Electron Microscopic Studies of the Liver Ⅱ

(3) Changes of Histochemical Finding of the Liver Cell in Rats Suffering from Ligation of the Common Bile Duct

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(Received October 12, 1966)

It is generally accepted that jaundice caused by the obstruction of the biliary tract, either extrahepatic or intrahepatic, is a result of regurgitation of bile from the biliary tract into the blood stream. As to the route of regurgitaion in the hepatic lobule, Aschoff, Ohno, Eppinger and others indivisually reported their original opinions. However their theories have not been widely accepted. Recently, as histochemical and electron microscopic studies have made great progress, there appeared a great deal of reports on the mechanism of obstructive jaundice on the basis of observations using these techniques.

In the previous report (8), the author reported the presence of canalicular-sinusoidal connections which have the possibility to be the route of regurgitation of the bile at the early stage of obstructive jaundice. This connection was clearly demonstrated by Monroe et al. (4) who examined the liver by histochemical method. In this paper, the localization of canalicular-sinusoidal connection in the lobule has been studied by demonstrating iron-reaction positive granules following injection of saccharated ferric oxide in the common bile duct. And several histochemicl studies have been also tried to demonstrate this connection in the hepatic lobule.

MATERIAL AND METHOD

Adult white rats, weighing about 250 gms, were used, and they were divided into two groups as follows.

Group A

Under ether anesthesia, the commom bile duct was doubly tied with cotton, and cut between ligatures. The liver specimens were obtained 24 hours, 48 hours and 72 hours after ligation respectively. Each specimen was fixed and stained as follows ; hematoxylin and eosin stain after fixation in 10% formalin, PAS stain after fixation in Carnoy's solution, alkaline phosphatase by the Takamatsu's method after fixation in aceton, acid phosphatase modified by Gomori

after fixation in cold aceton, 5'-nucleotidase and adenosintriphosphatase (ATP ase) by the Wachstein-Meisel lead method after fixation in cold aceton.

As a control, normal rat liver was stained with the same method.

Group B

Under ether anesthesia, the distal portion of the common bile duct was ligated tightly. Then 20 ml of saccharated ferric oxide solution was injected slowly with minimum pressure into the duct at the portion proximal to the ligation. Immediately after the injection, the liver was rapidly excised and fixed in 10 per cent formalin. Iron was demonstrated by Perls-Stieda's method. According to the interval between ligation of the duct and injection of saccharated ferric oxide, the rats were arranged in 4 subgroups as follows :

Subgroup I : Injection of saccharated ferric oxide immediately after common bile duct ligation.

Subgroup II : Injection of saccharated ferric oxide 24 hours after common bile duct ligation.

Subgroup III : Injection of saccharated ferric oxide 48 hours after common bile duct ligation.

Subgroup IV : Injection of saccharated ferric oxide 72 hours after common bile duct ligation.

All specimens of both group A and B were examined by light microscope.

RESULTS

Group A

Control liver

H. E. stain : There was no abnormality as shown in Fig. 1.

PAS stain : PAS positive granules were more numerous in the central area than in the peripheral zone (Fig. 2).

Acid phosphatase : Enzymatic activity was scatteringly seen in the liver cells (Fig 9).

Alkaline phosphatase : Staining for alkaline phosphatase activity was slightly found in the liver cells of the peripheral area and moderate activity was revealed in the stroma and the epithelium of the bile duct in the Glisson's capsule. (Fig. 12, 13).

5' - nucleotidase : Staining for 5'-nucleotidase activity was localized slightly in the cytoplasmic membrane of the liver cells and moderately in the strome of the portal area. Linear contiguity of staining between canaliculus and sinusoid was noted to occur from several to some dosen places per lobule. These canalicular-sinusoidal connections were recognized almost exclusively in the centrilobular and midzonal regions (Fig. 17).

ATP ase : Slight activity was found in the cytoplasmic membrane of the liver cells and in the wall of the central vein. The bile canaliculi were sharply and specifically stained (Fig. 21). In some areas, contiguity of reaction between canaliculus and sinusoid was noted in the centrilobular and midzonal area (Fig. 21). However, these connections were not so prominent as was demonstrated by the staining for 5'-nucleotidase activity.

