ANATOMIC PATHWAY OF BILE FORMATION

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Recent physiologic studies [1] have emphasized the limitation of knowledge concerning the anatomic pathways involved in bile secretion. It was considered desirable, therefore, to evaluate the concepts of the fine structure of the liver recently developed through the use of electron microscopy [2,3] and to ascertain if these might be applied to the problem of bile secretion. Paramount questions that arise include the mechanism of transfer of substances from the blood across the hepatic sinusoidal wall, the means by which these substances are incorporated in liver cells, and the manner and route of transfer of bile constituents to the excretory tracts, the bile canaliculi.

Material and Methods

In the course of other investigations, the livers of 24 normal adult rats and of 2 human subjects were examined by electron microscopy. The specimens were prepared by standard procedures for electron microscopy.[4] An RCA EMU-3 microscope was used. The observations to be described are derived from retrospective analysis of approximately 200 electron micrographs of these preparations.

Observations

Transfer Across the Sinusoidal Wall

Since the fine structure of the liver has been described by several observers, it will be given only brief consideration here. Greater emphasis will be placed upon those aspects which appear to be involved in the production of bile.

The endothelium lining the sinusoids presents certain features which appear to have major physiologic significance. The endothelial cytoplasm forms a very incomplete lining over the hepatic cells. It is interrupted by comparatively large spaces or pores measuring up to 2 μ in diameter (Fig. 1). Through these, there is a ready communication between the sinusoidal lumen and a distinct but narrow space which separates the hepatic cell from the endothelium (the space of Disse). The content of this space is similar to that of the sinusoids, except for the absence of red cells, leukocytes and platelets. Fine granular particles measuring

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about 150 Å, resembling similar elements in normal plasma, and, occasionally, lipid particles (chylomicrons) are found in the space of Disse. This space is occupied by many fine villous projections from the hepatic cells. No structure resembling a basement membrane is found in the wall of the sinusoid.

The anatomic pattern of the sinusoid is ideally suited to the unimpeded transfer of plasma fluid from the blood to the pericellular space. It seems justified to conclude that the hepatic cells are bathed continually in a fluid which has all the constituents of blood except the formed elements. The numerous, fine villous projections of hepatic cell membrane increase the interface with this cell-free, protein-rich fluid. The arrangement described provides an anatomic basis for the active participation of the liver in a wide variety of metabolic phenomena, for the rapid uptake of substances absorbed from the intestinal tract into the portal blood, and for the high content of protein which is found in hepatic lymph.[5]

Although in many pathologic conditions, the sinusoidal lining cells appear to be actively phagocytic, in the normal animal these cells do not contain significant amounts of particulate material. It is, therefore, believed that in the normal animal the endothelium merely serves as an incomplete lining of the sinusoids and may not be significantly involved in the removal of substances from the blood, nor their transfer to the hepatic cells, as has been suggested.[6]

Uptake by Hepatic Cells

The numerous fine villi which project into the spaces of Disse, and the formation of many small vesicles in the peripheral portion of the cytoplasm, suggest a high degree of membrane activity in hepatic cells. The cell membrane, bordering upon the space of Disse, engages in the production of numerous fine infoldings, forming pinocytotic cytoplasmic vesicles (Fig. 2). These draw fluid and small particles from the fluid environment into the cytoplasm, and serve to transport them through the hepatic cell. By this means, some of the constituents of bile gain entrance into the parenchymal cells, preliminary to secretion into the bile.

Observations upon the assimilation of chylomicrons[7] and of ingested latex particles measuring 2,200 Å,[8] indicate that the membrane of hepatic cells is highly active in the process of pinocytosis and imbibition of fluid and fine particles from the pericellular environment. This is in accord with the observation[9] that an energy-consuming mechanism plays an important role in bile formation. Such a mechanism of membrane transport is well known, having first been designated in tissue culture cells by Lewis[10] as pinocytosis, and recently correlated with electron microscopic findings by Bennett.[11]

There is considerable similarity between the mechanisms of uptake by hepatic cells and the intestinal absorption of lipid. The latter has recently been demonstrated with electron microscopy by Palay and Karlin.[12] There is a difference, however, in that absorbed particles in the intestinal epithelium remain encompassed by the membrane of the vesicle until discharged from the cell, while the membranes may disappear from around the incorporated material in the hepatic cell. This allows direct exposure of the material to the hepatic cytoplasmic elements. The imbibed substances apparently may be stored,[13] metabolized, or eliminated from the cell.

