

## An Electron Microscope Study on the Sinus Endothelial Cells of Lymph Node with Reference to their Relation to the Reticuloendothelial System\*

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### Introduction

Among the cells of the reticuloendothelial system widely distributed throughout the body, the sinus endothelial cell of lymph node is one of those which have extremely vague backgrounds. According to Aschoff<sup>3)</sup> and Kiyono<sup>16)</sup> who proposed the concept of reticuloendothelial system, this cell was essentially one of the representatives of the reticuloendothelial system and nothing but the reticulum cell arranged along the sinus wall. On the other hand, based on their extensive studies on this system, Akazaki<sup>2)</sup> and his co-workers considered the sinus endothelial cell in the direction of proper endothelia.

Therefore, it seems significant to study sinus endothelial cells from a cytological view point with the electron microscope. Many electron microscopic studies of lymph nodes have recently appeared<sup>4,5,7,11~14,18~20,26,29)</sup>, although the property of sinus lining cells has not always been emphasized. However, these studies failed to agree with each other; some recognized the presence of endothelial cells morphologically identical with the proper endothelia<sup>14,18,20,26,29)</sup>, while others insisted on the direct coverage of the sinus wall by the reticulum cells<sup>4,11,13,19)</sup>. The main causes for such disagreement are thought as follows; firstly the orientation of the sinus within the specimen is difficult in the electron microscopic study, leading to the false identification of the space outside the sinus as a sinus. Secondly the fixation for electron microscopy is rather difficult in the lymphatic tissues, resulting in the disorganization of fine structure so that the characteristics of them are hard to recognize. Finally at third, the lymph nodes have been physiologically subjected to various stimuli, causing some disorders in structure before any experimental procedures.

Taking all these points into consideration, vital fixations of the inguinal lymph nodes from 49 mice were carried out. Then utilizing the cortical sinuses as the main subjects, those without disorder in the sinus structures were carefully selected. Since on one side the cortical sinus showed a characteristic structure of the capsule, the sinus was distinctly recognized in most cases. Based on these observations, fine structures of the sinus endothelial cells were traced from the cortical sinus to the medullary one. Phagocytic activities of the sinus endothelial cells were also studied. Moreover, the appearance of sinus endothelial cells after repeated foreign body stimulations through the lymphatic channel was compared with that of control state. From the differences in fine structure and function

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between normal and stimulated condition, the cytological orientation of the sinus endothelial cells of lymph node in the concept of reticuloendothelial system was attempted.

### Materials and Methods

Healthy adult male mice of inbred Swiss strain were divided into three groups, and the inguinal lymph nodes of them were examined.

Group 1; control

Group 2; 0.2 ml of dextran iron solution (Meito's product, containing 50 mg of dextran iron in 1 ml solution) was once injected subcutaneously in hind footpads 30 to 60 minutes prior to sampling.

Group 3; 0.1 ml of 1% India ink in physiological saline was injected subcutaneously in hind footpads on 14 consecutive days followed with an injection of 0.2 ml dextran iron solution in the same manner as group 2.

The animals were maintained on laboratory diet for the entire course of the experiment.

Human materials; paragastric lymph nodes without metastasis from 3 patients of gastric cancer were also examined.

Inguinal lymph nodes of all experimental mice were exposed under ether anesthesia and covered by a few drops of 1% osmium tetroxide solution buffered to pH 7.4 to 7.6 with veronal acetate<sup>6)</sup>. After 5 to 15 minutes the lymph nodes were cut into small pieces with osmium blackened perilymphatic fatty tissues and placed into the same fixative for 2 hours at 4°C.

The extracapsular fatty tissues were black in unstained section. Accordingly the specimens with cortical sinus were easily selected utilizing the black extracapsular fat as a marker without any special staining. Because the cortical sinus is certain to lie between black fatty tissue and brown lymphatic parenchyma. This was a quite useful method to select the specimens from a few hundreds ones.

In the case of human materials, surgically removed lymph nodes were fixed for electron microscopy in the usual manner.

All specimens were dehydrated by cooled ethanol and embedded in Epon 812 according to Luft<sup>17)</sup>. They were cut with a Porter-Blum MT-1 ultramicrotome using glass knives. Sections were stained with uranyl acetate<sup>31)</sup> and examined by a Hitachi HS-7 electron microscope. Low magnification views at 1,500 to 2,500 diameters were usually taken and higher magnifications up to 15,000 were added when required.

Inguinal lymph nodes of another side obtained from the same experimental mice were fixed with Carnoy's solution and examined histologically after hematoxylin and eosin, Berlin blue, or methenamine silver stain to compare with the electron micrographs. Electron histochemical examinations for acid phosphatase activity were performed on a few materials by the method of Sabatini et al.<sup>24)</sup>.

## Observations

### I. *Normal structure of the sinuses and the sinus endothelial cells*

#### 1. *Structure of the cortical sinus*

Underneath the extracapsular fatty tissues, the capsule of lymph node lay as a sheath of collagenous fibers about  $3\mu$  width with a few fibrocytes and macrophages. A few afferent lymphatic vessels penetrating the capsule were seen. The walls of lymphatic vessels were composed of thinly flattened slightly electron opaque cytoplasmic processes with a small amount of cytoplasmic organelles. The nucleus was not occasionally seen. As Fraley and Weiss<sup>9)</sup> have reported, lymphatic vessels usually lacked a basement membrane. The junctions between the adjacent endothelial cells showed no tight junction structure. Micropinocytotic vesicles were present at the luminal and abluminal surfaces of lymphatic endothelia.

The cortical sinus was observed as a subcapsular lymphatic lumen which was frequently occupied by pseudopod-like cytoplasmic processes of reticulum cells and by some lymphocytes. The diameter of the lumina ranged from 3 to  $10\mu$ . In well fixed specimens, the lumina were filled with lymphoplasm. The trabeculae, projected bundles of collagenous fibers from the capsule, sometimes traversed the lumen.

#### 2. *Sinus endothelial cells of the capsular side.*

The inner surface of the capsule was lined with attenuated and elongated sinus endothelial cells. The cytoplasm of the cells was as thin as  $0.3\mu$  in width and slightly electron opaque. Several small rod-shaped mitochondria and Golgi apparatus were seen in the perinuclear portion of the cytoplasm. The nuclei shaped spindle and contained rather dense homogeneous nuclear substances and no nucleolus in most instances. Micropinocytotic vesicles were seen at the both surfaces and within the intervening cytoplasm.

Subjacent to the abluminal surface, a basement membrane was seen that separated the endothelial cells from the capsule. Tight junction structures were occasionally observed between the adjacent endothelial cells. No discontinuity among the endothelial cells was seen in the capsular side.

#### 3. *Sinus endothelial cells of lymphatic parenchymal side of the cortical sinus.*

The sinus endothelial cells which border the capsular side of the cortical sinus is said to turn the direction at the end of the sinus or go across the lumen associated with the trabeculae to cover the lymphatic parenchyma. Therefore, the endothelial cells of lymphatic parenchymal side should be essentially similar to those of the capsular side.

However, as a matter of fact, the endothelial cells of lymphatic parenchymal side appeared thicker and slightly more electron lucent than those of the capsular side. Endothelial cells sometimes appeared spindle in shape. Junctions between the adjacent endothelial cells usually showed complexed interdigitations, but typical tight junction structures were less frequently seen. The basement membrane, which has been clearly recognized as an about  $250\text{ m}\mu$  thick dense layer on the capsular side, was observed as an amorphous ground substance layer  $0.5$  to  $2\mu$  in thickness. This ground substance layer contained

some collagenous fibers and finer fibrills. Clark<sup>7)</sup> has mentioned this as a layer of reticulum fibers which consisted chiefly of collagenous fibrils, associated with a few elastic fibers and a finely fibrillar material. But after methenamine silver stain, even in the case when no reticulum fiber of reticulum cells was detected, this layer was stained as strongly as the basement membrane of the sinus endothelial cells of the capsular side.

In the lymphatic parenchymal side there often existed some gaps in 1 to 3  $\mu$  in width between the sinus endothelial cells. Each perforation was occluded by a reticulum cell or lymphocyte, lying partially in the sinus and partially in the lymphatic parenchyma, constricted by the wall of the sinus. After repeated stimulations, these gaps increased in number and diameter resulting in an increase of reticulum cells and lymphocytes within the sinus lumen.

