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The studies on electron-microscopic observation of fine structure of the liver sinusoid have been reported by a number of researchers,[1)–11)] yet, despite various attempts have been made, no uniform observation on the aspect of contiguity of innermost wall structure of the sinusoid or on the special reference to lattice fibers of the Disse's space were ever made known.[6) 9) 10) 11)] And then, as to the fine structure of the stellate cells hardly any systematic observation has been reported.[10)] The present paper deals with the report of above observation upon detail studies on fine structure of the wall of sinusoid as well as stellate cells and lattice fibers in rabbits, and upon the findings that the innermost sinusoid wall was not of contiguous nature and further, the Kupffer's stellate cells have some relation with the Disse's space.

METHOD

The animals used were healthy mature rabbits weighing roughly 2Kg. Upon ether anesthesia, laparotomy was performed and the liver was quickly removed. A liver tissue slice of 1 mm.^{3} was made and put in a fixing agent.

The following two fixing solutions were prepared:

A. A mixture of an equal amount of osmic acid 2 percent and phosphate buffer M/100 containing 0.5 M succrose. (Fixing solution I)

B. Palade's fixative:[12)] 1 percent osmium tetroxide buffered with veronalacetate to pH 7.3. (Fixing solution II)

The fixing is first made one to two hours in an ice box and then without washing[13)] dehydration was made beginning with 70 percent alcohol and increased its concentration. Following dehydration, it was embedded in plastic mixture which was made up with N-Butylmethacrylate and Methylmethacrylate in a proportion of 7 : 3 or 8 : 2, and added with Benzol peroxide 2.5 percent and was polymerized in a constant heater at 48°C.

Ultra thin sections were made by an ultramicrotome, Shimazu, Type K and the observation and photography was made with the electron microscope, Hitachi HU 10.

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RESULTS

With electron-microscopy, the author was able identify the following structual elements of the sinusoid.

1. Structural constituents of the sinusoidal wall.
   a. Endothelial cells.
   b. Hepatic microvilli.
   c. Disse's space.
   d. Argyrophilic fibers of the Disse's space.
2. Kupffer's Stellate Cells.
3. Blood Cells in the Sinusoid.

Because of the detail formed cellular elements of the sinusoidal boundary which are readily identifiable by means of the electron micrsocopy and the fact that the stellate cells which may have concerned with the sinusoidal cellular formation, it would perhaps be proper to consider separately from the structural constituents of the sinusoid.

As to the blood cells found in the sinusoid, these will be not mentioned in this paper inasmuch as these have been reported elsewhere[14) 15)] and correspond to present finding.

General Appearance of the Sinusoid

As shown in the photographs No. 1, 2, 3 and 4, the inner aspect of sinnsoidal wall consists of endothelial cells of blood vessels; the cytoplasm are relatively wider in the close proximity of nuclei but they appear to be decidely narrow as they are further away from the nuclei and having stretched out to both directions from the nuclei leaving rather indefinite periphery. These endothelial cells with the wider cytoplasm around the nuclei or with projected cytoplasm covered with so called microvilli, the villous projections from the liver cells, and formed a space between the hepatic cells and microvilli, the Disse's space is this very area of gap.

The Endothelial Cells of Sinusoid

Various cellular structures have been observed according to sections used as shown in the photographs 1, 2, 3, 4 and 5. Among those, the photograph 1 and 5 are the most common findings. The photograph 3 is presumably the picture of cross section of No. 2 photograph.

The nuclei are invariably larger when their cytoplasms were compared, most were either fairly spherical or oval shaped with occational shallow and dull indentation.

The nuclear membrane is made up of double layered structure as the photograph 6 shows, of which inner membrane is thicker than outer membrane studded with small granules of somewhat higher electron density. Both membranes are commonly seen to be parallel though occasional intrusion into cytoplasm created a space with inner membrane. At times, the parts of two membranes were completely

fused as the photograph 6 indicates.

The nuclear ground substances is relatively packed with fine granular particles but of even distribution.

The nucleolus was rarely observed. Ref. : photograph 1.

The cytoplasm is comparatively wider in the area of nucleus and elongated toward both ends which serves to provide the formation of inner cellular wall of the sinusoid. The wider area will be called peri-nuclear cytoplasm while the elongat d part is to be known as the cytoplasmic projection.

The small particulate components of Palade[16] were dispersed roughly in the peri-nuclear cytoplasm along with endoplasmic reticulums[17] [18] and mitochondrias.[19] [20] [21] [22] There were two varieties of the endoplasmic reticulums scattered, i. e., small vesicular smooth surfaced, and flattened, vacuolar rough surfaced. Frequently, smooth surfaced variety was seen to be formed by the cytoplasmic membrane buried into cell body. Ref. : photograph 7.

The mitochondrias were often of round, less round and rodshaped. Although these are abundant as observed in the photograph 4 but most of them as seen in the photographs 1, 2, 3, & 6, contained only a few. The cytoplasmic widening of perinuclear area seemed to contain more number of the mitochondrias as well as relatively noticeable development of the endoplasmic reticulum. Ref. photogrophs 5 & 6.

There are granules of which the size is same or larger than the mitochondria but of spherical or oval shaped and of higher electron density were seen as observed in the photographs 1, 5 and 6. The number is not fixed, usually one or several present.

The Golgi[23] [24] [26] complex was not observed.

The cytoplasmic protrusions which were cytoplasmic extentions from the peri-nuclear widened area as thin projections seemed to comprise the inner cellular boundary of sinusoidal structure. (photographs 2 and 3).

The thickness of the cell boundary was varied, having no uniformity ; it is as thin as a cell membrane and the thickest part resembled more like a part of peri-nuclear area where Palade's particulates were distributed sparingly besides endoplasmic reticulum and larger granules which were of high electron density. (photographs 3 and 8). Moreover, these sinusoidal walls were interrupted and lost of their continuity and formed of crevices. (photographs 8, 9 & 2).

The photograph 8 is a longitudinal section of sinusaidal picture, while the photograph 9 is of a picture of cross section. Interruption of the sinusoidal wall is definitely observed in either picture and the interrupted ends appear to be blunt but single cell membrane can be seen, presenting pore formation along the membranous wall.

Hepatic Microvilli

Some protoplasmic projections from an area of the hepatic cells are protruded

directly to sinusoidal wall these are also known as microvilli. (photographs 8, 4, 3 and 2). Most of them run right angle that is straight out to the sinusoid wall which is made up of endothelial cells, but others appear as cord like, some take small round shape, some appear to be elongated oval shape or horse shape type and with arborization, showing no uniformity of their course of direction. (photograph 8, 6 and4). Practically, most of them leave dead short space with endothelial wall of the sinusoid. Some of them are intimately lose to the endothelial wall are no longer distinguishable. Their size are usually 0.4–0 5 μ length with 0.045 μ width. (photograph 8).

Disse's Space

An existence of space formed by the liver cell with it's microvilli and sinusoidal lining made up of endothelial cells is interpreted as the space of Disse which has the same electron density as the sinusoidal space. (photographs 8, 2, 3 and 4). Occasionally, however, flocculent substances which were well distributed fine particulates were observed as shown in the photograph 13. As shown in the photograph 2, the granules which were oval to elliptic type possessed of moderate electron density were found but incidence of meeting such situation is very rare.

The photograph 10 shows the picture of spindle like figures which are the formed elements of cytoplasms could be found in the Disse's space. The rough endoplasmic reticulum is well developed here but true identity of cellular origin is difficult. As to the stellate cells, the pictures are highly suggestive of closed relation with the structure of Disse's space.

The photograph 15 shows that the stellate cells protruding themselves into the Disse's space through pores of inner sinusoidal wall closely approximate themselves with the liver cells.

These pores found in the sinusoidal wall which are formed by endoth lial projection make connection between the Disse's space and the sinusoid. As the photograph 8 indicates those pores have unusually larger diameter of 0.09–0.13 μ though they are variable as a rule: so with the width of Disse's space, none shows uniformity, as shown in the photograph 5, it is hardly recognizable, yet, some are as wide as 2.5 μ as the photograph 15 shows.

Fibrous Structures

The finding of fibrous structure is extremely rare.

The photograph 11 presents Disse's space immadiately under the sinusoidal endothelium including endothelial cells, perivascular connective tissue and hepatic cells. There are numerous microfibrils to be seen in the space, some present short or long linear appearance, while others appear to be in specks.

In the center of the space, these seem to follow long axis of the space, and none have crossed. On the other hand, these fibers which were close proximity with either superficial endothelial cells or connective tissue cells are running right

angle or angle nearly so. The hepatic cells seen in the neighborhood of these fibers are completely devoid of microvilli on the surface. In the photograph, they are seen to be either taking round or oval shape and free in the center of space or somewhat toward right upper aspect.

In observing the relationship of the fiber structure in reference to endothelial cell and connective tissue cell, it seems that the fiber structure is definitely connected with endothelial cell body but they communicate in right angle nearly so, as indicated in the arrow 1. The arrow 2 shows, also, the connection with the connective tissue cell, while, the arrow 3 indicates the endothelial cell protrusion which is connected with the fiber structure. These fibers have formed bundles each composed of a collection of several to scores; the bundles could be clearly seen in crossing also. As indicated in the arrow 4, these fibers out from the surface of connective tissue in the right angle take parallel course in the direction of long axis of Disse's space as they approach the central part. The width of each fiber was around 10 A°.

The photograph 12 has shown the fiber structure directly under sinusoidal endothelials space, showing connections between the two, the former taking their courses right angle into the space from cellular membrane or near the membrane.

The photograph 10 is another showing of the fiber structure present themselves into the space and these located at the right lower part from center of the space is the picture of cross section and those of the center is of vertical section; a part of the latter closely approximate itself with the liver cell. They formed bundles but not in right angle with the liver cell. An attention has been called that hepatic microvilli which are commonly present are seen least and none at all in some parts in the neighborhood of fibrous structure, which agrees with the previous finding. These fibers have average width of 50–80 A°.

In summarizing the findings of the structurs of the Disse's space:

(1) That these fibers have average width of 50 A° when ultra thin section made.

(2) They tend to form bundles, each composed of several or several tens collected fibers.

(3) Their directions are not uniform but many of them take right angle to endothelial membrane when closer to them.

(4) Commonest site for the fibrous structure to be seen were near endothelial cells.

(5) Occasionally, these fibers were seen to be connected with endothelial or connective tissue cells.

(6) Hepatic microvilli were least seen in the Disse's space where fibrous structure are present.