Evidence for Secretory Activity in Bile Production

Sometime prior to secretion, bilirubin is conjugated with glucuronides.[14] Unfortunately, the bilirubin molecule is not recognizable in electron micrographs, except when it is deposited in the cell or bile canaliculi as dense aggregates. Therefore, its transfer from the cell to the canaliculi cannot be visualized. The electron-dense particles commonly seen in or on oval microbodies probably represent bile which is temporarily stored in hepatic cells (Fig. 3). These aggregates are grouped around the bile canaliculi. They are frequently incorporated within Golgi substance which appears more prominently around the bile canaliculi than elsewhere in the cytoplasm. Since Golgi substance is known to be related to the process of secretion,[15] it appears highly probable that this is a site of active secretion in hepatic cells.

The bile canaliculi are demarcated only by the hepatic cell membrane, from which extend numerous microvilli (Fig. 4). It is assumed that conjugated bilirubin, cholesterol, alkaline phosphatase, bile salts, and other constituents which appear in the bile in concentrated form, pass from the hepatic cytoplasm across this membrane into the canaliculi. The same route is no doubt followed by bromsulphalein. This process could explain the high concentration of certain constituents of bile and implies an energy-consuming, secretory function by hepatic cells. The delay in appearance of bilirubin and bromsulphalein in bile, after their introduction into the blood, as compared with the more rapid appearance of diffusible compounds, such as lithium salts,[16] is readily understood in the light of these observations. The incorporation into the hepatic cell of protein-bound bilirubin or bromsulphalein, their conjugation with glucuronide, the transit of these substances through the cytoplasm, and their secretion into the canaliculi, would all be expected to require a relatively long time for completion. Saturation of the mechanisms of hepatic removal of bromsulphalein or bilirubin and the competition of these substances for removal[17] can also be explained by the inability of

the liver cells to perform more than a given quantity of work in a period of time.

Evidence for an Anatomic Site of Ultrafiltration in Bile Production

The parallel concentrations of sodium, potassium, chloride and other relatively small molecules in blood and bile [1] and the rapid appearance of K^{42} in the bile as compared with its slower appearance in the liver cells,[18] favor the existence of some form of bile canaliculi-blood contiguity, and the production of an ultrafiltrate of blood as a constituent of bile. Anatomic evidence that may shed light, therefore, on this phase of bile formation, is the existence and nature of possible communications between the canaliculi and the blood or lymph.

