Iron Metabolism and Cell Membranes

I. Relation Between Ferritin and Hemosiderin in Bile and Biliary Excretion of Lysosome Contents

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IT PREVIOUSLY HAS BEEN SHOWN that a decrease in total body and liver iron occurs when iron-replete animals are changed from diets containing excess iron to a standard diet.[1] The present study considers the cellular pathway of biliary iron excretion. Using chemical and electron microscopic techniques, we have been able to show that: (1) in the liver cells of iron-loaded animals, a large amount of the stored iron is concentrated as hemosiderin and ferritin within secondary lysosomes; (2) lysosome contents including acid phosphatase together with ferritin are present in bile canaliculi; and (3) lysosome contents and ferritin are present in the bile. It is suggested that excretion of lysosome contents is responsible for some of the ferritin present in the bile.

Materials and Methods

Iron Loading

Young male rats of the Holtzman strain were fed a high-fat, protein-deficient diet supplemented with iron for 3 weeks; they then were returned to a standard diet. The iron-loading diet consisted of: corn grits 77.4%, lard 19.4%, vitamin mixture 1.0%, choline 0.3%, and ferric citrate 1.0%. The standard diet consisted of: vitamin mixture 1.0%, choline 0.3%, glucose 66.7%, vitamin-free casein 18.0%, corn oil 10%, and salt mixture 4.0%. Control animals were fed the corn grits-lard diet without added iron. A group of normal animals of similar age and weight were fed the standard diet. All animals received water ad libitum and were weighed twice a week.

Collection of Bile

After 3 weeks on the diet containing excess iron, animals were placed on the standard diet. Studies were made from 1 to 8 days following the change of diets. Bile was collected as follows: The animals were lightly anesthetized and a polyethylene cannula inserted in the common bile duct. After the surgical wound was

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closed with sutures, the animals were placed in restraining cages and the bile collected and maintained at 0–4° C for 24 hr. Following collection periods, the animals were sacrificed by anesthesia, and pieces of the liver were removed for microscopic study and chemical analysis.

Chemical Analyses

Bile. Bile was analyzed for iron by a modification of the method of Forman.[2] In this procedure, 10% trichloracetic acid is substituted for 30% trichloracetic acid, and bathophenanthroline is extracted with *n*-octyl alcohol.

Acid Phosphatase. Aliquots of the 24-hr bile collections were analyzed for acid phosphatase according to the method of Appelmans, Wattiaux, and de Duve.[3] Inorganic phosphate was determined by the method of Fiske and SubbaRow.[4] In order to assess the stability of acid phosphatase in bile at 0–4° C, aliquots of a 100,000-g supernatant fluid from a liver homogenate in which the acid phosphatase was activated by Triton X-100 were incubated with bile. These studies revealed that acid phosphatase activity was not altered by 24-hr incubation with bile, even when 1 part of supernatant was diluted with 9 parts of bile.

Light Microscopy

The sections of liver were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin, or with Perls' iron stain. Light microscopic observations were also made of 0.5- to 1-μ sections cut from Epon blocks and stained with alkaline toluidine blue. For light microscopy, acid phosphatase and iron were demonstrated on the same section with Barka's simultaneous coupling pararosanilin method [5] following Perls' reaction for iron.

Electron Microscopy

Blocks of liver for routine morphologic analysis were fixed by immersion of approximately 1-mm cubes of liver in cold 1% osmium tetroxide buffered with *s*-collidine (pH 7.4) at 0–4° C. These were dehydrated, embedded in Epon, and examined in Hitachi HS-7 or HU-11 electron microscopes after en bloc staining with uranyl acetate. In some instances, double staining with uranyl acetate and lead citrate was performed.

Electron microscopic histochemical demonstration of acid phosphatase was accomplished as follows: Thin slices of liver were fixed for 2–4 hr in cold (0–4° C) 4% sodium cacodylate-buffered glutaraldehyde and washed overnight in cacodylate buffer containing 7.5% sucrose. Thin slices (approximately 50μ thick) or small cubes of tissue were then incubated according to a modified Gomori technique [6] for the demonstration of acid phosphatase; the ammonium sulfide step was omitted. The incubated tissues were then postfixed in osmium tetroxide, dehydrated, and embedded in Epon. These sections were examined unstained in the electron microscope.

