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ULTRASTRUCTURE OF THE LYMPH NODE OF THE GUINEA PIG

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Introduction

There is hardly another organ whose structure would have been investigated as often as that of the lymph node. This is not surprising, for changes in the lymph nodes — especially under pathological conditions — provide always useful indications for the histologist. It is due to the possibilities offered by the electron microscope that we are still able to point out new aspects in this connection.

The present experiments had the aim to supplement existing data regarding the structure of the sinuses and the capsule of lymph nodes by electron microscopic observations.

Material and method

Lymph nodes measuring 2 to 3 mm taken from the submandibular region of guinea pigs were fixed and thereafter minced in buffered (pH = 7.2) isotonic and isoionic 1 per cent osmium tetroxyde solution prepared according to Sjöstrand. After 2 hours, the specimens were dehydrated in ethanol and embedded in a 5 : 1 mixture of butyl and methylmethacrylate. Sections were prepared with a Porter-Blum type microtome and photographed by means of a Tesla BS 242 table electron microscope with 2500 to 9000-fold magnification. Most sections were stained with saturated uranyl acetate solution or 2 per cent $KMnO_4$ solution and covered with formvar membrane to reduce heating artifacts.

Results

Capsule (Fig. 1). It consists of lamellated connective tissue in which four layers can be distinguished: (1) lamellae of connective tissue; (2) fibrocytes; (3) fibres; (4) endothelium.

ad (1). This layer consists of flattened fibrocytes which form several superposed parallel lamellae (Fig. 1, Nos. 1—7). They showed often the phenomenon of pinocytosis in the form of minute vesicles produced by invaginations of the cell membrane. The lamellae had a thickness of 30 to 40 mμ at some, and one of 0.2 μ at other points. Round, large mitochondria could be seen in the cytoplasm and also osmiophilic bodies were perceptible in some cells. These bodies may have represented phagocyted particles. Connective tissue fibres were observed in the gaps between the cellular lamellae. Their thickness

varied between 90 and 300 Å. The thicker fibres were transversely striated, thus certainly collagenous fibres, while no striation could be demonstrated on the thin fibres. The thick ones appeared in bundles; the thin fibres appeared partly in bundles and partly isolated. Both kinds of fibres were seen to run in all directions in the narrow, flattened interlamellar interstices, although most of them were running parallel to the lamellae. The thickness of the fibres was found to grow with increasing depth.

ad (2). Large, characteristic fibrocytes were encountered in the second layer (Fig. 1, cc); their nuclei were large and revealed coarse chromatin structure. Large mitochondria were visible in the cytoplasm which contained a small number of ergastoplasmic sacs and ribosomes. This layer of the capsule has, because of the large size of its cells, a thickness of 5 to 6 μ, i. e. one exceeding that of the first layer.

ad (3). The third layer consists of fibres similar to those contained in the first; it is mostly composed of bundles formed by thick fibres (Fig. 1, k); these bundles are continuous with the trabeculae emitted by the capsule (Fig. 1, tr). The layer had a thickness of about 7 μ. Unstriated fibres of a homogeneous structure and a thickness varying between 60 and 200 μ were observed here and there among the collagenous fibres: they must have represented elastic fibres (Fig. 4). Hardly any cells were found in this layer.

ad (4). The fourth layer represents the endothelium of the marginal sinus (Figs. 1, 2a). It is a layer of flattened cells, the thickness of which does not exceed 10 mμ at certain points. The mitochondria were found to be smaller and more elongated than those in the fibrocytes, and there were few processes on their surface. Dark osmiophilic granules pointing to phagocytosis as also vacuoles could be observed in them. The cytoplasm of the cells contains smooth-surfaced endoplasmic reticulum and scattered Palade-granules. No endothelial cells could be seen on the cortexward surface of the marginal sinus (Fig. 2b). This wall of the marginal sinus was formed by densely packed lymphocytes and reticulum cells.

Sinuses (Fig. 3). Externally, their wall consists of a layer of collagenous or argyrophilic fibres which separates the endothelium from the substance of the lymph node. The fibres — partly scattered and partly in bundles — are situated in a space about 0.3 μ wide. No basement membrane was found beneath the endothelium of the sinuses. The thickness of the endothelial cells varied between 30 mμ and 2 μ. Minute, elongated mitochondria were observed in them. These cells emit sometimes microvilli into the gap below them, a phenomenon pointing to the activity of this surface. A great number of irregular villi — rather membrane-like curved structures — originate from the inner wall of the sinuses ("undulating membranes", Figs. 5, 6, 7). These membranes frequently surround large (about 0.5 μ) cavities; it is possible that the latter become subsequently vacuoles merging into the cytoplasm of the endothelial cells,

an assumption supported by the presence of several larger or smaller vacuoles in the cytoplasm. The processes of the endothelial cells form a sheath around the trabecules which transverse the sinuses. There is an endoplasmic reticulum of medium size in the endothelial cells. The ribosomes in them are either isolated or arranged in small groups. Endothelial cells are capable of phagocytosis and pinocytosis, as indicated by osmiophilic bodies and small vesicles in the cytoplasm. The endothelial cells form a continuous coat over the wall of the sinuses and the trabecules transversing them. Their edges are overlapping like tiles. Although there is no gap between them, in one case we saw a cell passing through between two neighbouring endothelial cells.

In a single case we succeeded in finding two centrosomes above the nucleus of an endothelial cell (Figs. 10, 11, c_1 and c_2). One of them, obliquely sectioned, was situated in the cytoplasm, the other, forming a basal body, was found immediately under the cell membrane. Characteristic ciliary filaments, issuing from the basal body and covered by the cell membrane, projected into the lumen. Numerous small vesicles could be seen near the centrosomes.

Reticulum cells (Figs. 2a, 2b, 7). These cells, well observable in the sinuses, show a variety of form. Their processes vary in shape; we saw a whole range of such forms, from short, cylindroid processes seen in the light microscope to long, thin and tortuous submicroscopic ones (Fig. 8). There were moreover sac-like invaginations between the microvilli, and also vacuoles of different size that had become detached into the cytoplasm. The cytoplasm therefore exhibited a spongy pattern. Bundles of connective-tissue fibres were often observed passing through the cytoplasm so that reticulum cells seemed to encapsulate the trabeculae as did the endothelial cells of the sinuses. There exists a close topographical connection between reticulum and endothelial cells. The long processes of contiguous reticulum cells are often suturewise interlaced, and may be likewise interlaced with the microvilli of the endothelial cells. The cytoplasm of the reticulum cells in the marginal sinus contains many round bodies with a homogeneous osmiophilic substance. They have different diameters up to 0.5 μ. In some instances, the centre of the osmiophilic substance appeared to be less opaque; in these cases, the body was enlarged and more compact formations could be observed in the interior of the rarified spots (Fig. 9). Even a thin delimiting membrane around the dense bodies could be seen in some cases. Such observations make it probable that the structures in question were the lysosomes described by DUVE (1959) and NOVIKOFF (1960). The cytoplasm includes numerous small vesicles of the endoplasmic reticulum as well as many ribosomes; the latter appeared either isolated or arranged in rosette form. They were sometimes joined to the endoplasmic reticulum forming rough-surfaced vesicles. The nuclei of the reticulum cells displayed various shapes; they were multilobular in some instances and provided with irregular indented processes in others.