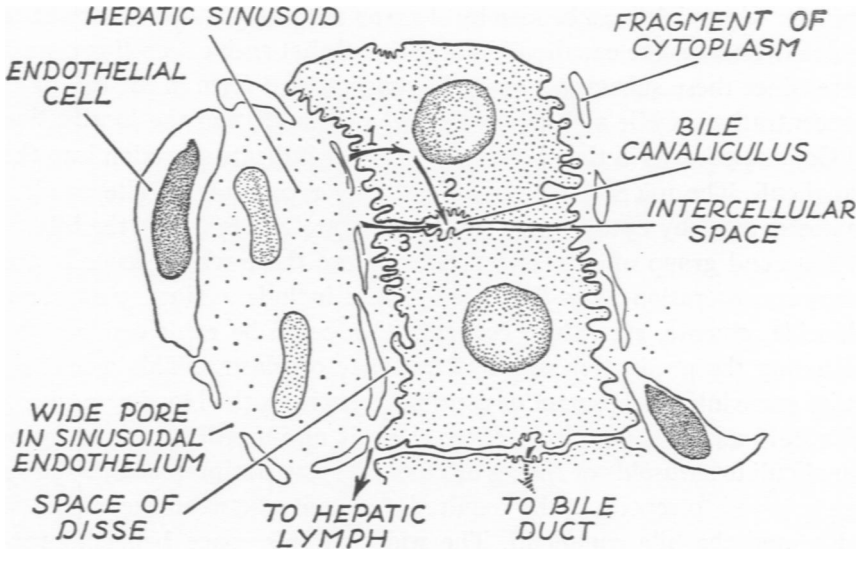
The bile canaliculi do not themselves approach the sinusoids or the spaces of Disse closely. On the other hand, there is a narrow but distinct intercellular space which forms a minute channel 100 Å in width, connecting with both the canaliculi and the space of Disse (Fig. 5). At the site of continuity between the space of Disse and the intercellular space there is usually found a short, wide channel between adjacent liver cells, representing an extension of the space of Disse. This extension is as wide as 1 μ, and may continue for a distance of several microns before it tapers into the 100 Å intercellular space (Fig. 6). The extension has not been observed, however, to communicate directly with the canaliculi without the intervention of the narrow intercellular space. The space is amply wide to permit a flow of water and solutes of small molecular size, but narrow enough that larger molecules, as albumin, would be withheld. Under normal conditions, the direction of flow must be from the spaces of Disse to the canaliculi. By this explanation, the parallel concentration of solutes of small molecular size in blood and bile and the rapid appearance of injected K^{42} in bile can be easily understood. It would also explain the fact that the concentration in the bile of those solutes which would appear in an ultrafiltrate is directly proportional to their plasma concentration. No saturation state in the secretion of these constituents in the bile can be demonstrated.

There is ample anatomic evidence that epithelial cells throughout the body are separated from one another by distinct intercellular spaces in which active movement of intercellular fluid occurs. In the intestinal epithelium, for example, the existence and continuity of such spaces can be readily established by electron microscopic study of the movement of particles in the process of absorption.[12] In some epithelium the intercellular spaces are sufficiently narrow that only protein-free filtrate would be expected to circulate. In others, such as stratified squamous

epithelium, comparatively wide spaces exist between the cells, so that protein-containing fluid could readily pass through these channels.[19]

Discussion

When the fine structure of the liver is evaluated in conjunction with physiologic observations, a reasonable basis for the route of bile secretion can be postulated (Text-fig. 1). The hepatic sinusoidal wall differs from other capillary structures in the body in the absence of a basement mem-



Text-figure 1. Schematic diagram to show the concept of anatomic pathway of bile secretion.

1. Transfer of materials from space of Disse to hepatic cell cytoplasm.
2. Secretion from hepatic cell into bile canaliculi.
3. Formation of ultrafiltrate from space of Disse through narrow intercellular channel into canaliculus.

brane and the existence of large openings in the cytoplasmic lining.[20] This allows a ready passage of plasma into the pericellular spaces of Disse. Thus, the plasma bilirubin, even though bound to albumin, is brought into intimate contact with the hepatic cell membrane and its fine villous projections. There is no evidence that the lining endothelium or Kupffer cells serve as intermediate carriers of bilirubin. Uptake of bilirubin by hepatic cells is postulated to represent the result of the activity of the hepatic cell membrane. Here, vesicles are visibly produced, transporting fluid and particles into the hepatic cell cytoplasm. This same pumping mechanism probably explains the hepatic uptake of chylomicrons, other small particles, and possibly fluid and solutes too

small for visualization even by electron microscopy. This is obviously an active, energy-consuming process, which may very well be one of the major limiting factors as far as the metabolic activity of hepatic cells is concerned. A certain analogy can be drawn between particulate absorption by intestinal epithelium and the uptake of substances by hepatic cells.

A subsequent step in bile secretion involves the elimination from the hepatic cell into the bile canaliculi of conjugated bilirubin and of other substances modified or synthesized by the hepatic cell. Although no transfer of particles can be seen by electron microscopy at the membrane which delineates the canaliculi, it is believed that active secretion occurs here, since these substances occur in concentrated form in the bile. The concentration of bile aggregates around canaliculi, and the localization of Golgi apparatus in the same region further indicate secretion into the canaliculi. The microvilli in canaliculi may represent the site of clasmatosis, whereby cytoplasmic constituents are released into the bile.

