Electron Microscopic Study on Uptake and Excretion of Biligrafin by the Hepatic Parenchymal Cells in Rats

Yasumaro KAJII

Department of Pathology,
(Director: Prof. S. Hosokawa)
Yamaguchi University School of Medicine. Ube, Japan
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The hepatic parenchymal cells are not so complicated in morphology, but have many functions such as glycogen metabolism, detoxication, production of the bile and others. Excretion of the harmful substances in the blood stream and of the intravenously injected materials is also one of the important functions of the liver cells. To examine such excretory activity is nowadays considered to be useful clinical aid in the interpretation of hepatic cell function.

Morphological studies on the production of bile and excretory mechanism in the liver cell are relatively scanty. Only Hampton[1], Ohkita[2] and Nango[3] investigated on these subjects either electron microscopically or histochemically. Hampton[1] showed that intravenously injected particles of colloidal mercuric sulfide and thorotrast were transported across the hepatic cells and discharged into the bile canaliculi. He also demonstrated that after retrograde injection of colloidal material into the bile duct colloidal particles were transported across the hepatic cells from bile to blood stream. After ligation of the common bile duct or following administration of cholagogue, Ohkita[2] and Nango[3] examined the rat liver cells electron microscopically and histochemically, and they pointed out the significant role of the Golgi complex in excretion of the bile. These investigations, however, are devoid of detailed description on the other cell organellae which may play some roles in excretory mechanism.

As a part of systemic morphological studies of the liver which have been continued in our department, the author performed electron microscopic study of the liver cells following intravenous injection of Biligrafin which is commonly used for intravenous cholangiography to human subjects. The purpose of this paper is to describe the mechanism whereby Biligrafin particles are transported from the sinusoidal surface to the biliary surface across the hepatic cells, and also to demonstrate the morphological alterations and functional significance of the cell organellae in the transfer of the material.

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MATERIALS AND METHODS

Adult albino rats were used. They were fed a stock diet and were injected 1 ml. of Biligrafin into the common iliac vein. Under ether anesthesia, the animals were sacrificed for study at each time intervals such as, 5, 15, 30, 45, 60 and 120 minutes following injection. Small pieces of the liver tissue were immediately fixed with one per cent osmium buffered with veronal acetate for approximately two hours at 40°C. Then tissues were washed and dehydrated with ascending alcohol and finally embedded in methacrylate or Epon 812. Thin sections were cut with Porter-Blum ultramicrotome employing glass knives. Thin sections were stained with uranyl acetate or doubly stained with uranyl acetate and lead citrate according to Reynald's method.[4] Stained sections were examined with a Japan Electron Optics LAB. JEM-5HS electron microscope.

A part of the liver was also fixed with 10 % formalin and examined with light microscope after staining with hematoxylin eosin and periodic acid Schiff reaction.

As a control, the liver of the rats which were fed the same diet was likewise examined both light and electron microscopically. To avoid the difference of distribution of Biligrafin in the liver, the specimens were taken from the same portion of the right inner lobe.

RESULTS

I) ELECTORN MICROSCOPIC FINDINGS

I) Endoplasmic Reticulum

(A) : *Smooth-Surfaced Endoplasmic Reticulum*

In the control group, the smooth-surfaced endoplasmic reticulum was commonly vesicular in shape and occasionally reticular or tubular. It was situated among the rough-surfaced endoplasmic reticulum and was more developed near the cellular surface (Fig. 1).

In the experimental group, the smooth-surfaced endoplasmic reticulum was well developed and showed dilatation of the lumens. These changes were most prominent at five minutes following injection of Biligrafin. The smooth-surfaced endoplasmic reticulum situated at the sinusoidal surface was distended up to two or five times in diameter comparing to that in the control liver cell. The cytoplasm was almost filled with the enlarged smooth-surfaced endoplasmic reticulum and mitochondria (Fig. 2, 3). At the canalicular site, the smooth surfaced endoplasmic reticulum was moderately developed, but distension of their lumen was less prominent (Fig. 14). Such alteration of the smooth-surfaced endoplasmic reticulum gradually subsided and returned to the similar morphology

of the control liver cell at 120 minutes after injection.

At 60 minutes following injection of Biligrafin, a few granules were observed in the lumen of the smooth-surfaced endoplasmic reticulum. These granules were almost round and had an approximate diameter of 40 to 80 mμ and showed high electron density (Fig. 5, 6). The smooth-surfaced endoplasmic reticulum containing these granules was evenly distributed throughout the cytoplasm, but revealed the tendency to be more developed at the sinusoidal site. The endoplasmic reticulum at the canalicular site also contained the similar granules (Fig. 13, 15).

(B) : *Rough-Surfaced Endoplasmic Reticulum*

In the control group the rough-surfaced endoplasmic reticulum showed lamellar or ribbon-like arrangement and the lumen was quite narrow (Fig. 1). In some areas in the cytoplasm, it was arranged in parallel fashion.

In the experimental group, the rough-surfaced endoplasmic reticulum showed the tendency to be less prominent. In 30 minutes after injection of Biligrafin, the rough-surfaced endoplasmic reticulum as well as the smooth-surfaced form was mostly distended (Fig. 2, 3). Such distended form was partially devoid of the Palade's granules. In some areas, anastomosis of the rough-surfaced endoplasmic reticulum with the smooth-surfaced one was seen, and the rough-surfaced reticulum was arranged in lamellar fashion (Fig. 2).

At 60 minutes following injection of Biligrafin, the rough-surfaced endoplasmic reticulum revealed no enlargement of the lumen and scatteringly distributed in the cytoplasm occasionally showing lamellar arrangement. There was no granules, which were observed in the smooth-surfaced form, in the lumen of the rough-surfaced endoplasmic reticulum.

2) *Golgi Complex*

In the control group, identification of the Golgi complex was difficult in most hepatic cells. In some cells, however, the Golgi complex was rather small and consisted of a few lamellae, vesicles, and vacuoles. The Golgi complexes were scarcely visible at the sinusoidal site, and mostly situated near the nucleus at the canalicular site or at the vicinity of the bile canaliculi.

At 5 minutes after injection of Biligrafin, the Golgi complexes were hardly discernible. At 30 to 45 minutes, one or two Golgi complexes were observed, but they were morphologically almost similar to those observed in the control liver cells (Fig. 4).

At 60 minutes following injection, the Golgi apparatus were markedly developed and its three elements (lamellae, vesicles and vacuoles) were easily discernible even at the low magnification (Fig. 7, 8). They showed various morphological appearances and were located at the sinusoidal site, in the vicinity of the nucleus (Fig. 7) or near the bile canaliculus (Fig. 8). In a single liver cell two or three Golgi areas were frequently seen.

Characteristic findings observed in these Golgi complexes was appearance of the same granules as those seen in the smooth-surfaced endoplasmic reticulum.

The granules were recognized in the lamellae, vesicles and abundantly in the vacuoles (Fig. 7-12). The lamellae were varied in shape, some had distended tips or some showed cystic distension at the middle portion (Fig. 11, 12). Mostly individual vesicle contained only one granule.