Kupffer's Stellate Cells

The stellate cells are seldom observed with the electron microscope, It is not

an easy matter to describe definitely as their morphological variation can be frequently observed. It is true that the polymorphous nature of the stellate cells signify functional difference, nevertheless, basic morphological peculiar to the stellate cells have been observed and respective description of pictures which showed common characteristic have been reported.

The photograph 13:

The cell body of stellate cell is unusually large having a dumb-bell shape, extending to the both sinusoidal wall. Although a part of cell attached to the sinusoidal wall but no shifting of their protoplasm to be found between endothelial projections and the stellate cell, and each has its own limiting membrane.

The cells are large and possessed of small projections with their nuclei situated at one end but what appear to be a large phogocytosed material closely resembled nucleus could be seen at the symmetrical point. The area of cytoplasm between them has high electron density, also an oval shaped granule with high electron density which is somewhat larger than mitochondria was found. It has clear boundary though no limiting membrane can be seen. There were granules with relatively high electron density numbering 11 arranged in a manner of wheel axis to be seen. The endoplasmic reticulum could be identified well in the cytoplasm. The Palade's particulate were roughly distributed in the area toward periphery and with round or short rod shaped mitochondrias disseminated in the peri-nuclear area. The endoplasmic reticulum are either tubular or saccular, and appear to be rough surfaced and many small vesicular smooth surfaced ones are observed. Smooth endoplsmic reticulum which are enlarged and unusually large and round can be seen near left end of cytoplasm, containing phagocytized substances with fine granules containing high of electron density.

These large phagocytized substances are filled with granules with relatively higher electron density than nuclear gronnd substances. The limiting membrane has a higher electron density, some parts are appear to be stratified having unusually wide space between two membranes but rough unlike double layed structure of nuclear membrane. In some areas, there are collection of small granules of high electron density between the two limiting membranes, these are presumably the phagocytosed substances.

Golgi complex are found in the area where nuclei are sunk; three constituents, i. e., Golgi lamella, Golgi granula and Golgi vacuole were observed. The nucleus is oval shaped and having shallow depression, the nuclear membrane showed a parallel double membranous structure. The nucleolus contained evenly distributed fine particles. One fine nucleole was found. Nucleus has round substances which have lower electron density in the center than nucleole, with neither limiting membrane nor granules but its periphery has clear nuclear ground.

The Photograph 14.

Here, the stellate cells are situated at the sinusoidal junction, having their cytoplasm traversed to the sinusoidal wall, but separated by a cellular membrane.

No shifting picture is observed though a part is attached to the sinusoidal wall.

The cell is large taking a triangular shape containing small projections. The nucleus usually is located at one side. Palade's granules are roughly distributed and closely packed with fine granules of high electron density larger than Palade's granules are found in the center of cytoplasm.

The endoplasmic reticulum is much developed than those seen in the photograph 13, and more numerous in the wider area. Smooth surfaced endoplasmic reticulum is most marked showing both tubular or saccular appearance, while rough surfaced endoplasmic reticulum is lesser presenting vacuolar or tubular shape. The mitochondrias are seen to be away from the nuclei a d looked short rod shaped or oblong.

The substance of large size readily taken for phagocytosed substance are to be found in the smooth surfaced endoplasmic reticulum in the lower dilated area of the cell. They are grouped themselves having a higher electron density than the nuclear ground. A non-granular foreign substance which is of an equal size but with irregular elect on density can be found at the immediate right of the nucleus. There are smaller substances which may be phagocytized foreign materals or changed substances have been observed. The relation of these substances and the endoplasmic reticulum is not known. There are also another type of round granules observed in the lower right besides other two mentioned substances. All these have comparatively higher electron density. Those with right of nuclei are irregular in size but with under them are regular. None has related with endoplasmic reticulum but found in cytoplasmic ground substance.

The nucleus is round and has double membraneous structure. In some area the outer membrane is swollen and elevated into the cytoplasm. Fine granules are evenly distributed in the nuclear ground substance. One nucleole with two other uneven, irregular substances without limiting membrane can be observed. This is presumably the identical substance already seen in the nuclear ground of the photograph 13. Golgi complex is faced with a wider area of the cell near the nucleus.

The Photograph 15.

The stellate cell is seen to be extended to both sinusoidal wall. As seen previously, the cells have their membranes and cytoplasmic shifting could not be observed. However a part of this cell have entered the Disse's space through sinnsoidal pores. A part have reached the hepatic cell and entered like a wedge. In the space, there frequently seen a part of free stellate cell.

The nucleus is seen in one side of cytoplasm. High electron density fine granules which are larger than Palade's granules are coarsely distributed and grouped themselves irregularly were seen in the center of cytoplasm. Moreover, fibrous structure which at times closely imitate rough surfaced endoplastic reticulum are seen, under the nucleus c n be seen tubular or endoplasmic reticulum which a part has been dilated. On tha whole, lamellar structure has been noticed. Smooth

surfaced endoplasmic reticulum with a smaller number of particulates occupy throughout. In a wider area of the cell, small vacuolar type of smooth surfaced endoplastic reticulum is abundantly distributed but practically none found in the center.

The mitochondria is either rod shaped or oval and some were closely attached to the endoplasmic reticulum. The wider part of the cell is filled with numerous uniform oval granules with various electron density of higher degree. The presence of limiting membrane is not definite. The granules with low electron density have become higher in the periphery as to present itself a limiting membrane. These granules could be clsssifiable according to variation of electron density.

1. Extremely high electron density.
2. Moderately high electron density without uniform structure.
3. Comparatively low electron density, at times showing limiting membrane.

Golgi complex is found in a wider cytoplasmic part adjacent to the nucleus and with Golgi vacuole arranged like a petal.

The nucleus is round but one side presents many irregular indented margin with double nuclear membrane which runs parallel. A well developed nucleole is found, showing a complex mass.

In the nuclear substances, aside from the nucleole, were found a substance which is clearly visible in center as shown in the photographs No. 13 and 14, and which showed lower electron density than the nucleole without having a limiting membrane and surrounded with clear area which lacks granules.

The Photograph 16.

In this picture, the stellate cell was found free in the sinusoidal space. The nucleus is nearly oval and indented superficially.

The cell has short projections but indented deeply in the upper margin as if presenting a picture of engulfing a small cellular piece. Palade's granules are coarsely arranged but interspersed with the minute granules of high electron density throughout the cytoplasm. Oval shaped relatively large granuled are also present, those are having less electron density and irregular. There are other irregular substances besides those granules of various shapes from oval to round of varied electron density. These granules as well as non-granular substances with their shifting appearances may be the phagocytic substances or from an effect of such process and unrelated with endoplasmic reticulum.

Mitochondrias are oval and short rod like, with a limited number. The endoplasmic reticulum is well dispersed, presenting vacuolar type of smooth surfaced reticulum though some are long tubule like or cystic like roughened surfaced and with less number.

In the left end zone of the cell were seen collected smooth surface endoplasmic reticulum of either long or cystic tubules. Some of these were attached with membrane a small number of granules which may be taken for roughened surfaced endoplasmic reticulum of shifting kind ; and the cell membrane is connected with tubular smooth surfaced reticulum at the lower left margin.

The nuclear membrane has a double parallel membraneous structure. The nuclear substances are evenly distributed with minute granules. One nucleole can be seen.

The Golgi complex can not be observed.

The Photograph 17.

The picture presents a part of the stellate cell. A point of interest is that here again nuclear membranes are doubled but a part of outer membrane is swollen; in some areas, nuclear ground substance like substances are trapped between external and internal membranes. The swollen parts in some area have been made free within the cytoplasm and presenting a picture that there have changed into widened endoplasmic reticulum which held a substance which appear to be identical with the nuclear substances.

The cell contained Palade's granules sparingly, while larger sized high electron density granules were disseminated widely.

A round phagocytosed substance which is somewhat larger than the mitochondria was seen in the left side and unrelated with the endoplasmic reticulum.

The electron density of the limiting membrane is not uniform. The mitochondrias are mostly irregular. Most of the endoplasmic reticulum showed changing picture between smooth to rough surfaced reticulum. The granules attached to the membrane are varied and the endoplasmic reticulum is connected with cellular membrane at the left upper margin.

The Golgi complex is found near the nucleus and all the essential constituents, i. e., Golgi vacuole, Golgi lamella and Golgi guranula can be seen.

Thus, observation made of the stellate cells can be summarized as follows:

1. The stellate cells are commonly found not only in the close proximity of the sinusoidal wall but also are found free in the sinusoidal space.

2. Some cells have seen to be invaded into the Disse's space through the pore of sinusoidal wall a part of their cellular structurs, while, others are intimately related with hepatic cells.

3. Many of these cells are unusually large, so much so that the cellular bodies have bridged over the other sinusoidal wall.

4. Because of morphological variability, these cells seem to posses flexible nature.

5. These cells are frequently seen to have minute projections in their cellular peripheray, while, other show indentations and at times, showing a figure of holding some other substance.

6. Occasionally, the cellular membrane presents a picture of direct connection with endoplasmic reticulum.

7. These endoplasmic reticulums exhibit relative difference in their development, distinguish themselves into smooth or rough surfaced types; the former seemed to be well developed characterized by vacuolation and cystic or tubular reticulums have grouped themselves frequently although some displayed lamellar structure.

8. The mitochondrias are found to be either rod or oval shaped, or occational dumb-bell have been observed. They are widely dispersed but often the nuclei are pushed to one side, facing wide opposite area.

9. The Palade's granules are roughly distributed in the cytoplasmic ground substance. Besides these, there are large with high electron density, widely scattered or forming irregular collections which showed at times rough surfaced endoplasmic reticulum within the formation.

10 The cells contained large sized substances which are readily taken for phagocytosed foreign body, and when engulfed substances are of large ones, they are often found in dilated smooth surfaced endoplasmic reticulum.

11. Other than above, small sized oval and well formed smaller granular substances are present. Many of them are from electron density of various ones but uneven.

12. Among these substances, are found shifting type, that is, they are smaller than the larger roughened phagocytosed substances but have irregular shape. These are definitely granular in appearance and have uneven electron density. When they become smaller in size, they appear like oval shaped granules mentioned above and there will be more of those shifting types in presence signifying those are in their process of transformation into the oval shaped granules.

13. Golgi complexes are located adjacent to the nuclei which are found near nuclear depression or wider cytoplasmic areas. The three contituents, i. e., Golgi vacuole, lamella and granula are observed. In some cells, only the Golgi vacuoles seemed to have developed.