24 hours after ligation

H. E. stain : The central veins were slightly dilated. Small vacuoles, which might be fatty droplets, were scatteringly seen in the cytoplasm of the liver cells. The bile duct in the Glisson's sheath was slightly dilated.

PAS stain : PAS positive granules in the liver cells were decreased, especially at the perilobular zone (Fig. 4).

Acid phosphatase : The intensity of staining for acid phosphatase activity was slightly increased and this tendency was more prominent at the canalicular site of the liver cells (Fig. 10) and also in the central area of the lobule.

Alkaline phosphatase : There was no remarkable change in the staining pattern for alkaline phosphatase though the stroma and the epithelium of the bile duct in the Glisson's sheath showed slight increase in intensity of staining for this enzymatic activity. In the peripheral zone of the lobule, slight decrease in intensity was observed (Fig. 14).

5'-nucleotidase : Staining of the cytoplasmic membrane of the liver cells and of the wall of the central veins for 5'-nucleotidase was almost similar to that observed in the normal liver. However, canalicular-sinusoidal connections revealed increased intensity and appeared to be linear, tortuous or rod-shaped, but no increase in the number of canalicular-sinusoidal connections was noted (Fig. 18).

ATP ase : Staining for ATP ase activity was slightly reduced in the wall of the central vein and at the hepatic cell membrane. Canalicular-sinusoidal connection showed increased activity of this enzyme but there was no increase in the number of this connection (Fig. 22).

48 hours after ligation

H. E. stain : There was no remarkable changes except for the slight increase of small vacuoles in the liver cells comparing to the specimens 24 hours after ligation.

PAS stain : PAS-positive granules in the liver cells were somewhat increased than were recognized in the liver 24 hours after ligation. But comparing to the normal liver, the granules were decreased especially at the peripheral area.

Acid phosphatase : Staining for acid phosphatase activity of the cytoplasmic membrane showed moderate increase. This tendency was more prominent in the vicinity of the canaliculus (Fig. 11).

Alkaline phosphatase : The intensity of cytoplasmic staining for alkaline phosphatase activity was reduced at the peripheral area. The Glisson's capsule showed markedly reduced staining for this enzyme (Fig. 15).

5'-nucleotidase : The staining pattern for 5'-nucleotidase activity resembled that after 24 hours of ligation. Although there was no increase in the intensity of staining, the number of canalicular-sinusoidal connection apparently increased (20 to 30 per 5 micron lobule-section) (Fig. 19).

ATP ase : The cytoplasmic membrane and the wall of the central vein showed slight decrease in the intensity of staining for this enzyme. The cytoplasm of the liver cells occasionally revealed the presence of this enzymatic activity even at the sinusoidal aspect. Canalicular-sinusoidal connection demonstrated by the staining for ATP ase activity was slightly increased in number comparing to that 24 hours after ligation. (Fig. 23).

72 hours after ligation

H.E. stain : Dilatation of the central vein and bile duct was almost similar to that observed in the liver 24 and 48 hours after ligation. There was mild infiltration of small round cells in the Glisson's capsule. Kupffer's cell were slightly enlarged and the liver cells showed occasional vacuolization of the nucleus (Fig. 3).

PAS stain : PAS-positive granules were more abundant than were recognized 48 hours after ligation and were almost identical with those of the normal control.

Acid phosphatase : The staining pattern for this enzyme essentially resembled that of 48 hours after ligation.

Alkaline phosphatase : Marked decrease in the intensity of staining for alkaline phosphatase activity was observed. The liver cells in the perilobular area and the Glisson's sheath were devoid of this enzymatic activity (Fig. 16).

5'-nucleotidase : The cytoplasmic membrane of the liver cells and the wall of the central vein revaled almost the same intensity of staining for this enzyme as that observed 48 hours after ligation. The number of canalicular-sinusoidal connection, as demonstrated by staining for 5'-nucleotidase activity, approximated that of 48 hours after ligation (Fig. 20).

ATP ase : The staining pattern for ATP ase activity was almost similar to that of 48 hours after ligation (Fig. 24).

Group B

In the control animals, there was no iron reaction positive granules in the liver.