3) *Lysosomes*

In the experimental group, the lysosomes were slightly increased in number. They were markedly pleomorphic and some of them contained ferritin-like particles or a little large particles. The electron density of the matrix of the lysosomes were also varied. They were limited by the single membrane and were round or oval in shape scattered throughout the cytoplasm. At the canalicular site, the lysosomes contained the similar granules to those seen abundantly in the Golgi complex (Fig. 16).

4) *Mitochondria*

Comparing to the control group, there was no morphological changes of the mitochondria following infusion of Biligrafin. The matrix and cristae mitochondriales showed no marked alteration. However, in some areas where dilatated endoplasmic reticulum assembled, indentation of the membrane of the mitochondria was observed.

5) *Nucleus*

The nucleus showed no remarkable changes, but in the experimental liver there was a tendency that the nuclear pore was clearly visible.

6) *The Surface of the Liver Cell and the Bile Canaliculi*

The microvilli on the surface of the hepatic cells showed no morphological changes. In both control and experimental livers, the liver cells were confronted each other having the intercellular space of 100 to 150 A, and the invagination of the cell memebrane was occasionally found.

In the experimental group, the bile canaliculi were varied in morphology. Some showed distended space, some revealed loss of the microvilli, and some canalicular spaces were filled with the microvilli. But in general, there was no specific granules in the canalicular space. The microvilli at the canalicular surface demonstrated the similar electron density to that of the cytoplasm and revealed no alterations such as swelling or elongation (Fig. 8, 14, 15, 16).

II) LIGHT MICROSCOPIC FINDINGS

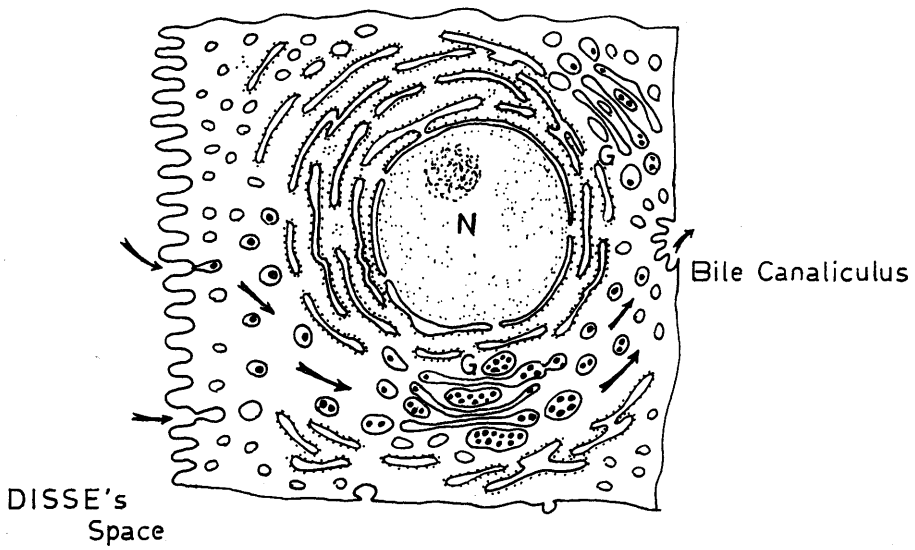
In the light microscopic studies, no significant changes were obtained after intravenous injection of Biligrafin. No aggregates of Biligrafin particles were found in the hepatic cytoplasm.

DISCUSSION

Biligrafin is N, N'-adipine. di-(3-amino. 2. 4. 6. triiodopylvate) methygultamate containing 30 to 50 per cent iodine and has been reported to have sufficient electron density to be recognized with electron microscope[5]. Even if the granules observed in this experiment could not necessarily identified with Biligrafin particles themselves, it would be reasonable to regard the particles with high electron density to be mainly derived from injected Biligrafin. Furthermore, organellar changes in the liver cells would be caused by infusion of this substance. Therefore, it would be of significance to discuss the mechanism and pathways implicated in the transfer of Biligrafin particles in the hepatic parenchymal cells.

From the results of electron microscopic findings observed in this experiment, following speculation would be suggested as to uptake of Biligrafin by the liver cells and excretion of its particles into the bile capillaries.

Biligrafin particles taken up at the plasma surface of the hepatic parenchymal cells were enveloped by the smooth-surfaced endoplasmic reticulum and were transported to the Golgi complexes, where the substance was stored temporarilly. The granules of Biligrafin were then enveloped mainly with the smooth-surfaced endoplasmic reticulum and / or occasionally with the lysosomes, and were finally discharged into the bile capillaries. The schema of the transport mechanism is shown in Text-Figure. Detailed discussion is made about this speculation.



Text-Figure. Diagramatic representation of transport-mechanism of Biligrafin in the liver cell. Round granules indicate Biligrafin particles.
(N: nucleus, G: Golgi complex)

1) *Smooth-Surfaced Endoplasmic Reticulum and Uptake of Biligrafin.*

At five minutes following intravenous injection of Biligrafin, the smooth-surfaced endoplasmic reticulum showed marked development and distension of the lumen. These two morphological changes were more prominent at the sinusoidal site, but gradually returned to the similar morphology of the control liver with the lapse of the time. Such changes of the smooth-surfaced endoplasmic reticulum is presumed to be intimately related with uptake of Biligrafin by the liver cells.

Popper and Schaffner[6] have reported that the single membrane vesicles at the sinusoidal surface have close relation to pinocytosis and they have designated these vesicles "pinocytotic vesicles". However, morphological differentiation between the pinocytotic vesicles and smooth-surfaced endoplasmic reticulum is very difficult. According to the definition of the smooth-surfaced endoplasmic reticulum[7][8], these vesicles located near the cell surface should be included in the smooth-surfaced endoplasmic reticulum.

In this experiment, the granules with increased electron density were observed in the smooth-surfaced reticulum. Noro[9] and Aschworth[10] described the appearance of the similar granules in the smooth-surfaced endoplasmic reticulum in the liver cells at the time of lipid absorption. After intravenous administration of HgS and ThO_2 in the rat, Hampton[1] observed these colloidal particles within the tubular structures and small vesicles in the liver cells. Oudea[11] reported that mercuric sulfide taken up by the rat liver cell was seen within the pinocytotic vacuoles. In addition, Palay and Karlin,[12] and Onoe[13] found lipid droplets within the smooth-surfaced endoplasmic reticulum of the intestinal epithelial cells. Considering these observations, it would be probable that the granules observed within the smooth-surfaced endoplasmic reticulum in this study are chiefly derived from infused Biligrafin.