#### 4. *Endothelial cells of the medullary sinus.*

The endothelial cells appeared swollen and the cytoplasm became more electron lucent than in the lymphatic parenchymal side of the cortical sinus. Gaps between the endothelial cells also increased. In only rare instances tight junction structures between the endothelial cells were recognized. In the cases when the gaps became as wide as 10  $\mu$  or more, it was difficult to identify the reticulum cells occluding the gaps from the sinus endothelial cells. It seems to be one of the reasons to make disagreements about the features of sinus endothelial cells.

#### 5. *Reticulum cells in the sinus and lymphatic parenchyma.*

Reticulum cells were sometimes exceptionally observed as elongated cells but in most cases showed stellate shape with numerous complicated cytoplasmic processes. The matrices of these projections were thin without any cytoplasmic organelle, like the pseudopods of neutrophilic leukocytes as have been reported by Senda et al.<sup>25)</sup>

In the cytoplasm around the nucleus, abundant organelles with numerous phagosomes in various size and shape were seen. The mitochondria of reticulum cells were round and about 0.5  $\mu$  in diameter. They had transparent mitochondrial matrices and radially situated short cristae which did not intersect the entire length of diameter. These mitochondria were differ from those of endothelial cells which were oval and had darker matrices with rather long cristae running transverse the shorter diameter.

The nucleus of reticulum cell was larger than that of endothelial cell and was round in configuration. The central nucleoplasm was less dense than the peripheral one and the latter formed a narrow dark rim about the edge of the nucleus. A nucleolus was occasionally seen in each nucleus.

Reticulum cells were in contact with each other by simple cell-to-cell abutment or by complexed cytoplasmic intertwinings. No tight junction or desmosome structure was recognized. Recently Swartzendruber<sup>27,28)</sup> has reported the presence of desmosomes among the reticulum cells in the germinal center of mouse spleen. In the present investigation, reticulum cells having desmosomes were frequently observed in the germinal center of human lymph node. These structures of reticulum cells in germinal center were also recognized in human tonsils (K. Kawaguchi, personal communication). However, at least in the reticulum cells in the sinus or lymphatic parenchyma, no such structure was seen.



As Clark<sup>7)</sup> has pointed, reticulum cells in the sinus did not contact directly to the basement membrane or the collagenous fiber sheath.

## II. *The fate of injected iron particles.*

Histologically, dextran iron was detected in the cortical sinus after Berlin blue stain within 30 minutes after injection. The iron particles were observed electron microscopically in high condensation in the lymphoplasma adjacent to the cytoplasmic processes of reticulum cells in the sinus lumen. They were also recognized at first within the pinocytotic canals of reticulum cells and then condensed and aggregated into electron dense phagosomes around the nucleus.

On the contrary, the sinus endothelial cells had neither cytoplasmic process, pinocytotic canal, nor iron containing phagosome in their cytoplasm.

## III. *Sinus endothelial cells after stimulation.*

As shown in the light micrographs in Figs. 12 and 13, differences between the phagocytic activities of the sinus endothelial cells in experimental group 2 and 3 were quite evident. Namely in group 2, when single dose of dextran iron was given 60 minutes prior to sampling, the iron particles were phagocytized only by the reticulum cells situated in the sinus or in the periphery of lymphatic parenchyma. On the other hand, in group 3, when dextran iron was given after the 14 consecutive days injections of India ink, the sinus endothelial cells also phagocytized the iron particles as strongly as the reticulum cells. In these two experiments, the doses of injected iron solution were same. Electron microscopically, in the latter group, the endothelial cells of capsular side of the cortical sinus which had been flattened as the proper endothelia of blood vessels, swelled and contained a lot of phagosomes in their cytoplasm.

The endothelial cells of lymphatic parenchymal side of the cortical sinus and those of the medullary one which had already swollen under unstimulated condition, swelled more evidently and phagocytized the iron particles as abundantly as the reticulum cells. However, at the same time the cytoplasm of these endothelial cells were also occupied by a large number of vacuoles.

In the additional observation of regional lymph nodes from 3 patients of gastric cancer which were considered to have suffered more prolonged stimulations, the sinus endothelial cells were found to be swollen markedly and bulged into the lumen. The cytoplasm of them were occupied by numerous phagosomes and vacuoles so that they resembled closely to the reticulum cells. But an attention must be paid in the point that the sinus endothelial cells always embraced the basement membrane even in a part. Furthermore, the sinus endothelial cells did not lose the complexed intercellular junctions or tight junction structures between the adjacent endothelial cells. This may be the reason why they are usually recognized histologically in syncytial state under the condition of so-called sinus catarrh. The features of mitochondria of the endothelial cells were, as stated above, also differ from those of the reticulum cells.

## Discussion

As is well known, the concept of reticuloendothelial system was at first proposed by

Landou et al. under the direction of Aschoff in 1914 as a specific type of cells playing an important role in the metabolism of cholesterol. In 1924 Aschoff<sup>3)</sup> established the concept of this system as the cell group of mesenchymal origin with positive activities for supravital stain. Aschoff listed the following cell group as the reticuloendothelial system in a narrow sense.

1) Reticulum cells of spleen. Reticulum cells of lymph nodes and other lymphatic tissues.

2) Reticuloendothelia of sinuses of spleen and lymph nodes. Reticuloendothelia of capillaries of liver lobules (Kupffer's stellate cells), bone marrow, adrenal cortex, and anterior pituitary.

The reticuloendothelia in the concept of Aschoff were considered to be fixed reticulum cells facing to a blood or lymph stream.

In 1914, Kiyono<sup>16)</sup> verified a specific mesenchymal cell group with marked phagocytic activity as "histiocytic cell system". According to Kiyono, both reticulum cells and reticuloendothelial cells turn to histiocytes by being free from the cell syncytium and vice versa.

Hence it follows that according to Aschoff and Kiyono, the reticuloendothelial system is, in the essential meaning, the reticulum cell system or histiocyte system. It must be noted that the system was considered to consist of one kind of cell origin.

On the other hand, Akazaki<sup>1)</sup>, who has been studying this system since the early 1940's, considered the sinus endothelial cells in the direction of proper endothelia from the following points; firstly the sinus endothelial cells of lymph node show lower activity for the vital staining in comparison with the reticulum cells. Embryologically at second, they seem to develop from the endothelia of lymphatic vessels. Thirdly, in pathological condition they tend to be isolated from the sinus wall in histological syncytial state. And fourthly their cytoplasmic processes have no close relation to the reticulum fibers.

From these reasons Akazaki omitted the sinus endothelial cell of lymph node from the reticuloendothelial system. But as an exception, he reserved those of mesenteric lymph node in this system. In mesenteric nodes, the sinus endothelial cells showed positive phagocytic activities after birth, whereas in fetal state they did not show phagocytic activity. Akazaki interpreted this phenomenon as that the sinus endothelia of mesenteric lymph nodes had no phagocytic activity in their nature, but when stimulated after birth by endotoxin or cell debris which were produced by the increased intramesenteric bacteria, they became phagocytic. This interpretation is quite important. Because, according to Akazaki the concept of reticuloendothelial system is reconstructed as a functional entity of at least more than two cell systems.

In the present electron microscopic observations, the sinus endothelial cells of lymph node had a basement membrane along with their abluminal surfaces. Besides they showed complexed intercellular connections with tight junction structures<sup>15)</sup> between adjacent cells as Watanabe<sup>30)</sup> has reported in the sinus endothelial cells of bone marrow. Thus the sinus endothelial cells of lymph node were quite similar in fine structure to the proper endothelia<sup>23)</sup> of blood vessels.

Furthermore, when iron particles were injected via lymphatic channel, the sinus endo-

thelial cells did not show phagocytic activity. Then the sinus endothelial cells of lymph node resemble to the proper endothelia of blood vessels not only in fine structure but also in function. Those who showed marked phagocytic activities to the injected iron particles were the reticulum cells standing in the sinus lumen. As iron particles were observed in high condensation close to the pseudopod-like cytoplasmic processes of reticulum cells, it may be considered that these cells were able to move freely in the sinus lumen toward the foreign bodies and make them condense waving their cytoplasmic processes as ameba does.

