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Absorption into the rabbit articular cartilage

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INTRODUCTION

Recent work has shown that the superficial cells in articular cartilage from immature and young adult rabbits are healthy: there is little or no evidence that with use of the joint there is loss of substance from the surface of the cartilage (Davies, Barnett, Cochrane & Palfrey, 1962). This has been confirmed in the mouse (Silberberg, Silberberg & Feir, 1964). There must therefore be a pathway by which nutrient substances reach these cells and by which their products of metabolism can be removed. The effect of friction in synovial joints has been studied extensively in recent years (Barnett & Cobbold, 1962): these studies suggest that, during movement of the joint, synovial fluid is released from the cartilage into the joint cavity and that this is reabsorbed when movement is completed. It may be that this movement of synovial fluid in and out of the matrix of the cartilage plays at least a part in the mechanism by which the superficial cells are supplied with metabolites.

Stockwell & Barnett (1964) have studied the absorption of silver proteinate into rabbit articular cartilage immediately after removal from the living animal and were able to demonstrate that silver had penetrated 30–170 μ into the cartilage. It was thought desirable to repeat this work as an *in vivo* experiment and to extend it as an electron microscopic study. 'Thorotrast' was chosen as the substance for injection, as it is readily identified under the electron microscope.

MATERIALS AND METHODS

Rabbits of either 3 or 8 months of age were used in this study: the former were immature animals while the latter were young adults. In each rabbit the left knee was injected under general anaesthetic with 0·5 ml. of thorotrast, which had been diluted with an equal volume of physiological saline. The animals were allowed to recover from the anaesthetic and to take active exercise in the laboratory. Specimens of cartilage were taken from both femoral condyles, 1 hr., 2 hr., 24 hr., and 48 hr., after injection, those from the right side acting as controls. The animals were anaesthetized, 0·5 ml. of fixative was injected into both knees and after 1 min. the joint cavity was opened. A shaving was removed from the surface of the cartilage and cut up in fixative so that no dimension of the specimen was greater than 0·5 mm. The fixative was osmium tetroxide buffered to a pH of 7·3 (Palade, 1952). The material remained in fixative for 2hr., was dehydrated in alcohol, passed through propylene oxide (Luft, 1961) and embedded in Araldite (Glauert, Rogers & Glauert, 1956; Richardson, Jarett & Finke, 1960). Sections 1 μ thick were cut from the Araldite block and stained with Azur II (Richardson *et al.* 1960): thin sections were stained for 1 hr. with a saturated solution of uranyl acetate in absolute methyl alcohol.

RESULTS

The changes seen in the cartilage are similar in the material from immature and from young adult rabbits, and are described together. Specimens taken from the un-injected knee joints show appearances similar to those which have already been described (Davies *et al.* 1962; Barnett, Cochrane & Palfrey, 1963).

Within two hours of injection

The specimens taken at 1 and at 2 hr. after injection showed similar appearances. With the electron microscope it can be seen that only a small amount of thorotrast is absorbed into the cartilage and the particles are either on the surface or not more than 0·2 μ deep to it (Fig. 1). Under the light microscope this thorotrast is too small to be seen as the particles are less than 100 Å. in diameter; a small number of red cells are scattered over the surface of the cartilage together with occasional white cells (Fig. 2). The cartilage cells in the superficial 20–30 μ have in many cases shrunk away from the surrounding matrix, though no subcellular detail is visible. In the deeper middle zone of the cartilage, occasional cells exhibit small vesicles, but the general appearance is that of normal articular cartilage.

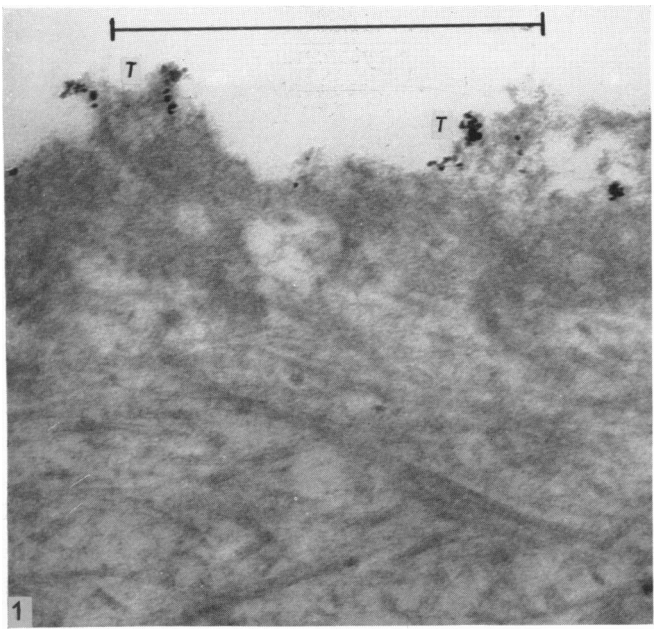
Under the electron microscope the cells of the superficial zone are found to vary considerably in appearance. Some are normal, with rounded nuclei, well-formed mitochondria, scattered granular cytomembranes and a complete cell membrane exhibiting occasional short processes. Others show a cytoplasm almost filled with fine fibrillae (Fig. 3), each about 80 Å. in diameter and arranged in bundles parallel to one another. In such cells the nucleus is normal but the matrix of the mitochondria is filled with a granular electron-dense material amongst which the cristal structure can be identified. In some cells irregular vesiculation can be seen in the cytoplasm: in other parts of the cytoplasm dense bodies are found in the centre of a mass of amorphous material: the cytomembranes cannot be identified in these cells. Other cells in the superficial zone show complete degeneration (Fig. 4). Neither nucleus nor cell membrane can be recognized: the cell space contains an assorted mass of structures from 300 to 2000 Å in diameter. In some of these structures the typical cytomembrane triplet can be identified, though no true myelin figures are seen: in some the centre of the structure is dense but in others it is translucent. These bodies are suspended in an irregular meshwork of electron dense material in which discrete filaments cannot be identified.