In the rats, which were injected saccharated ferric oxide into the common bile duct, iron reaction positive granules were found in the liver as a linear or rod-shaped deposit, occasionally forming irregular clumps. Such deposits were more numerous in the central area and most of the granules were phagocytosed by stellate cells of Kupffer (Fig. 5-8). Occasionally small granules were found in the cytoplasm of the liver cells. In some area, the granules were deposited as a linear shape connecting the bile canaliculi with the sinusoid. Such linear deposition was also seen in the bile canaliculi between the adjascent liver cells from several to some dosen places per lobule.

In subgroup I, the connections, as demonstrated by the deposition of iron reaction positive granules, appeared as a straight lines (Fig. 5). In subgroup II, the connections had increased width and were more clearly visible (Fig. 6). These connections were evenly distributed in the lobule and were recognized in several to some dosen places in a lobule.

In subgroup III and IV, these connections were increased in number (about 20 to 30 per 5 micron lobule-section) and in their width, and appeared as rod-shaped deposition (Fig. 7, 8). There was no marked difference in the distribution of the connection in a lobule. In the Glisson's capsule, iron reaction positive granules were observed in the bile duct and also in the blood vessels.

DISCUSSION

Rouiller (1) demonstrated the presence of the space between the adjascent liver cells electron microscopically and he reported that this space might be the channels communicating canaliculi and Disses' space. Monroe et al. (4) histochemically showed the direct connections demonstrable by staining for 5'-nucleotidase activity between canaliculi and sinusoid, and they designated this channel as canalicular-sinusoidal connection. Schatzki (9) also demonstrated the direct communication between canaliculi and sinusoid by staining for ATPase activity. In the previous paper (8), the author reported the presence of these communications from the results of electron microscopic examination of the rat liver following ligation of the common bile duct. Electron microscopic examination, however, has too small visual field to precisely demonstrate the location and number of the connections in a lobule. Therefore, in this paper, the author tried to demonstrate the connection histochemically. Furthermore, saccharated ferric oxide was injected in the common bile duct at the various intervals after ligation,

and then the possibility that the bile could be regurgitated into the blood stream passing through the canalicular-sinusoidal connection has been suggested. Saccharated ferric oxide is chemically stable and the particles are as small as 150 A in diameter possessing colloidal character. This compound is commonly administered to human subjects intravenously and is not so harmful as to damage the hepatic parenchyme or to disrupt the intercellular connection. Furthermore, this material can be more easily demonstrated by iron reaction than other histochemical procedures.

Small round cell infiltration in the Glisson's capsule, which was encountered in H. E. preparation 72 hours after ligation, would be a manifestation of mild cholangiolits. In PAS staining, PAS-positive granules were markedly decreased 24 hours after ligation. As duration of observation continued, PAS-positive granules gradually increased and after 72 hours of ligation, the number of these granules approximated that of the normal liver. The marked decrease of PAS-positive granules, namely that of glygogen granules, immediately after ligation would be the effect of operation. As the effect of operation subsided, PAS-positive granules gradually increased to such an extent as that of normal liver.

The intensity of staining for acid phosphatase activity increased after 24 hours of ligation especially in the vicinity of the canaliculi and then decreased during the subsequent 48 hours. This finding was almost similar to that reported by Monroe et al. (4), who described that granules staining for acid phosphatase activity and components of the Golgi apparatus increase at the canalicular aspect of the liver cells following ligation of the common bile duct.

Staining for alkaline phosphatase activity was localized in the peripheral area especially in the epithelial cells of the bile duct in the control animals. Slight increase in the intensity of staining for this enzyme was observed 24 hours following ligation when there was no increase in the number of canalicular-sinusoidal connections. Then marked decrease of its intensity was recognized after 48 hours of ligation. It may be presumed that such rapid change of alkaline phosphatase activity following common bile duct ligation would be due to transfer of this enzyme to the blood stream with resultant hyperalkalinephosphatemia. It is said that such transfer of alkaline phosphatase would be due to the mechanism of active transport (10).