The vesicles located at the periphery of the cytoplasm were found very adjacent to the hepatic cell surface. Some vesicles retained narrow openings at the cell surface and the others were devoid of them. These findings suggest the direct continuity of the smooth-surfaced endoplasmic reticulum with the cytoplasmic membrane, or invagination of the cell membrane. In addition, the findings indicate that uptake of Biligrafin by the hepatic parenchymal cells is performed according to membrane flow theory mentioned by Bennet[14], or through the opening of the cell membrane as reported by Uchino[15]. Both two theories confirm the direct continuity between the smooth-surfaced endoplasmic reticulum and the cell membrane. Palade[8][16], Robertson[17], Dempsy[18], Pease[19] and Yamada[20] also have reported the same result. This connection can serve as a route for exchange of the intracellular and extracellular substances, and the smooth-surfaced endoplasmic reticulum may play a significant role as an important intracellular transport system of the substances.

2) *Golgi Complex*

The Golgi complex contained the same granules as those observed in the smooth-surfaced endoplasmic reticulum, and in the Golgi complex the granules were observed in the form of an aggregate. This finding will suggest that the particles taken up by the smooth-surfaced endoplasmic reticulum have been accumulated in the Golgi complex. Similar mechanism has been reported to operate in absorption of lipid by the intestinal[12)13)] and duodenal epithelial cells[21)], where transfer of the lipid droplets from the smooth-surfaced endoplasmic reticulum to the Golgi apparatus has been observed.

Palade[8)] has reported that the Golgi membrane is actually a specialized form of the endoplasmic reticulum and he has demonstrated the morphological continuity between them. He has also considered that the Golgi apparatus is the membrane "depot" into which the membranes of pinocytotic and phagocytotic vacuoles flow. Palay and Palade[22)], and Freeman[23)] have mentioned that the Golgi complex is a special aggregation of the smooth-surfaced membrane. Robertson[17)] has described the continuity between the cisternae of the Golgi complex and the endoplasmic reticulum. Observing two functional hepatomas in rats, Essner and Novikoff[24)] have reported that smooth surfaced derivatives of the endoplasmic reticulum become refashioned into the Golgi membrane. Dalton and Felix[25)], on the other hand, have reported that the Golgi apparatus differs both morphologically and functionally from the endoplasmic reticulum. Thus, the exact relationship between the Golgi complex and the endoplasmic reticulum still remains unknown.

In this study, the direct communication of the endoplasmic reticulum with the Golgi complex has not been demonstrated, but in some areas, the endoplasmic reticulum was located close to the Golgi lamellae. In addition, the membrane structures which contained dense granules were difficult to be identified if they were the endoplasmic reticulum or the Golgi lamellae. From these findings, the author considers that there exsists a connection between the endoplasmic reticulum and the Golgi complex and through this connection the particles in the endoplasmic reticulum is transported to the Golgi apparatus. As have been reported by many investigators[25)27)28)], the three elements of the Golgi complex transform each other. In some cases, the Golgi lamellae are continuous with the Golgi vesicles, and the vacuoles are located adjacent to them. By transformation of three elements of the Golgi complex the substance is transferred from the lamellae to the vacuoles. Remarkable increase or prominent appearance of the Golgi complex following administration of Biligrafin would be one of morphological changes favourable to excretion of Biligrafin into the biliary system.

It is still undetermined what kind of chemical reactions occur to the Biligrafin particles within the Golgi complex. There are abundant reports about function of the Golgi complex. Participation of the Golgi complex in the formation of

zymogen granules in the pancreatic exocrine cells have been studied in detail[23)29)30)]. It is widely accepted that the Golgi complex in the secretory cells serves as a site for concentration of the materials produced at the other sites in the cytoplasm. As to the functional significance of the Golgi complex in the hepatic cell, however, there are only a few reports. Ohkita[2)] and Nango[3)], using the parabiotic rats, have examined the liver cell following administration of cholagogue. After ligation of the common bile duct of the one rat, they examined the liver of the non-ligated rat. Observing marked development of the Golgi apparatus and appearance of the granules within the vacuoles, they concluded that these granules would be the substances related with the bile components. Furthermore, they have reported that the Golgi complex plays an important role in concentration of the bile. Appearance of aggregates of dense particles in the Golgi complex following intravenous administration of Biligrafin suggests condensation and storage of Biligrafin in the hepatic cells. It is probable that these particles are transported across the cytoplasm being enveloped mainly by the smooth-surfaced endoplasmic reticulum and are finally discharged into the bile capillary.

3) *Lysosomes*

From the evidence that both the Golgi vacuoles and vesicles reveal increased intensity of staining for acid phosphatase and the lysosomes similarly show increased intensity of staining for this enzyme, Novikoff and his associates[31)32)33)] have reported that some lysosomes are derived from the Golgi vesicles and they have designated them as primary lysosomes. In addition, Essner and Novikoff[24)] have considered that the Golgi apparatus makes secretory vacuoles and lysosomes.

Originally, the lysosomes was biochemical designation applied by de Duve[34)35)] to a group of cytoplasmic particles which were obtained by differential centrifugation and characteristically contained about eight kinds of hydrolytic enzymes such as acid phosphatase. Morphologically, the lysosomes are small round bodies with the approximate diameter of 0.4 to 1.0 μ, surrounded by a single limiting membrane.

In the hepatic parenchymal cells, the lysosomes have been variously named, such as peribiliary body[36)], dense body[31)] or microbody[37)]. In the phagocytic cells, on the other hand, they have been called phagosomes[38)]. It is generally thought that the lysosomes participate in digestion of some kinds of materials taken up by pinocytosis or phagocytosis, and also in secretion of the substances. Hampton[1)] has described that mercuric sulfide and Thorotrast administered intravenously are taken up by the pinocytotic vesicles into the liver cells and then these vesicles aggregate and transform into the lysosomes. According to Oudea[11)], colloidal mercuric sulfide were taken up into the rat liver cells by the pinocytotic vacuoles, and after gradual concentration of the substance a kind of lysosomes is formed. Following injection of neutral red into the rat, Nango[3)] and Ohkita[2)] have found that the organellae with increased electron density are

markedly increase in number and gradually assemble in the vicinity of the bile canaliculus. They have also reported that administration of cholagogue causes proliferation of the lysosomes around the bile capillary. In this experiment, however, increase of the lysosomes was not observed, but the lysosomes near the bile capillaries occasionally contained small granules. Most granules probably derived from Biligrafin were recognized within the smooth-surfaced endoplasmic reticulum at the canalicular site in the cytoplasm. Therefore, intravenously administered Biligrafin would be discharged into the biliary system, being enveloped chiefly by the smooth-surfaced endoplasmic reticulum, though the lysosomes may also play some roles in secretion of Biligrafin.

SUMMARY

After intravenous injection of Biligrafin, the changes of the cytoplasmic organellae in the liver cells of the rats were examined electron microscopically and the following results were obtained. Discussion on uptake and active transport of substance from plasma to bile is also made.

1) At five minutes following injection of Biligrafin, the smooth-surfaced endoplasmic reticulum showed marked development and distension of the lumen which contained some dense granules with an approximate diameter of 40 to 80 mμ. Proliferation of the endoplasmic reticulum was more prominent at the sinusoidal area in the liver cell.