14. The nucleus are indented superficially usually found at the one end of cell. They show double membraneous structure with occasional protrusions into the cytoplasm, at times, they either appear to hold some inclusion substance or protruded membrane have detached itself and transforming into what appear to be endoplasmic r ticulum.

15. The nuclear ground substance contains fine particulates of even distribution. One nucleolus is commonly situated slightly away from the center of nucleus.

16. Aside from nucleolus, there are found circular substances without definite limiting membrane and of low electron density but devoid of granular structure. They are commonly found one or more clearly visible in the center, and with clear surrounding area of number ground substance which lacks granular presentation. An average diameter of the substance is 0.4 μ.

DISCUSSION

Disse's Space.

It has been considered that the Disse's space is latent in character and observable only upon the condition of agonal asphyxia or edematous state.[9] Under the method of electron microscopy, Braunsteiner et al.,[3] and Furuta[8] were not suc-

cessful in identifying the Disse's space, but Fawcett[7] and Parks[4] were able to show. Their reports were subsquently clarified by Takagi,[6] Onoe,[5] Matsuo,[10] Wasserman [9] and Cossel[11] and the other. The authors has made a similar observation and made cleared of its closer structure, not only of the hepatic cell microvilli in the space and fibrous structure, flocculent substance though seldom observed but also a part of the stellate cell which had entered from the sinusoidal space and a certain undetermined cellular part as shown in the photograph 10.

The width of Disse's spacee is varied, those of the photographs 4, 14, 5 and 2 are rather wide, while thse of the photographs 1 and 5 are narrow. The photographs 3 and 8 demonstrate the common picture of the space which is 0.7 μ. The difference of a width, however, should not be construed as the result of artificial product in the preparation of section specimen. The photographs 15 and 14 show frequent situations observed of wider or shrunken spaces lying side by side; the variation may account for some functional reason in view of cell preserved cellular intergrity with least sign of injury.

When the space is contracted, the microvilli are usually shruken and packed, and space is lost between them as shown in the photograph 5, which is not the case with widened space, and here, the distance between the sinousoidal wall and microvilli is definitely separated, but the later had elongated. It may be that this shortening or extension of microvilli speaks for their morphological adaptation for functional necessity which may be required in other organs as well.

An interesting appearance is that of undetermined substance which are seen in the widened space shown in the photograph 2, while the photograph 15 shows invasion of the stellate cell; these findings may apply specific function in this area of space.

According to Mölbert and Guerritore,[28] in acute hypoxemia the Disse's space becomes narrower but in the administration of tissue poison such as cyanide or malonic acid, the space would be widened due to the state of existing hypoxydase.

Wassermann has assumed presence of associated substance with microvilli and fibers in the space by means of PAS reaction and demonstrated a stainable substance of pink, and that dispresed the entire space is equivalent to basal membrane to all intents and purposes.[9]

The present author, on the other hand, contents that the Disse's space should be interpreted as an empty space or an aperture because of the findings of stellate cells invasion into the space through the sinusoidal interestices and the fact that electron density of the space is same as that of the inner space of sinusoid and a very fact that the microvilli always projected into space, hence, it is hardly possible to assume that the Disse's space should be regarded as the same as the basal membrane as seen in the renal tubules or pulmonary alveolar epithelium.

On the presence of aperture seen on the sinusoidal wall.

On the subject of the contiguity of endothelial cell, Parks[4] said nothing but Fawcett[7] had recognized its discontinuity and presumed the definite communication

between sinusoid and the space of Disse's. While, Wasserman[9] maintained that contigual nature of the sinusoid wall and that discontinuous area is the result of endothelial detachment and what remained only forms transient small holes but these soon will be obstructed. In this connection, Matsuo[10] reports strongly of the existence of these small holes, which is backed by Takagi.[6] As discussed previously, the present writer has observed of an entity of the stellate cells had entered the Disse's space through small hole like aperture which are directly contiguous with hepatic cells, proving their identity. The margin of these holes are dull as Matsuo[10] reported and together with the presence of limiting membrane and absence of broken down tissue which might be separated from the cells around small holes make us believe that these could never be a picture of artificial damage. (The photograph 5, 8, 9 and 17)

The Stellate Cells.

The report on the electron microscopic observation of the stellate cells has not been come to attention due perhaps to the less frequent observation in normal liver. Parks[4] and Onoe[5] had only reported simple description, while, Sampaio[29] and Uchino[30] reported on them on the observation of phagocytic character. Matsuo,[10] on the other hand, classified into four types by injecting Indian ink or inoculating tuberculous bacilli and observing growth process of the stellate cells which apperded in the sinusoid. Present investigation is limited to the study of stellate cells to be seen in normal liver.

The morphological observation of these cells in normal liver tissue seemed to be varied markedly according to the cellular function. Taking of the phagocytic action of the stellate cells as an index of functional importance, it seems that when the phagocytosed substance is large and roughened, it is in the stage of early period of pr cess, and when the cell undergo changes, they becames smaller in size and the shape became more regular. The photograph 13 shows those cells which have engulfed rather large and roughed foreign bodies and none are smaller. The photograph 14 showed a medium sized roughened foreign body and small regular shaped substance. The photograph 15 shows numerous small granular substances. It would be proper to say granules inasmuch as their are of uniform shape. The photograph 16 showed a medium sized foreign body mixed with many granules of even size, of which many showed transition picture. As the photographs 13, 14, 15 and 16 show there phagocytosed foreign substance had become smaller gradually. A fact should be noted here is that where phagocytosed bodies are fresh and large, less number of small foreign granules; on the contrary, when cells possessed of small granules, the no larger foreign body to be found. Thus, cnece the stellate cells have undergone phagocytic action, no longer will they show ability to digest that whole energy being spent for changing foreign body into small granules. The photograph 13 and 14 show those cells trapping the fresh substance are called the phagocytosis type, but those of 15 and 16 are called digestion types because of their high digestibility having many small granules.

Although no marked difference in the organelle between digestive type and phagocytised typus, the former had developed little more of the endoplasmic reticulum and has tendency to form tubular type of endoplasmic group.

An interesting fact is that those stellate cells of digestive type have inserting themselves directly into the liver cells in a wedge shape through the Disse's space indicating closer relations though it is difficult to explain its significance at this time. Nevertheless, the attachment of stellate cells to sinusoid tissue had to be admitted. As to the development and specialization of these cells, Tanaka[35] is of an opinion that these will grew out from a specific endothelial cell. Omori[32] and Onishi[33] interpreted that these are the result of specific growth of sinusoidal endothelials. Wassermann[9] had reported a picture that the endothelial cells have slipped from the sinusoidal wall and that the stellate cell or free phagocytic cells are derived from endothelial cells.

The author's findings have revealed that the definite cellular membrane does separate the stellate cell from inner sinusoidal wall in all observation and none to show protoplasmic shifting between the two nor a picture of endothelial detachment and free in the sinusoid.

Matsuo[10] however, reported the absence of such findings and that stimulation of the stellate cells with the administration of Indian ink have caused migration of free immatured cells in the sinusoid and have seen the picture of immature type of cells transforming into mature cells and denied of direct relationship between the stellate cells and endothelials.

The small round substances found in the nucleus

The round but irregular substances of low electron density, having approximate width of 0.3–0.4 μ, found in the nuclei of the stellate cells require further study as no reference is available on this substances. Ref. Diagram 1.

Endothelial Cells

The electron microscopic studies of endothelial cells are less characteristic, the cellular boundaries that is, the demarcation of protoplasmic contact are not clear and whether it is a syncytial nature is not settled.

Observation made as that of the photograph 7 is frequently met, it is the findings of smooth surfaced endoplasmic reticulum connects with cellular membrane. Palade's[34] presence is a sign of pinocytosis.[35]

Besides unusual smallness of mitochondrias of the endothelial cells, Wassermann[9] pointed out that the chromatin material often observed near nuclear membrane which is the characteristics of endothelials was not detected. The high electron density granules of either round or oval shape found in the endothelials were closely resembled the small phagocytosed granules seen in the digestive stellate cells, or under the digestive stage of phagocytosis. (The photograph 1, 3, 6 & 8)

Although Matsuo[10] has reported that the endothelial cells have an ability to phagocytose to some extent but this has never been proved by the author on the fact that a substance which could be taken for a foreign body or relatively large

substance within the endothelial cell so that fixation of granules would be hardly poss ble.

Lattice fiber

Recognition of lattice fiber has been reported by many investigators by means of optical microscopy, yet, with respect to electron microscopic observation despite many attempts made, the literature is limited other than those of Wassermann,[9] Cossel,[11] Ruttner and Togel.[36] Takagi[37] has mentioned something similar but failed to described exact structure of this particular fiber. Matsuo[10] admits strange situation why electron microscopic observation could not identify lattice fiber. Fiber like structure has been found in Disse's space in the present study but limited to only few instances. Wassermann,[9] however, attributed that a reason for undectability might be due perhaps to marked sensitivity of fibers against osmic acid or resin used an caused damage to the tissue.

Presence of fine fiber structure in the Disse's space and the endothelial cells has been observed by Cossel[11] in the tissue of human and mouse and Wassermann[9] on the rat tissue. It is true that these fine fibers vary in width according to the animal specie but it does not seem to related with the kind of size or morphology of animal.

It was found that the width of rat's fiber 200 to 600 A°,[9] mouse' 60 to 400 A° and that of human fiber was 100 to 500 A°.[11] The author's finding of rabbits' was 10 to 80 A°. The difference does vary greatly not only of different specie but also the same kind of animals.[11] The findings observed by various observers are indicated in the Table 1.

It is the writer's assumption based on the observation that these fibers are related to the endothelial cells of sinusoid and cellular membrane, that is, shifting of the cellular content so to speak; in another word, these fibers appear to be originated in the endothelials, but the fibers were never found in the latter. Further, these fibers were found to join with connective tissue cells of perivascular area aside from sinusoidal endothelials, coinciding with what Cossels[11] have found. And it was also found that the lattice fibers are intimately related with the sinusoidal endothelials and perivascular connective tissue.

Lipophagic Cells

A special attention was made to observe so called lipophagic cells which Itoh[38] has mentioned, but no such cells could be identified. Matsuo[10] has described the endothelial like cells which has taken up fat granule under the cellular projected part of endothelials, that is, under the sinusoidal wall, and also found the cells loaded with fat in the area intervened by the adjacent liver cells, but stated his difficulty to admit these cells as what designated by Itoh[38] as lipophagic cells.

