ABSORPTIVE FUNCTIONS OF THE SYNOVIAL MEMBRANE

BY

W. COCHRANE, D. V. DAVIES, AND A. J. PALFREY

From the Arthritis and Rheumatism Council Electron Microscopy Unit, St. Thomas's Hospital Medical School, London

Absorption from the joint cavity has been studied sporadically for a long time, but we are still far from a complete understanding of the mechanisms involved in the exchange between the synovial fluid, tissue fluid, synovial membrane, and the vascular spaces. Substances in solution are removed from synovial joints mainly through the capillary bed, colloidal particles including proteins are removed from the joint more slowly, mainly by the lymphatic stream, while particles of 100 μ or more in diameter are removed exceedingly slowly and are mainly deposited in the subsynovial tissues. Some of the larger particulate matter is taken up by synovial cells, some is trapped in fibrin clots at the joint surfaces, and some enters directly from the joint into the tissue spaces of the subsynovial layer where it is removed by phagocytes (macrophages).

The problem of the transport of substances in and out of the synovial cavity is of importance in connexion with the formation and dispersal of joint effusions, for the nutrition of the articular cartilage, in connexion with the vascular changes which occur in the synovial membrane in rheumatoid conditions, and in respect of the permeability of the synovial membrane and the accessibility of the joint cavity to drugs (Bianchi, 1953, 1954; Spector and Willoughby, 1962; Sharp, 1963).

In the present investigation the absorption of Thorotrast (thorium dioxide) from the joint cavity has been studied by electron microscopy.

Material and Methods

Four rabbits, 2 males 8 months old and 2 females 8 and 3 months old have been used. Under intraperitoneal Nembutal anaesthesia, supplemented by a minimal amount of ether, each received an injection of 0·5 ml. of a 50 per cent. dilution of Thorotrast in physiological saline into one knee joint, the opposite joint serving as a control. The animals were anaesthetised with chloroform, 1, 2, and 24 hours respectively after the injection and 0·5 ml. fixative was injected into both knee joints; portions of synovial membrane less than 1 mm. diameter were removed. The fixative was osmium tetroxide buffered to a pH of 7·4 (Palade, 1952). The blocks remained in fixative for 2 hours, were then dehydrated in alcohol, passed through propylene oxide, and embedded in Araldite (Glauert, Rogers, and Glauert, 1956). Sections 1μ thick were cut from the block and stained with Azur II (Richardson, Jarett, and Finke, 1960); thin sections were then cut, mounted on uncoated copper grids, stained with a saturated solution of uranyl acetate in absolute methyl alcohol, and examined in the EM6 electron microscope.

Results

1 Hour after Injection.—There was much free Thorotrast in the joint cavity; there was an abundance of Thorotrast particles in the synovial cells, in some of the cells of the subsynovial tissue, and in the interstices of both the intimal and subsynovial layers of the synovial membrane. The particles, each 50 to 100 Å in diameter, quickly penetrated the gaps between the synovial cells and became widely dispersed in the subsynovial tissue.

Vacuolated and non-vacuolated synovial cells, corresponding to Types A and B of Barland, Novikoff, and Hamerman (1962) and Chapman, Muirden, Ball, and Hyde (1962) could be distinguished, but many transitional forms occurred. The most prominent accumulations of Thorotrast granules occurred in vacuolated cells; they were contained in membrane-bound vacuoles, sometimes in association with a flocculent precipitate. One hour after injection many of the vacuoles were completely filled with Thorotrast, others were incompletely filled, and some contained none (Fig. 1).
In the sybsynovial tissue were highly vacuolated cells, presumably histiocytes, with a considerable accumulation of Thorotrast in many vacuoles. In both synovial cells and histiocytes, a few scattered granules of Thorotrast occurred in the general cytoplasm outside the vacuoles.