Examination of particles within the bile was accomplished as follows: After removing 1-ml aliquots for the 24-hr chemical analyses, the remaining bile (usually 10–15 ml) was suspended in 0.3 M sucrose and centrifuged at 78,500 *g* for 60 min. The resulting pellet was fixed in cold 1% osmium tetroxide as above, embedded in Epon, and examined in the electron microscope after thin sectioning. Other portions were studied by diluting 0.1 ml of the pellet in 0.9 ml ion-free water, spraying aliquots on a carbon-coated grid with a nebulizer, and examining them unfixed and unstained in the electron microscope. In some cases particles of colloidal thorium dioxide were added to the suspension to facilitate focusing.

Results

Chemical Studies

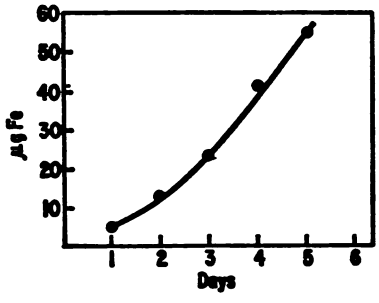
Biliary Iron

After return of the animals to the standard diet, iron content in the bile was measured at 24-hr intervals. The following measurements were made: total iron (micrograms), volume of bile (milliliters), and concentration of iron in the bile (micrograms per milliliter). The data obtained (Table 1; Text-fig 1) indicated that the daily amount

Table 1. Biliary Iron Excretion Showing Mean Values and Standard Deviation of Concentration, Volume, and Total Iron Values for Study Period

Days unloading	Animals (No.)	Iron excretion in bile		
		Iron conc (μg/ml)	Bile volume (ml/24 hr)	Total iron (μg/24 hr)
Normals				
0	6	0.65 ± 0.29	11.32 ± 2.71	7.09 ± 4.29
Experimentals				
1	3	1.00	9.20	9.00
2	8 (9*)	0.79 ± 0.26	8.80 ± 1.31	6.87 ± 2.15
3	12	1.25 ± 0.35	8.90 ± 2.02	11.18 ± 4.05
4	12	1.81 ± 0.63	10.00 ± 1.94	18.27 ± 7.94
5	7 (8*)	1.42 ± 0.53	8.90 ± 1.31	12.64 ± 5.12
8	3	1.48	10.8	16.24

* One animal rejected by Chauvenet's criterion.

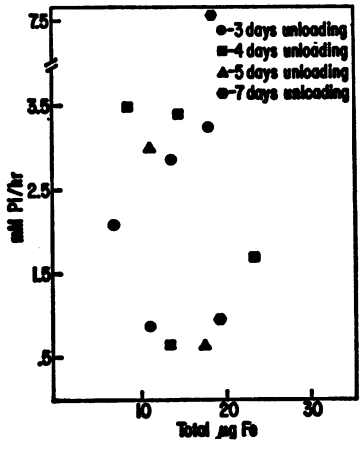


Text-fig 1. Total cumulative output of biliary iron is expressed per 24-hr time period. Each point represents a mean value of several animals. Number of animals and standard deviations are given in Table 1.

of iron excreted increased sharply after the first 2 days of unloading, and reached a maximum at 4 days. After this, the total biliary iron excretion gradually decreased, but was still above control levels at the end of 8 days. The marked increase in total iron output in the bile, measured over the 5 days following dietary change, is the result of an increased concentration of iron in the bile, as there was little change in volume.

Acid Phosphatase

Total acid phosphatase activity was measured in the bile per 24-hr period of bile collection during the first 7 days of unloading. When acid phosphatase activity was plotted against total biliary iron, there was no correlation between acid phosphatase excretion and biliary iron excretion per 24-hr time period (Text-fig 2).



TEXT - FIG 2. Total acid phosphatase in bile collected from individual animals as expressed in millimoles of inorganic phosphate released per hour per 24-hr period of time. Total iron excreted in the bile per 24-hr period is expressed as micrograms of iron. There is no correlation between acid phosphatase activity and excretion of biliary iron.

Light Microscopy

Paraffin sections of untreated control animals rarely showed blue peribiliary cytoplasmic granules in periportal cells with Perls' method for iron (Fig 1). Sections from animals fed corn grits-lard diets without added dietary iron showed an increased number of blue peribiliary granules (Fig 2). The livers from animals treated with corn grits-lard diets with added iron for 3 weeks had the most iron-containing granules of all liver sections studied (Fig 3). These consisted of dense blue granules within each hepatic cell, and the granules were localized in peribiliary regions. In addition, many hepatic parenchymal cells had a diffuse bluish cytoplasmic haze. When acid phosphatase was demonstrated simultaneously, the picture was striking. Many peribiliary granules stained blue-red, suggesting the presence of both iron and acid phosphatase in the granules. A few granules within each cell stained only red for acid phosphatase, or blue for iron. Quantitatively, blue particles were the most numerous.