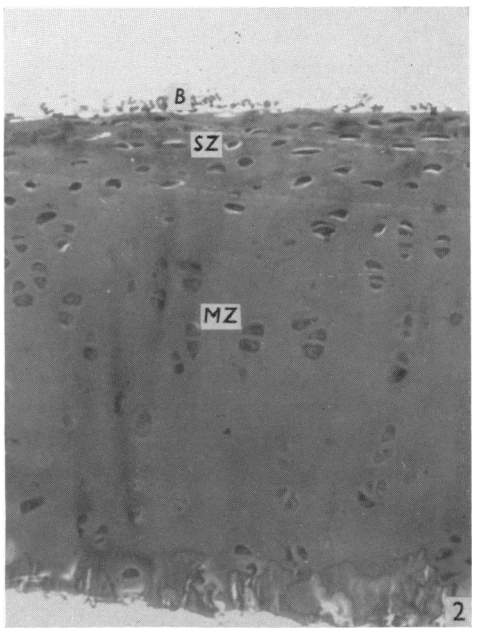
All sections are embedded in Araldite: light micrographs are stained with Azur II, electron micrographs with uranyl acetate. The scale marker in the electron micrographs represents one micron.

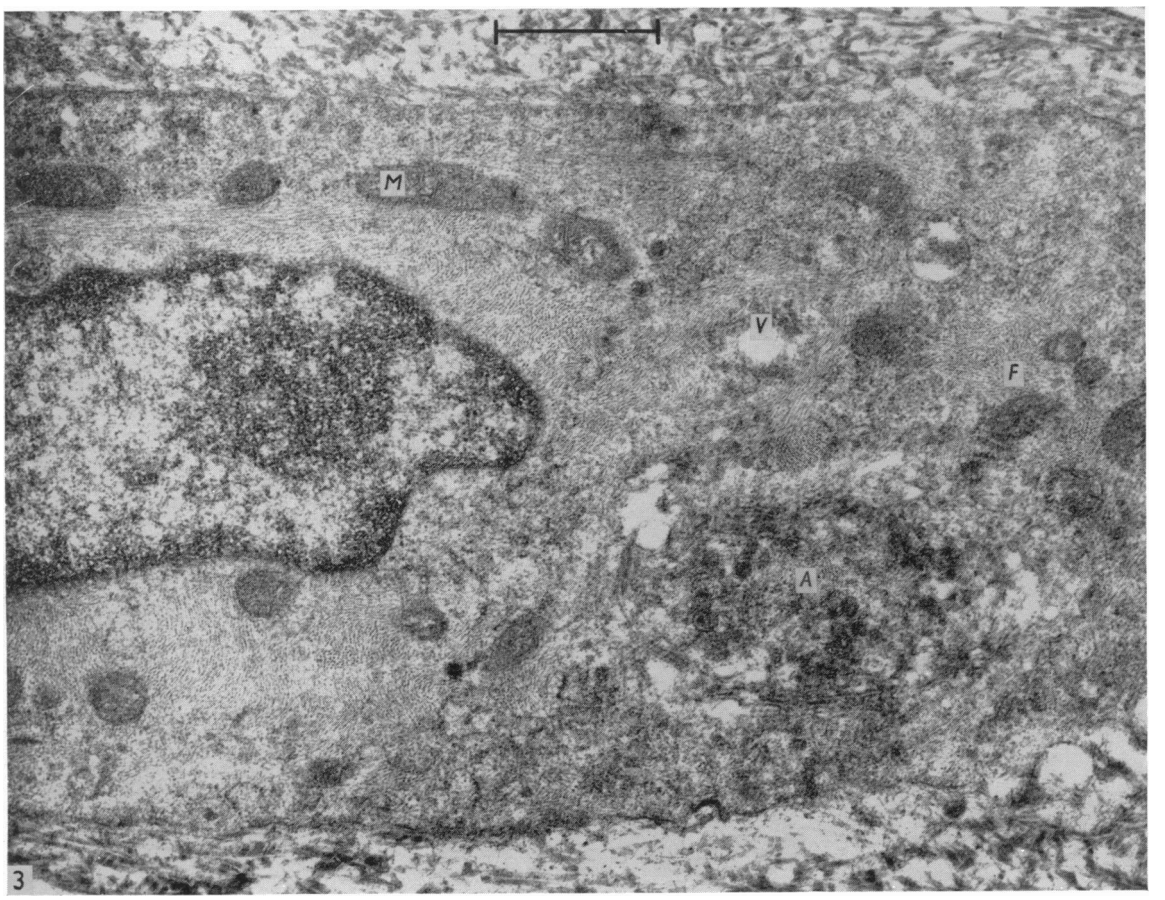
Fig. 1. The surface of the articular cartilage 1 hr. after the thorotrast injection. Occasional scattered particles (*T*) are seen within 0·2 μ of the surface. Electron micrograph. × 60,000.