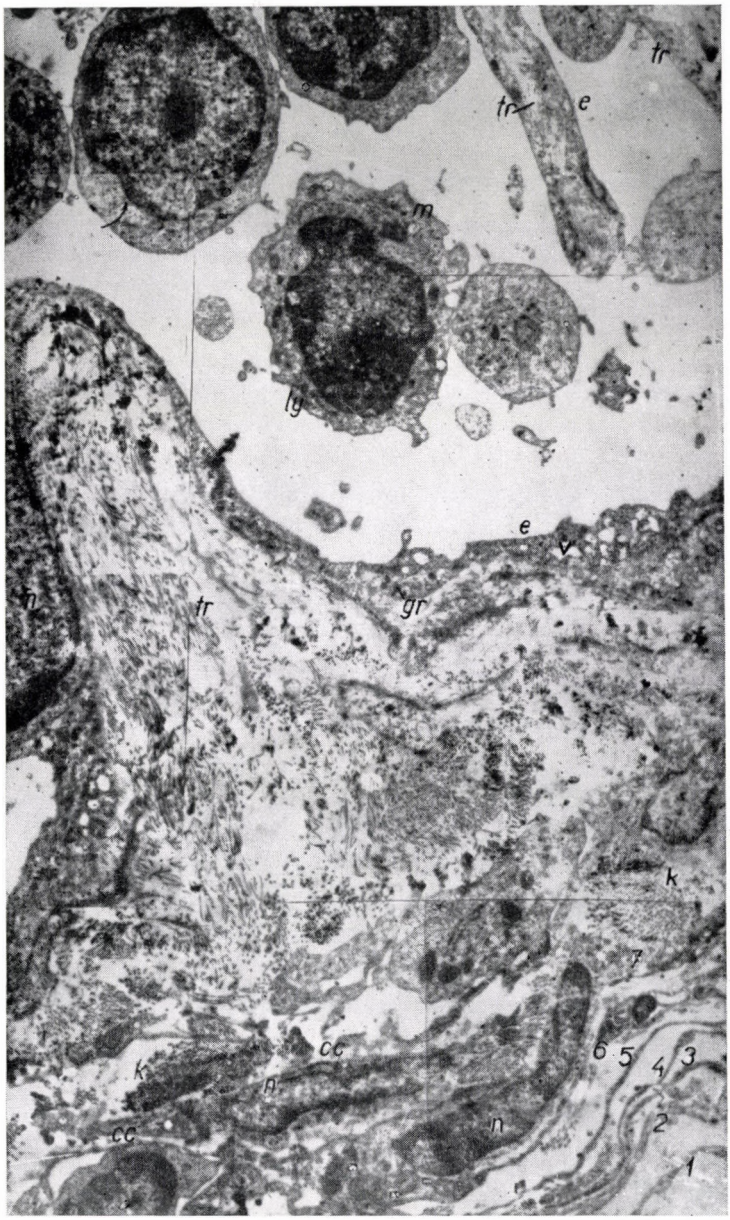

Fig. 1. Capsule of lymph node and marginal sinus. Strongly flattened cellular lamellae (1—7) and bundles of connective-tissue fibrils between them, which constitute the outer layer of the capsule

Fibrocytes (*cc*), containing flattened nuclei, large mitochondria, vesicles of the endoplasmic reticulum, and ribosomes, are found in the next layer. The frame of the capsule is formed by thick bundles of collagenous fibrils (*k*) which may project into the interior of the lymph node in the shape of trabeculae (*tr.*) Delicate elementary fibres and elastic fibres may be seen between the collagenous bundles. The inner surface of the capsule is lined by the flat endothelium of the sinus (*e*) which contains, beside the nucleus, vacuoles (*v*) of varying size, dark spherical granules (*gr*), mitochondria and ribosomes

ly = lymphocytes; *m* = mitochondrium; *n* = nucleus

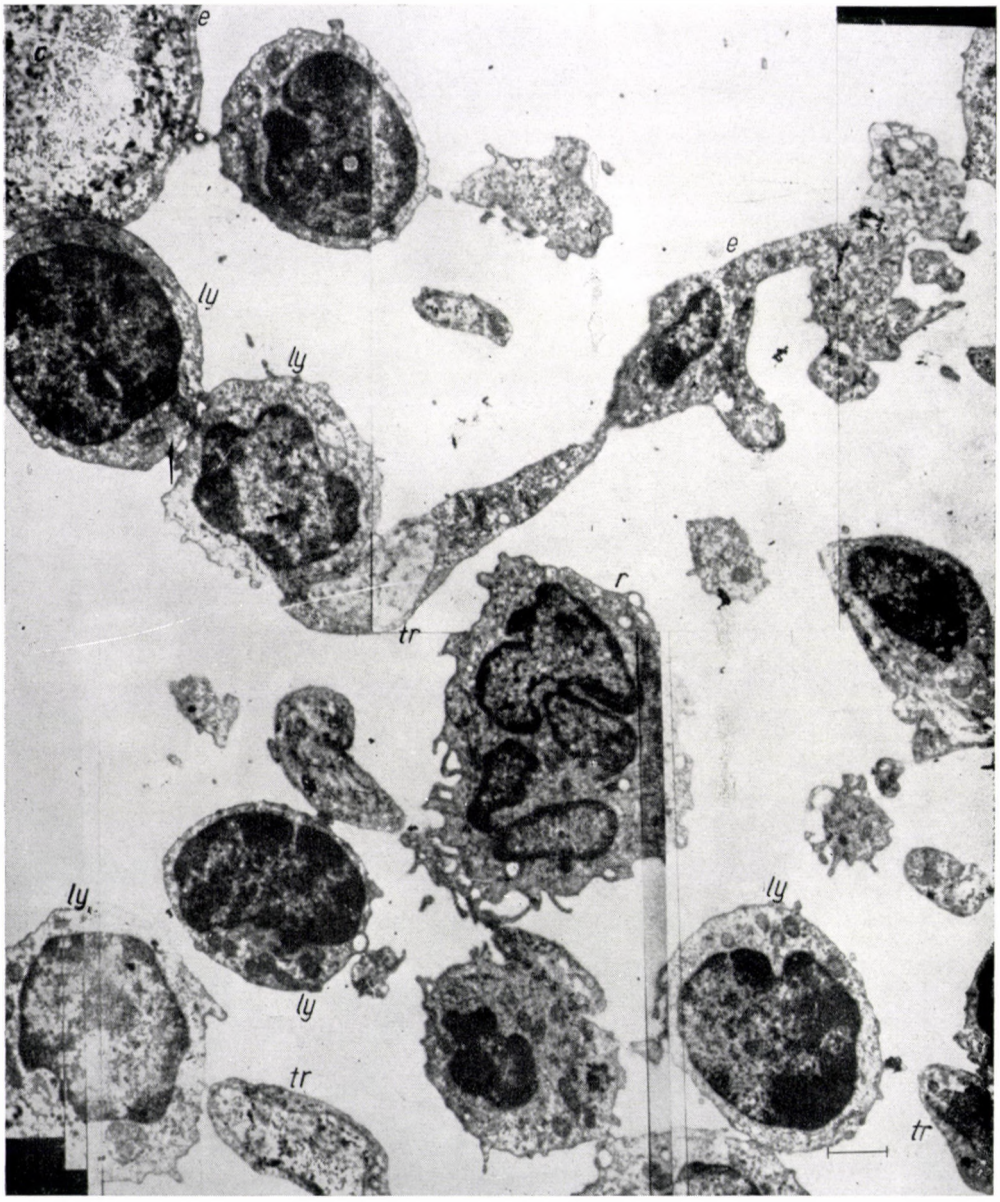

Fig. 2a. External region of the marginal sinus. Its outer wall is formed by the connective-tissue capsule of the lymph node (*c*) and the covering flat sinal endothelium (*e*)