In the normal liver, ATP ase activity was localized at the canalicular aspect of the liver cells. As duration of obstruction continued, increased activity was found at the sinusoidal aspect. As to this transfer of activity, Monroe et al. (4) reported that ATP ase might play a role in active transport of substances across cell membrane.

Staining for ATP ase and 5'-nucleotidase activities was occasionally found in the canalicular-sinusoidal connection even in the normal liver. At 24 hours after

ligation of the common bile duct there was an increase in the activity of these enzymes and at 48 hours marked increase in the number of the connections was observed. Monroe et al. (4) reported similar findings and pointed out that this connection would be a route for the regurgitation of the bile following the experimental bile duct ligation in the rat. In this study, iron reaction positive granules were found in the canalicular-sinusoidal connectins even immediately after the common bile duct ligation, and as duration of ligation continued, marked increase in the number and the width of the connection became apparent. Therefore, it may be presumed that the bile may regurgitate into the blood stream through the increased number of canalicular-sinusoidal connection when the physiological flow of the bile is disturbed.

Although there are many reports as to the route of regurgitation of the bile in case of obstructive jaundice, they are divided into two main groups. The first one is that the rupture of the bile canaliculi occur following obstruction and the bile regurgitate into the blood through this ruptured part (5, 6, 7). In the previous experiment (8), the author could not find any rupture of the bile canaliculi even after 72 hours of ligation. However, it may be presumed that the canaliculi may possibly be ruptured when marked increase of intrabiliary pressure occurs. In fact, there was such findings which suggested the rupture of the bile canaliculi following retrograde injection of saccharated ferric oxide with great pressure. According to the second theory (2, 3), the bile regurgitates into the sinusoid passing through the physiological route through which the bile is secreted into the canaliculi. This theory is based on the concept of active transport of the material across the cell membrane. As to the mechanism of active transport Schazki (9) presumed that the passage of the bile across the cell membrane is not only due to the increase of the intrabiliary pressure but the result of change of the bile components following common bile duct ligation. In this experiment, iron reaction positive granules were occasionally found in the liver cells, which suggested one of the possible route for regurgitation of the bile in case of obstructive jaundice.

On the other hand, there are some authors who express doubt as to the presence of canalicular-sinusoidal cennection as previously described in detail (8). Schatzki (9) demonstrated rare communications between the sinusoid and the bile canaliculus and he suggested the possible transfer of the enzymatic activity through this communication under the condition of the common bile duct obstruction. However, he considered that such rare communication could not account for the transportation of abundant volume of the bile. Monroe et al. (4), on the other hand, could not find any rupture of the bile canaliculi even when a significant hyperbilirubinemia was present after 24 hours of common bile duct ligation. And they suggested that the canalicular-sinusoidal connections

could therefore serve as a route for the regurgitation of canalicular contents following the marked increase of intrabiliary pressure.

Recently, Yodaiken (11) clearly demonstrated a direct communication between the sinusoid and the bile canaliculi using a combination of lead and gum acacia as a tracer for electron microscopy. Describing that even desmosomes does not act as barriers to the passage of lead-gum particles, he suggested the presence of a pathway which permits not only fluid but macromolecular substance to travel from the bile canaliculi to the sinusoid.

In conclusion, morphologic pathway between the sinusoid and the bile canaliculus, that is canalicular-sinusoidal connection, do exsist and is the space between two adjascent liver cells. On the basis of the results reported here, it is presumed that canalicular-sinusoidal connections, which exsist under normal condition, act as a route for the regurgitation of the bile at the early stage of biliary obstruction. However, the author dose not maintain that canalicular-sinusoidal connection is a single pathway for the retrograde passage of the bile under condition of obstruction of the bile duct. Active transport of the material across the cell membrane mainly based on adenosinetriphosphatase and the rupture of the bile canaliculi following elevated intracanalicular pressure must be also considered at the same time.

SUMMARY

Morphological changes of the liver of the rats which had sustained common bile duct ligation for twenty-four to seventy two hours were studied by staining with hematoxylin-eosin and Periodic acid Schiff reaction. Histochemical stainings for enzymatic activity such as acid phosphatase, alkaline phosphatase, 5'-nucleotidase and adenosintriphosphatase were also applied. In other rats, saccharated ferric oxide was injected in the common bile duct at various intervals after common bile duct ligation and then liver sections were stained with iron reaction. Following results were obtained.