Fig. 2. A light micrograph of the articular cartilage 2 hr. after the thorotrast injection. Scattered blood cells (B) are seen at the surface: the chondrocytes in the superficial part of the cartilage (*SZ*) have shrunk away from the surrounding matrix, whereas those in the middle zone (*MZ*) are normal in appearance. × 160.

Fig. 3. A chondrocyte from the superficial zone 1 hr. after injection of thorotrast. The cytoplasm is filled with fibrillar material (*F*) and the mitochondria (*M*) have an electron dense matrix; on the left irregular vesiculation (*V*), a few dense bodies and amorphous material (*A*) can be identified. Electron micrograph. × 20,000.







Figs. 1–3. Legends on facing page. Key to lettering on p. 375.

The cells of the middle zone are normal in appearance. The nucleus is well defined though slightly irregular. Occasional vesicles up to 0·6 μ in diameter are present and in some a Golgi zone may be recognized. The granular cytomembranes are well marked in a small proportion of these cells. Mitochondria are scanty and slightly swollen. Pinocytotic vesicles are numerous in many of these cells—a common finding in middle zone cells of normal articular cartilage.

The matrix around the cells in both superficial and middle zones shows collagen fibres arranged as in normal articular cartilage. The standard collagen banding of 640 Å has been demonstrated.

Twenty-four hours after injection

After 24 hr. a more extensive penetration of the surface of the cartilage by the thorotrast particles can be seen with the electron microscope. This is confined to the most superficial layer of the cartilage, to a maximum depth of about 3 μ. The thorotrast seems to occupy a high proportion of the cartilage but measurement shows that between 20 % and 30 % of the substance along randomly chosen lines consists of thorotrast particles: this corresponds to not more than 3 % by volume, but to about 20 % by weight. The identity of these dense particles as thorium dioxide has been confirmed by electron diffraction. In some sections (Fig. 5) the most superficial part of the cartilage is splitting away but the new 'surface' still contains a small proportion of thorotrast particles, though the majority are in that part of the matrix which is being abraded.

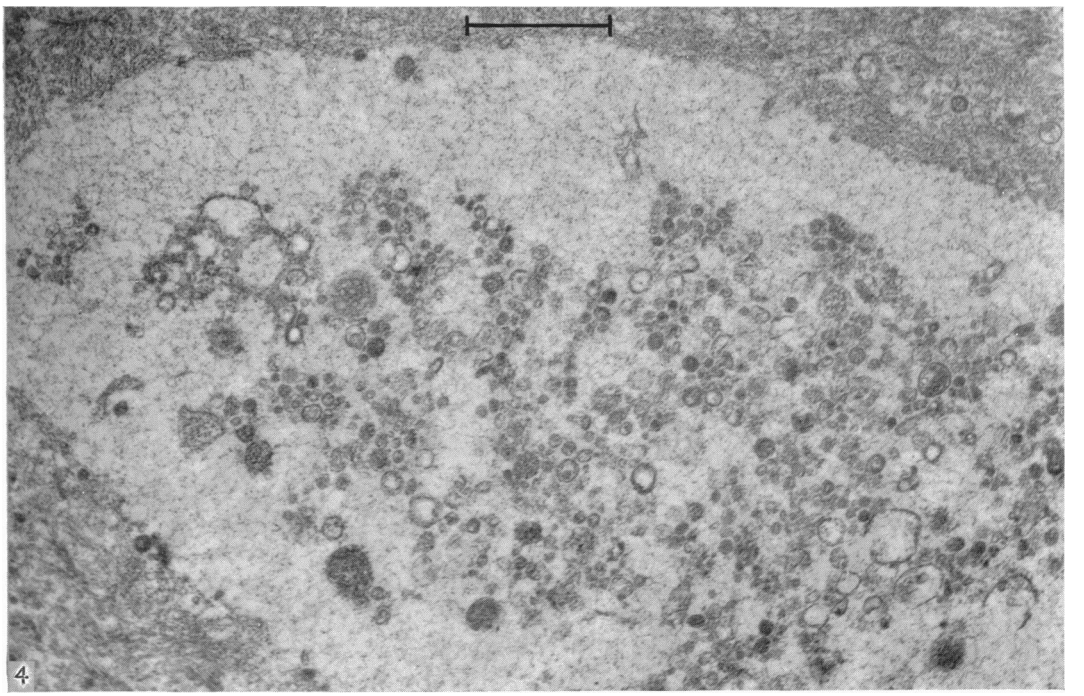
The irregularity of the surface can be seen with the light microscope, and a few red cells can be identified lying free on the articular surface. In the superficial zone of the cartilage the cells have shrunk away from the surrounding matrix. In the middle zone the cells appear normal and again occasional small vesicles can be seen.