Pericyte

These cells were observed as shown in the photograph 18 but rarely seen. These cells were observed in the lateral wall of the Disse's of sinusoidal aspects. The fine structure of the cells resembled vascular endothelials. Wassermann[9] had

proved their existence.

CONCLUSIONS

The electron microscopic observation of the liver sinusoid of rabbits was studied and the following conclusions were obtained.

1. The structual constituents of the sinusoidal wall are made up with vascular endothelial cells and protruded cell bodies constitute the innermost wall and the Disse's space is the space formed between the above innermost wall and the hepatic cells. From the liver cells, numerous microvilli projected into the space and the lattice like fibers are found. The projected endotheli l cells which make up the sinusoidal inner most wall have small holes through which a part of the stellate cell can protrude itself and making contact with the liver cells, which in turn, make fixation of stellate cells.

2. It is assumed that the stellate cells are changeable t adapt for functional condition. The width of the Diss 's space is found to be variable.

3. The stellate cells are ploymorphous by nature and the common characteristics are that the nucleus is located to one side and holding the phagocytosed foreign body. Besides necleolus, it holds round bodies of low electron density in the center of intranuclear area. The Golgi complex is clearly visible. And it is possible to classify the stellate cells into two types, one is phagocytised and the other is digestive type.

4. The lattice like fibers are joined with connective tissue of privascular area and the endothelial cells.

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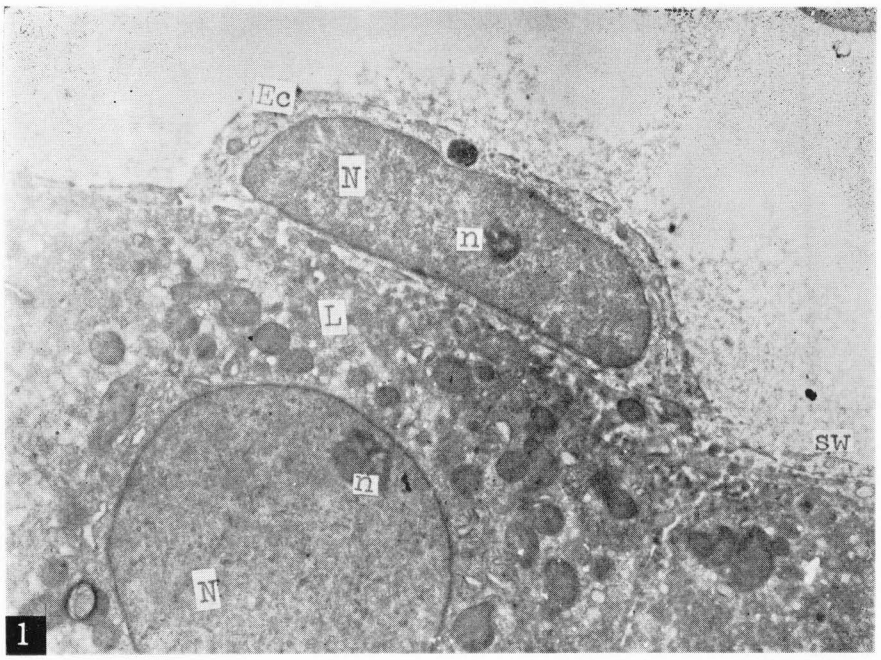
Abbreviation for Photograph.

Ec	Endothelial cell	li	Lipid granule
L	Liver cell	gc	Bile capillary
N	Nucleus	e	Endoplastic reticulum
n	Nucleolus	mv	Microvilli
sw	Sinusoidal wall	G	Golgi's complex
S	Sinusoid	g	Granule
D	Disse's space	af	Fibrous structure in the Disse's space
p	Pore of the sinusoidal wall		
m	Mitochondria	pc	Pericyte



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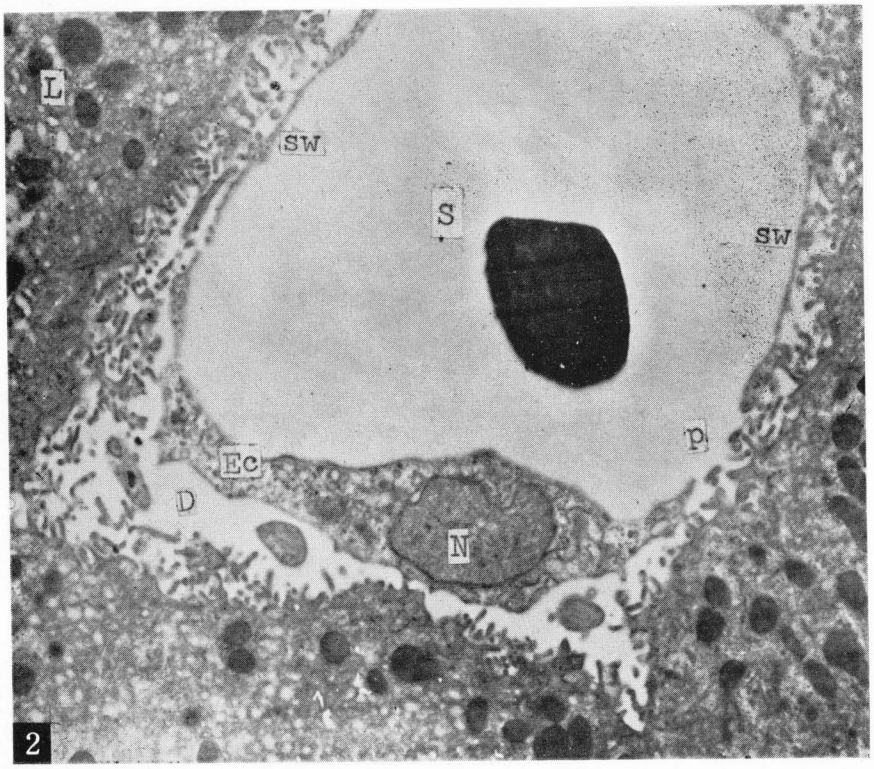

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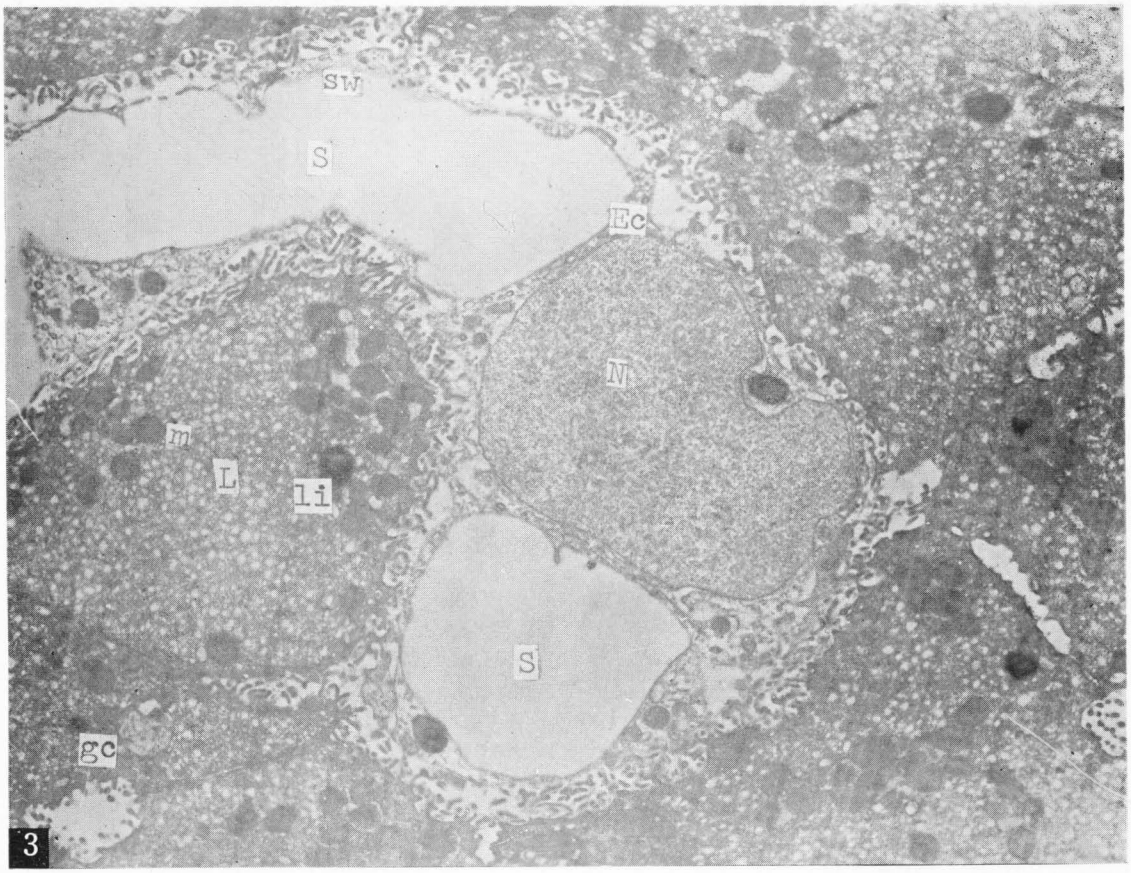

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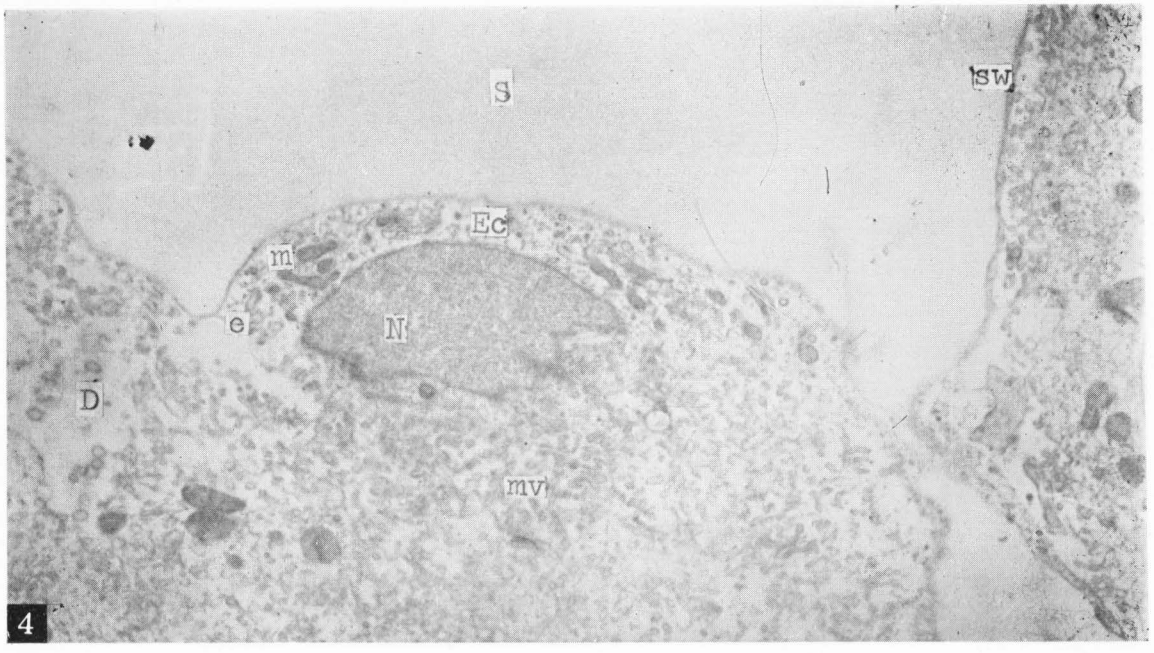