A second group of constituents of bile are those which occur in the same concentration as in the blood. These include sodium, potassium, chloride, glucose, etc. Their concentration could be explained best by assuming the production of an ultrafiltrate of plasma. This would require some intimate contact or continuity between the blood or pericellular fluid and bile canaliculi. Since there is no immediate proximity of canaliculi to sinusoids or spaces of Disse, the narrow intercellular spaces are believed to represent the required continuity between the spaces of Disse and the bile canaliculi. The width of this space being approximately 100 Å, it is well disposed for the exclusion of protein molecules while allowing the free passage of water and solutes of relatively small molecular size. The problem of pressure relationships has to be considered before such a route for the flow of ultrafiltrate can be accepted. The hydrostatic pressure of sinusoidal blood, transmitted to the space of Disse, would well serve as the main driving force for filtrate formation. It is known that variations of perfusion pressure in isolated liver preparations are accompanied by parallel changes in bile flow. Further, the flow of bile can be reversed by raising the pressure within the biliary system sufficiently, causing the bile to disappear from the bile ducts and canaliculi.[21] This would require, and could logically be explained by, a patent connection between canaliculi and sinusoids, and a flow in the reverse direction from normal. It is difficult, however, to explain the observation that even after increasing the bile duct pressure in perfusion experiments to the point where it exceeds perfusion pressure, bile flow continues for a period of time.[21] This might indicate that the bile flow mechanism is dependent upon other factors besides hydrostatic pressure

of blood. It is probable that secretion from the hepatic cells may add to the driving force of bile flow, as may also the motility of the microvilli in bile canaliculi and activity of bile duct epithelium.

It is perhaps significant that there are certain similarities between the anatomic pathway of glomerular filtrate production and that proposed for the ultrafiltrate in the liver. In the epithelium covering glomerular capillaries, foot processes of epithelial cytoplasm are applied to the external surface of capillary walls. Between these foot processes, or pedicels, are narrow channels (slit pores) of approximately 100 Å width.[22] These are analogous, in some respects, to the narrow intercellular spaces between liver cells, described above. They appear to perform a similar function to that proposed for the intercellular spaces in hepatic laminas, namely, the flow of a filtrate of blood, devoid of its protein content.

Summary

The present state of knowledge of the pathway of bile secretion is incomplete. The mechanism of production and the anatomic route of what appears to be an ultrafiltrate of plasma as one of the constituents of bile has not been demonstrated. Examination of electron micrographs of normal rat and human liver reveals an anatomic pathway which is reconcilable with the known physiologic aspects of bile secretion. The plasma of hepatic sinusoidal blood is found to have ready access to the pericellular spaces of Disse through wide defects or pores in the endothelial lining. An active pinocytotic process presumably occurs at the hepatic cell membrane lining the space of Disse, permitting plasma constituents to be transported into the hepatic cells by an energy-consuming mechanism. Secretion of certain concentrated bile constituents into the canaliculi from the hepatic cell is postulated. Narrow spaces, 100 Å wide, are shown to extend between the cells from the space of Disse to the bile canaliculi. This provides a route through which protein-free ultrafiltrate can be transported from plasma to bile canaliculi. The driving force of this flow can be attributed to the hydrostatic pressure of sinusoidal blood.

The mechanism described would explain the highly concentrated components of bile, as well as the constituents which occur in concentrations approximately equivalent to those of plasma. It would also explain the absence of protein in bile secretion. Pathologic and physiologic aspects of bile secretion appear to be clarified by this proposed mechanism.