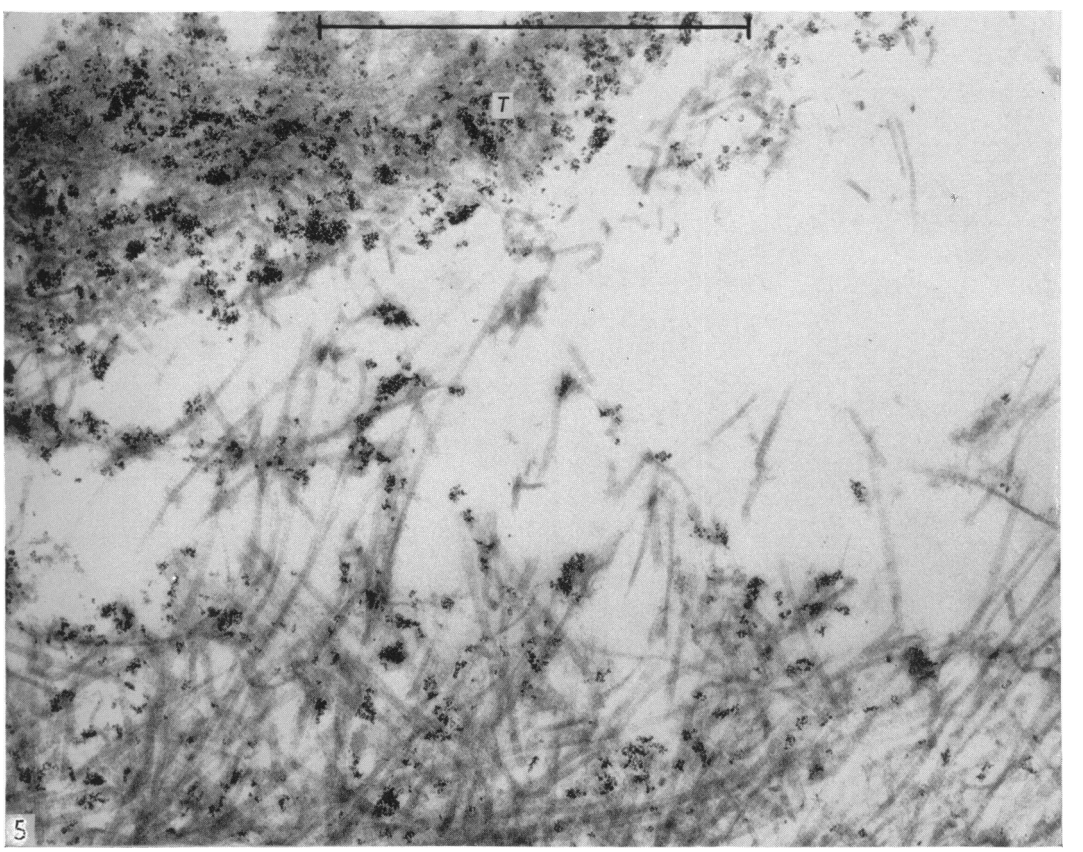
Under the electron microscope both red and white blood cells have been seen near the articular surface. The polymorphonuclear leucocytes show absorption of thorotrast into some of their vacuoles although the cells are otherwise normal; some of the thorotrast has been absorbed into vesicles which also contain other electron dense material; in such cells the thorotrast forms a cuff around the other material.

The cells in the superficial part of the cartilage again vary widely in appearance. Some are normal, except that their surface processes are unusually scanty. Others retain a very similar overall shape (Fig. 6), but their mitochondria are denser and their internal structure is not clearly visible. In the cytoplasm of many cells a normal Golgi apparatus is well marked—an unusual finding in the cells of the superficial zone: there is also an early stage of the fibrillar change in the cytoplasm. In others degeneration is complete (Fig. 7), and fully formed myelin bodies are seen. The 'cell space' also contains occasional fibres interspersed with the remains of the organelles: in some of these typical collagen banding can be identified.

Fig. 4. The debris formed from a chondrocyte in the superficial zone seen in an electron micrograph of a specimen taken 1 hr. after the thorotrast injection. × 18,400.

Fig. 5. The surface of articular cartilage 21 hr. after a thorotrast injection. The considerable invasion of thorotrast particles (*T*) is evident and the superficial part can be seen to be breaking away. Electron micrograph. × 60,000.