1) In the normal liver, canalicular-sinusoidal connections were more numerous in the central area than in the peripheral zone of the lobule. The number of the connections ranged from several to some dozen per 5 micron section of a lodule.

2) The width of canalicular-sinusoidal connections was increased after 24 hours of common bile duct ligation and both the number and width of the connections showed increase after 48 hours of ligation.

3) Under conditions of common bile duct ligation and administration of saccharated ferric oxide by intrabiliary injection, iron reaction-positive granules were found in canalicular-sinusoidal connections as a linear deposit.

4) Main route for the regurgitation of biliary contents at the early stage of obstructive jaundice is presumed to be canalicular-sinusoidal connections.

ACKNOWLEDGEMENT

Greateful acknowledgement is made to Prof. S. Hosokawa for his kind guidance and careful review of manuscript. Thanks are due to Dr. F. Uchino for his constant guide and advice and also to Mr. M. Yamashita for his assistance.

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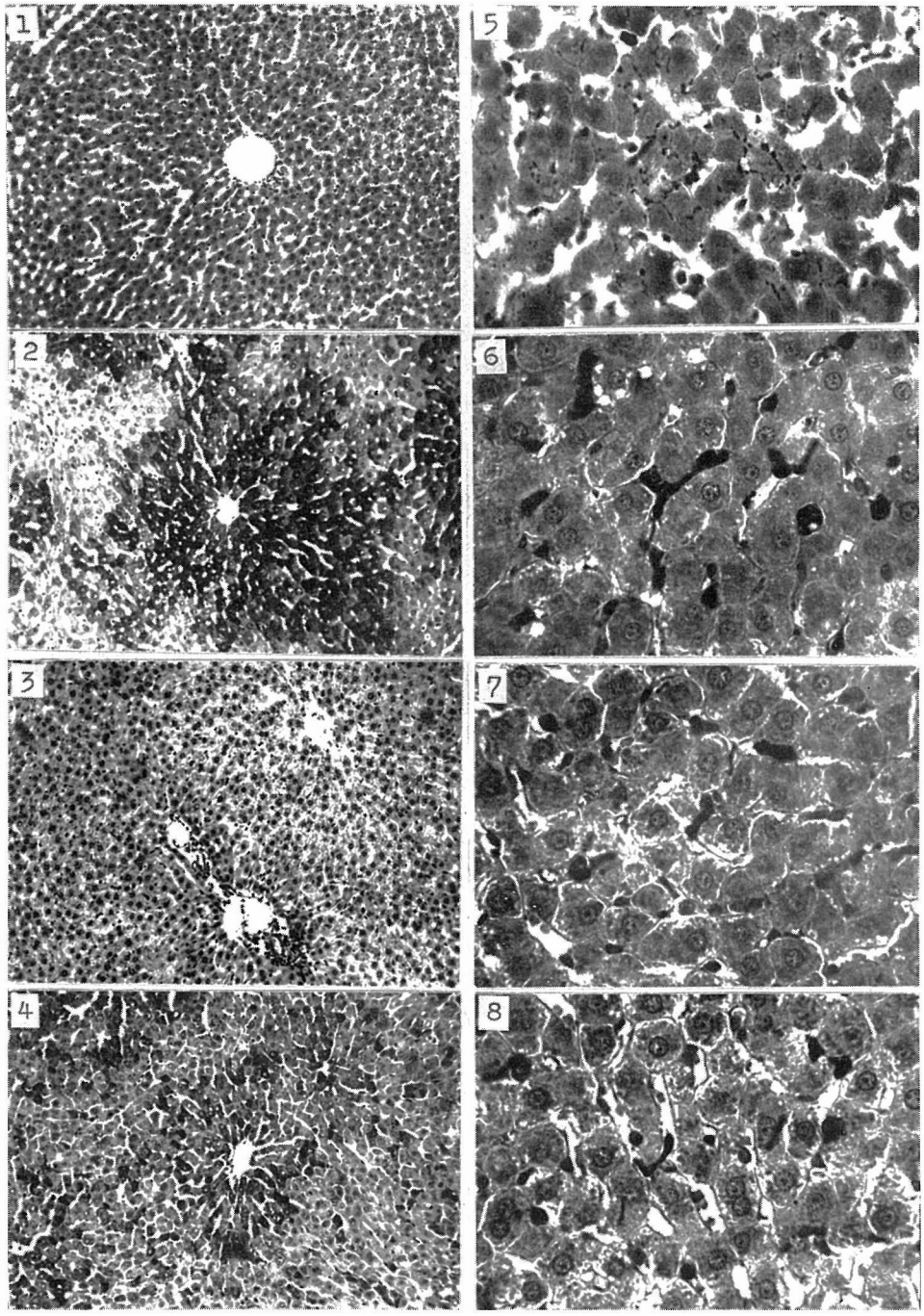
EXPLANATION OF FIGURES