At the free surface of the synovial cells an abundance of Thorotrast could be seen adherent to the plasma membranes. In several places this lay in bays and invaginations of the plasma membrane, some of which extended deeply into the cell body. Folds of the plasma membrane or filopodia were present but were not a prominent feature. It would appear that particles became included in the cytoplasm by a process of invagination of the plasma membrane and the cutting of the resulting bay by approximation of the walls. Some possibly became enfolded by membranous folds or filopodia. In
other places Thorotrast particles appeared to enter the cytoplasm by another method; they adhered to the cell surface, the plasma membrane underwent some modification, perhaps dissolution, and the cytoplasm flowed around and incorporated the particles. In these regions the plasma membrane could not be defined, whereas elsewhere it remained intact and could be readily resolved in the electron micrographs (Fig. 2).

Even one hour after injection, polymorphonuclear leucocytes could be seen infiltrating the synovial tissues, but these cells contained no Thorotrast particles nor had any granules crossed the basement membrane of blood capillaries and entered the endothelium.

Only small accumulations of Thorotrast were seen in fibrocytes; most of these cells had not ingested any particles (Fig. 3). No Thorotrast entered fat cells, even where the surrounding tissue was heavily infiltrated with particles.

2 Hours after Injection.—There was still abundant Thorotrast in the joint cavity. There was deeper penetration and an increased amount of it in the synovial membrane; the individual cells did not display any obvious increase in the amount ingested. Much of the material was contained in membrane-bound vacuoles, some of which were still only partially filled. Some vacuoles were devoid of granules. Some Thorotrast appeared in dense bodies resembling lysosomes; some of these lay close to, and appeared to be derived from, the Golgi apparatus, which now appeared to be more prominent and more frequently encountered than at the 1-hour stage (Fig. 4).
Many cells in the tissue showed some separation of the inner and outer laminae of the nuclear membrane. One cell displayed the appearance of the extrusion of nuclear material through the membrane (Fig. 5).

In one case (Fig. 6) the outer lamina had been raised into a bleb which contained a flocculent precipitate.

Several cells in the intimal and subsynovial layers contained complicated multivesicular bodies. These varied in diameter, the largest measuring up to 4μ. They were bounded by a triple-layered membrane about 130 Å thick, enclosing smaller vesicles which usually contained a flocculent precipitate; around and between the vesicles was an electron-dense coarsely granular material (Fig. 7). These complicated bodies occurred in both synovial cells and histiocytes. Some of the cells were peppered with discrete Thorotrast particles, some contained aggregations of granules, while still others were devoid of Thorotrast particles, at least in the sections showing these complex bodies.
Numbers of polymorphonuclear leucocytes were present in the subsynovial tissue, especially around blood vessels. They contained few or no Thorotrast granules. The endothelium of the blood capillaries and fat cells was also devoid of Thorotrast.

**24 Hours after Injection.**—The distribution of Thorotrast in the synovial cells and histiocytes showed little change. Some small accumulations of Thorotrast occurred in fibrocytes (Fig. 8), and there was possibly less in the intercellular matrix,
Fig. 5.—Surface cell containing accumulations of Thorotrast. Note the widened interval separating the inner and outer laminae of the nuclear membrane (NM) and apparent extrusion of nuclear material through the nuclear pores (NP) × 47,335.
though the medium was still present in the joint cavity and adherent to the free surfaces of the synovial cells.

In most regions, in synovial cells, histiocytes, fibrocytes, and lymphocytes, the endoplasmic reticulum was more prominent and was studded with R.N.A. granules. The Golgi apparatus was much enlarged in many cells (Fig. 9).

Some synovial cells contained only a minimal amount of Thorotrast and some none at all. The largest accumulations were in the histiocytes in the subsynovial tissues. Polymorphonuclear leucocytes were more numerous in the synovial membrane. They did not phagocytose the Thorotrast in any quantity; a few isolated granules only were seen in some cells. The cellularity of the synovium was increased and rough-walled endoplasmic reticulum was a conspicuous feature in most cells. Lymphocytes were present but contained an occasional Thorotrast granule.

Cells with multivesicular bodies were more numerous than at 2 hours. These bodies occur both in the synovial cells and deeply in the subsynovial tissues (Fig. 10).

In some synovial cells a fibrous material appeared in association with some of the Thorotrast accumulations; elsewhere an electron-dense secretion was accumulating in the vesicles in these cells (Fig. 11).