2) At 60 minutes the Golgi complex showed a prominent appearance, and the vesicles and vacuoles contained many dense granules. Near the bile canaliculi, similar granules were recognized mainly within the smooth-surfaced endoplasmic reticulum. Though there was no increse of the lysosomes, they occasionally contained the same granules especially in the vicinity of the bile capillary.

3) The mitochondria and the nucleus revealed no remarkable changes.

4) From these findings, the following conclusion has been obtained as to the mechanism whereby the hepatic parenchymal cells move Biligrafin particles across the cytoplasm from plasma to bile. Biligrafin particles taken up by pinocytosis and / or phagocytosis at the sinusoidal surface are enveloped by the smooth-surfaced endoplasmic reticulum, transported to the Golgi complexes where the materials may be concentrated, transferred chiefly to the smooth-surfaced endoplasmic reticulum and finally discharged into the bile capillaries.

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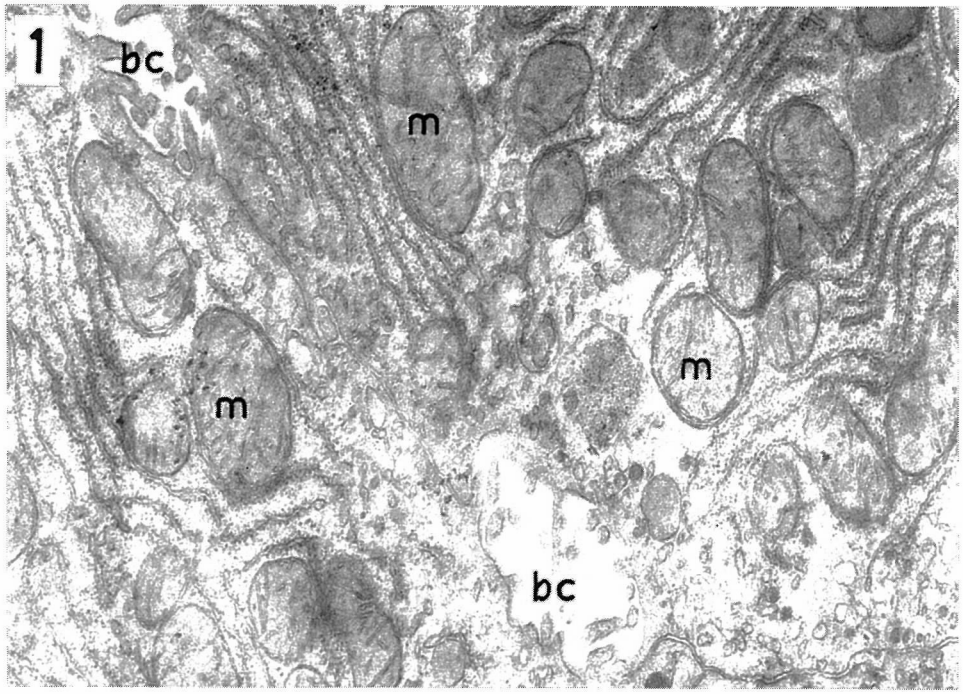
EXPLANATION OF FIGURES

Key to abbreviation

N : nucleus, G : Golgi complex
D : Disse's space, bc : Bile canaliculus,
ser : Smooth-surfaced endoplasmic reticulum
rer : Rough-surfaced endoplasmic reticulum
m : Mitochondria

Fig. 1. Control liver cells. The bile capillaries are seen between two adjacent liver cells. The rough-surfaced endoplasmic reticulum is moderately developed. × 28,000

Fig. 2. At five minutes after injection of Biligrafin, well-developed smooth-surfaced endoplasmic reticulum is seen in the central area of this photograph. At the left side, the rough-surfaced endoplasmic reticulum shows lamellar arrangement. × 24,500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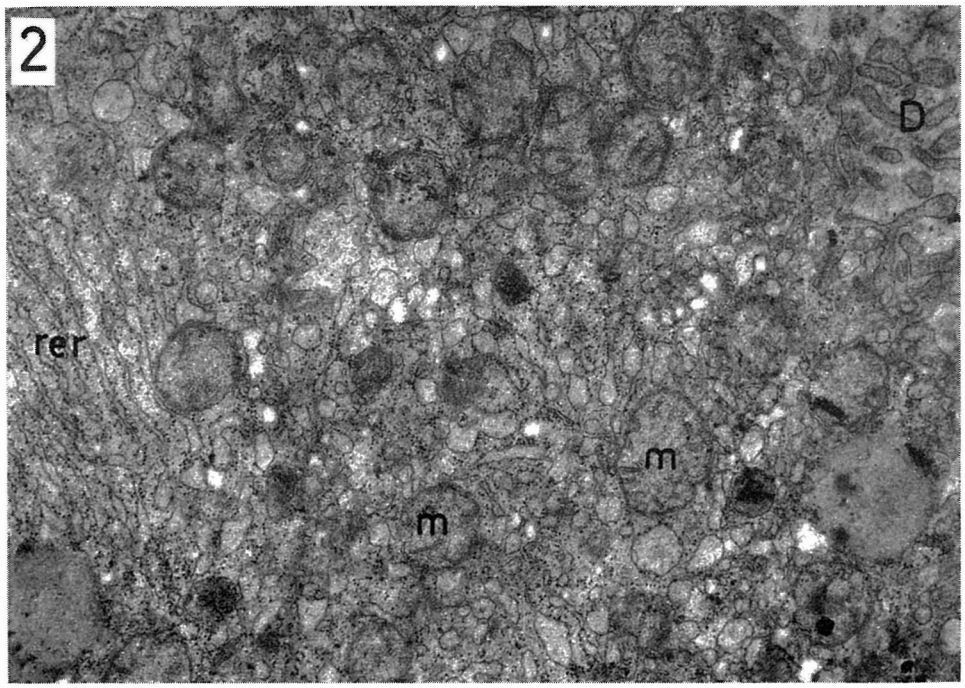
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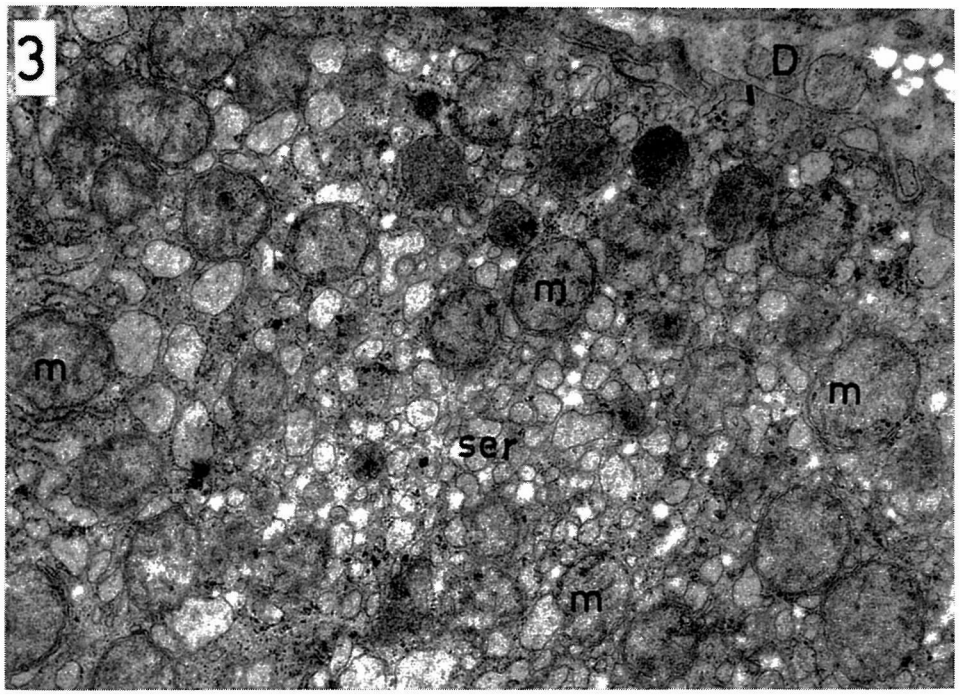
Fig. 3. At 15 minutes following injection of Biligrafin, the smooth-surfaced endoplasmic reticulum in the sinusoidal area of the liver cell is markedly developed. It has distended lumen and is situated among the mitochondria. × 18,400