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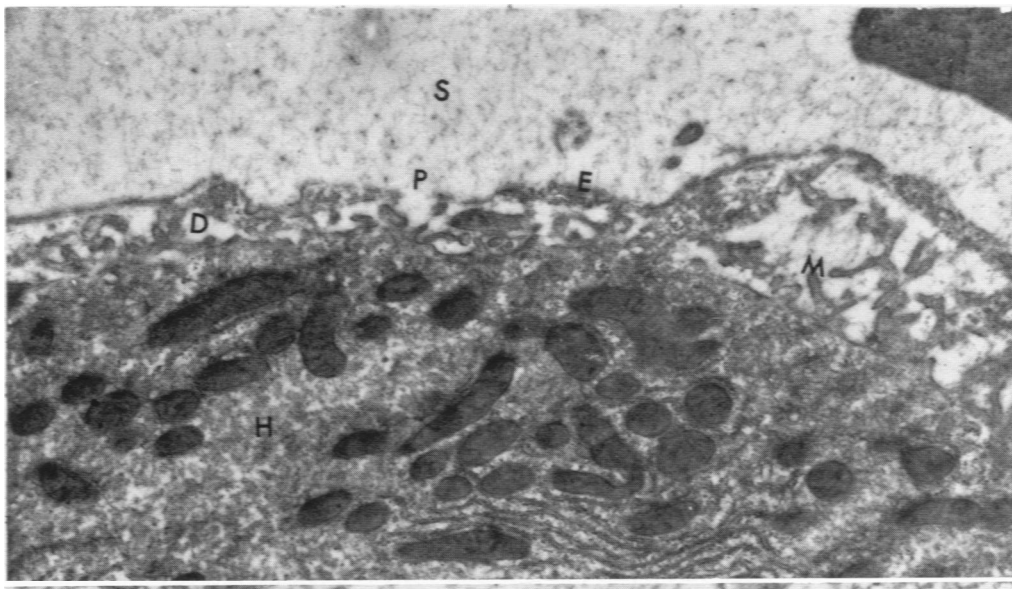
[*Illustrations follow*]

Legends for Figures

FIG. 1. Electron micrograph of a hepatic sinusoid (S) and a portion of a hepatic cell (H). The sinusoid is lined by a thin layer of endothelial cytoplasm (E) which contains numerous large defects or pores (P). The space of Disse (D) is apparent, and many microvilli (M) of the hepatic cell project into the space. × 11,200.

FIG. 2. The peripheral portion of a hepatic cell (H) borders on a sinusoid (S). The pinocytotic vesicles (V) are numerous and prominent in this portion of the cytoplasm. × 16,000.

FIG. 3. A bile canaliculus (C) is shown. Intercellular spaces (X) communicate with the canaliculus through approximately 100 Å openings. Bile pigment granules on dense microbodies (B) are grouped around the canaliculi. × 8,000.



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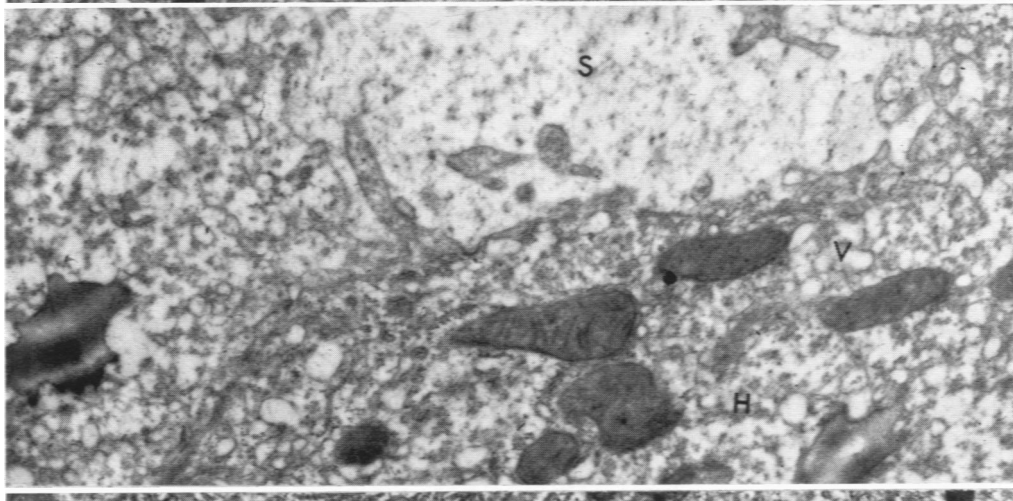