Fig. 1. Normal rat liver. The central vein is seen in the center. H. E. X 100
Fig. 2. Normal rat liver. PAS-positive granules are numerous in the central area. PAS X 100
Fig. 3. Seventy-two-hour ligation. Small round cell infiltration is seen in the Glisson's capsule. H. E. X 100
Fig. 4. Twenty-four-hour ligation. Marked decrease of PAS-positive granules is recognized. PAS X 100

Figures 5 to 8 are microphotographs of liver sections injected saccharated ferric oxide at various intervals following common bile duct ligation and stained with iron reaction.

Fig. 5. Injection of saccharated ferric oxide immediately after ligation. Iron reaction-positive granules are linearly deposited in the canalicular-sinusoidal connections. In the cytoplasm of the liver cells, such granules are occasionally seen. X 400
Fig. 6. Injection of saccharated ferric oxide after 24 hours of ligation. X 600
Fig. 7. Injection of saccharated ferric oxide after 48 hours of ligation. X 600
Fig. 8. Injection of saccharated ferric oxide after 72 hours of ligation. X 600

In figures 6 to 8, most of the iron reaction-positive granules are seen in the sinusoid being phagocytosed by Kupffer's cells. In some area, band-like deposit of iron reaction positive material is seen continuously from canaliculus to sinusoid.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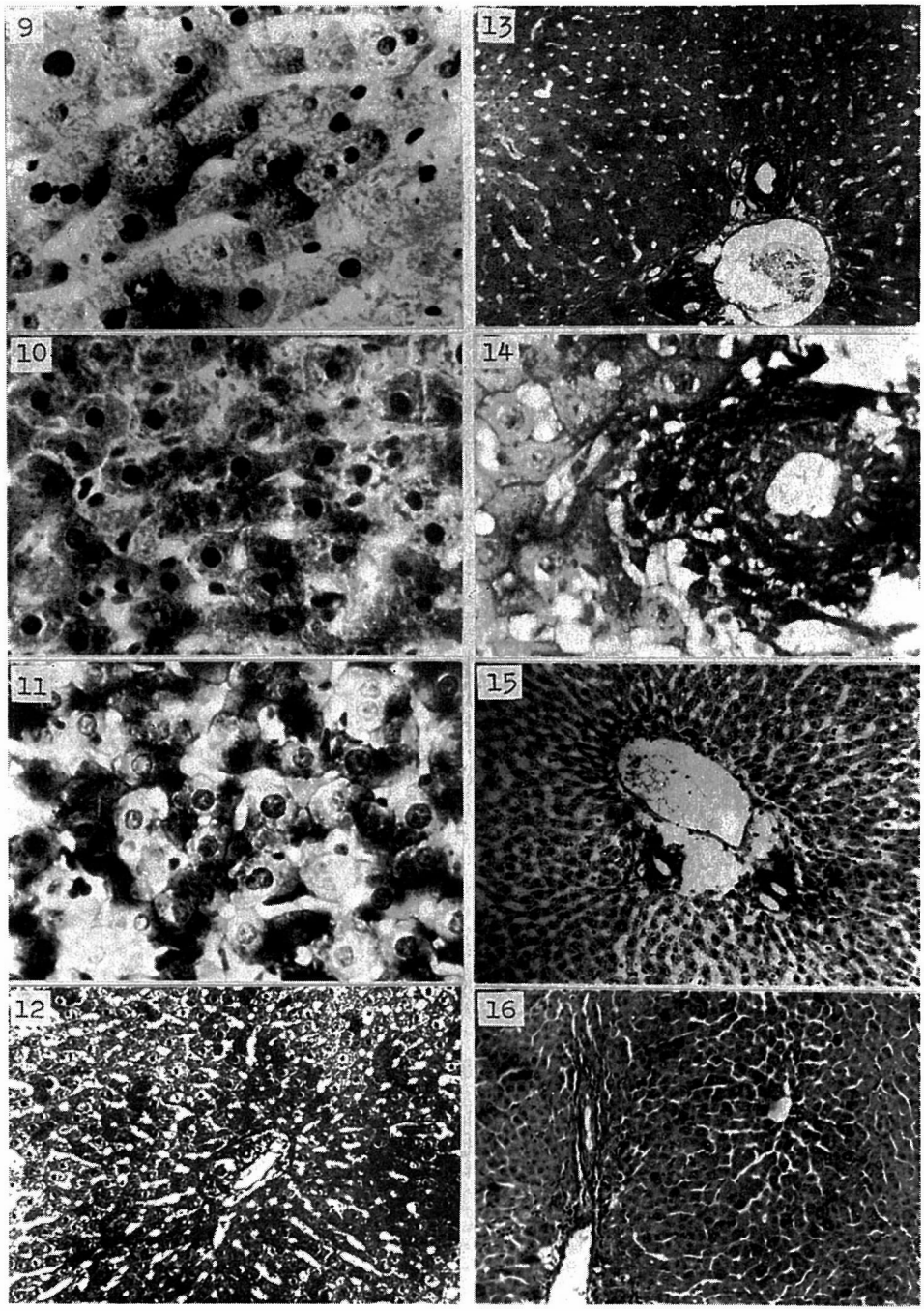
Figures 9 to 11 are microphotographs of liver sections incubated for acid phosphatase activity, counterstained with hematoxylin.