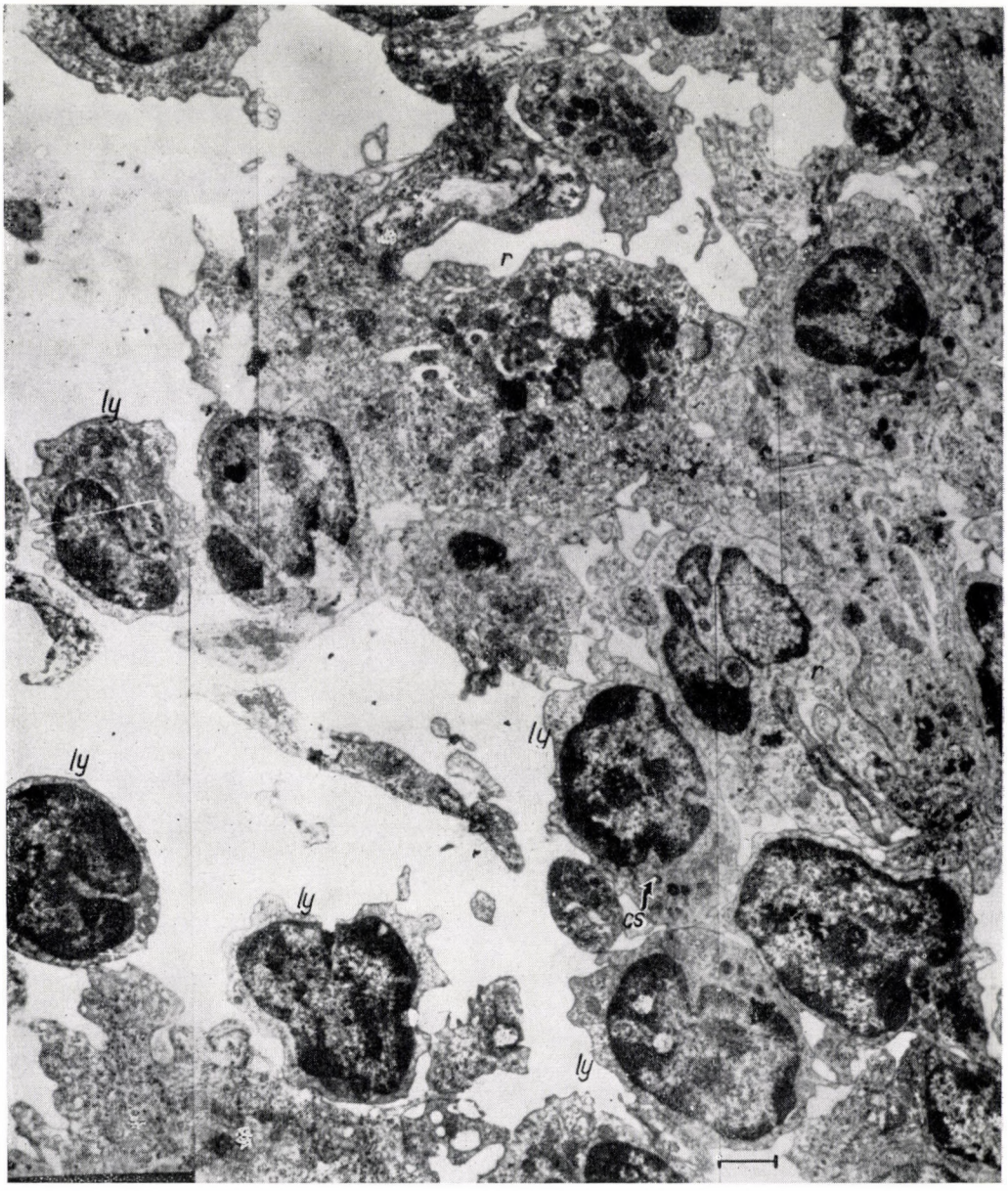

Fig. 2b. Internal portion of the marginal sinus (continued from Fig. 2*a*). The sinus has no sharp boundary towards the substance of the lymph node, and the characteristic endothelium is absent. The wall consists, essentially, of densely packed cells, reticulum cells above all
c = capsule; *e* = endothelium of the sinus; *r* = reticulum cell; *tr* = trabecula; *ly* = lymphocyte; *cs* = centrosome

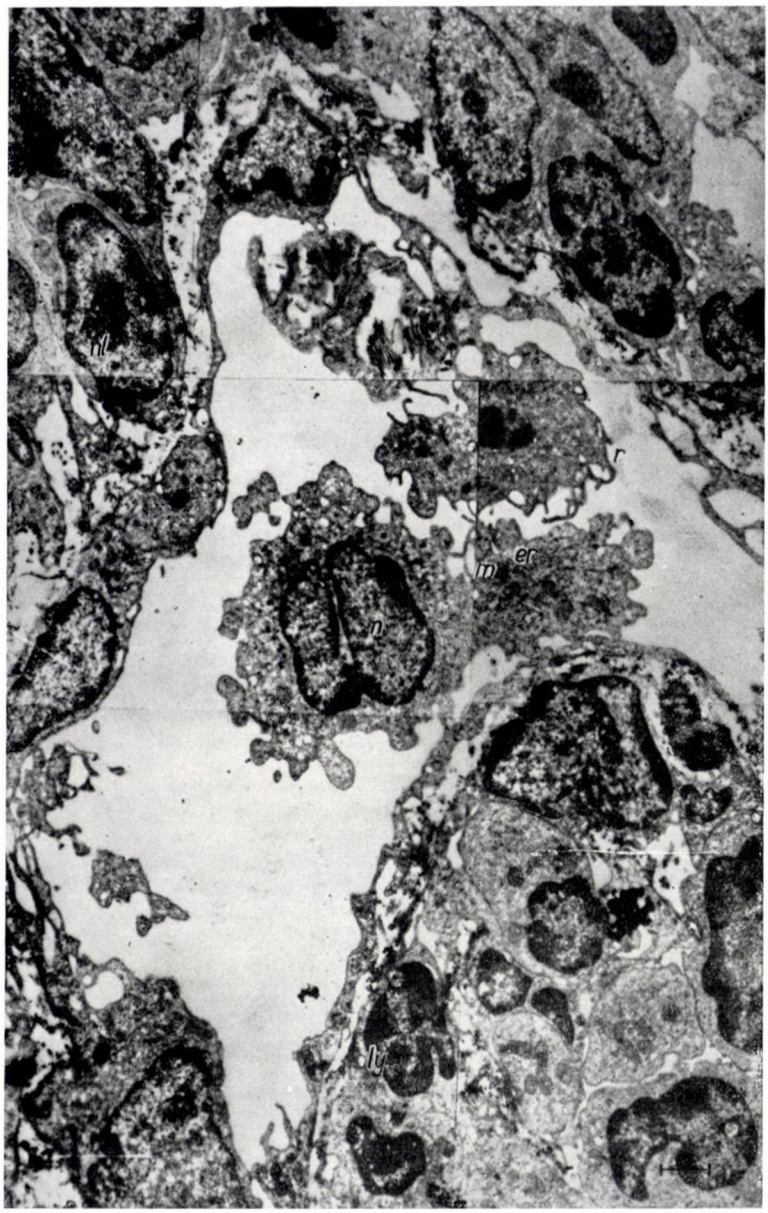

Fig. 3. Medullary sinus. Its wall is formed by flat endothelium of variable surface, and — beneath it — a loose layer of connective tissue fibrils. The endothelium engulfs the fibre bundles traversing the sinus (*tr*). Two reticulum cells are visible in the sinus (cf. Fig. 7). *e* = endothelium; *r* = reticulum cell; *n* = nucleus; *er* = ergastoplasm; *f* = bundle of fibrils; *nl* = nucleolus; *cs* = centrosome