On the contrary, since the sinus endothelia are fixed tightly to the capsule or the sinus wall and connected with each other by complexed intercellular junctions or tight junction structures, they should be unable to move freely. Moreover, as they possess no cytoplasmic process, it may be quite reasonable that they show no phagocytic activity.

The mechanism of vital stain, that is the characteristic feature of the cells of reticuloendothelial system, has been pointed out electron microscopically by Onoé and Tsukada<sup>22)</sup> as nothing but an intensified form of pinocytosis in an unselective fashion in a significant magnitude. In this meaning, it may be concluded that the sinus endothelia of lymph node must be omitted from the categories of reticuloendothelial system under normal condition.

But it is quite interesting that these sinus endothelia became distinctly phagocytic when iron particles were given after the consecutive India ink injections for 2 weeks. This phenomenon is not considered to occur as a result of secondary effect from the blockade of reticuloendothelial system, as Cotran<sup>8)</sup> has observed in the proper endothelia of blood capillaries in cardiac muscle. Because light microscopically, the injected India ink particles are observed in only some of the reticulum cells in or underneath the sinus. Furthermore, the total dose of India ink which was contained in the physiological saline of 14 days injections is not more than 0.25 mℓ. When iron particles were given after single injection of 0.25 mℓ India ink, sinus endothelia did not phagocytize them. Therefore, this increase, or aquirement in precise meaning, of phagocytic activities may be considered as that the endothelia turned to the reticuloendothelia with prolonged foreign body stimulations. This result suggests a possibility that the reticuloendothelia are nothing but the proper endothelia which are situated in an irritative circumstance. But it must be also taken into consideration that the sinus endothelia of lymph node have already had some phagocytic activities in a masked state, because they turned easily to the reticuloendothelia by foreign body stimulations for only 2 weeks. On this point, another experiment utilizing the proper endothelia of blood vessels is now in progress.

On the other hand, the sinus endothelia which turned to phagocytic showed a marked increase of vacuoles in their cytoplasm. In the case of so-called sinus catarrh, the cytoplasm of sinus endothelia were almost occupied by numerous vacuoles with increased phagosomes. In such cases they seemed as if they were degenerated and showed a tendency to fall into the sinus lumen. These degenerative changes have been reported by Imai<sup>14)</sup> in the sinus endothelial cells of lymph node after a treatment of zymosan. They are interpreted as the dissociation of the activities between the phagocytosis and the digestion. Concerning the difference of digestive activity between the cells of reticuloendothelial system and sinus endothelial cells of lymph node, Miura<sup>18)</sup> has observed that

the phagosomes of sinus endothelial cells were not condensed even after 1 week following the foreign body injection, whereas those of reticulum cells were rapidly condensed. Frankel et al.<sup>10)</sup> have observed in the liver that the condensation of injected carbon particles were recognized in the Kupffer cells within 48 hours after the injection. Electron histochemically as well as histochemically, acid phosphatase activities were demonstrated in the phagosomes of reticulum cells as strongly as Onoé et al.<sup>21)</sup> has shown in the Kupffer cells. However, the sinus endothelial cells showed no such strong acid phosphatase activity although they turned to phagocytic. In these aspects, further investigations about the establishment of the concept of reticuloendothelial system may be required from a viewpoint of not only the phagocytic activity but also the digestive activity of cells.

On the other hand, the sinus endothelia of lymph node lose the complete basement membrane and tight junction structures inversely as they acquire the phagocytic activities. Same relationship between the basement membrane structures and phagocytic activities has been observed in my electron microscopic study on the endothelia of blood vessels in adrenal cortex (unpublished data). Namely in zona glomerulosa, where the endothelia showed trace phagocytic activity, they had a complete basement membrane. Therefore, they were separated from the adrenal cells by two layers of basement membrane, one from parenchymatous cells and one from themselves. On the contrary, in zona fasciculata where the endothelia showed marked phagocytic activity, they sometimes lacked a basement membrane. In such cases, the endothelia of blood capillaries were separated from the parenchyma by only an incomplete basement membrane of adrenocortical cells. It was also observed that the cortical cells were stretching numerous microvilli toward the endothelia. This is quite similar to the relation between Kupffer cells and parenchymatous cells in the Disse space of liver.

It may be suggested that the endothelia are able to turn to phagocytic not only by foreign body stimulation but also by an intimate interrelation between the parenchymatous cells adjacent to them.

### Summary

1. A study was made on the fine structure and function of the sinus endothelial cells of lymph node to know more precisely about the features of them with reference to the relation to the reticuloendothelial system.

2. In normal condition they showed no phagocytic activity whereas reticulum cells in the sinus showed marked phagocytic activity.

3. Electron microscopically they were identical with the proper endothelia of blood vessels. Namely they had a basement membrane and connected with each other by tight junction structures.

4. These results led to the conclusion that the sinus endothelial cells of lymph node had to be omitted from the categories of reticuloendothelial system.

5. However, they turned to phagocytic after the foreign body stimulation for 2 weeks.

6. This phenomenon showed a possibility that the reticuloendothelial cells were not specific cells but only the proper endothelia which have been situated in an irritative

circumstance.

7. The significance of these results on the concept of reticuloendothelial system was briefly discussed.

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### Explanation of figures

- Figs. 1 and 2** The capsule of lymph node is seen in the upper parts, and the cortical sinus occupies the lower halves of the electron micrographs.
- The capsule are composed of intermingling bundles of collagenous fibers (C) with some fibrocytes (F) and smooth muscle cells (SM).
- The sinus lumen (S) is occasionally filled with some lymphocytes and numerous cytoplasmic processes (P) of the reticulum cells. The sinus walls are bordered by attenuated cytoplasm of the endothelial cells (E), which are somewhat electron opaque in comparison with the matrices of reticulum cells. Tight junction structures (T) between the adjacent endothelial cells are usually seen and no discontinuity is recognized in the capsular side. Fig. 1  $\times 6,000$ , Fig. 2  $\times 11,000$ .
- Fig. 3** In somewhat higher magnification electron micrograph, the sinus endothelial cells show a basement membrane (BM) along with their abluminal surfaces in the capsular side of the cortical sinus.
- Micropinocytotic vesicles (V) are seen in the both surfaces and within the intervening cytoplasm. No phagosome are recognized.  $\times 30,000$ .
- Fig. 4** The endothelial cells (E) of the lymphatic parenchymal side of cortical sinus are essentially similar in structure to those of the capsular side. However, they are observed slightly swollen. The nucleus is spindle in shape and the nucleoplasm disperses rather homogenous. No nucleolus is recognized in most cases.  $\times 4,500$ .
- Fig. 5** The basement membrane of sinus endothelial cells is seen as an amorphous ground substance layer (BM)  $0.5-2 \mu$  thick in the lymphatic parenchymal side. This layer sometimes contains collagenous fibers (C) or finner fibrils.  $\times 9,000$ .
- Fig. 6** The wall of cortical sinus is sometimes perforated in the lymphatic parenchymal side. Junctions between the adjacent endothelial cells usually show complexed interdigitations but tight junction structures are less frequently seen than in the capsular side.
- Gaps between the endothelial cells (arrows) are usually occluded by lymphocytes (L) or the cytoplasmic processes of reticulum cells (R).  $\times 3,500$ .
- Fig. 7** Gaps between the endothelial cells increase in number and diameter in the medullary sinus. Occasionally, large gaps as wide as more than  $10 \mu$  in diameter are found. In these cases discrimination of the sinus endothelial cells from the reticulum cells occluding the gaps are rather difficult. This may be one of the reasons to make disagreement about the features of sinus endothelial cells. The endothelial cells of the medullary sinus swell slightly and the cytoplasm is seen somewhat electron lucent than those of the cortical sinus.
- Injected iron particles are seen within the cytoplasm of reticulum cells (R) in and underneath the sinus, while the sinus endothelial cells (E) show no phagocytic activity.  $\times 4,500$ .
- Fig. 8** Schematic illustration of the low magnification electron microscopic survey picture of the structure of the sinuses and the sinus endothelial cells.
- Fig. 9** Desmosome structures (D) are frequently observed among the cytoplasmic processes of reticulum cells in germinal center of human lymph nodes, whereas no desmosome nor

tight junction structure is recognized between the reticulum cells within the sinus or in the periphery of lymphatic parenchyma. Those showing tight junction structures in the lymph node are the endothelial cells of the sinuses and those of blood and lymphatic vessels.  $\times 14,000$ .