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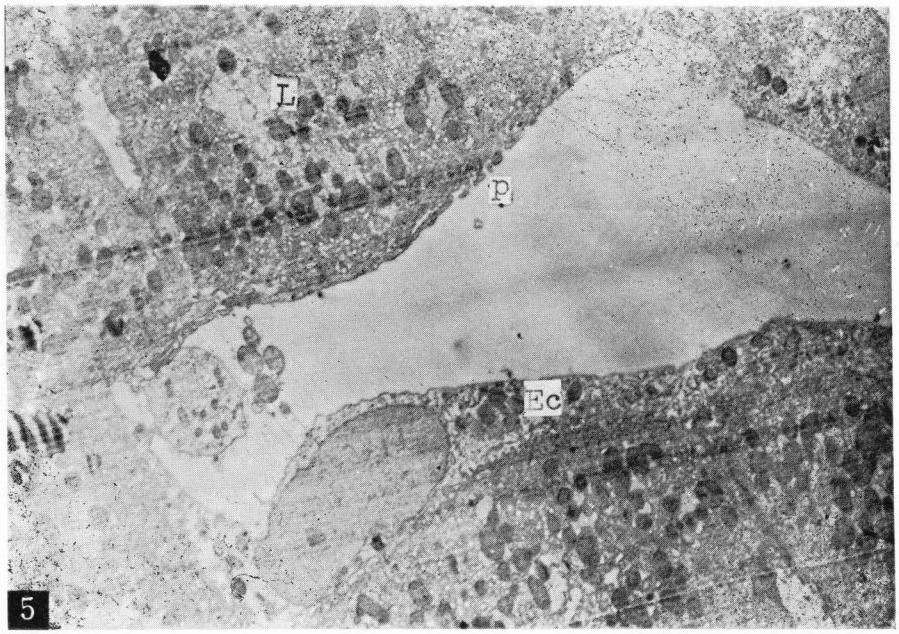

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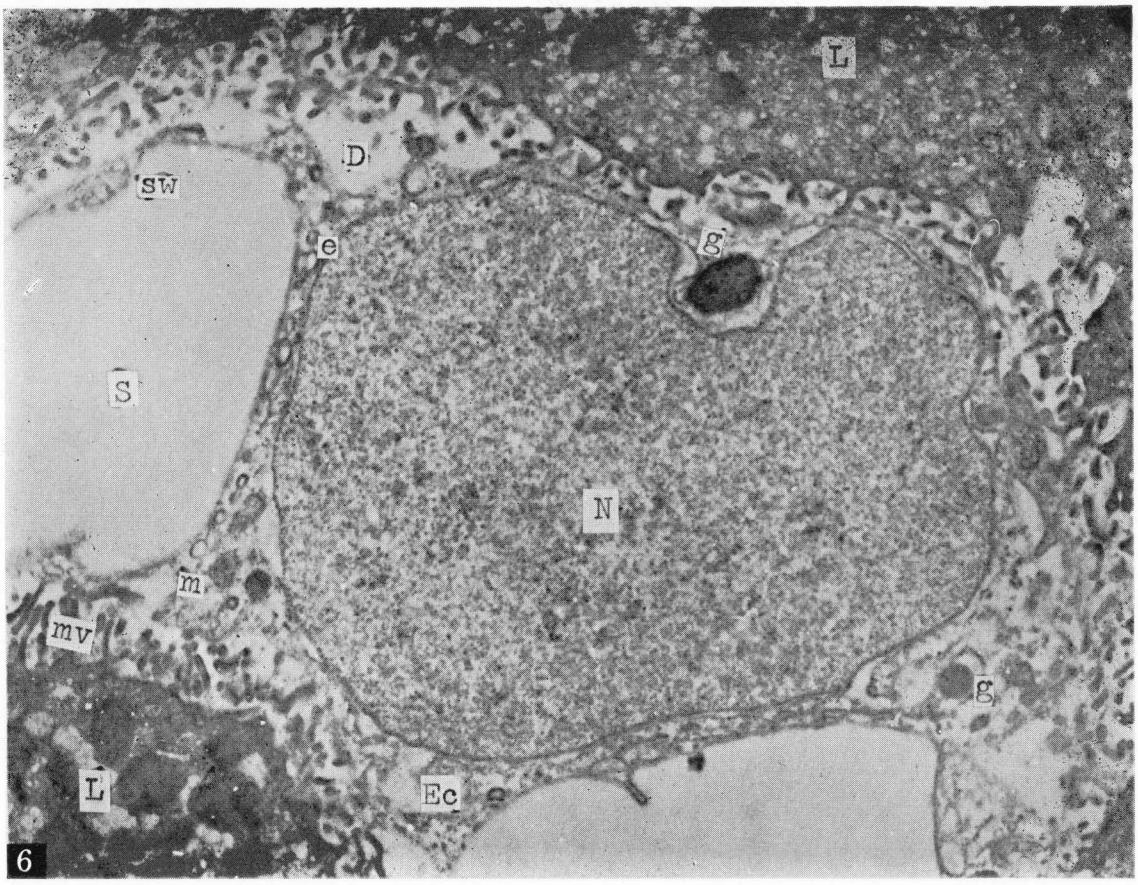

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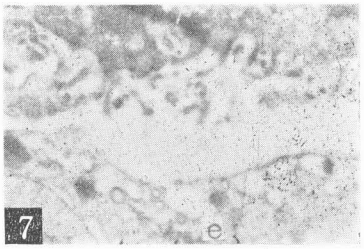

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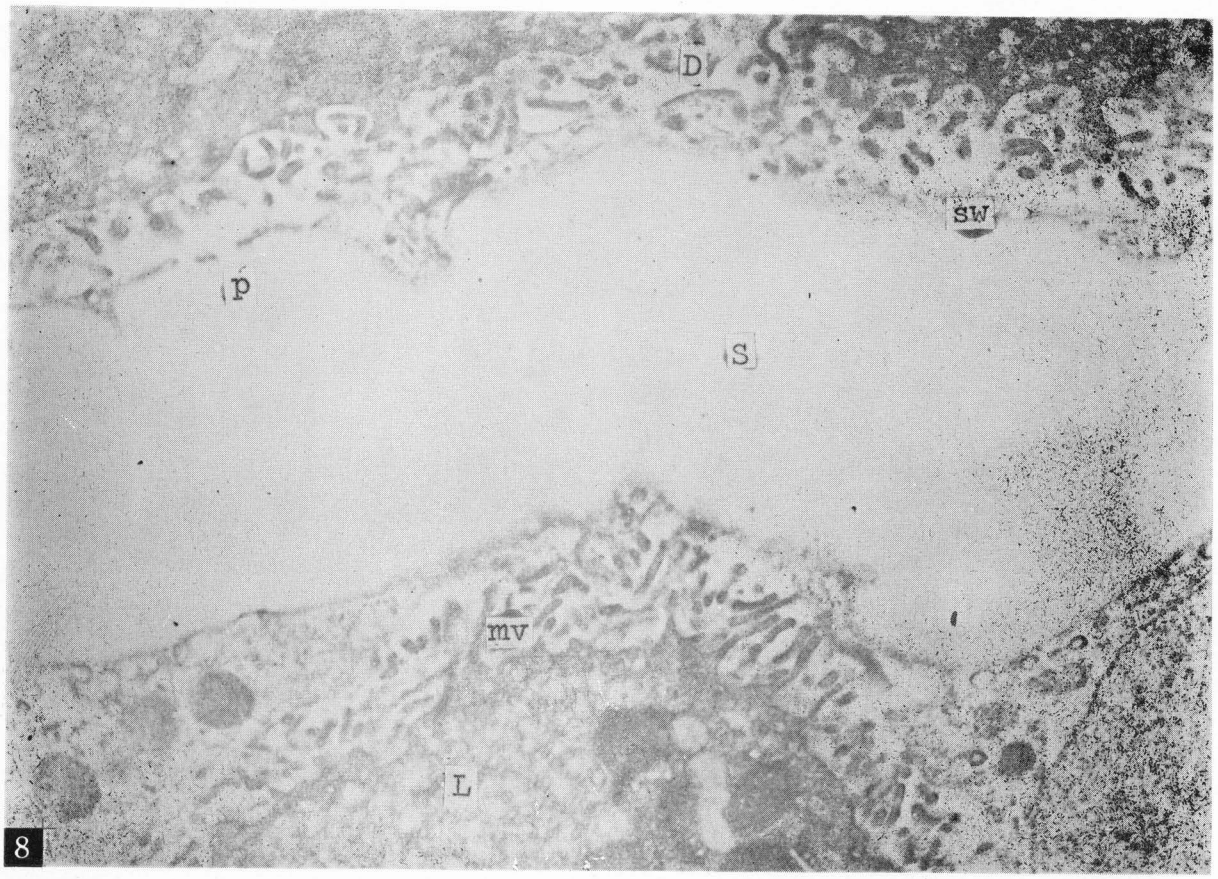