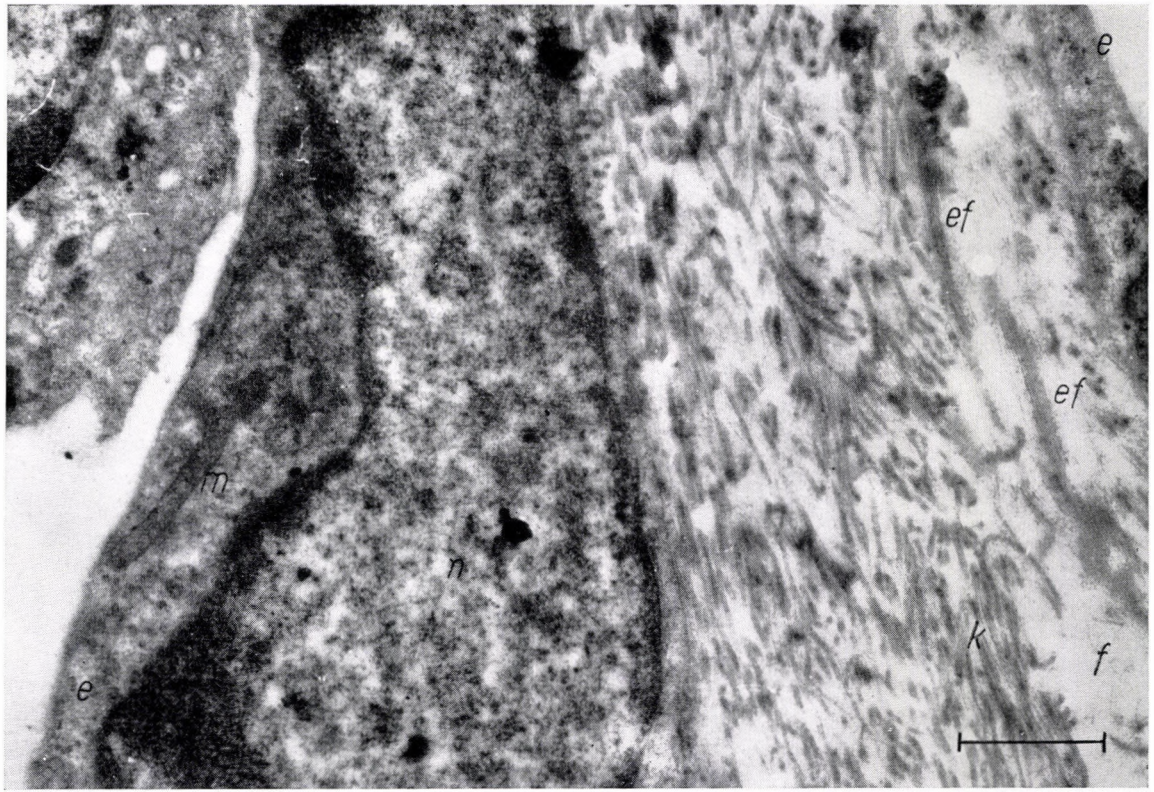

Fig. 4. Detail of a trabecule projecting from the capsule into the marginal sinus
The bundle of connective-tissue fibres is covered by endothelium on both sides (*e*). It is composed of striated collagenous fibrils (*k*), very thin elementary fibres (*f*) and thicker (elastic) fibres of homogeneous appearance (*ef*)
n = nucleus; *m* = mitochondrium

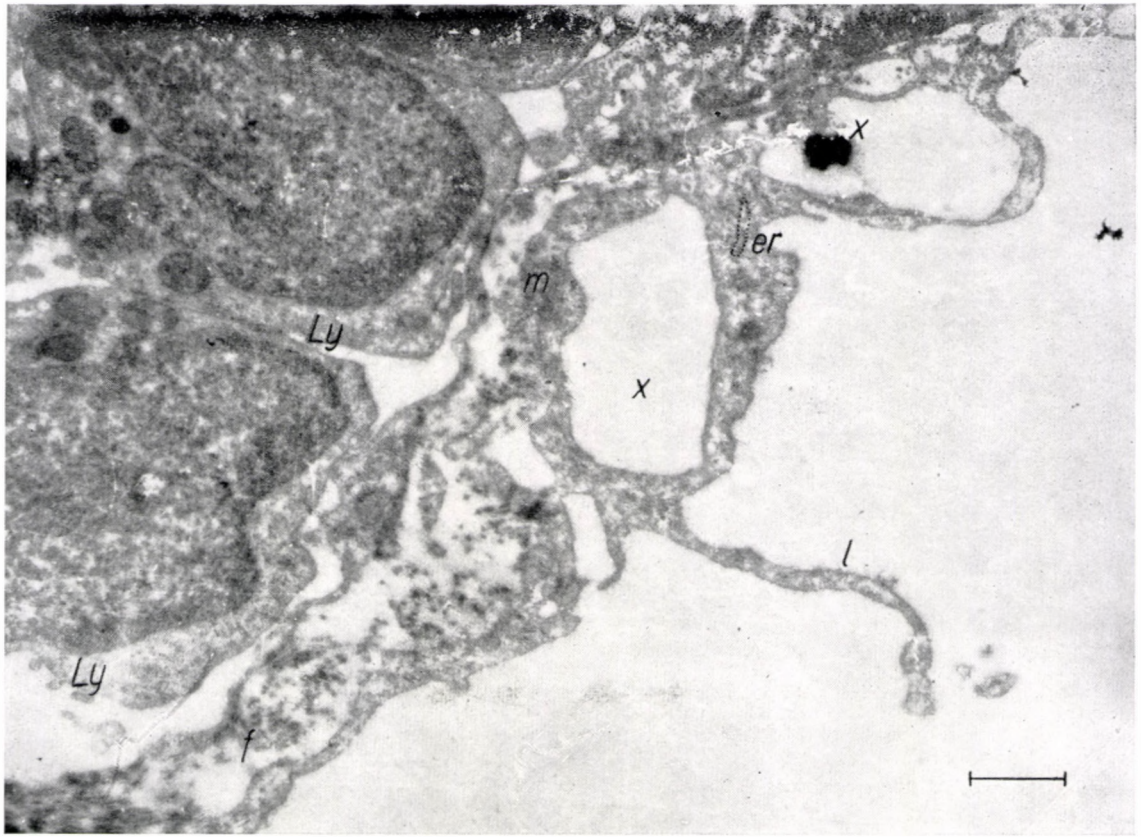

Fig. 5. Detail from the wall of a medullary sinus. Note the irregular inner surface of the endothelium: it emits lamellar processes (1) which, converging, form cavities (*x*) at several points. A few elements of the endoplasmic reticulum (*er*) and mitochondria (*m*) are visible in the endothelial cells. The endothelium has no basement membrane. Beneath the endothelial cells there is only a space of varying width which contains connective-tissue fibrils (*f*)
ly = lymphocytes