Fig. 9. Normal rat liver. The cytoplasm of liver cells are slightly stained. X 600
Fig. 10. Twenty-four-hour ligation. Intensity of staining in the liver cells shows slight increase. X 600
Fig. 11. Forty-eight-hour ligation. The canaliculi are stained intensely for acid phosphatase activity. X 600

Figures 12 to 16 are microphotographs of liver sections incubated for alkaline phosphatase activity, counterstained with hematoxylin.

Fig. 12. Normal rat liver. The liver cells in the peripheral area are moderately stained. X 100
Fig. 13. Normal rat liver. Stromal and epithelial cells of the Glisson's sheath are stained. X 100
Fig. 14. Twenty-four-hour ligation. Stromal cells in the Glisson's sheath show moderate intensity of staining. X 600
Fig. 15. Forty-eight-hour ligation. Staining of the liver cells in the peripheral area is considerably diminished as compared to Fig. 12. X 100
Fig. 16. Seventy-two-hour ligation. There is significant decrease in stainig for alkaline phosphatase and cytoplasmic staining is almost absent. X 100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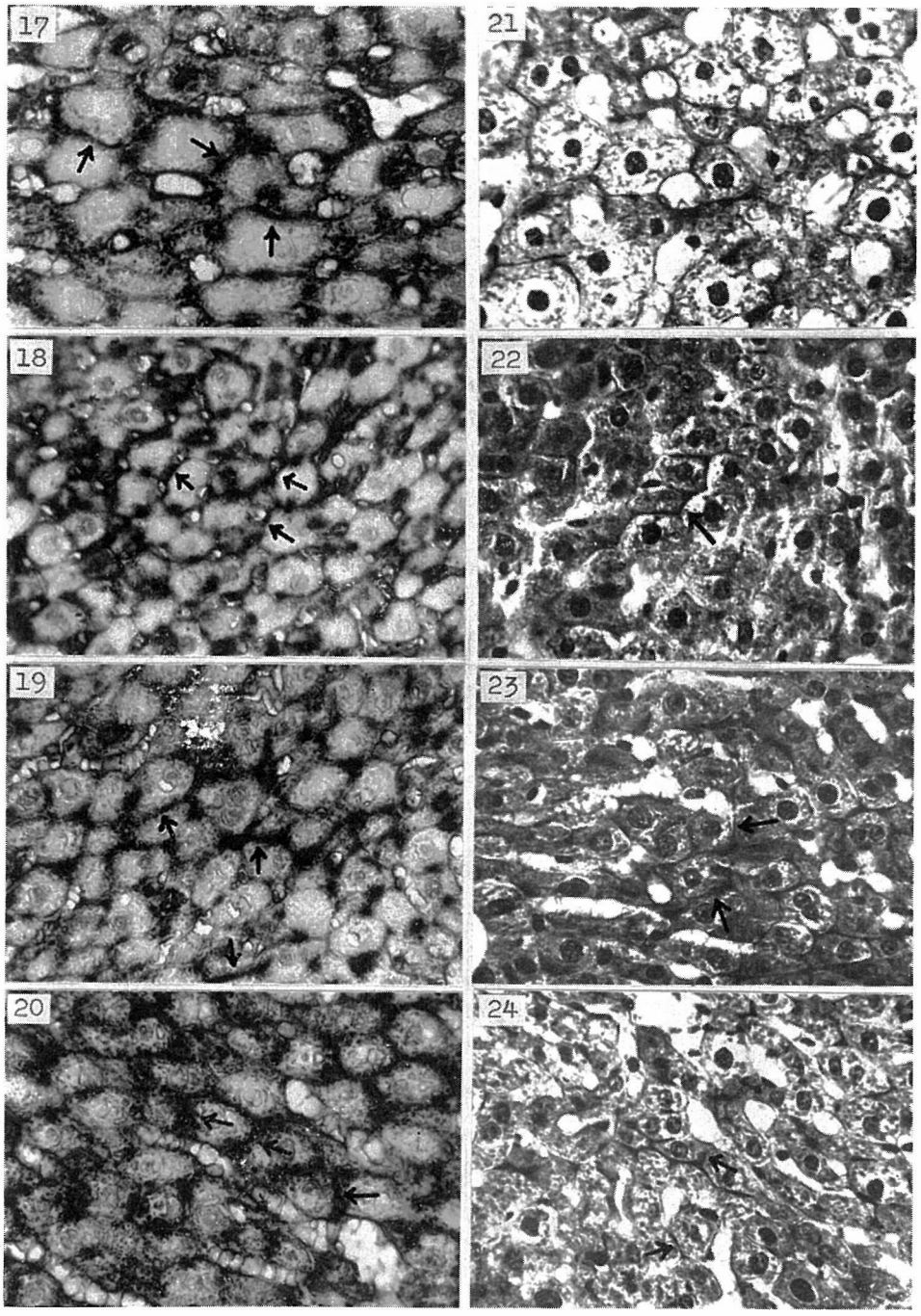
Figures 17 to 20 are microphotographs of sections incubated for 5'-nucleotidase activity, counterstained with hematoxylin.

Fig. 17. Normal rat liver. The cytoplasmic membrane is slightly stained and canalicular-sinusoidal connectin shows intense staining (arrow). X 700
Fig. 18. Twenty-four-hour ligation. Canalicular-sinusoidal connections (arrow) are apparently seen. X 400
Fig. 19. Forty-eight-hour ligation. Canalicular-sinusoidal connections (arrow) show increase in their number and intensity of staining. X 600
Fig. 20. Seventy-two-hour ligation. Canalicular-sinusoidal connections (arrow) are clearly demonstrated. X 700

Figures 21 to 24 are microphotographs of liver sections stained for adenosinetriphosphatase activity.

Fig. 21. Normal rat liver. Apparent canalicular-sinusoidal connection is seen. X 600
Fig. 22. Twenty-four-hour ligation. Marked canalicular staining with occasional branching (arrow). X 600
Fig. 23. Forty-eight-hour ligation. Canalicular-sinusoidal connection (arrow) shows increase in its width. Canaliculi are sharply outlined. X 600
Fig. 24. Seventy-two-hour ligation. Sharply outlined canaliculi with little branching. (arrow) X 600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