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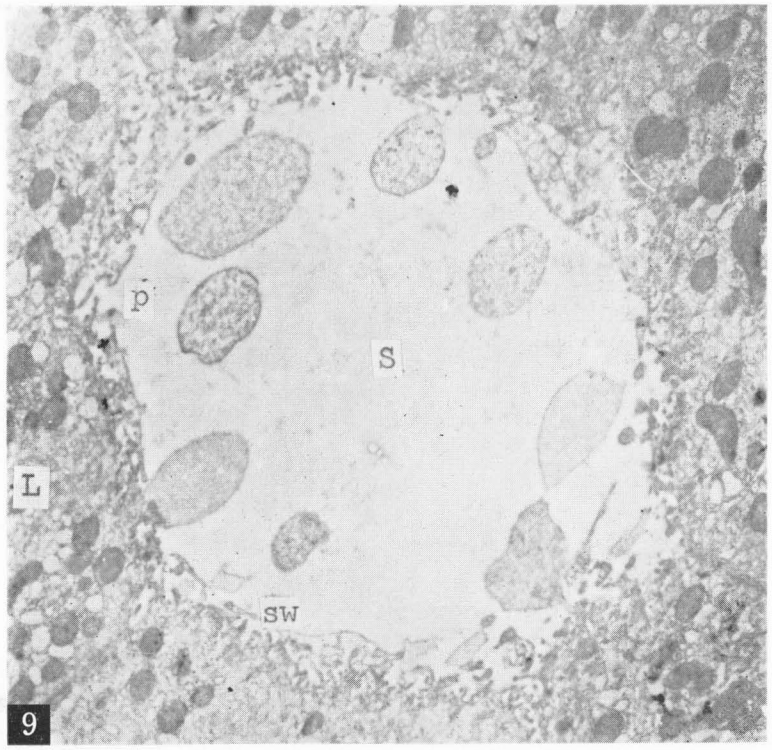

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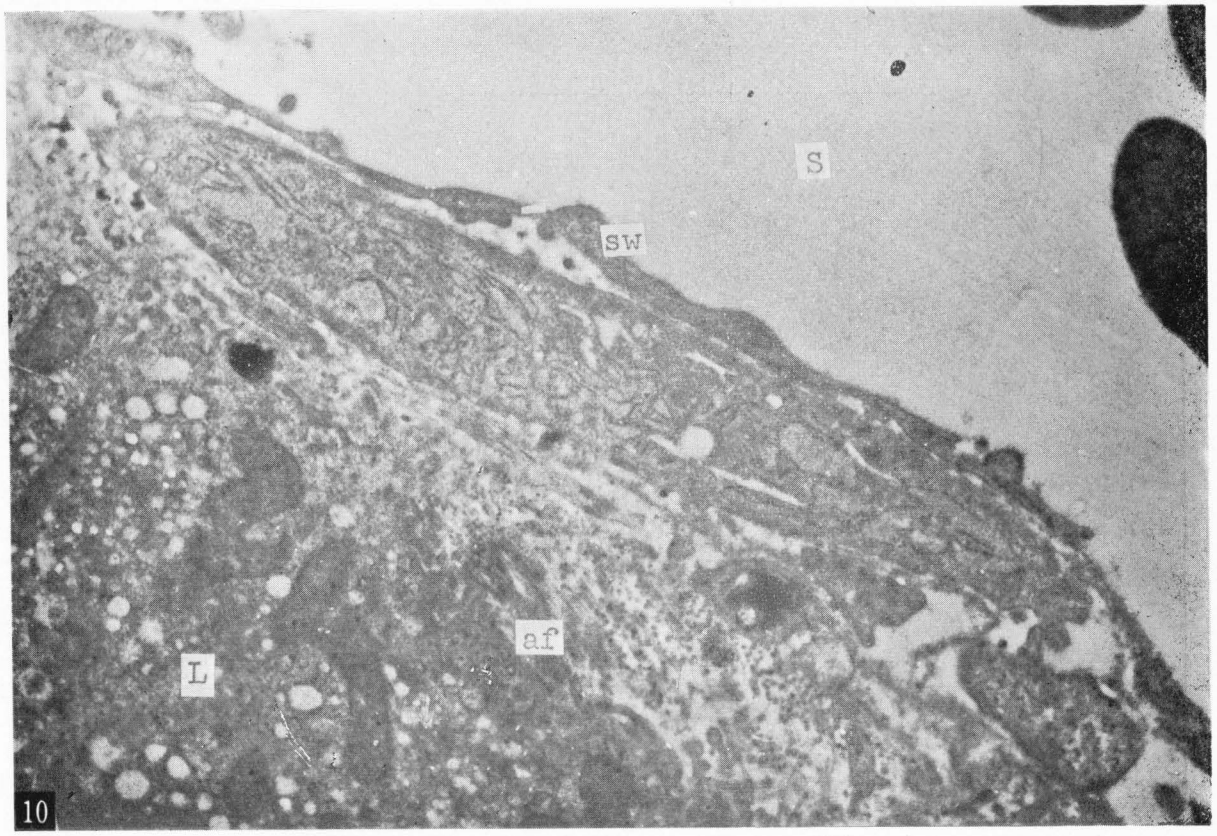

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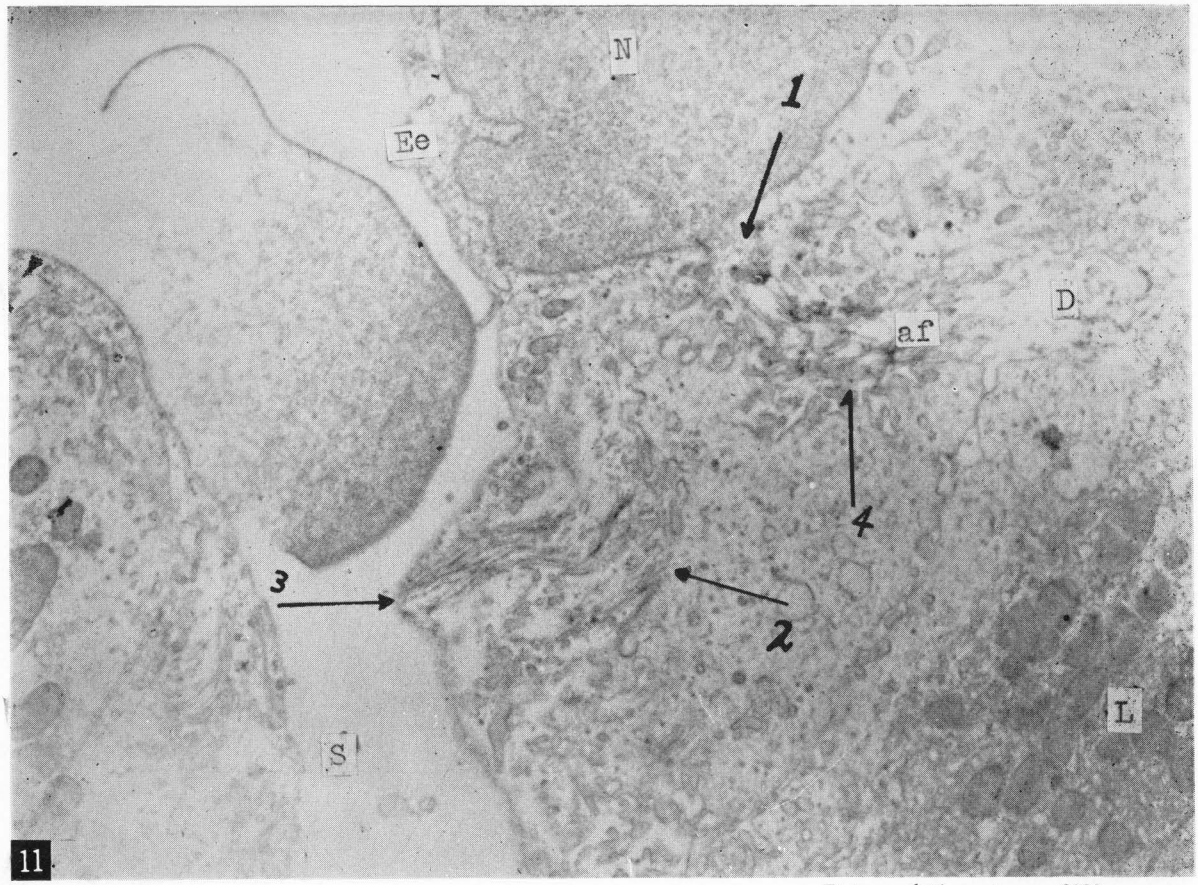

11

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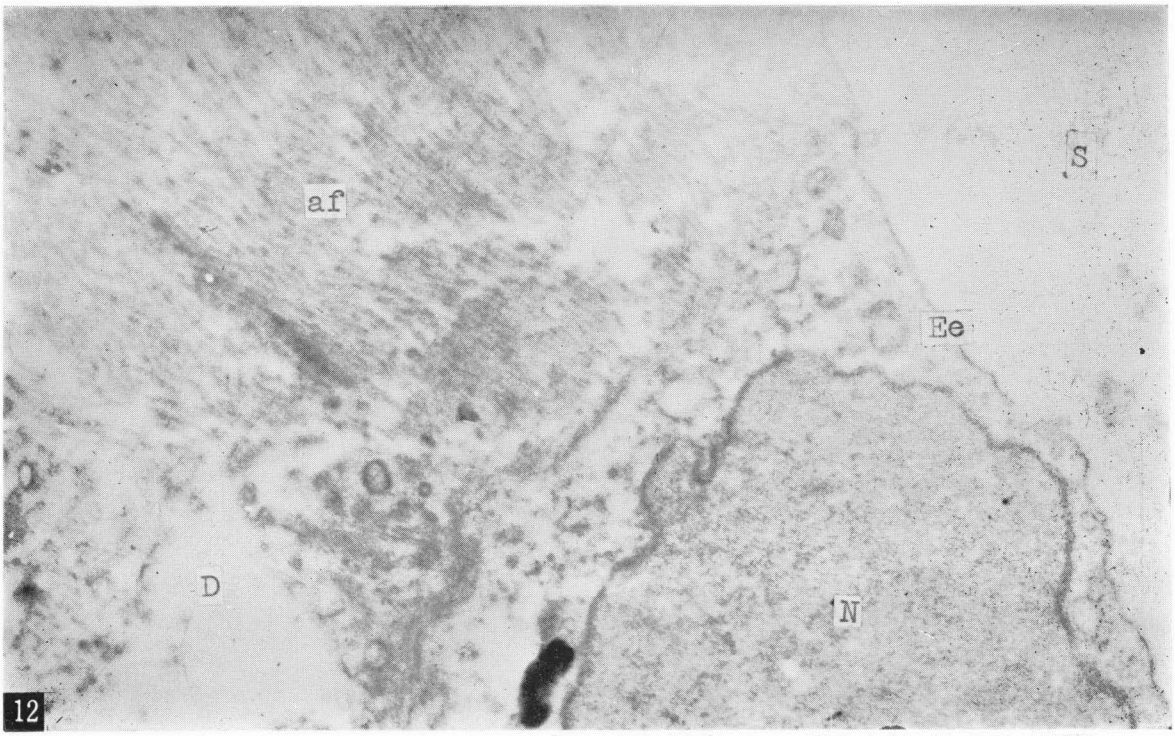