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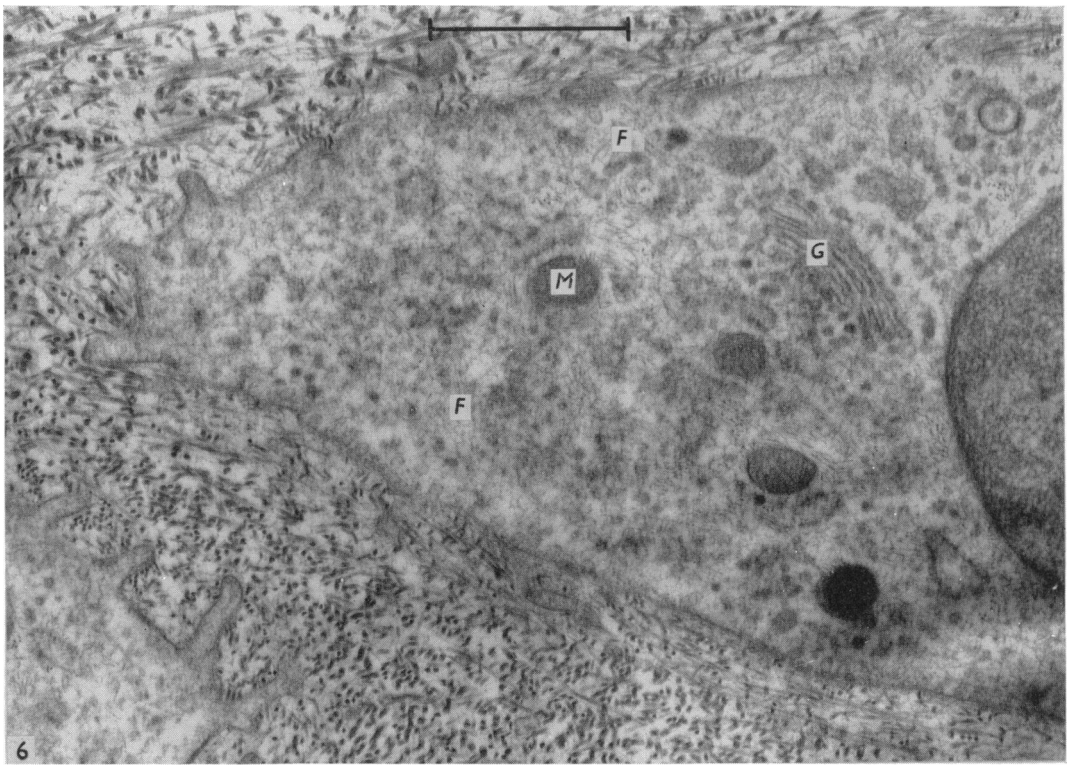
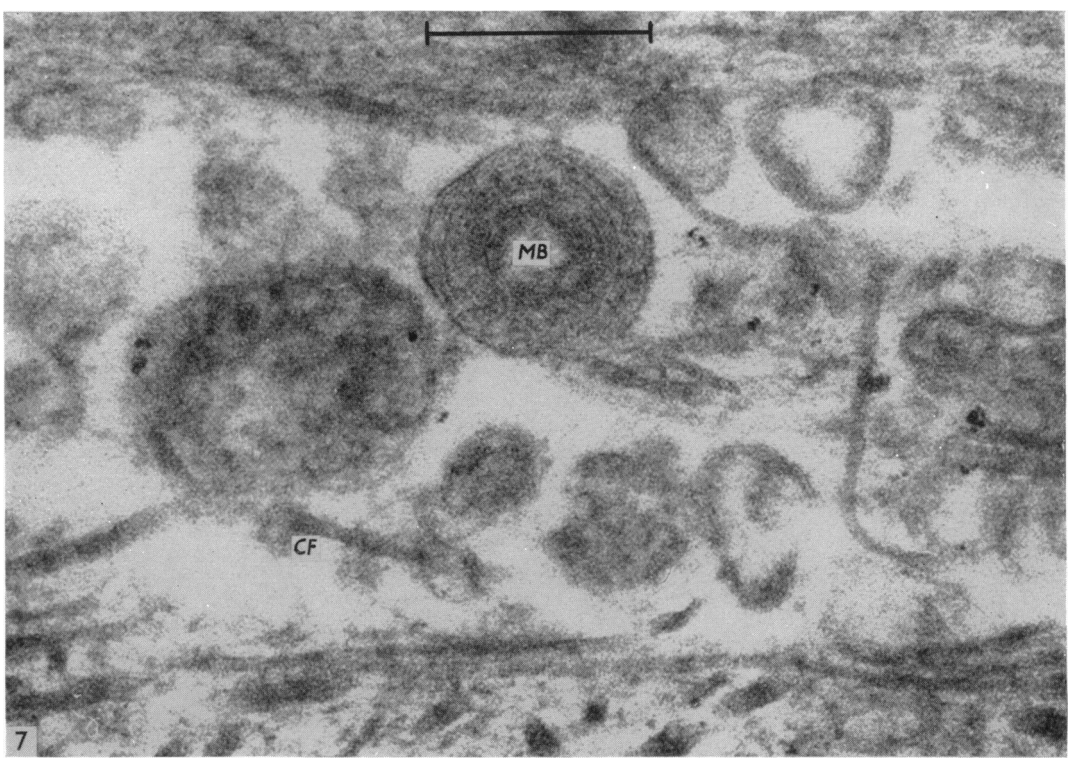




Figs. 6–7. Legends on facing page. Key to lettering on p. 375.