**Fig. 10** Somewhat higher magnification view of a desmosome structure between the adjacent cytoplasmic processes of reticulum cells in germinal center from human lymph node.  $\times 21,000$ .

**Fig. 11** The basement membranes of sinus endothelial cells are clearly demonstrated in the light microscopic preparations after methenamine silver stain as strongly as those of blood vessels, while the reticulum fibers of reticulum cells are scarcely or not stained.  $\times 200$ .

**Fig. 12** Injected iron particles via lymphatic channel are recognized in the lymphoplasm within the cortical sinus 30 minutes after injection.

They are rapidly phagocytized by the reticulum cells situated in the sinus lumen. They are at first found in the pinocytotic canals and then condensed into electron dense phagosomes (P) around the nucleus. No phagosome is seen in the cytoplasm of the endothelial cells (E).  $\times 7,000$ .

**Fig. 13** Light micrograph of the cortical sinus from mouse lymph node 30 minutes after the injection of dextran iron solution.

Phagocytized iron particles are seen only within the cytoplasm of the reticulum cells (R) standing in the sinus lumen, while no iron particle is recognized in the sinus endothelial cells (E). Hematoxylin and eosin stain.  $\times 600$ .

**Figs. 14 and 15** When single dose of dextran iron solution was injected after non-immunological foreign body stimulations for 2 weeks, the sinus endothelial cells (E) turned to phagocytic as strongly as the reticulum cells (R) in the sinus lumen.

Light micrograph after hematoxylin and eosin stain (Fig. 14) and low magnification electron micrograph (Fig. 15) show marked phagocytic activities of the endothelial cells lining the sinus. The sinus endothelial cells which contain numerous phagosomes in their cytoplasm seem to be degenerative. Fig. 14  $\times 1,500$ , Fig. 15  $\times 2,700$ .

**Fig. 16** Under the condition of so-called sinus catarrh, since the sinus endothelial cells (E) swell markedly and turn to phagocytic, they are closely resemble to the reticulum cells.

But they always embrace the basement membrane (BM) even in a part and the features of mitochondria are differ from those of the reticulum cells (R).

The cytoplasm of them are occupied by large vacuoles (V), while those of the reticulum cells rarely have vacuoles.  $\times 7,000$ .

**Figs. 17 and 18** Electron micrographs of the endothelial cells of blood capillaries (E) in zona glomerulosa (Fig. 17) and zona fasciculata (Fig. 18) from normal mouse adrenal cortex.

The endothelial cells are strongly phagocytic in zona fasciculata while those of zona glomerulosa are scarcely phagocytic.

In the former, they show no basement membrane along with their abluminal surfaces, whereas the latter have typical basement membrane (arrows). Fig. 17  $\times 6,000$ , Fig. 18  $\times 7,500$ .