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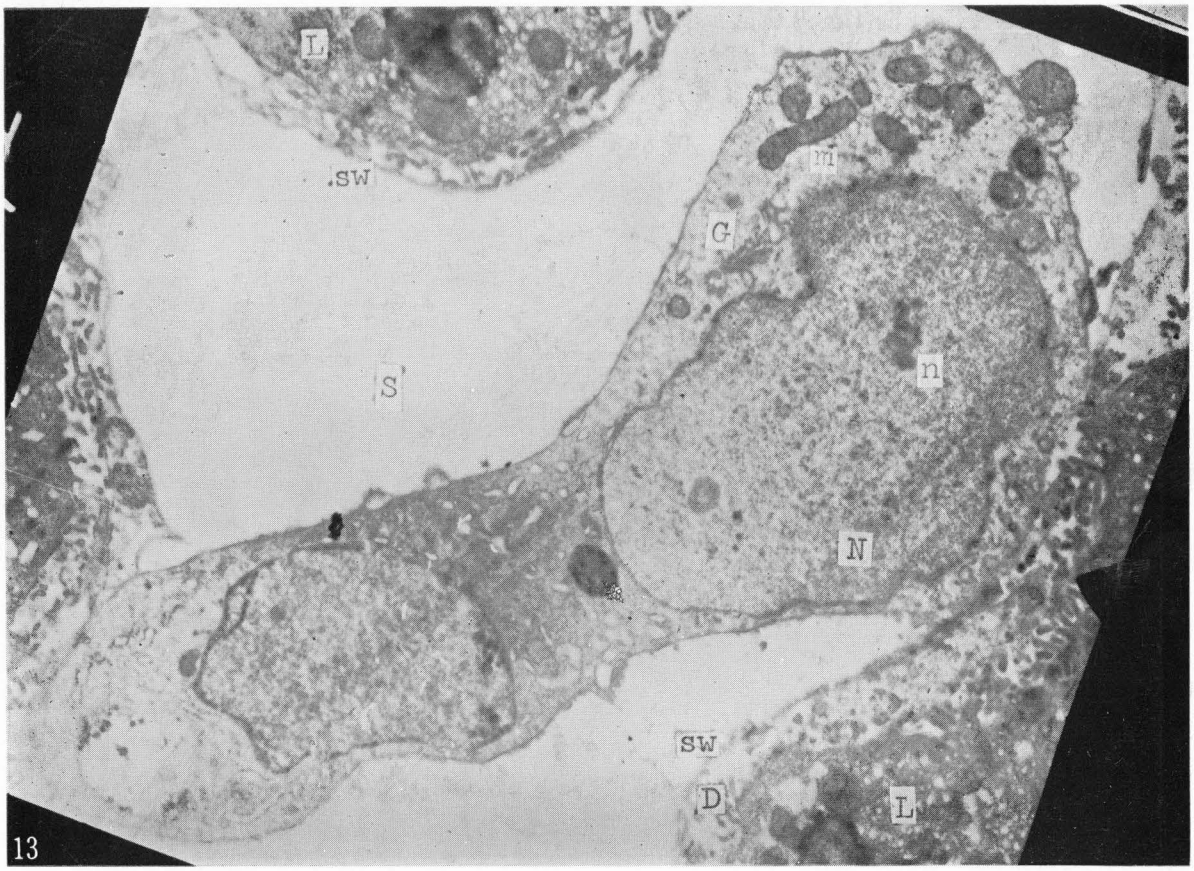

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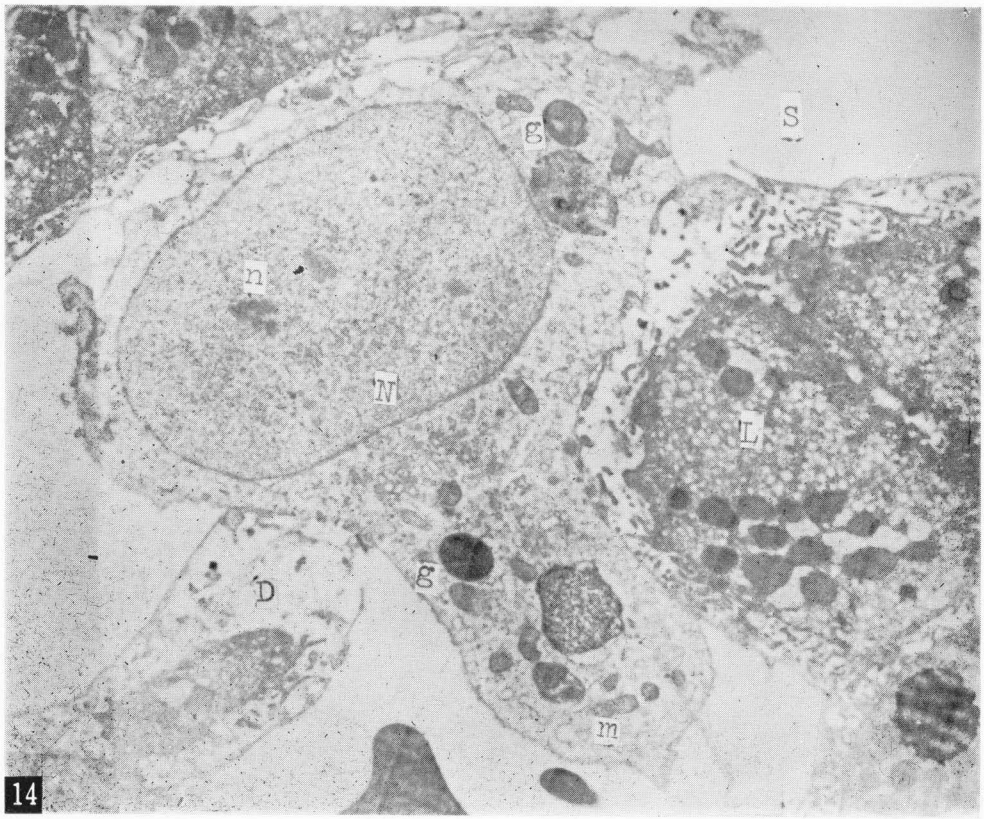

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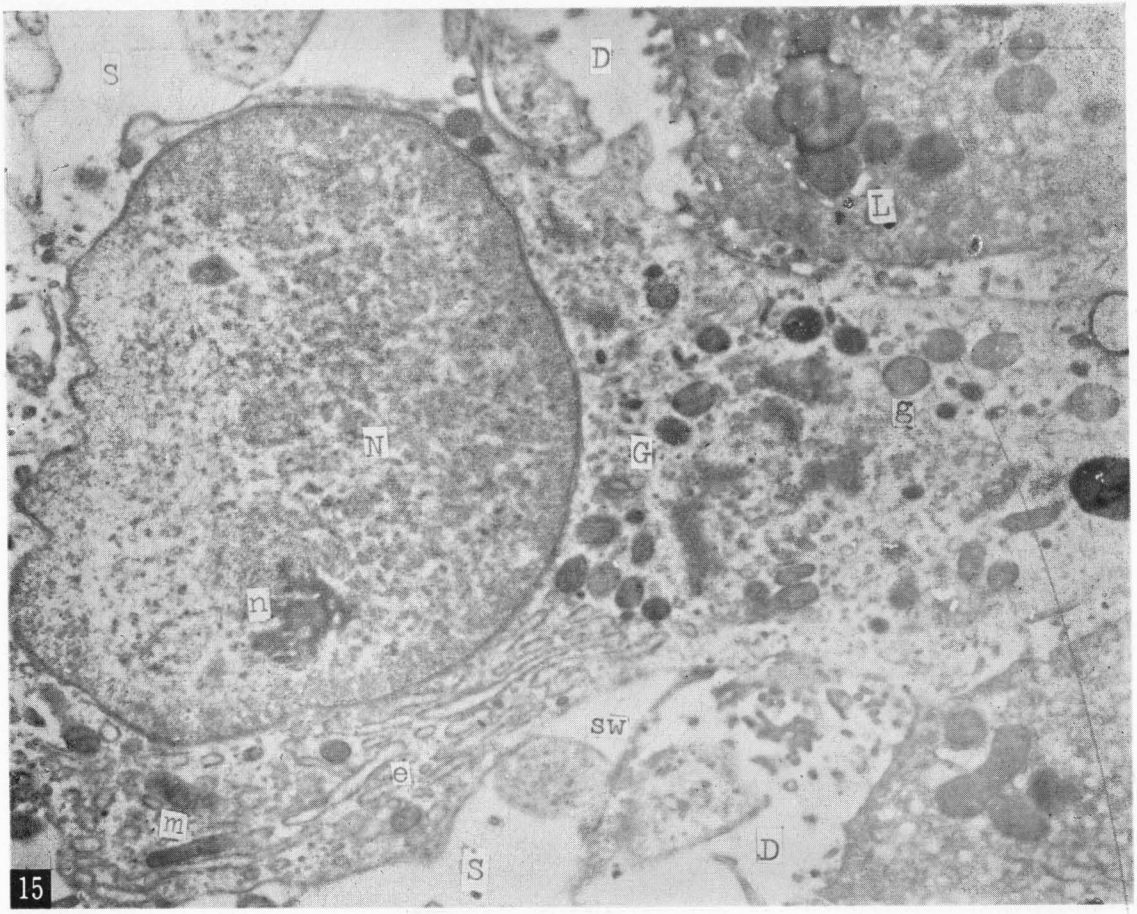

