with lipid.  × 9,200.

No evidence was found to suggest an association between lipid loading of the matrix and the development of the cartilaginous lesion of osteo-arthritis.

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Lipide extra-cellulaire dans la matrice du cartilage articulaire humain

Résumé
Le cartilage articulaire humain et certains autres cartilages contiennent, en plus de lipide intra-cellulaire, un lipide qui est extra-cellulaire et se trouve, sous la forme de particules, dans la matrice.
Dans le cartilage humain cela est très accentué immédiatement sous la surface où les particules lipidées sont souvent concentrées près des chondrocytes de la couche superficielle des cellules. Cette formation s'observe le mieux dans les cartilages intacts, comme ceux qui couvrent la tête de l’humérus chez des patients adultes.
La nature de ce lipide n’a été identifiée que par son acceptation de l’huile rouge O liposoluble et par son apparence au microscope électronique. Quelques particules révèlent une structure membranaire et consistent probablement en lipoprotéine d’origine cellulaire.
Bien qu’une partie du lipide libre vienne de la désintégration des cellules cartilagineuses, la plupart de ce lipide extra-cellulaire est située au voisinage des cellules saines et intactes et on propose l’hypothèse qu’il dérive de leurs processus cytoplasmiques expulsés.
On n’a rien trouvé qui puisse indiquer une association entre la charge lipide de la matrice et le développement de la lésion cartilagineuse de l’ostéo-arthrite.

**Lípido extra-celular en la matriz del cartílago articular humano**

**SUMARIO**

El cartílago articular humano y ciertos otros cartílagos contienen, además del lípido intra-celular, un lípido que es extra-celular y se encuentra, en forma de partículas, en la matriz.

En el cartílago humano esto se destaca muy cerca de la superficie, donde las partículas lípidas están a menudo concentradas cerca de los condrocitos de la capa superficial de células. Esta formación se observa lo mejor en los cartílagos intactos, como los que cubren la cabeza humeral en pacientes adultos.

La naturaleza de este lípido fue identificada sólo por su aceptación del aceite rojo liposoluble y por su apariencia al microscopio electrónico. Algunas partículas acusan una estructura membranosa y consisten probablemente de lipoproteína de origen celular.

Aunque una parte del lípido libre proviene de la desintegración de células cartilaginosas, la mayoría de este lípido extra-celular está situada cerca de células sanas e intactas; se propone la teoría que éste origina de sus procesos citoplasmicos expulsados.

No se encontró indicación de una asociación entre la carga lipida de la matriz y el desarrollo de la lesión cartilaginosa de la osteoartritis.
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