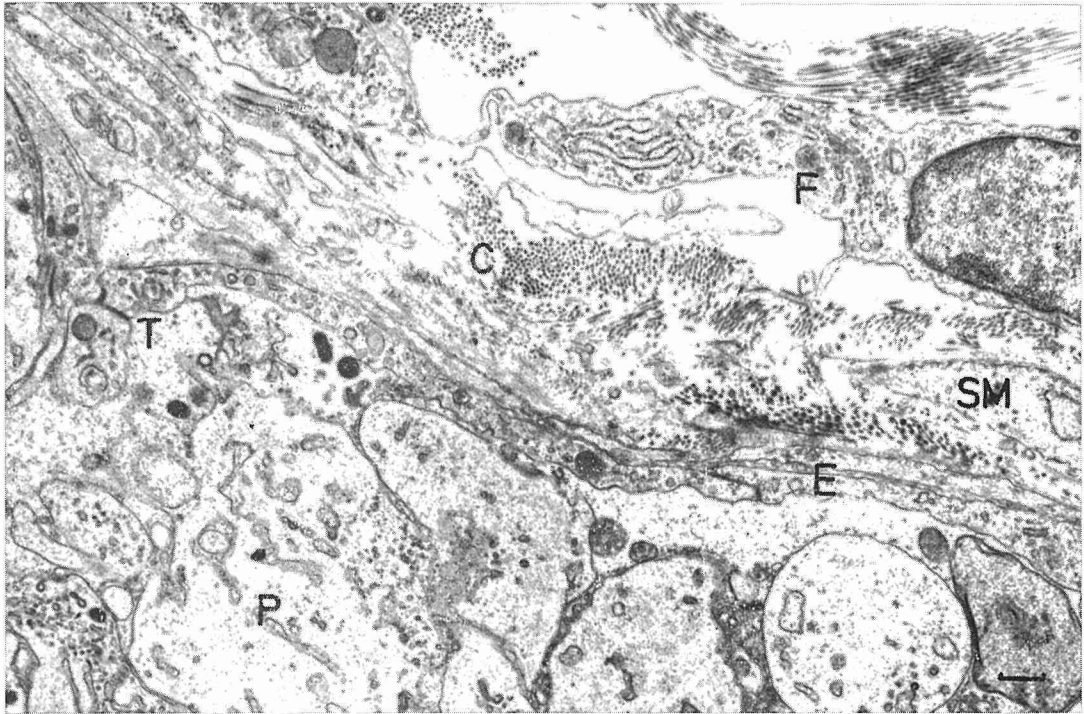


Fig. 1

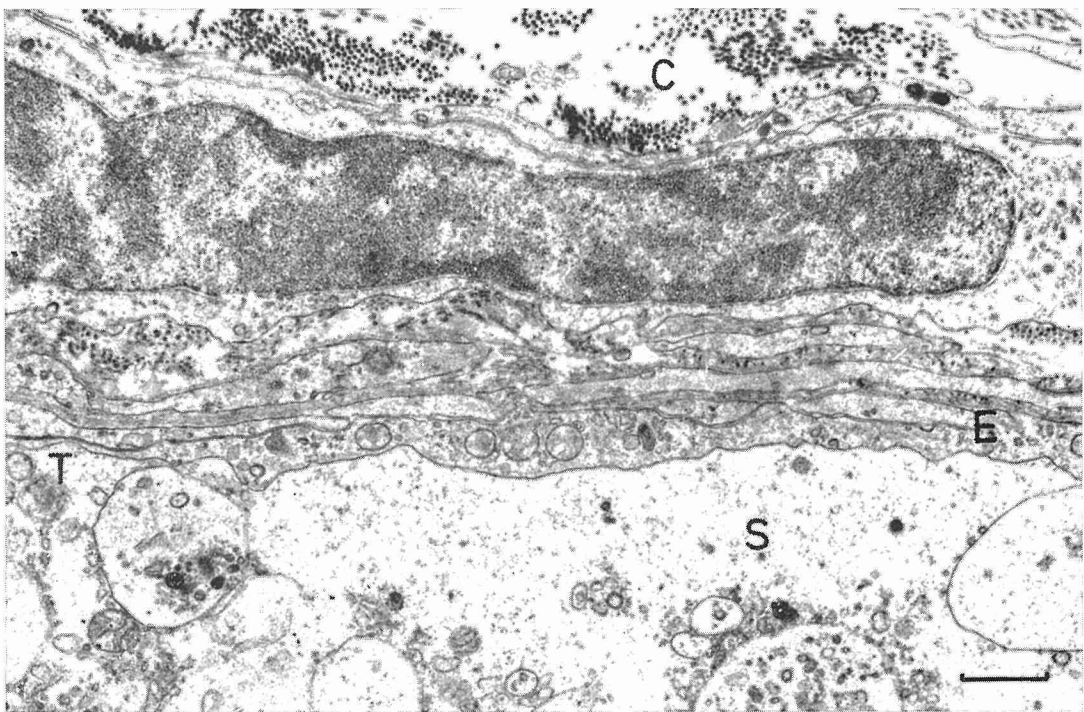


Fig. 2

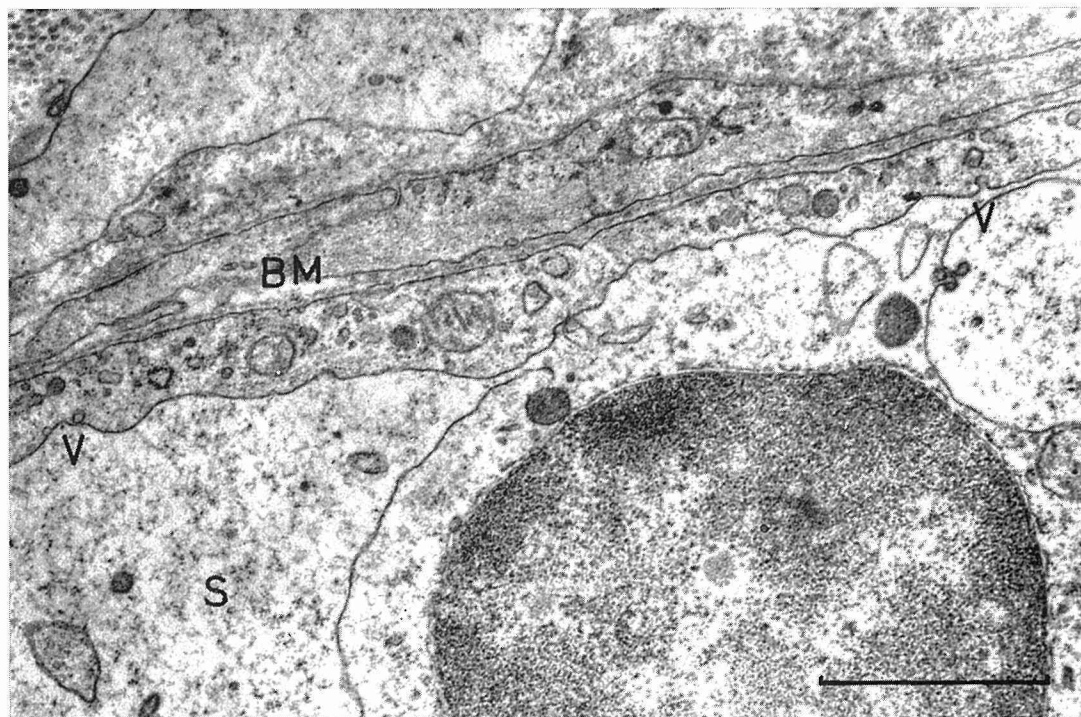


Fig. 3

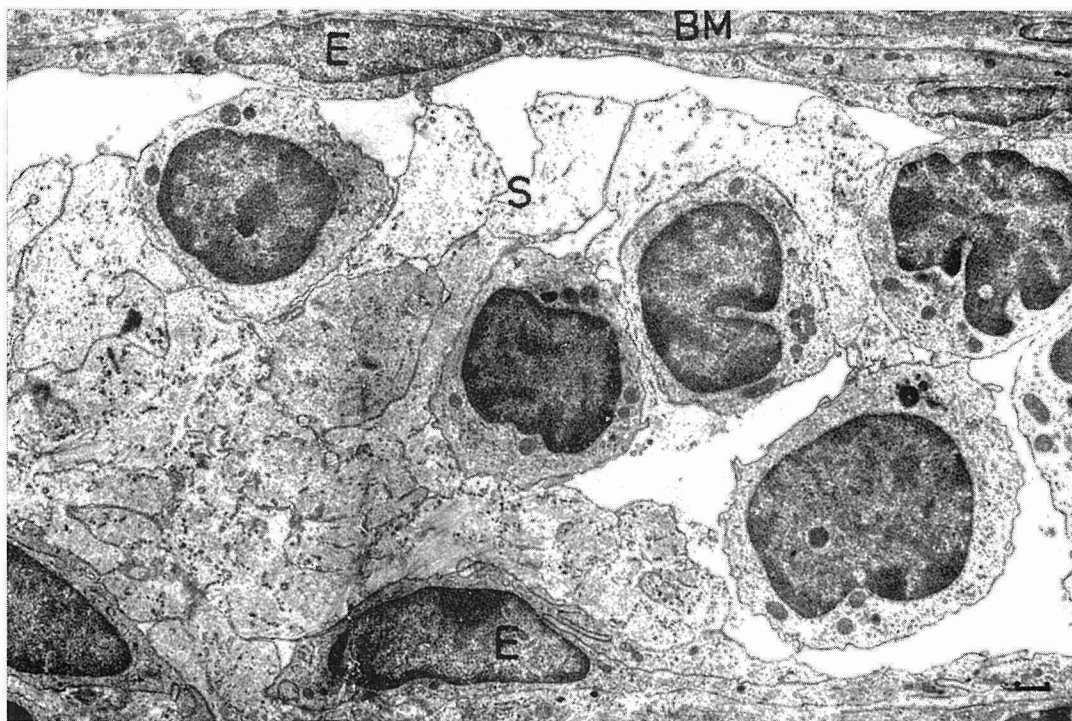


Fig. 4



Fig. 5