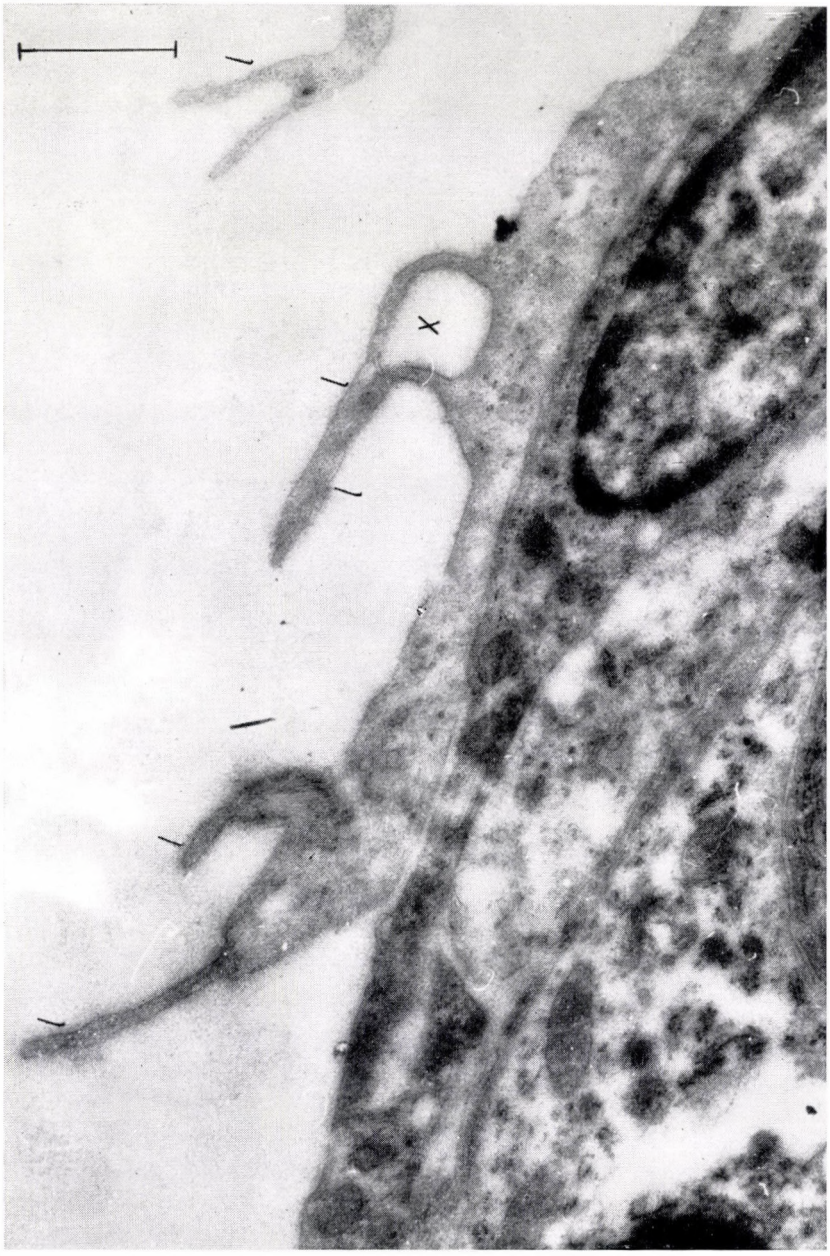

Fig. 6. Detail of a sinus wall. From the inner surface of the endothelium lamellar processes (*l*) are arising — they adhere closely to each other and form cavities at several points (*x*)