Fig. 4. The Golgi complex is not so prominent at 30 minutes after injection of Biligrafin. Well-developed smooth-surfaced endoplasmic reticulum is seen around the Golgi complex. × 21,000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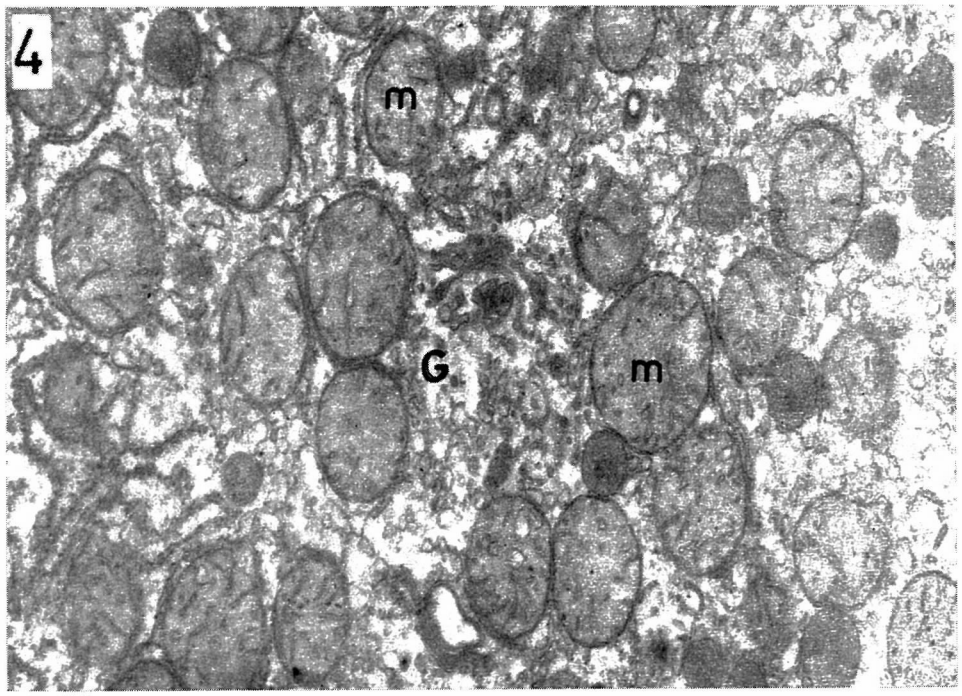
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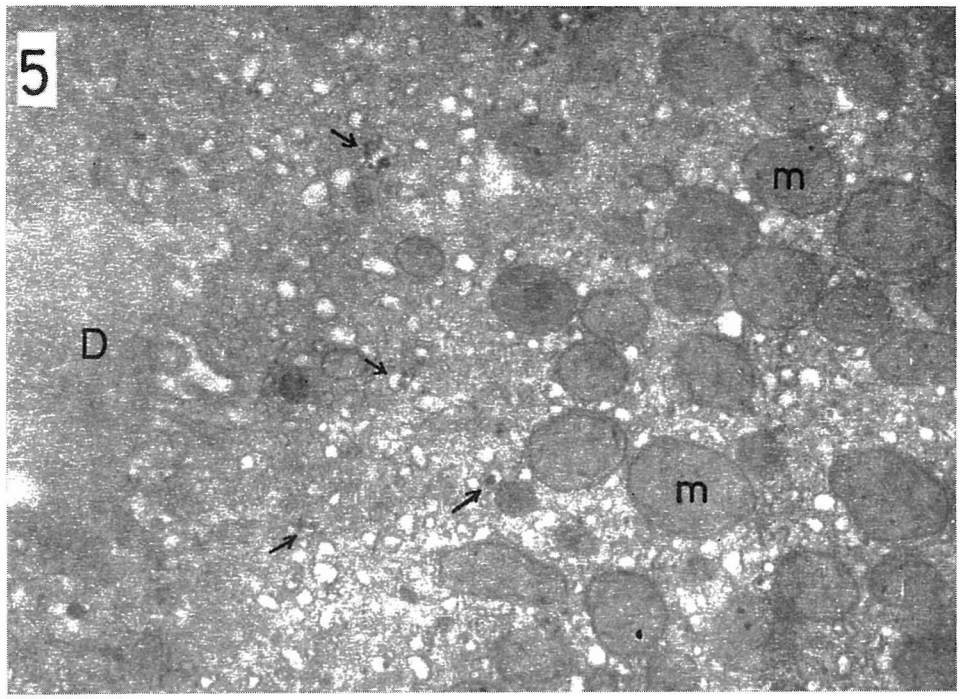
Figures 5 and 6 show the sinusodial part of the liver cells at 60 minutes following injection of Biligrafin.