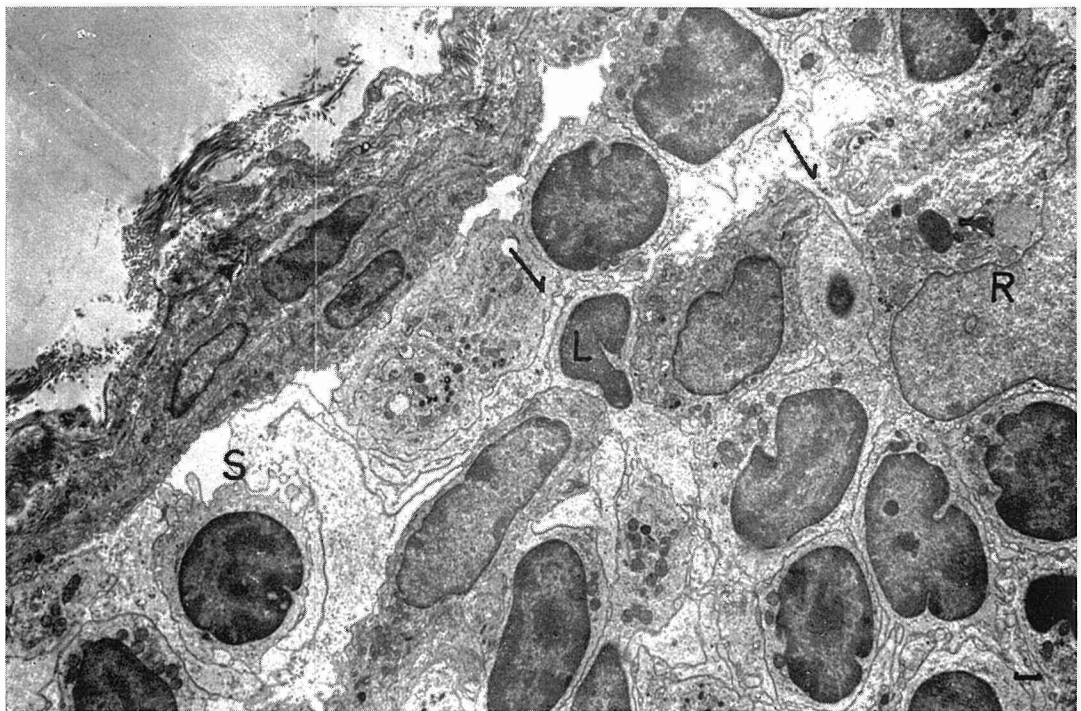


Fig. 6



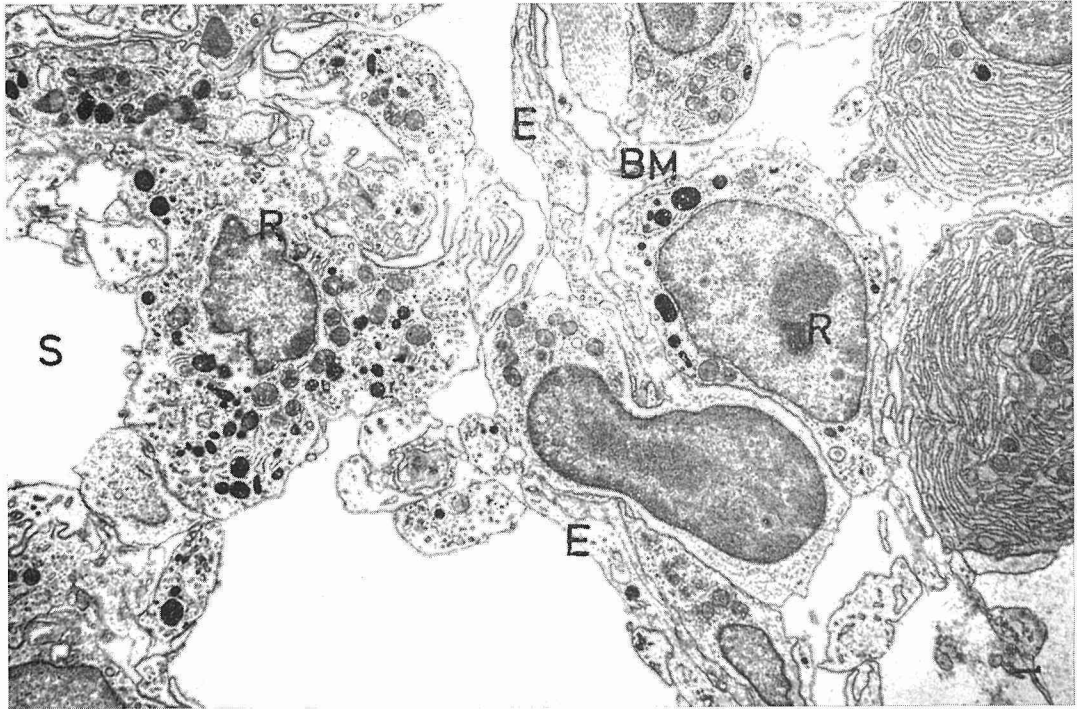


Fig. 7

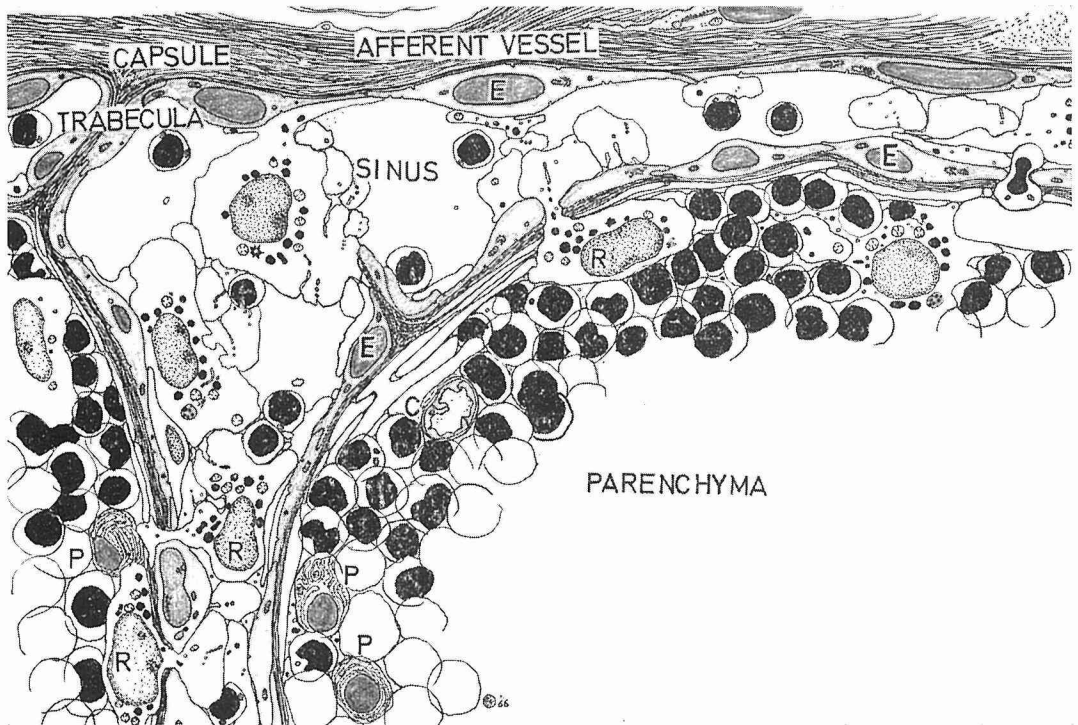


Fig. 8

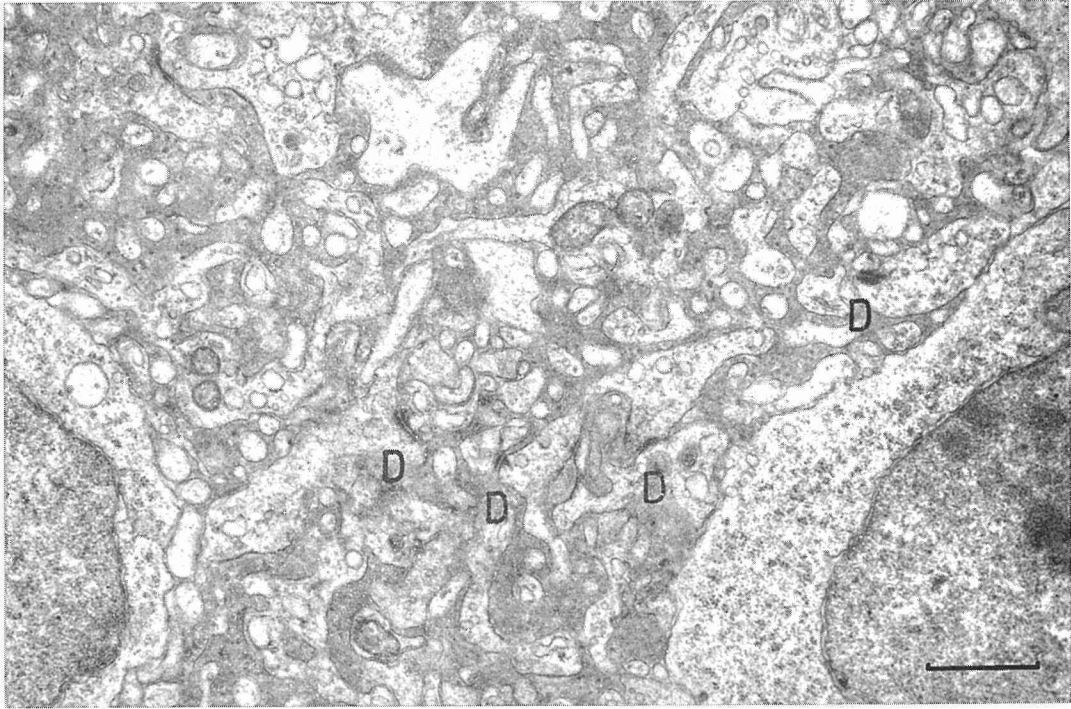


Fig. 9

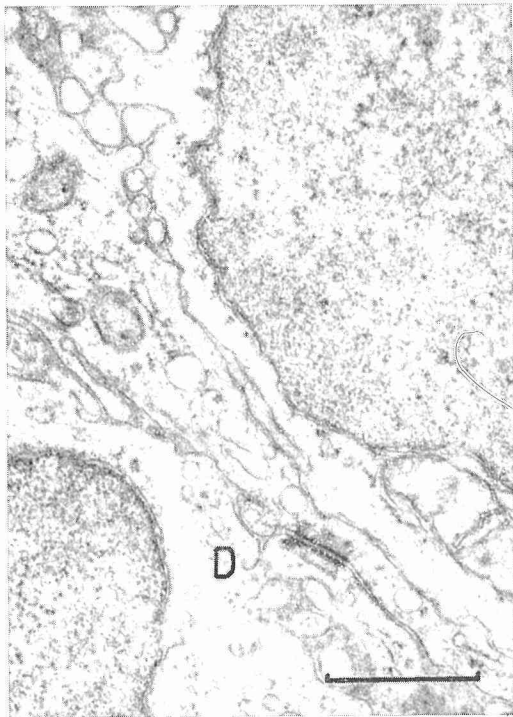