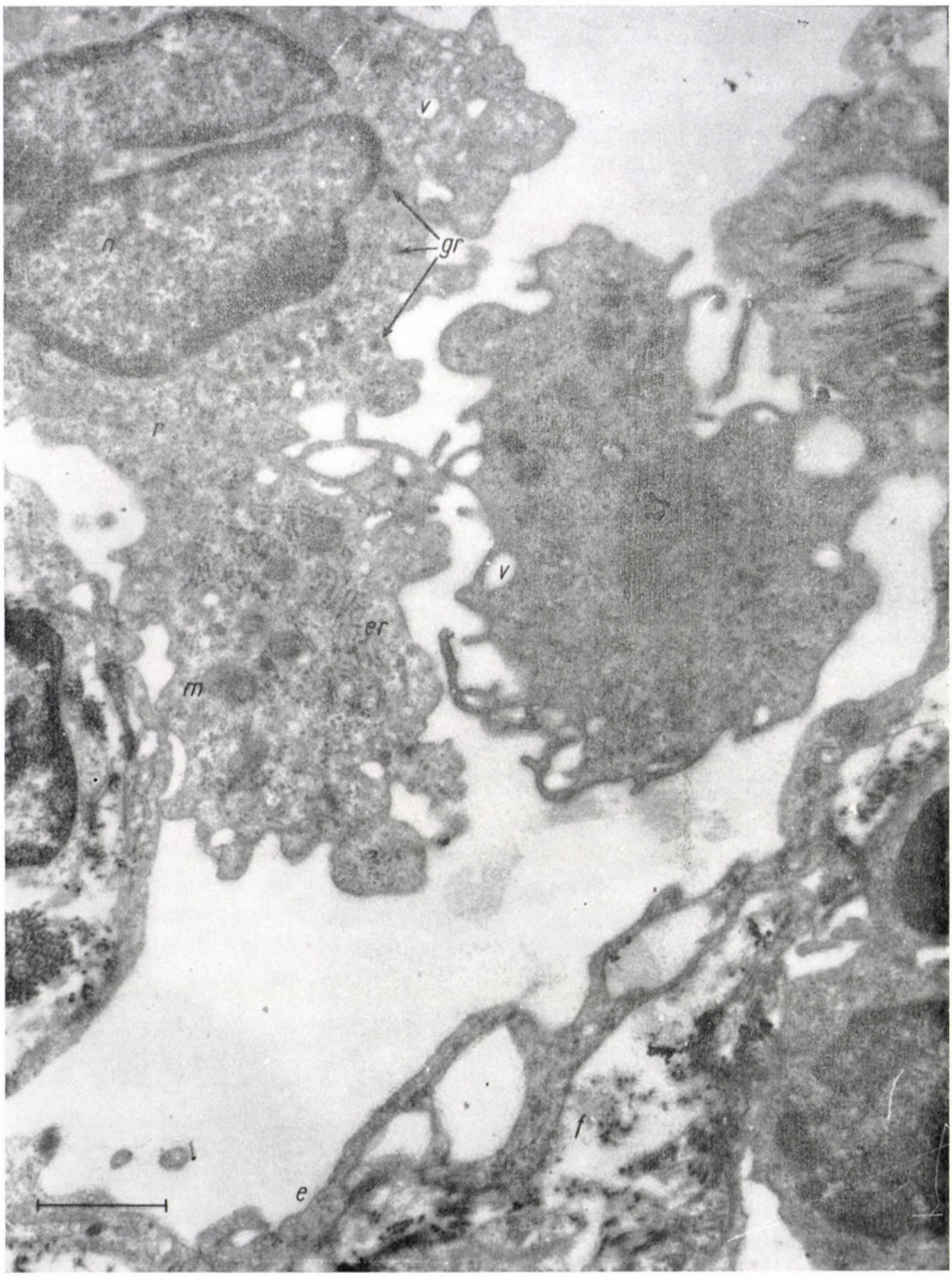

Fig. 7. Detail of a medullary sinus. Its wall consists of flat, irregular endothelium (*e*) and underneath, a layer of delicate connective-tissue fibrils (*f*). Two reticulum cells are seen in the cavity of the sinus. One of them emits numerous long microvilli. Their cytoplasm is well structured: it contains mitochondria (*m*), ribosomes (*r*) (Palade's granules), flattened ergastoplasmic vesicles (*er*), several minute, round, homogeneous osmiophilic granules (*gr*), and clear vacuoles (*v*); one of the cells has a multilobular nucleus

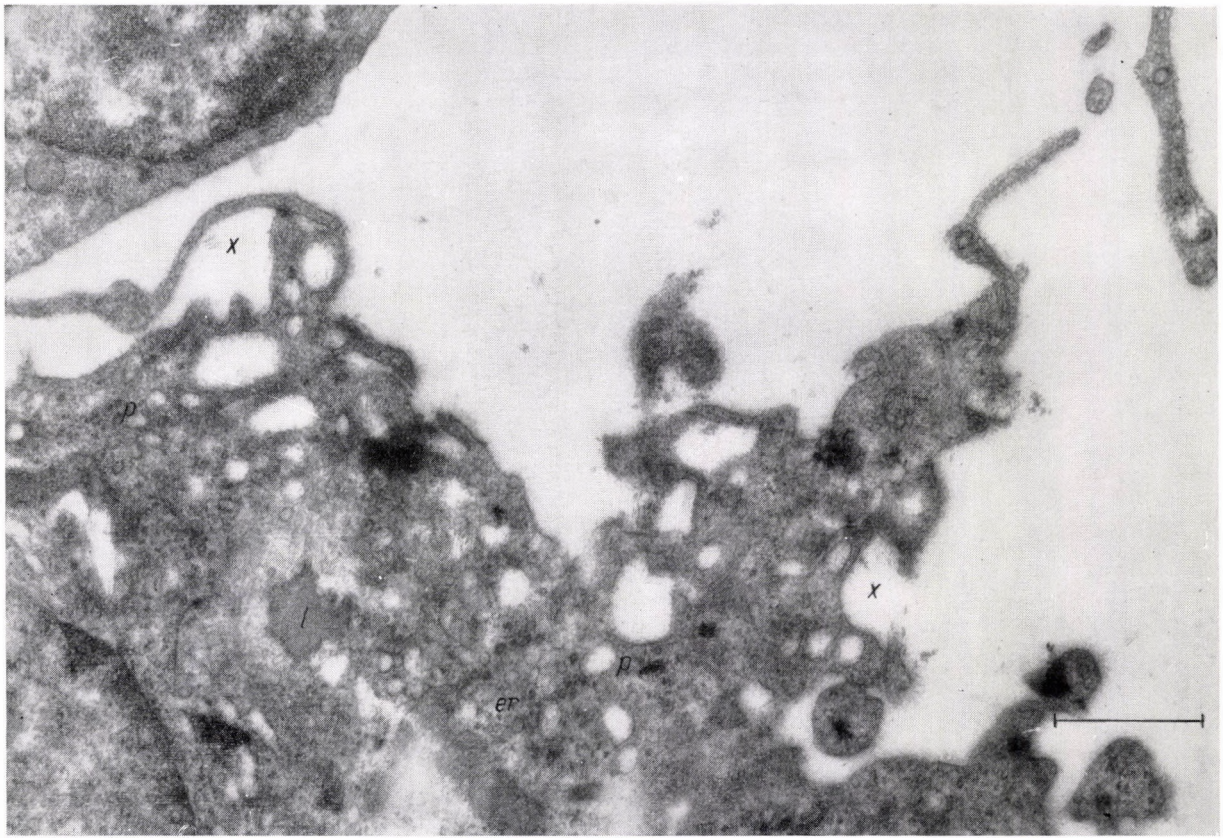

Fig. 8. Surface of a reticulum cell. The cytoplasm emits numerous villous or lamellar processes which form cavities (*x*) at several points. Several vacoules (*p*), bounded by a membrane similar in thickness to the cell membrane, with clear contents, can be seen near the surface of the cytoplasm; they are presumably products of pinocytosis. Numerous small vesicles (*er*) can also be observed in the same area of the cytoplasm; they probably belong to the endoplasmic reticulum
l = lipid droplet