Similar changes are seen in the cells of the middle zone. As before some are normal in appearance but in others the fibrillar change in the cytoplasm is well developed and is usually perinuclear in position (Fig. 8): the mitochondria are filled with electron dense material and their internal structure is less apparent: some cells also show considerable hypertrophy of the Golgi apparatus. In other cells the fibrillar change in the cytoplasm is more marked and the fibrils are arranged in whorls: the endoplasmic reticulum is well marked, as is usual in cells of the middle zone. In others again, the cell membrane and nucleus are clearly defined, but in some areas the cytoplasmic structures are breaking down and small dense bodies are forming: these appearances are those seen in superficial zone cells soon after the thorotrast injection.

Forty-eight hours after injection

Forty-eight hours after injection, it can be seen with the light microscope that there are many red cells and a good deal of debris on the articular surface (Fig. 9). In the cartilage there is an absence of the flattened cells of the superficial zone, and the matrix appears to blend with the substance of the nearest red cells. In the middle zone cells the number of vesicles is greater than usual, and the individual vesicles are larger.

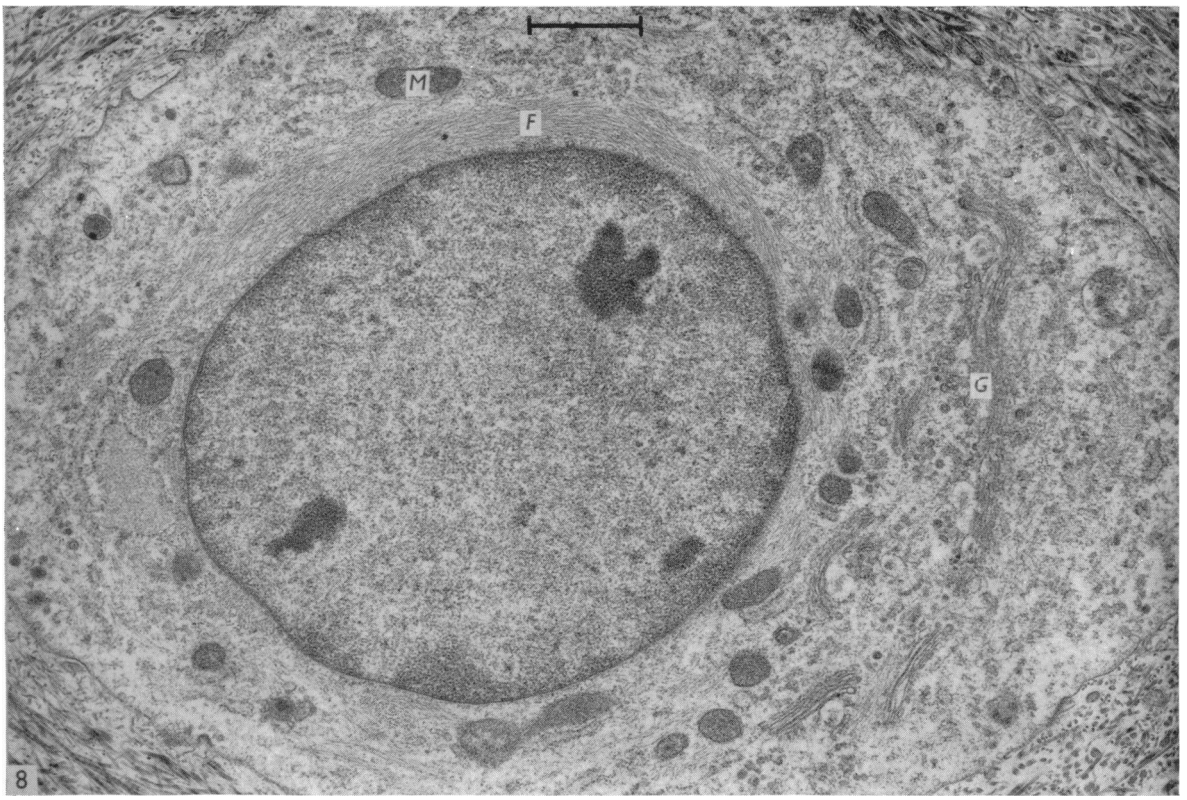
Under the electron microscope occasional thorotrast particles can be seen at the surface; erythrocytes are adherent to the matrix of the cartilage. Between these red cells there are occasional irregular masses of electron dense material, suggesting that the more superficial red cells are also held to the surface of the cartilage by a clot. Degenerate cells are still seen near the surface with an intermixture of cell debris and collagen fibres: the collagen fibres are fine, having a diameter of about 200–300 Å. In the middle zone some completely degenerate cells are seen, though here the cell debris has not been invaded by collagen fibres. The nuclei in most cells retain a distinct membrane (Fig. 10), and the surface membrane of the cell shows normal branching processes. The mitochondria are filled with electron dense material so that their internal structure is less evident. The endoplasmic reticulum is sparse and the members of each membrane pair are widely separated by electron dense material, an appearance which is common in middle zone cells of normal cartilage. Pairs of smooth cytomembranes are seldom seen, though vacuoles of sizes varying from 0·2 to 3–4 μ are common; all are clearly limited by a smooth membrane. Filamentous change is again seen in the cytoplasm though this is less extensive than at 24 hr. after injection.