Fig. 5. The smooth-surfaced endoplasmic reticulum contains a few granules (arrow). × 20,000

Fig. 6. Aggregate of dense granules (arrow) is seen in the distended smooth-surfaced endoplasmic reticulum. × 24,000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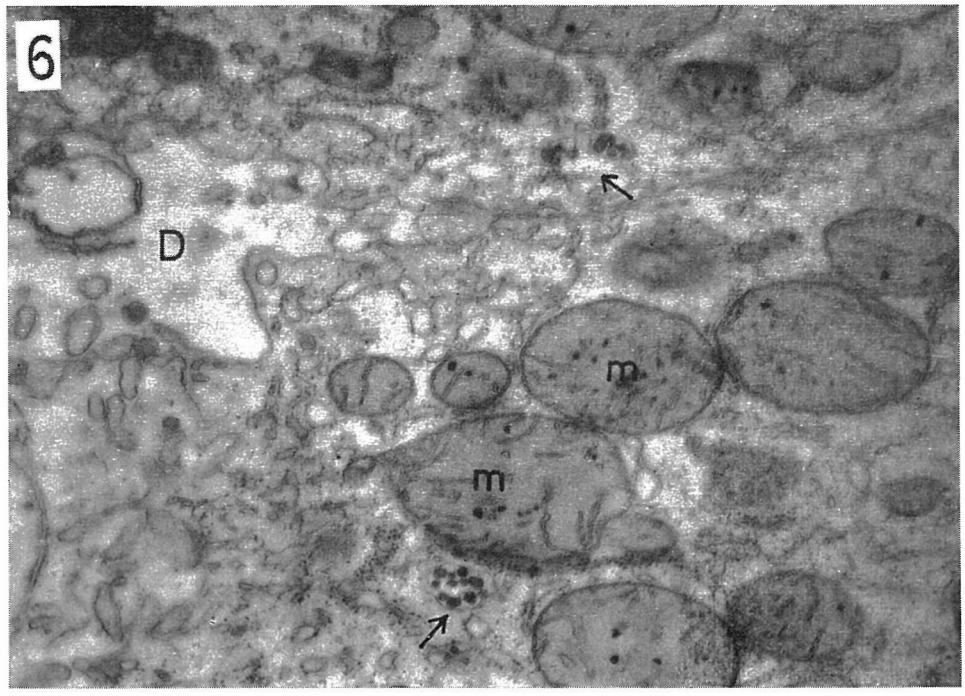
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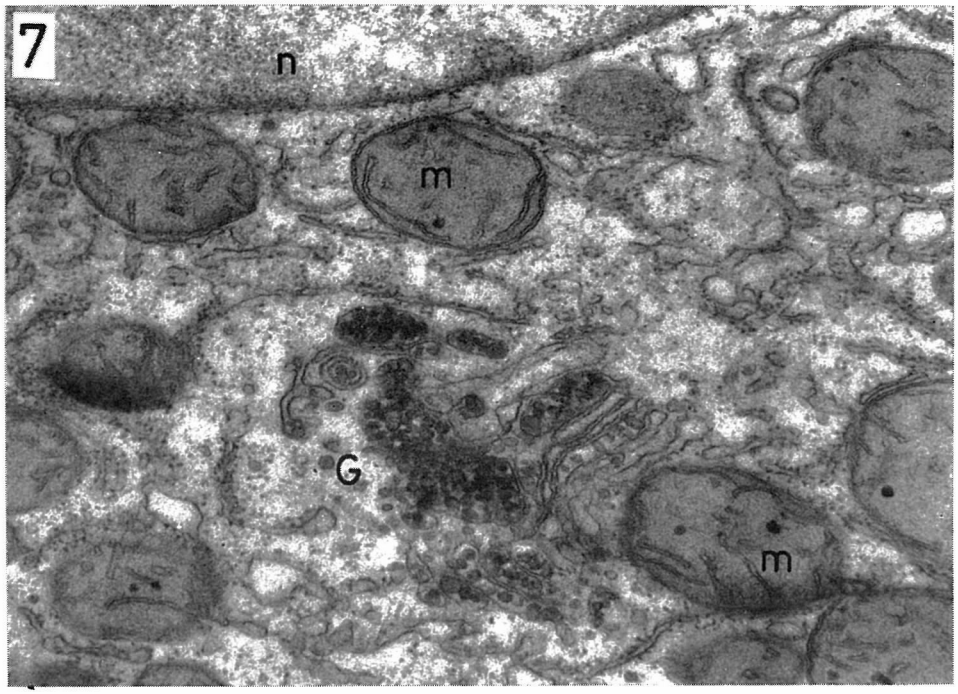
Figurees 7 and 8 demonstrate the liver cells at 60 minutes after injection of Biligrafin.

Fig. 7. Well-developed Golgi apparatus near the nucleus contains many granules. These granules have an approximate diameter of 40 to 80 mμ. × 32,000

Fig. 8. The Golgi complex at the vicinity of the bile capillary. Similar granules are visible within the Golgi vesicles and vacuoles. × 24,000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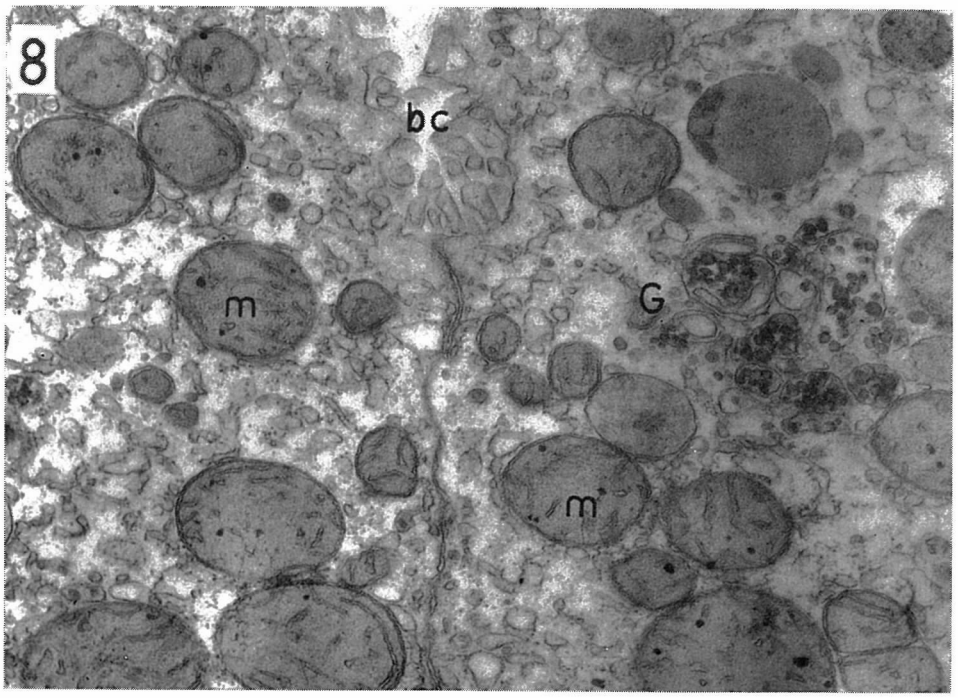
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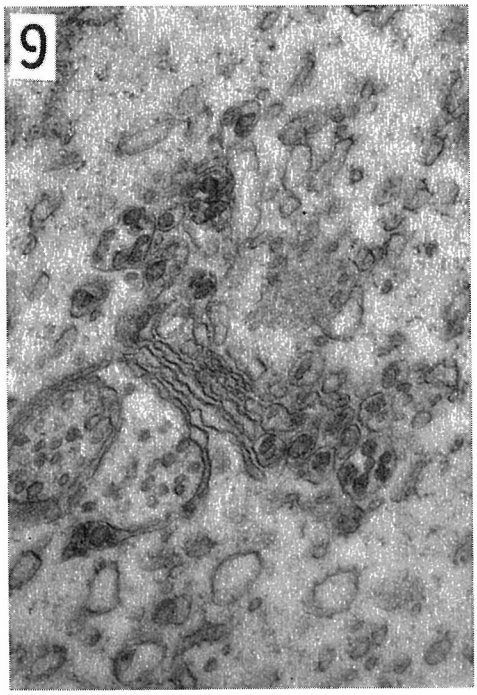
Figures 9 to 12 show high magnification of the Golgi complex at 60 minutes after injection of Biligrafin.