Fig. 10

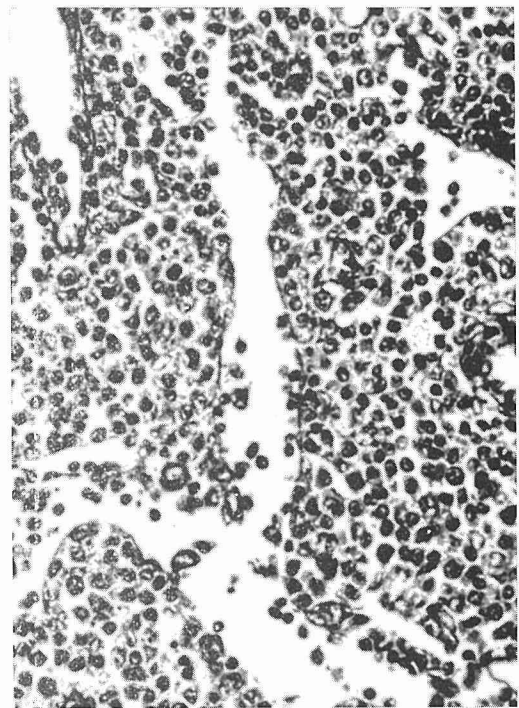


Fig. 11

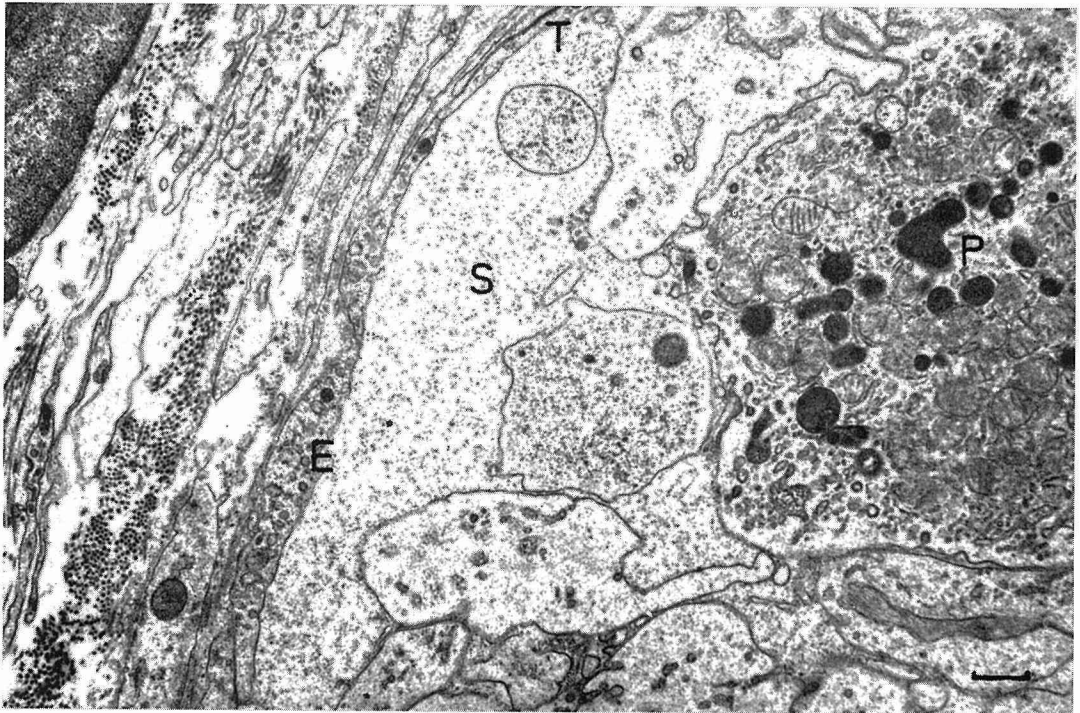


Fig. 12

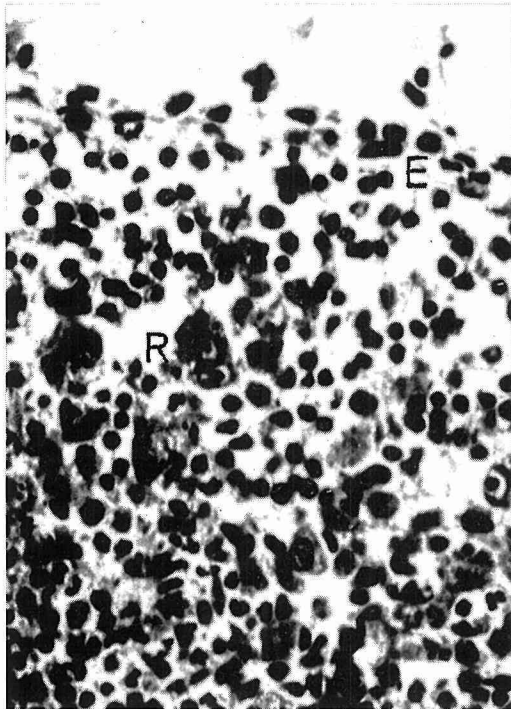


Fig. 13

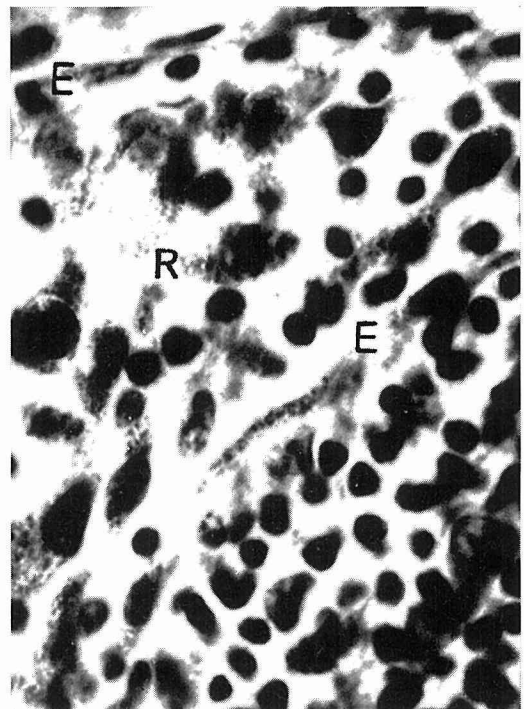