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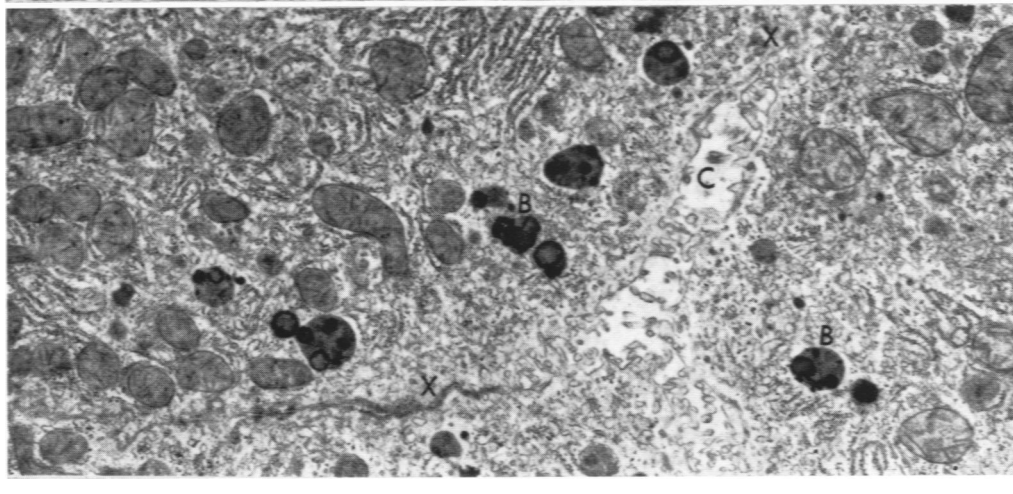

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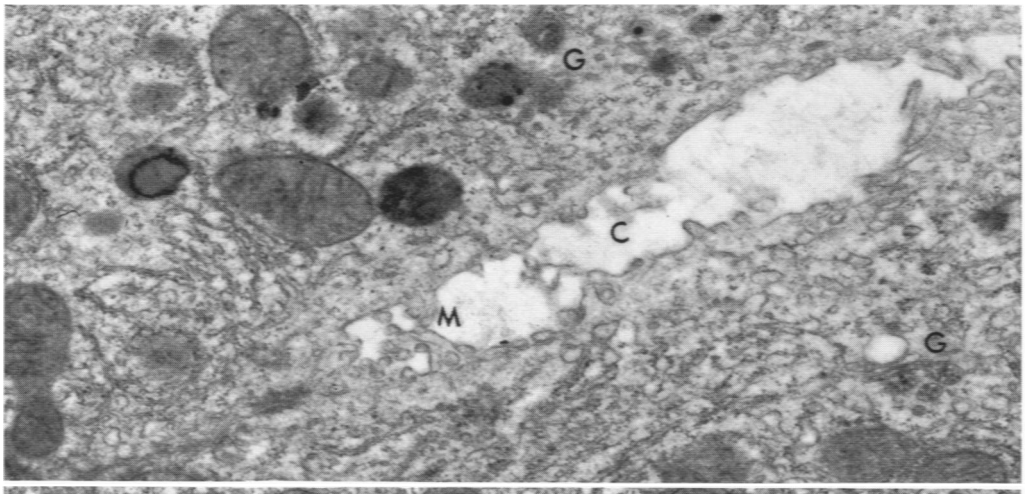
FIG. 4. In a bile canaliculus (C), prominent microvilli (M) project from the hepatic cell membrane into a canaliculus. The canaliculus is devoid of walls, being demarcated only by the hepatic cell membranes. Golgi material (G) is observed in the hepatic cytoplasm near the canaliculus. × 16,000.

FIG. 5. A sinusoid (S) is lined by a perforated endothelial cytoplasmic membrane (E). A triangular extension (T) of the space of Disse extends between two adjacent parenchymal cells, and is continuous with the intercellular channel (X). The channel measures approximately 100 Å in width. A dense plaque (P) or desmosome is seen in the cytoplasm on either side of the intercellular space at the point where the channel communicates with the canaliculus (C). × 11,200.