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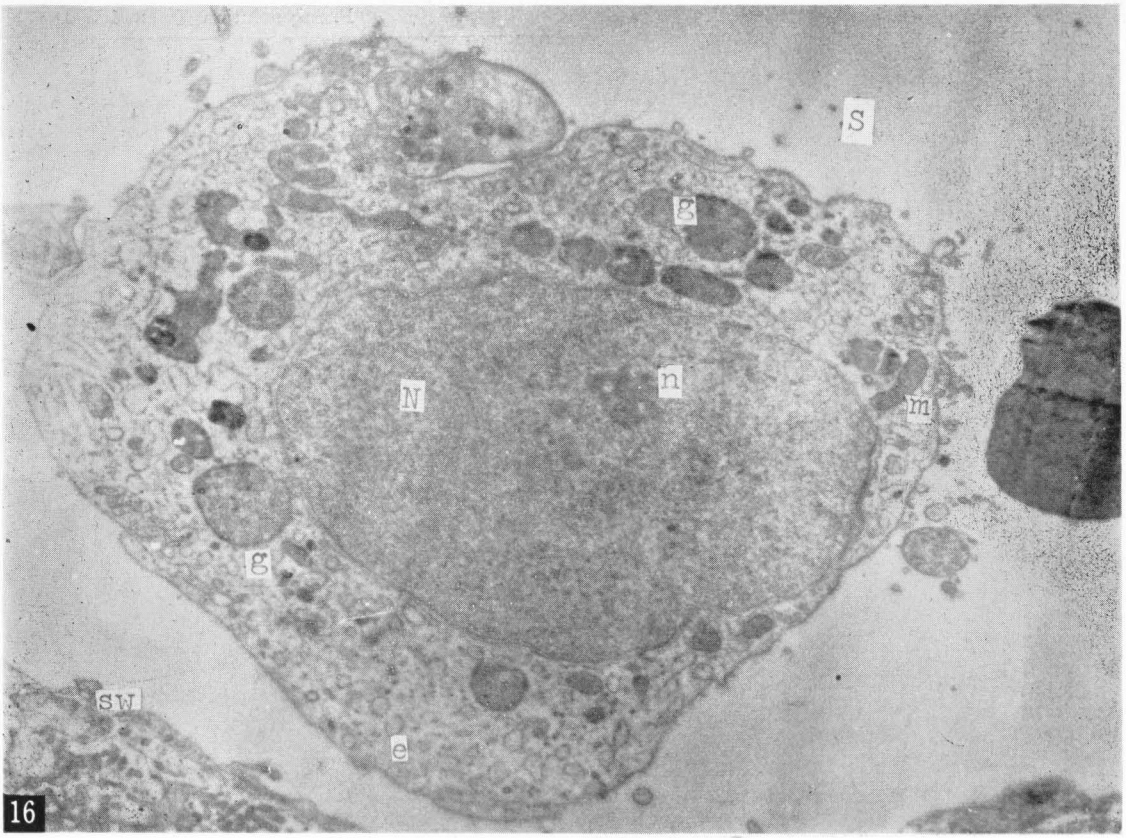

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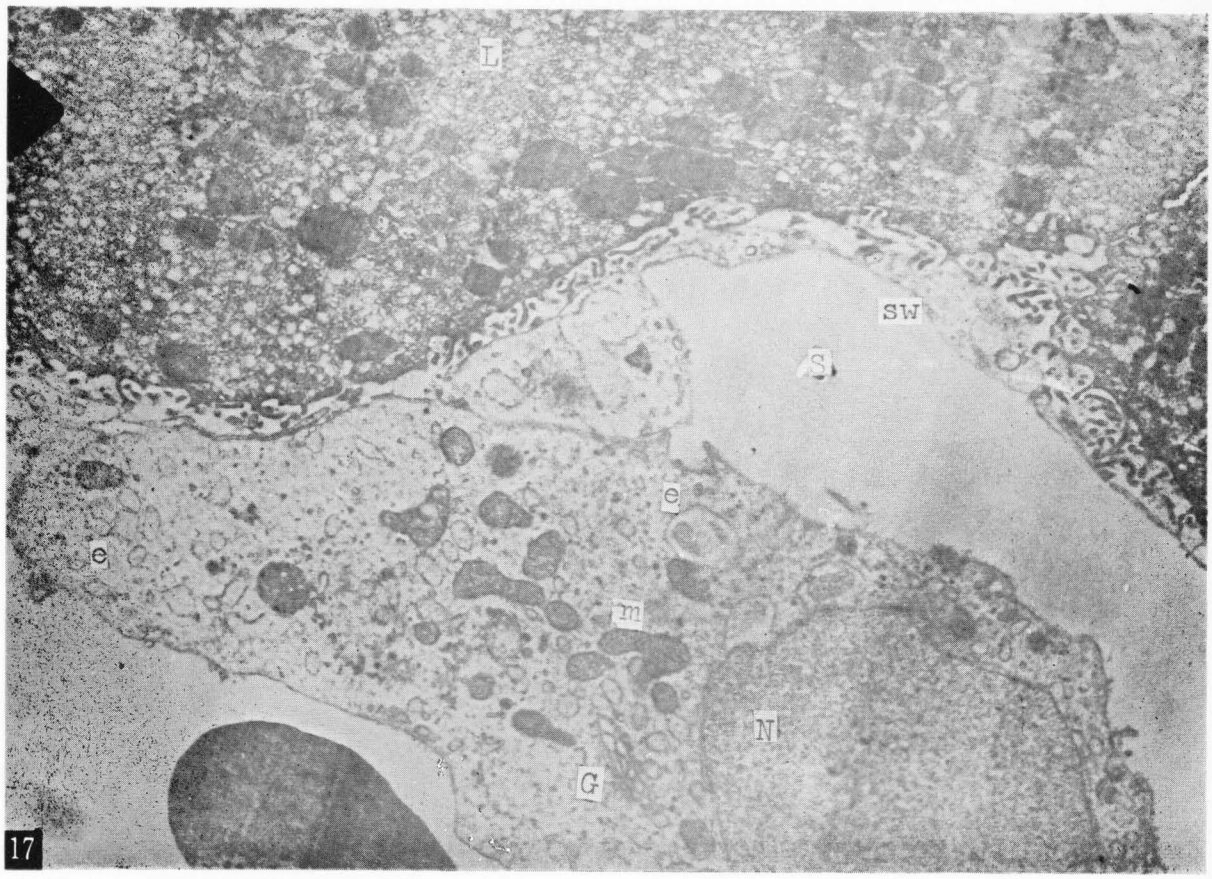

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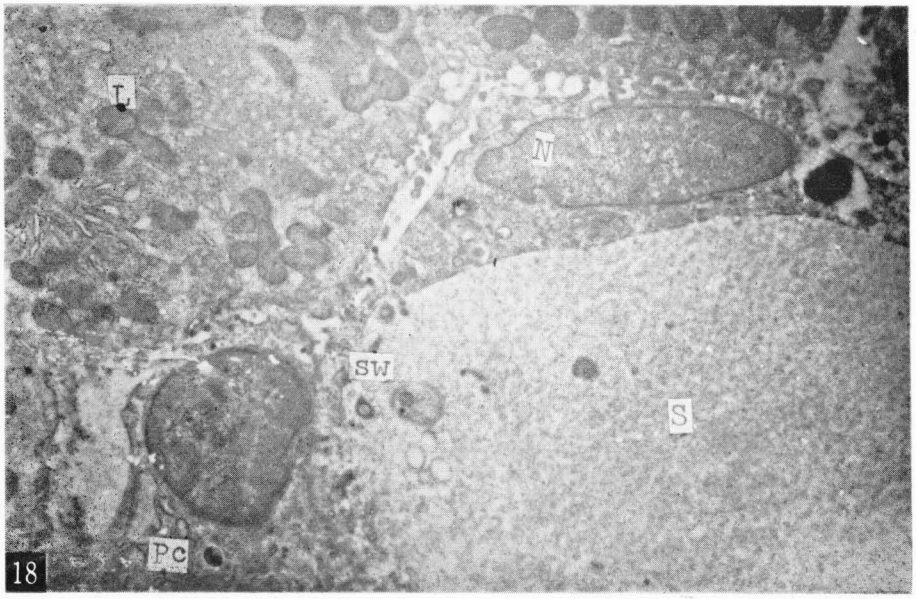

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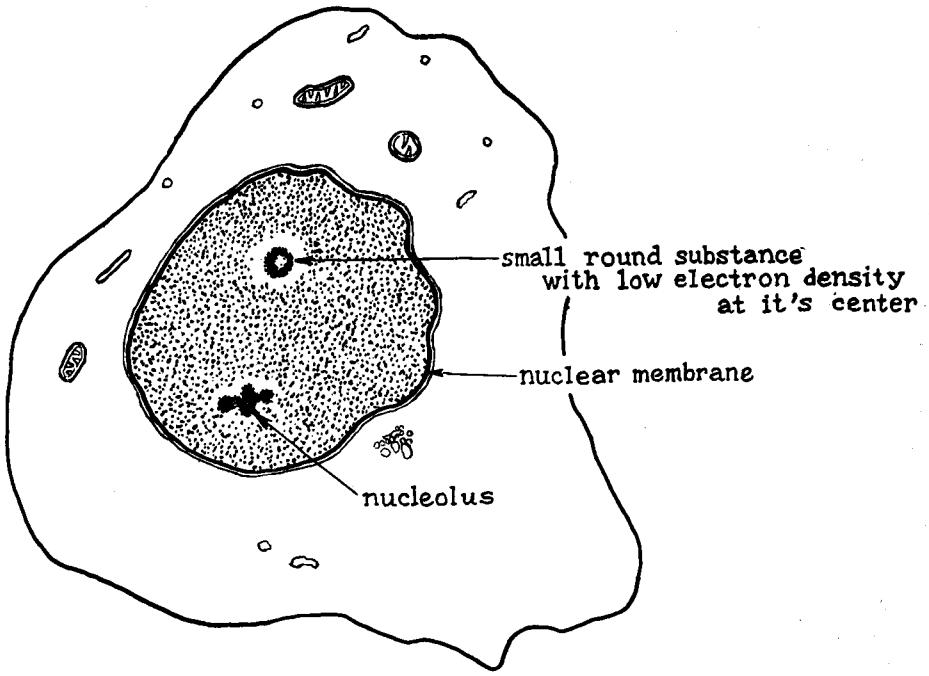

Diagram 1

Table 1

Reporters	Animals	Width of fine fiber	Direction of fine fiber	Relation with adjacentissue
Horiuchi	Rabbit	10- 80A°	To every direction	Joined with cell membrane of sinusoidal endothelials
Wasserman	Rat	200-500A°	To every direction	Presence of fiber in the endothelials
Cossel	Human	100-500A°	To every direction	Presence of fiber in the endothelials
Cossel	Mouse	60-400A°	To every direction	Presence of fiber in the endothelials