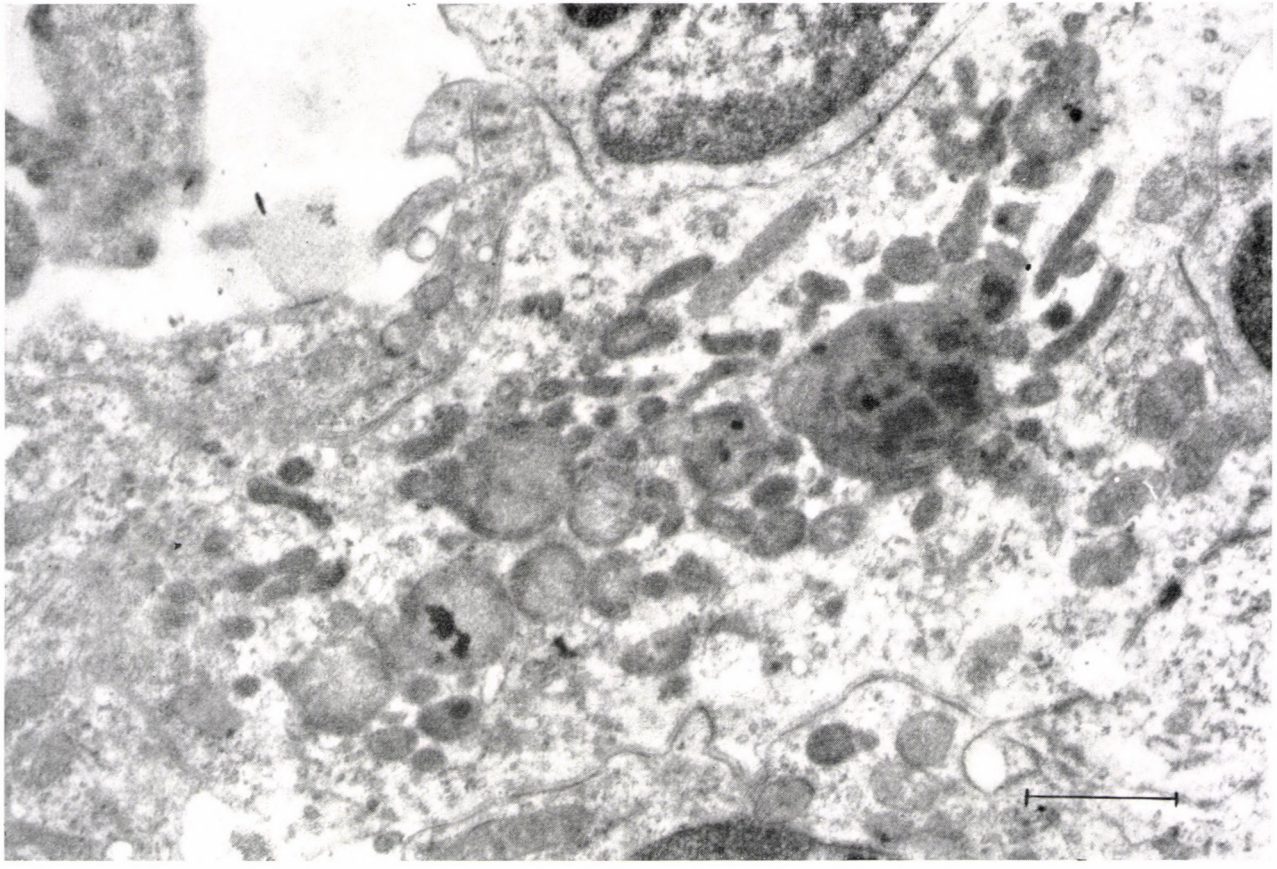

Fig. 9. Reticulum cell. Spherical osmiophilic particles, presumably surrounded by membranes, are visible in the cytoplasm. The central part is rarefied in some of them, while more compact osmiophilic elements are contained in others (lysosomes, phagocyted particles?)

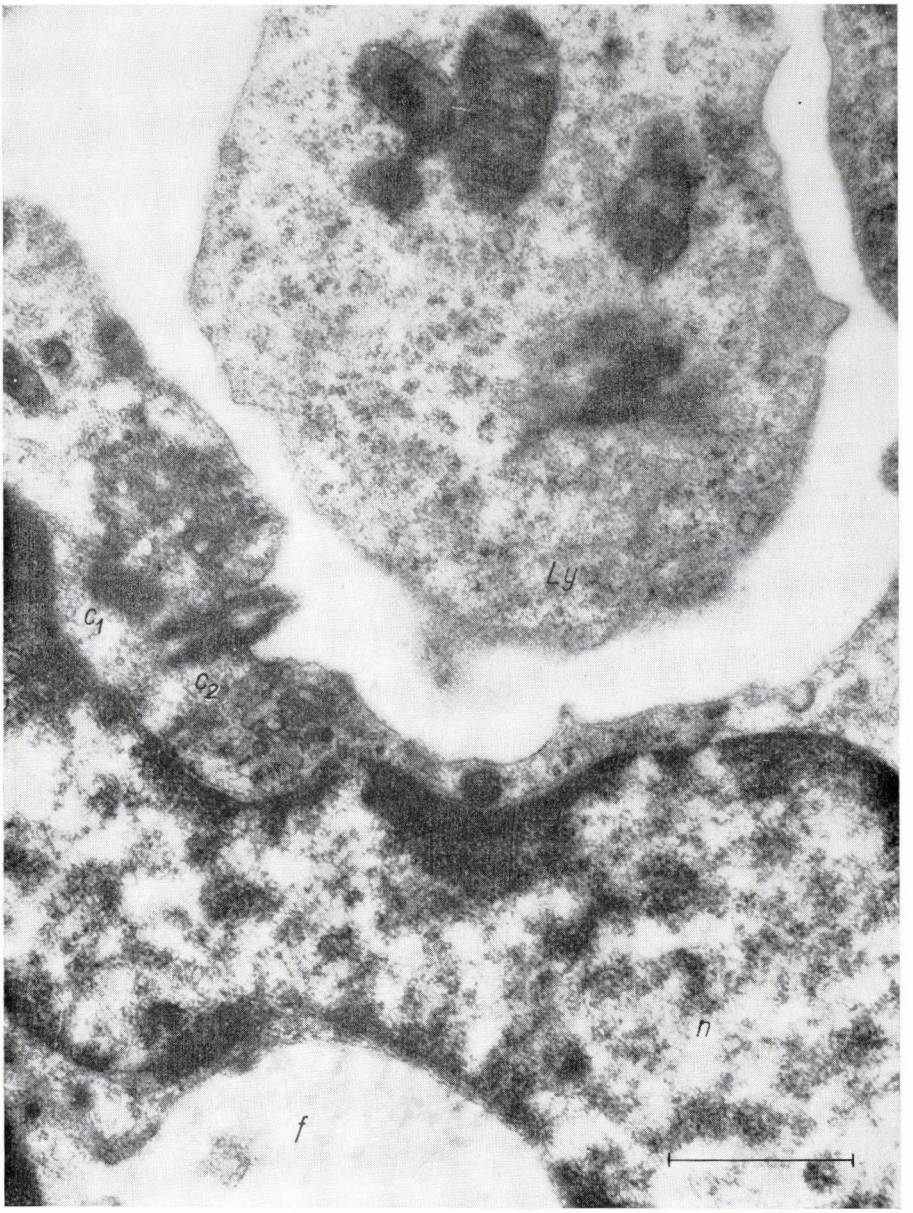

Fig. 10. Endothelial cell from a sinus. The major part of the cell is occupied by the flattened nucleus (*n*). Two centrosomes are seen near the nucleus, towards the lumen of the sinus. One is obliquely sectioned (c_1), the longitudinal axis of the other (c_2) is perpendicular to the cell membrane and forms a regular basal body. A cilium emerges from the latter which is covered by the cell membrane (oblique section)
ly = tangential section of lymphocyte; *f* = collagenous fibrils

The endothelium of the sinuses differs in certain respects from that of the blood vessels. The latter has a smooth outer surface and there is always a basement membrane underneath; also its inner surface is smooth, having only several minute microvilli. There were some pinocytotic vesicles in the endothelial cells of the blood vessels, but the structure of the cytoplasm did not display the spongy pattern found in the sinal endothelium. The endothelium of the blood capillaries may be so swollen in the lymph node that it sometimes occludes the lumen.

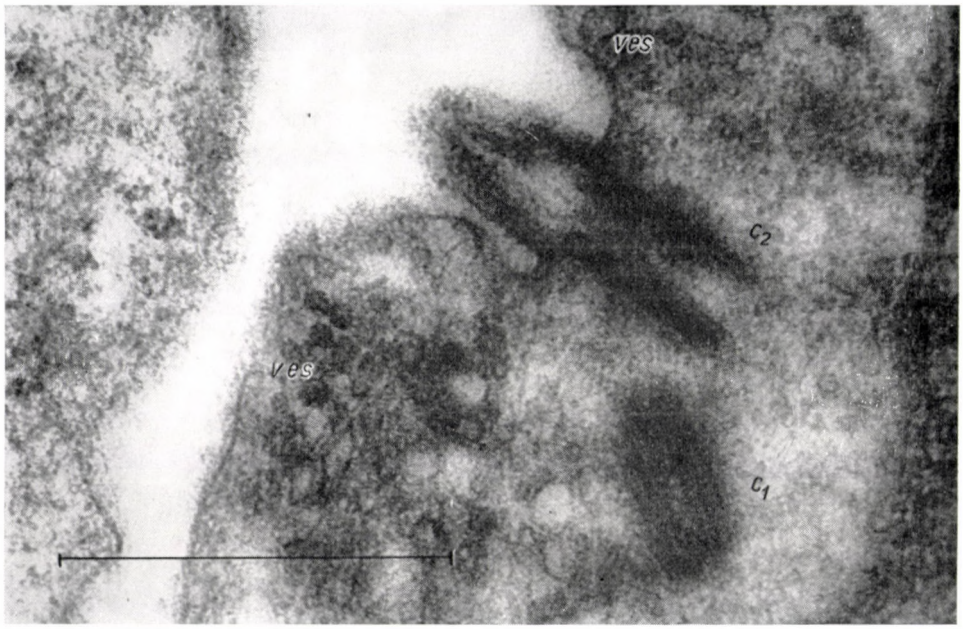

Fig. 11. Enlarged picture of a centrosome and the basal body shown in Fig. 10. Note the numerous minute vesicles (*ve*) around the centrosome