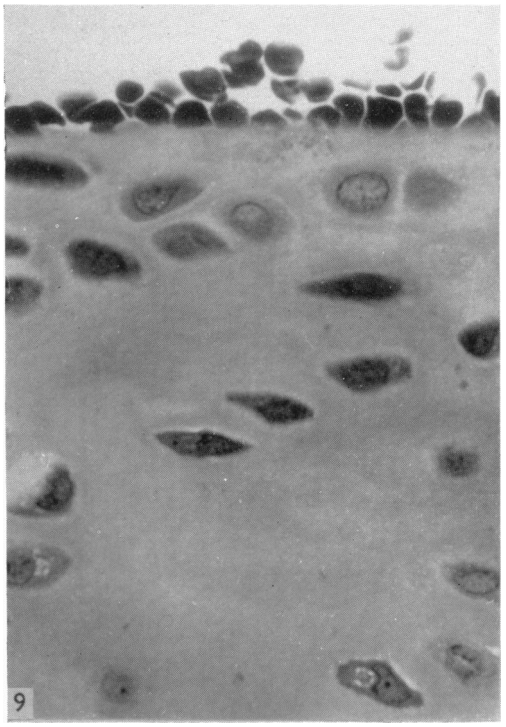
DISCUSSION

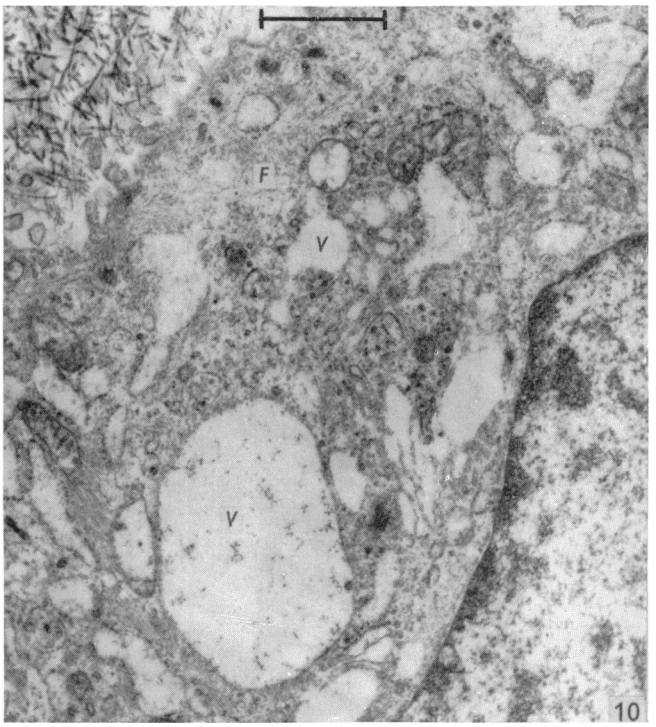
The thorotrast particles have been shown to penetrate the surface of the cartilage and to invade its substance to a depth of about 3 μ; they occupy no more than 3 % of the volume of the invaded part of the cartilage. This invasion begins immediately

Fig. 6. A chondrocyte from the superficial zone 24 hr. after a thorotrast injection. The mitochondria (*M*) are dense and the Golgi apparatus (*G*) is hypertrophied. An early stage of the filamentous change (*F*) can be identified. Electron micrograph. × 27,000.

Fig. 7. The remains of a chondrocyte from the superficial zone 24 hr. after a thorotrast injection. A myelin body (*MB*) has formed and collagen fibres (*CF*) can be seen among the cell debris. Electron micrograph. × 144,000.







Figs. 8–10. Legends on facing page. Key to lettering on p. 375.

after the injection but is maximal after one day; the invaded part of the cartilage subsequently breaks away from the surface, the separation being complete on the second day.

The depth to which the cartilage is penetrated by the thorotrast particles is much less than that reported for protargol and for many other substances (Stockwell & Barnett, 1964). Thorotrast particles vary between 50 and 100 Å in diameter, whereas the diameter of the protargol molecule, if it be spherical, is probably about 40 Å; the lack of penetration may simply be due to the larger particle size: on the other hand it may be due to a physical or chemical interaction with the collagen fibres or with the substances lying between these fibres.

The blood cells seen at the surface of the cartilage are thought to result from the operative procedures on the joint (Davies, 1945): in a parallel investigation (Davies & Palfrey, 1965) it has been shown that there is little change in the vascularity of the synovial membrane as a result of these injections.

Degenerative changes have been seen in the cartilage cells of the superficial and middle zones. These changes begin within 1 hr. of the injection but are still progressing on the second day: they proceed most rapidly in the cells of the superficial zone where they are complete on the first day. In the middle zone the degenerative changes are only beginning on the first day and are progressing on the second day.