Fig. 9. Three elements of the Golgi complex (lamellae, vesicles and vacuoles) are easily recognized. Dense granules are seen in the vesicles and vacuoles. × 32,000

Fig. 10. The Golgi vacuoles have remarkably distended lumen which contains aggregate of the granules. X 32,000

Fig. 11. The Golgi lamellae are arranged in parallel fashion. Some lamellae show cystic distension, in which a few granules are seen. × 40,000

Fig. 12. High magnification of the Golgi complex which contains many dense granules. × 44,000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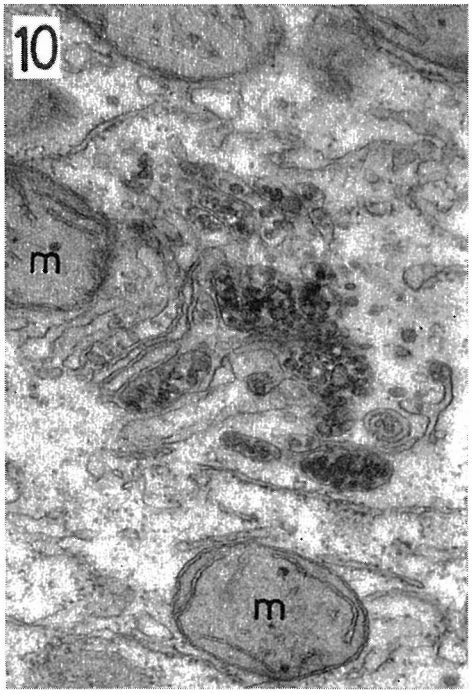
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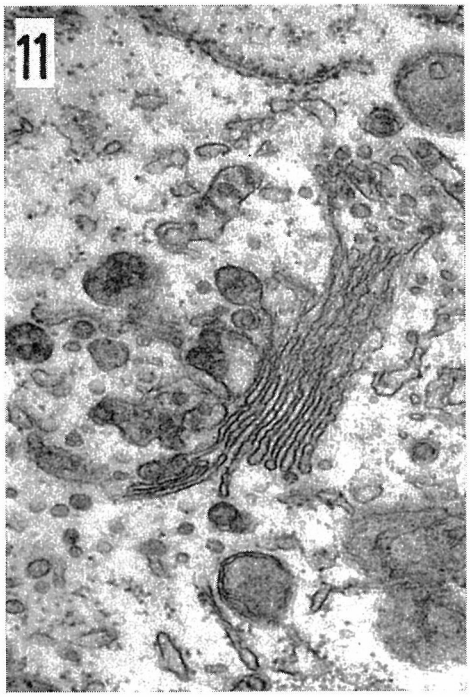
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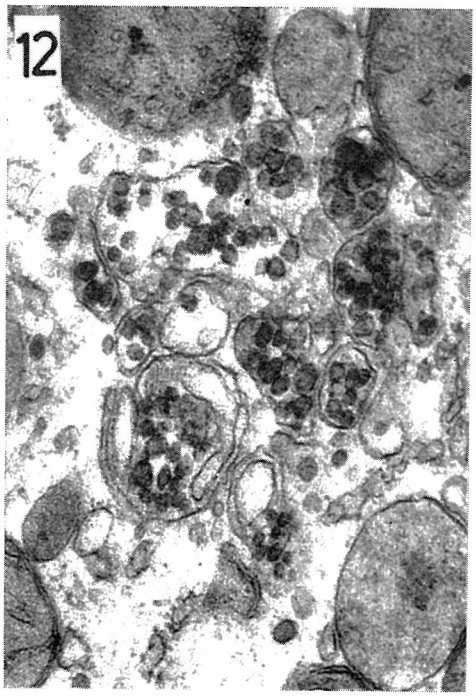
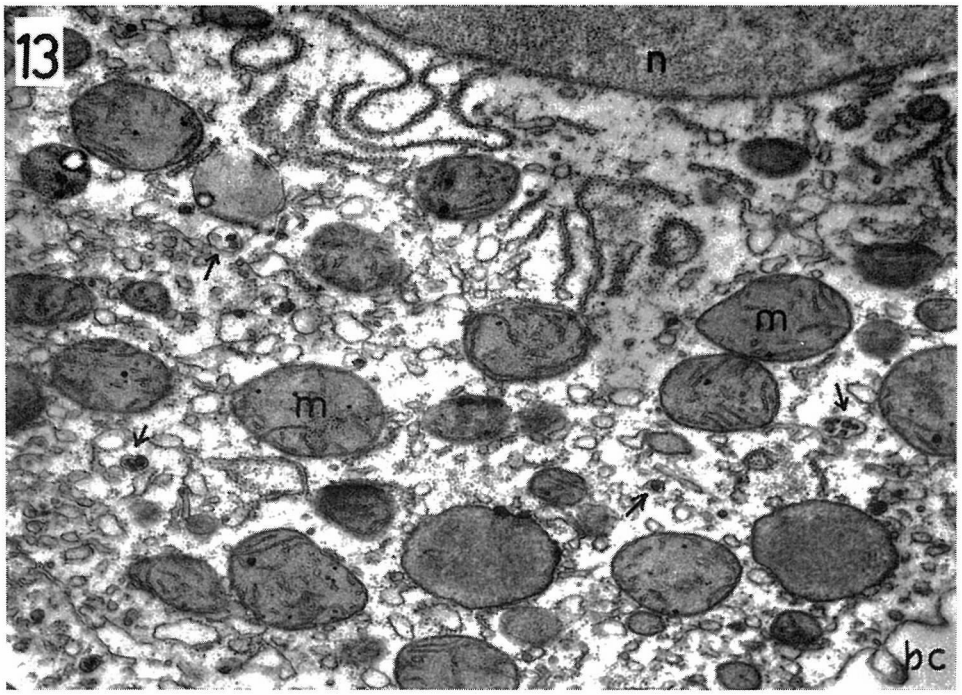

12

Fig. 13. Canalicular area of the liver cell at 60 minutes after injection of Biligrafin. The smooth-surfaced endoplasmic reticulum which contains a few granules (arrow) is scatteringly seen in the cytoplasm. × 18,400