When animals on iron-loading diets for 3 weeks were returned to standard diets, there was a marked decrease in the number of iron-positive granules in the first 5 days following dietary change. Animals off the iron-loading diet for 3 days (Fig 4), 1 week (Fig 5), and 2

weeks (Fig 6) showed progressive decrease in number and size of peribiliary iron-containing granules. At 1 and 2 weeks following return of iron-loaded animals to standard diet, acid phosphatase and iron were demonstrated simultaneously. At these later times a definite decrease in number and size of blue granules was noted. The acid phosphatase-positive red granules showed no change during the unloading phase.

Electron Microscopy

Untreated Normals

Examination of the hepatic cells in untreated normal animals revealed the usual ultrastructural features which are summarized in the figure legends, many of which have been described previously in the literature [7] (Fig 7). The secondary lysosomes in these animals contained a variety of dense granular and homogenous materials but very few particles identifiable as ferritin were evident. When liver from animals fed only a standard diet were incubated for the demonstration of acid phosphatase, deposits of lead phosphate reaction product were found occasionally within single-membrane-limited lysosomes and within bile canaliculi (Fig 29).

Animals Fed Corn Grits-Lard Diet Without Added Iron

Examination of hepatic cells in these animals revealed an increase in the size and number of peribiliary lysosomes. When compared with those of normals, these lysosomes contained slightly increased amounts of dense, irregular particles identified as hemosiderin, as well as particles identified as ferritin. Very few autophagic granules were noted. In addition, cells contained numerous lipid droplets and showed extensive dilatation of the endoplasmic reticulum and of the Golgi cisternae and vacuoles (Fig 8). The Golgi vesicles contained homogeneous rounded bodies of moderate density presumed to represent lipoproteins or triglycerides. The mitochondria tended to show swelling of the inner compartment and, in many instances, paracrystalline densities were noted within the matrix.

Animals Fed Corn Grits-Lard Diet With Added Iron

After 3 weeks, the hepatic cells in these animals were essentially identical with the hepatic cells of the animals on corn grits-lard diet without added iron, except for the appearance of the secondary lysosomes. The lysosomes, however, were significantly different. They were large, numerous, and contained an abundance of dense, granular material. Some of this material was composed of small particles approx-

imately 70 Å in diameter, which at high magnification showed the characteristic structure of the iron hydroxide miceller ferritin cores. The remaining portion of the lysosome contents consisted of course clumps of dense material. These clumps were presumed to represent iron in other forms or were associated with lipid-containing or other noniron-containing proteins and will be referred to as hemosiderin (Fig 15).

When livers from these animals were incubated for the demonstration of acid phosphatase, deposits of lead phosphate reaction product were found within the single-membrane-limited lysosomes containing ferritin and hemosiderin (Fig 23 and 24). The ferritin could be distinguished readily from lead phosphate reaction product as follows: Ferritin particles were uniform in size (70 Å) and had a typical micellar core. They were relatively low in density in sections stained only with uranyl acetate. The lead phosphate reaction product had a markedly different morphology. The smallest particles were ~300 Å in diameter; the particles were elongate rather than circular, often had a tortuous outline, and were always extremely dense.

Particles of ferritin also were observed within cell sap and occasionally within components of the Golgi apparatus and the endoplasmic reticulum. The details of intracellular distribution form the subject of another paper and will not be discussed here.

Animals Fed Iron-Loading Diets for 3 Weeks and Subsequently Standard Diets

Livers from animals were studied at 1, 2, 3, 4, and 5 days following return to standard diet. Biochemical data and light microscopy showed progressive decrease in iron content. As the ultrastructural changes were similar, differing only quantitatively, they will be discussed together. After 1 day, the appearance of the liver cells was essentially identical with liver cells during the loading phase. Most of the hepatic secondary lysosomes in the peribiliary region were large and contained numerous dense, granular deposits of hemosiderin and ferritin (Fig 9–11). Although the majority of secondary lysosomes were found in the peribiliary region, occasional smaller, single-membrane-limited bodies containing ferritin were seen near the microvilli lining the space of Disse (Fig 25). In addition, bile canaliculi often contained small clusters of dense, granular material resembling that found within secondary lysosomes. After 2 days of unloading, quantitative and qualitative changes were seen which clearly distinguished the loading and unloading phases. From Day 3 through Day 7 of unloading, the number and density of iron-containing lysosomes in the peribiliary region decreased

(Fig 12–14). Examination of the lysosomes at Days 4 and 5 of unloading indicated that, in addition to a decreased number of lysosomes, the concentration of ferritin and other forms of iron hydroxyphosphate-containing small particles within these lysosomes appeared to decrease markedly. While losing much of their ferritin, these lysosomes seemed to retain hemosiderin (Fig 14 and 16). Coarse clumps of dense material presumed to be hemosiderin (Fig 16) were distinguishable from particles of more uniform size and density present in iron loading (Fig 15). In addition, the background matrix of the lysosomes appeared relatively clear (Fig 14). At the later intervals, especially after Days 3, 4, and 5 of unloading, more granular material was found within the lumens of bile canaliculi (Fig 17–19). These deposits were not surrounded by a limiting membrane and were composed of ferritin particles mixed with coarsely granular material presumed to be hemosiderin. They were indistinguishable from the contents of lysosomes described above.