No lymphatic capillaries were identified with certainty in these tissues, but at 24 hours there was a vessel with electron-dense contents and very vesiculated cell lining in which Thorotrast seemed to be accumulating and passing into the lumen by a
Fig. 7.—Part of a synovial cell containing large multivesicular bodies. The cell is peppered with Thorotrast granules. × 49,700.
process suggestive of apocrine secretion. No Thorotrast was found in blood vessels.

**Discussion**

The speed and ease with which particulate matter was found to enter the intimal and subsynovial layers of the synovial membrane is in agreement with previous investigations with the light microscope. While drawing attention to the presence of filopodia and pinocytotic vesicles in synovial cells, Chapman and others (1962) add that "evidence is insufficient to indicate with certainty the mechanism of entry". While the engulfment of particulate matter by folds and filopodia and its entry via pinocytotic vesicles and bays at the cell surface seems a probability, this investigation also suggests another mode of entry, that of "membrane flow" or "rhopheocytopsis" as suggested by Policard and Bessis (1962). The injected Thorotrast is incorporated principally in the vacuolated type of synovial cell (Type A of Barland and others, 1962); this agrees with the findings of Chapman and others (1962). Almost all the Thorotrast within the cell is aggregated into vacuoles by the 1-hour stage. A few isolated granules can be identified in the general cytoplasm around the vacuoles and elsewhere. This suggests that the material is either incorporated into the cell mainly as aggregates or that the transport and aggregation of discrete granules in the cytoplasm is extremely rapid.
Fig. 9.—A cell in the subsynovial layer 24 hours after injection, with abundant endoplasmic reticulum and a large and diffuse Golgi apparatus (G). Only one accumulation of Thorotrast is seen in the cell. × 30,420.
Fig. 10.—Histiocyte with a multivesicular body in the subsynovial tissue at 24 hours after injection. There are accumulations of Thorotrast granules in vesicles elsewhere in the cytoplasm and some within the electron-dense material in the multivesicular body.  × 33,000.
Fig. 11.—Synovial cell at 24 hours after injection, showing the development of fibrous material close to accumulations of Thorotrast. Note also the electron-dense secretion in some intracytoplasmic vesicles. × 33,000.
The entry of material between the synovial cells and its dispersal through the subsynovial tissue is also rapid, even in the animal maintained under anaesthesia from the time of injection until killed. While earlier work suggests that, with particulate matter, movement is an important factor promoting absorption from the joint cavity, other factors must be operative. In the subsynovial tissue the particles are transported in the interfibrillar matrix from which they are incorporated into histiocytes or enter lymphatics. The state of the matrix, in particular of the mucopolysaccharide component, is important in this respect. The molecular size of the mucopolysaccharide, the state of the aqueous component of the tissue, and the electrical charges on the various constituents may well be involved.

Thorotrust particles do not cross the basement membranes of blood vessels though they quickly evoke a polymorphonuclear leucocytic reaction. They do, however, transgress the basement membrane of lymphatics and enter their endothelial cells. From here they appear to be released into the lumen by a method akin to apocrine secretion. Each particle, or group of particles, is released in an envelope of cytoplasm.

The identity of the phagocytic cells in the synovial membrane presents a problem. In the early stages most, if not all, of the intimal cells are synovial cells and the deeper phagocytic cells are histiocytes. In later stages the distinction between these two types cannot be made easily on the basis of their topography, though the subsynovial cells at this stage are principally histiocytes. Histiocytes are also present amongst the intimal cells and display little morphological difference from Type A synovial cells. Polymorphonuclear leucocytes play only a minor role in phagocytosis, fibroblasts and lymphocytes accumulate only small amounts of material, and fat cells play no part in the process.