Fig. 14. At 15 minues after injection of Biligrafin, the smooth-surfaced endoplasmic reticulum are well developed even at the canalicular site. The bile canaliculus shows no significant change. × 18,400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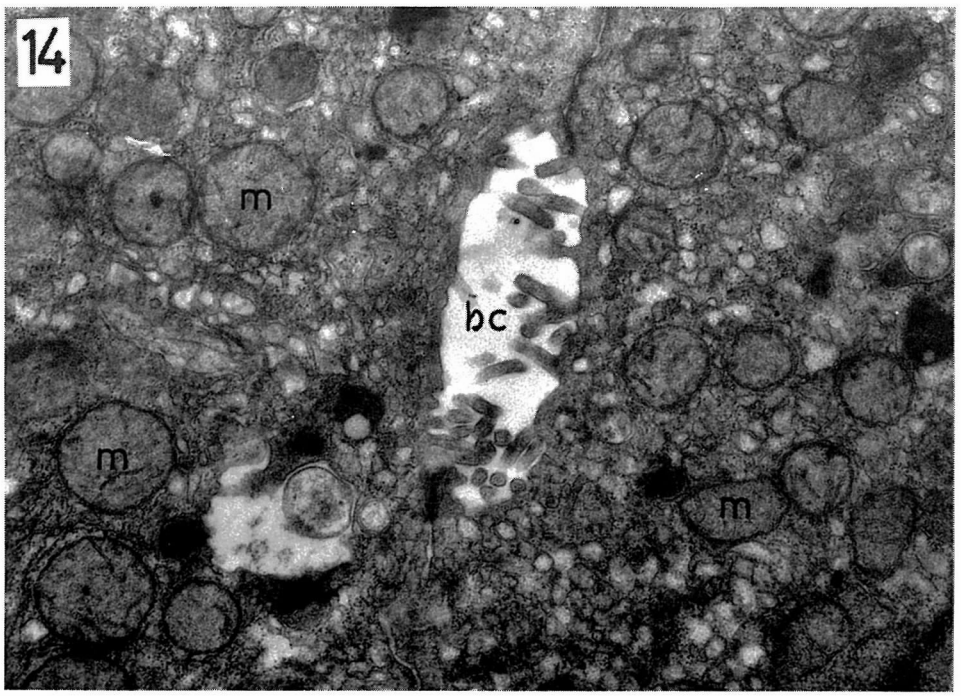
14
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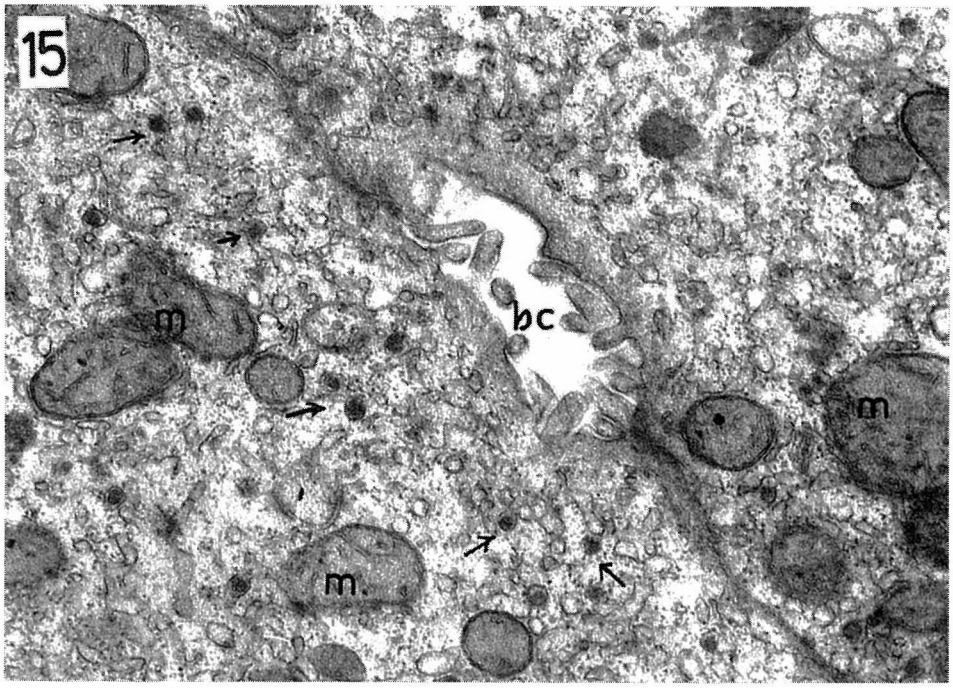
Figures 15 and 16 show the canalicular area of the liver cell at 60 minutes following injection of Biligrafin.

Fig. 15. The smooth-surfaced endoplasmic reticulum which contains dense granules (arrow) is scatteringly seen near the bile capillary. × 18,400

Fig. 16. The lysosome in the vicinity of the bile canaliculus also contains dense granules (arrow). × 32,000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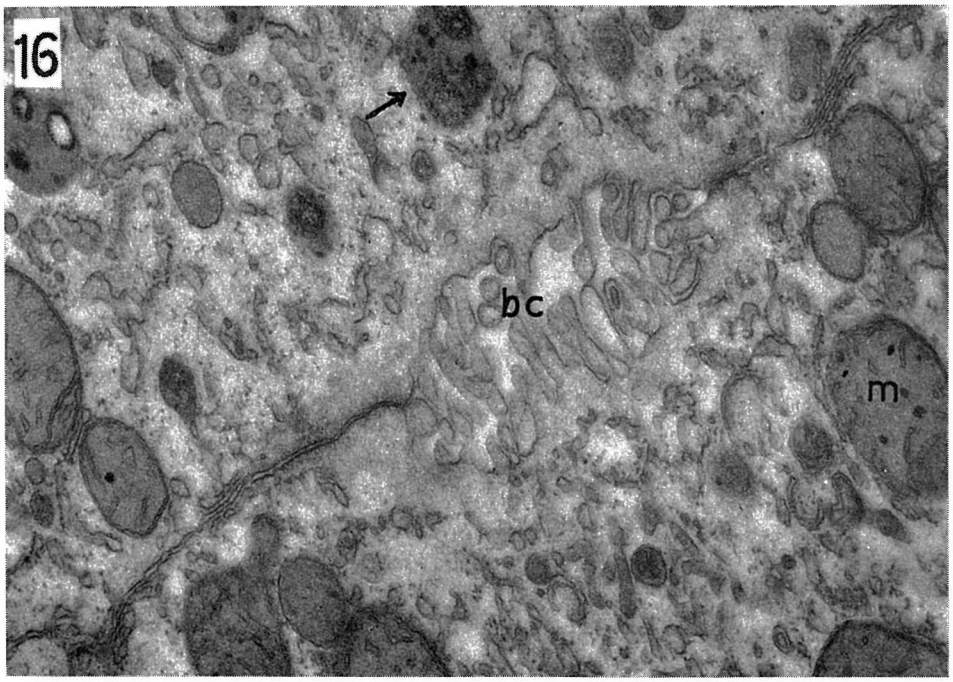
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