Thorotrast is not a simple aqueous suspension of thorium dioxide, since it contains, in addition to 25% colloidal thorium dioxide, 25% of dextrin and 0·15% of methyl-*p*-hydroxy benzoate; in these experiments it was diluted with an equal volume of physiological saline. Both dextrin and methyl-*p*-hydroxy benzoate in this dose are normally regarded as virtually non-toxic substances (Spector, 1956); the effects of intra-articular injection are being investigated. If the thorium dioxide is the only active ingredient it is conceivable that it acts as a mechanical block to the passage of metabolites, that its radioactive breakdown causes radiation damage, or that it acts as a chemical poison.

The damage to the cartilage cells begins before the mechanical invasion of the cartilage and continues in the middle zone cells after the blockage has largely been removed by abrasion of the surface of the cartilage. Mechanical blockage is thus unlikely to be the mode of action.

If radiation damage is responsible the effects may be considered by postulating a layer of thorium dioxide on the surface of the cartilage some 1 μ thick; calculation then shows that a cell, of diameter 8 μ, at a depth of 20 μ from the surface, will be hit by an alpha particle once in about every 20 hr. Observations show that the thorotrast in fact forms not more than 3% of the superficial 3 μ of the cartilage; if this is taken to reduce the incidence of alpha particles by a factor of 10, each cell will be hit

Fig. 8. A cell from the middle zone 24 hr. after thorotrast injection. Peri-nuclear filamentous material (*F*), dense mitochondria (*M*), and the hypertrophied Golgi apparatus (*G*) can be seen. Electron micrograph. × 14,700.

Fig. 9. A light micrograph of the surface of the cartilage 48 hr. after a thorotrast injection. Note the red cells blending with the surface, the absence of the flattened cells of the superficial zone and the vesicle formation in the middle zone cells. × 480.

Fig. 10. The cytoplasm of a middle zone cell 48 hr. after a thorotrast injection showing multiple vesicles (*V*); filamentous change (*F*) in the cytoplasm can also be identified. Electron micrograph. × 21,300.

on the average once every 200 hr. The damage should also be limited to the maximum range of alpha particles from thorium and its daughters in tissue, which is known to be about 30 μ. Since the incidence of alpha particles is too small and the cartilage is in fact damaged to a greater depth than would be expected, it is unlikely that this is the mechanism for the damage.

The toxicity of thorium dioxide was investigated by Irwin (1932), who gave intravenous injections of 250–1250 mg./kg. to rabbits: the particles were concentrated in the cells of the reticulo-endothelial system, but under the light microscope there was no evidence of any toxic reaction between 48 hr. and 4 months. The toxicity of soluble thorium compounds has been studied by McClinton & Schubert (1948) who found an LD50 in rats of 68 ± 12 mg/kg: but made no histological studies of the effects of thorium. Scott, Neuman & Bonner (1952) injected thorium sulphate intravenously in rabbits and found that it acted as an insoluble compound; but again no histological studies were made. Thorium dioxide is stated to be an insoluble compound (Hodgman, 1949) but 60 mg. of this compound in the restricted volume of a rabbit's knee joint may be sufficiently soluble in synovial fluid to cause the damage observed.

The invasion of the matrix by thorotrast particles occurs during the second day after injection, and by that time many of the cells are degenerate, particularly in the superficial zone. It is possible that the invasion is in some way facilitated by the degeneration of the cells, or that the processes of invasion and degeneration are unconnected. This problem can only be resolved if other substances are injected and produce invasion without degeneration: these experiments are in hand.

SUMMARY

A dilute suspension of thorotrast particles was injected into the knee joint of immature and young adult rabbits: the animals were exercised and specimens of the femoral articular cartilage taken for electron microscopy up to 2 days after injection. At both ages the thorotrast particles penetrated only about 2–3 μ into the cartilage: cells are not normally present at this level, so the particles were seen only in relation to the collagen fibres of the matrix and were not absorbed into the chondrocytes. After 24 hr. this superficial 2–3 μ appeared to be breaking away from the underlying matrix. Some of the cells in the superficial zone of the cartilage (occupying a depth of about 20 μ) showed break-up of the endoplasmic reticulum, electron-dense granules, myelin bodies and irregular vesiculation: others showed complete degeneration. Specimens taken 48 hr. after injection showed similar changes in the middle zone of the cartilage. Thorotrast interferes with the normal metabolism of the surface cells producing degeneration: this is thought to be the result of the chemical toxicity of the injection.

We are indebted to Professor D. V. Davies for the provision of the material on which this study was performed, to the Arthritis and Rheumatism Council for the establishment of the Electron Microscope Unit and to Messrs J. King, J. S. Fenton, and G. Maxwell for technical assistance and to Miss F. M. Fildes for secretarial assistance.

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KEY TO LETTERING

A	amorphous material	*MB*	myelin body
B	blood cells	*MZ*	middle zone
CF	collagen fibres	*SZ*	superficial zone
F	fibrillar material	*T*	thorotrast particles
G	Golgi apparatus	*V*	vesicles
M	mitochondria		