Interesting changes occur in the cells, probably as a result of phagocytosis. The inner and outer lamellae of the nuclear membrane become more widely separated than in the normal, and the outer lamella may be raised into a bleb. The Golgi apparatus increases in size, there is an increased amount of rough-walled endoplasmic reticulum, and multivesicular bodies appear in many cells. None of these changes is described by Chapman and others (1962). They may be due to the toxicity of the injected Thorotrust, and the question arises whether intracellular changes of a similar nature occur under similar circumstances but with different types of particulate matter. Few foreign substances have been investigated; in most studies on the absorption of particulate matter from joints Indian ink suspensions have been used. According to Key (1926), using carbon particles in suspensions, the macrophages never get rid of the carbon particles, which are said to remain within the cell until it dies or disintegrates, when the material is again set free into the tissues or the joint cavity. The degeneration of these macrophages has not been described.

A greatly extended study using a variety of different materials could materially help to elucidate some of these problems and those of cell pathology in general.

Summary

The fine structure of the synovial membrane of the knee joint of young adult rabbits has been studied between 1 and 24 hours after the injection of a suspension of Thorotrust (thorium dioxide). Within 1 hour of the injection, particles were found in vacuoles of synovial cells and histiocytes, and there were free particles in the subsynovial tissue. After 2 hours the amount of thorium dioxide had increased; some of the cells containing ingested particles now showed a prominent Golgi apparatus, separation of the constituent layers of the nuclear membrane, and complicated multivesicular bodies; the tissue showed a polymorph infiltration, but these cells did not absorb the particles. By 24 hours after injection the cells showed further enlargement of the Golgi apparatus and the endoplasmic reticulum was hypertrophied. The mechanism by which synovial cells absorb particulate matter is discussed; the later changes within the cells may be due to the toxicity of the injected material.

The authors thank the Arthritis and Rheumatism Council for the use of the electron microscope, Messrs. J. S. Fenton and G. Maxwell for technical assistance, and Miss F. M. Fildes for secretarial help.

REFERENCES


—— (1954). Ibid., 9, 166.


Absorptive Functions of Synovial Membrane


Fonctions d'absorption de la membrane synoviale

Résumé
La fine structure de la membrane synoviale de l'articulation de genou de jeunes lapins adultes fut étudiée entre une et 24 heures après l'injection d'une suspension de dioxyde de thorium. Une heure après l'injection on trouva des particules dans les vacuoles des cellules synoviales et des histiocytes et on observa des particules libres dans le tissu synovial. Après 2 heures la quantité de dioxyde de thorium se trouva augmentée; quelques cellules contenant des particules ingerées révélaient un appareil de Golgi prononcé, la séparation des couches constituant de la membrane nucléaire et des corps multivesiculaires compliqués; le tissu était infiltré par des polymorphes, mais ces cellules n'absorbaienst pas les particules. Vers 24 heures après l'injection l'appareil de Golgi cellulaire était encore plus grand et le réseau endoplasmique se trouvait hypertrophié. On discute le mécanisme par lequel les cellules synoviales absorbent de la matière sous forme de particules; les altérations intracellulaires tardives peuvent être dues à la toxicité du matériel injecté.

Funciones de absorción de la membrana sinovial

Resumen
La fina estructura de la membrana sinovial de la articulación de la rodilla de jóvenes conejos adultos fue estudiada entre la una y las 24 horas después de la inyección de una suspensión de dióxido de torio. Dentro de una hora de la inyección, partículas fueron halladas en las vacuolas de las células sinoviales y de los histiocitos y partículas libres se vieron en el tejido sinovial. A las dos horas, la cantidad de dióxido de torio se vio aumentada; algunas células con partículas ingeridas revelaron destacadamente el aparato de Golgi, la separación de las capas constituyentes de la membrana nuclear y cuerpos multivesiculares complicados; el tejido se vió infiltrado por polimorfos, pero las células ya no absorbían las partículas. A las 24 horas de la inyección el aparato celular de Golgi fue aún mayor que antes y el retículo endoplasmático fue hipertrofiado. Se discute el mecanismo por el cual las células sinoviales absorben materia en forma de partículas; alteraciones intracelulares tardías pueden deberse a la toxicidad del material inyectado.
Absorptive Functions of the Synovial Membrane

W. Cochrane, D. V. Davies and A. J. Palfrey

Ann Rheum Dis 1965 24: 2-15
doi: 10.1136/ard.24.1.2

Updated information and services can be found at:
http://ard.bmj.com/content/24/1/2.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/