Fig. 14

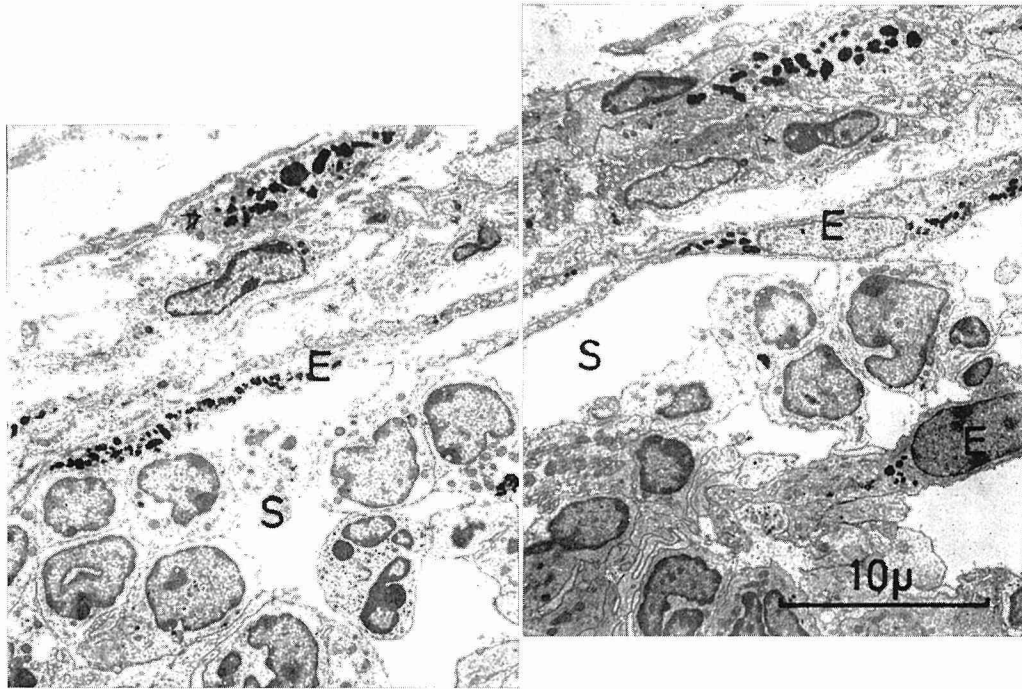


Fig. 15

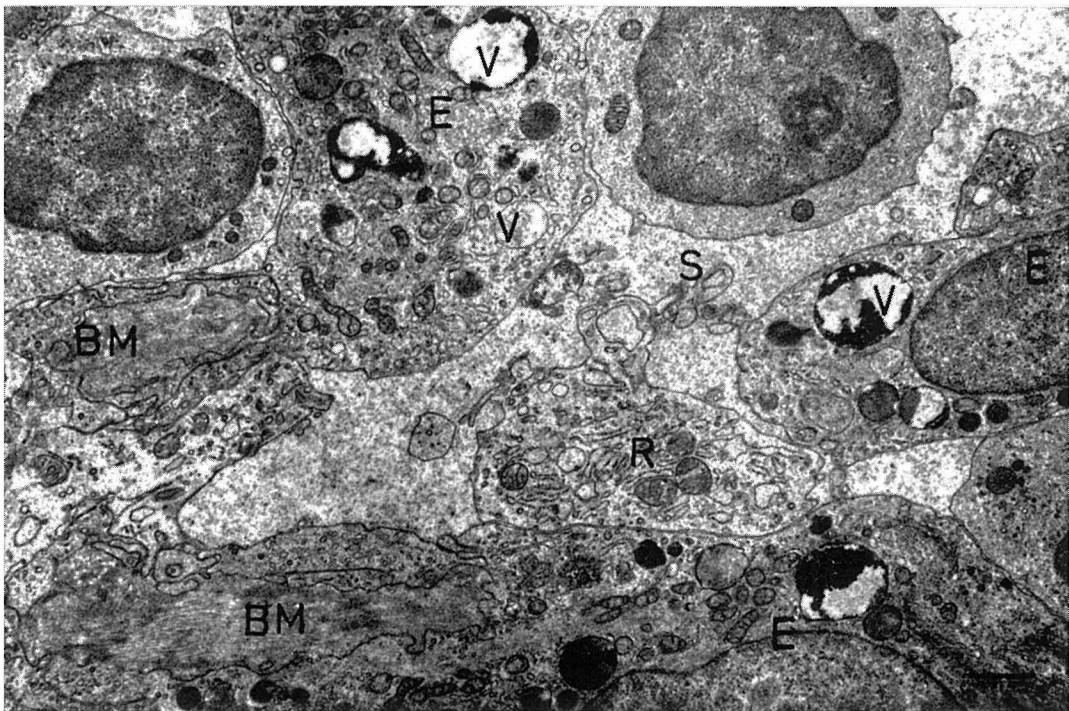


Fig. 16



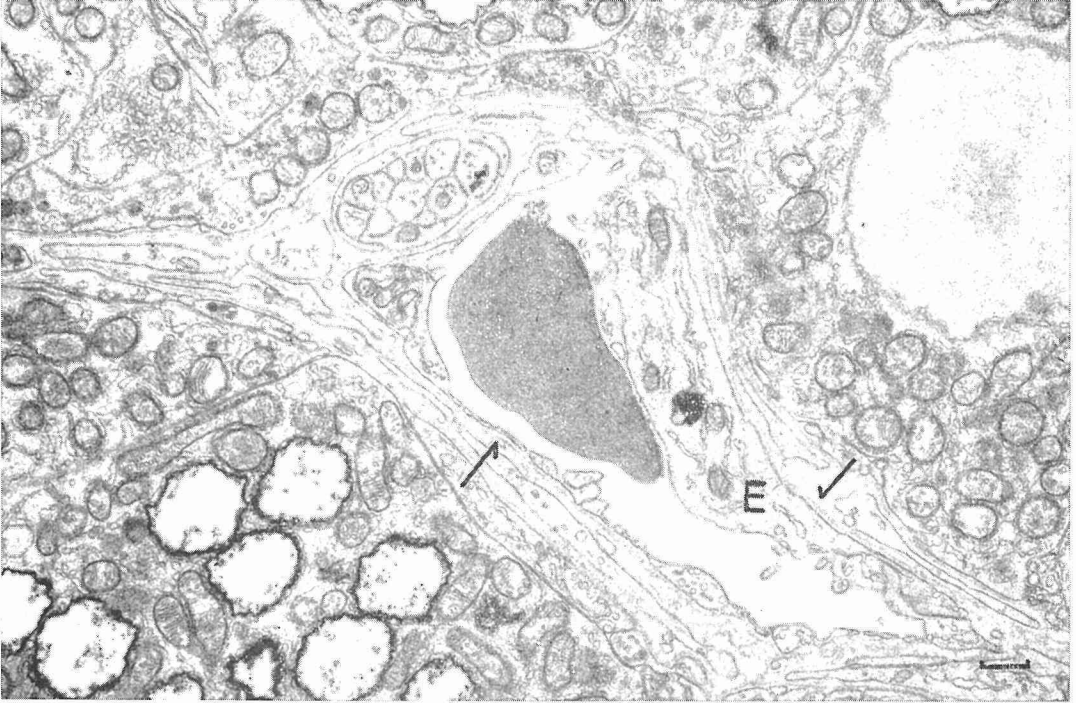


Fig. 17

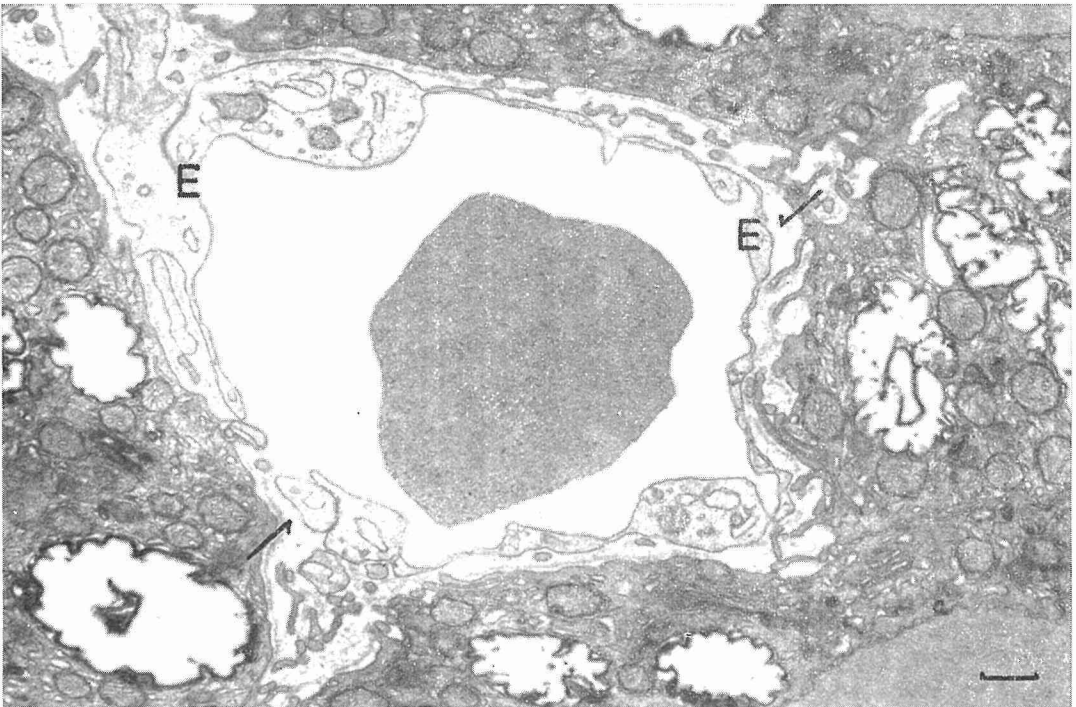


Fig. 18