Discussion

As regards the structure of the capsule, it has been found to consist of lamellated connective tissue. Going inward, one finds more and more fibres. The number of collagenous fibres increases towards the cortex. Forming bundles they project into the interior, and constitute the frame of the lymph nodes. There are few elastic fibres and smooth muscles are absent altogether. The structure of the capsular fibrocytes allows the conclusion that they play no role in the essential functions of the lymph nodes.

We have failed to demonstrate endothelial cells on that surface of the marginal sinus which faces the cortex. In the mesenteric lymph node of the mouse, Moe (1960) found an intercellular gap 1 to 2 μ wide between the inner

endothelial cells of the marginal sinus, the like of which he failed to find in the endothelium of the intermediary sinuses. The evidence of our finding in guinea pigs supports the assumption that the wall of the sinus is formed here by the cells of the cortex itself, the lymphocytes and various forms of reticulum cells, so that the lymph is free to pass from the marginal sinus between and along the cortical cells without flowing along preformed pathways.

The structure of the medullary sinuses offers several points of interest. These sinuses are invariably lined by endothelium which is separated from the substance of the lymph node by a wider or narrower space, and the separation is made still more distinct by the presence of delicate fibres. The endothelial cells may jut out of the wall and bulge into the sinal cavity at several points. Microvilli and numerous membrane-like processes emerge from the sinusward surface of these cells which lend a labyrinthic character to the sinus. These processes may act as "undulating membranes", thus becoming factors in the morphological changes of the endothelial cells and in the transport of the contents of the sinus. Numerous vacuoles render the cytoplasm of the endothelial cells sponge-like and similar to the reticulum cells which have the same surface and inner structure. There exists thus a striking similarity between the reticulum cells and those of the sinal endothelium. Transitory forms can be seen. One may regard the reticulum cells as detached, activated endothelial cells, and the latter as flattened resting reticulum cells. This view seems to be sup ported by the structure of the cytoplasm and the occurrence of pinocytosis and phagocytosis.

Development of cilia on the endothelial cells of the sinuses may have been induced by certain local factors in which case they should be regarded as an aberration. It is worthy of note that also other endothelial cells of mesodermal origin may be provided with cilia, such as are found, for instance, around the nephrostomes in vertebrates of the lower orders.

Reticulum cells are pervaded by numerous wider or narrower passages and show, therefore, a spongy structure so that it is over a large surface that they are in contact with the lymph (phagocytosis, pinocytosis !). The presence of dense bodies (lysosomes) especially in the interior of the reticulum cells of the marginal sinus points to the possibility of enzyme synthesis, a morphological phenomenon indicative of processes of digestion occurring in the sinuses of lymph nodes. SORENSON (1960) has described these bodies as densely packed irregular or ovoid structures; and found, in addition, some crystalline particles.

The lumen of the sinuses has a veritably labyrinthine character on account of the manifold and interlacing processes of the endothelial and reticulum cells. The inner structure is, moreover, constantly changing owing to the motion of the cytoplasm. Such a labyrinthine structure makes it difficult for the lymphocytes to pass through the lymph nodes, and it is by means of cytoplasmic movement and mechanical influences (blood vessels, muscles, *i. e.* external forces) that lymph flow is maintained. The labyrinthine character of the

sinuses is so pronounced that a mechanical massage is necessary to ensure a mass flow of lymphocytes from the lymph node to the vas efferens. In agreement with HAN (1960), we have failed to find the syncytium of reticulum cells. Our observations were otherwise in harmony with those of MOE (1960) who studied the mesenteric lymph nodes of mice, as also with those of HAN (1960) who studied the mesenteric lymph nodes of rats. Our investigations concerning lymphocytogenesis, plasmocytes, etc., are in progress.

The above-described structure justifies the conclusion that the lymph, after having gained access to the marginal sinus, flows hence into passages with walls of constantly changing structure ("intermediary sinuses") into the medullary sinuses and keeps seeping forth in the channels formed by the cytoplasm of endothelial and reticulum cells. The lymph flow is probably promoted also by the movement of the "undulating membranes" and processes also of the endothelial and reticulum cells. Natural congestion of lymph in the interior of the nodes creates favourable conditions for absorption, enzymatic activity, the synthesis of immune substances, pinocytosis, phagocytosis, in brief, for all the manifold processes occurring in lymph nodes. The sinuses are probably no permanent structures, since a mutual transformation of endothelial and reticulum cells may cause a disappearance or reconstruction of the lymph sinuses, phenomena revealing processes of inner dynamic adaptation which could explain why we find lymph nodes with different structures in different parts of the body.

Summary

The structure of the capsule and the sinuses of lymph nodes has been studied in guinea pigs. Thorough observations of the endothelial and reticulum cells of the sinuses, as seen in electron microscopic pictures, lead to the conclusion that the lymph sinuses are no preformed structures but functionally determined and constantly changing cavities of sinusoid architecture. There is no fundamental difference between endothelial and reticulum cells; depending on functional requirements, they may transform one into the other.

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ДАННЫЕ ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОГО ИССЛЕДОВАНИЯ СТРУКТУРЫ ЛИМФАТИЧЕСКОГО УЗЛА

И. ТЁРЁ и П. РЕЛИХ

Авторы сообщают о своих наблюдениях в связи со структурой капсулы и синусов лимфатического узла морской свинки. Подробно излагается электронномикроскопическая картина синусового эндотелия и ретикулярных клеток. Авторы придерживаются того мнения, что лимфатические синусы не представляют собой детерминированные образования а являются функционально индуцированными, постоянно меняющимися щелями синусовой структуры. Эндотелий и ретикулярные клетки являются функционально преобразовывающимися друг в друга клеточными формами тех же клеток.

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG DER LYMPHDRÜSEN-STRUKTUR

I. TÖRŐ und P. RÖHLICH

Die Struktur der Kapsel und der Sinuse der Lymphdrüsen von Meerschweinchen wurde untersucht. Das elektronenmikroskopische Bild des Sinusendothels und der Retikulumzellen wird ausführlich beschrieben. Die Meinung wird vertreten, daß die Lymphdrüsensinuse keine determinierten Gebilde, sondern funktionsbedingte, sich ständig ändernde Spalten sinusartiger Struktur darstellen. Das Endothel und die Retikulumzellen sind Zellformen der gleichen Zellen, die funktionell ineinander umwandeln können.

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