FIG. 6. Portions of liver cells show the communication of the intercellular channel (X) with a bile canaliculus (C). × 16,000.



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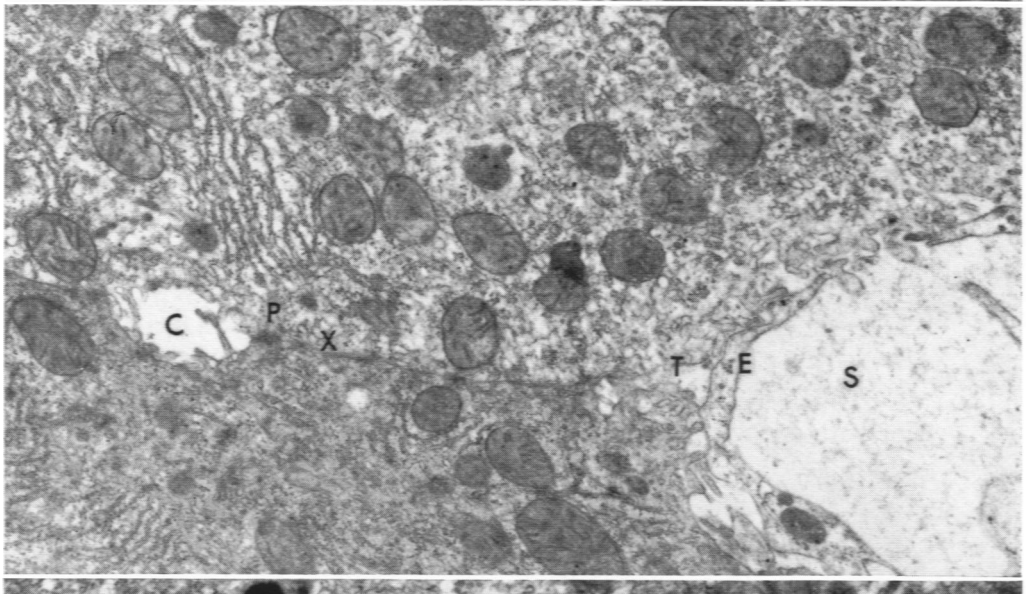

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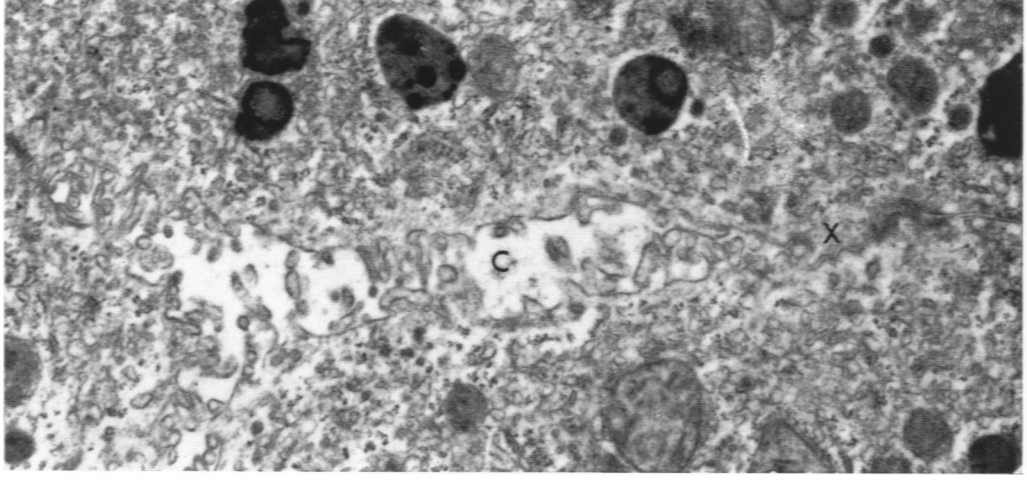

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