Examination of Bile

Examination of thin sections of the bile sediment on the second, third, and fourth days following dietary change revealed clusters of electron-dense material (Fig 20–22). These clusters were identical in appearance with the dense material found within the hepatic lysosomes and with the material found within the lumens of the bile canaliculi. These clusters were mixed with a variety of materials, including membranous debris and particles of ferritin; in no case were the clusters of this material limited by a membrane. In addition to these dense particle clusters, the bile sediment contained numerous membranous profiles, some of which resembled myelin figures. These presumably represented effects of bile salts on cell debris within the bile.

Examination of the dispersed preparations prepared by spraying bile sediments on electron microscope grids revealed numerous particles with the typical appearance of ferritin [8,9] (Fig 27 and 28). The diameter of the dense central core was approximately 60–70 Å. There appearance was identical to that observed in sprays of commercially obtained ferritin processed in the same fashion (Fig 26).

Discussion

It has been shown that there is marked deposition of iron in the liver of experimental animals fed a corn grits-lard diet with excess iron.[10] In the liver cell, iron is concentrated as ferritin and hemosiderin within secondary lysosomes in the peribiliary region. When animals are changed

from an iron-loading to a standard diet, a marked reduction in liver iron occurs. Of iron stored in the liver, 47% is lost within 3 days, and 56% of the liver iron is lost over a 14-day period following change from an iron-loading to a standard diet.[1]

The experiments reported above suggest that biliary loss of iron results, at least in part, from discharge of lysosomal contents into the bile canaliculi. Such a mechanism has been postulated by previous observers on the basis of morphologic findings of images resembling lysosomal content within the bile canaliculus.[7,11-13] However, our report presents the first definitive evidence that such a process, in fact, exists. The evidence presented is as follows: (1) Aggregates identical with those found within the lysosomes were observed within the bile canaliculi in liver sections, and these aggregates were not surrounded by a membrane. (2) Similar clusters of dense material were found in the bile when sections of bile pellets were studied. (3) Numerous particles of ferritin and hemosiderin were observed in preparations made by spraying bile on electron microscope grids. (4) The increased biliary excretion of iron, measured chemically, coincided with the demonstration of ferritin in the bile by ultrastructural techniques.

The exact mechanism whereby ferritin and hemosiderin cross the plasma membrane at the canaliculus cannot be stated. Images of actual fusion of lysosomes with cell membranes were not observed in this study. The most likely mechanism whereby macromolecules can cross the plasmalemma is reverse pinocytosis; images of fusion of secretory granules with plasmalemma have been observed in pancreatic acinar cells.[14] However, the number of secretory granules within the pancreatic acinar cell exceeds the number of peribiliary lysosomes in the hepatic cell and, thus, they are more likely to be seen in the process of fusion.

Examination of hepatic lysosomes after 3 days' unloading indicated that they were markedly different in appearance than lysosomes studied earlier in the unloading phase. They also differed from lysosomes in the fully iron-loaded animal. Closer examination revealed that the lysosomes in late stages of unloading contained more hemosiderin than ferritin. In effect, they appeared to have lost much of their ferritin content during unloading. Accordingly, this observation suggests a selective loss of some lysosome contents. Such incomplete discharge could occur if a portion of the lysosome containing only ferritin particles "pinched off" and subsequently fused with the plasmalemma at the canaliculus. In this way small amounts of ferritin may be released, while larger aggregates of hemosiderin remain.

The lack of correlation between acid phosphatase activity and content

of iron in the biliary samples described above suggests that the release of iron and acid phosphatase are independent. Although the explanation for this relative selectivity in rate of release cannot be given, it may be similar to the difference in rate of release between hemosiderin and ferritin. Such selective release of materials from hepatic lysosomes has not been suggested heretofore.

These experiments establish the presence of a lysosomal excretory pathway in mammalian hepatic parenchymal cells. In theory this type of mechanism could provide an alternative to digestion for release of material from secondary lysosomes. There is precedent for such a pathway from studies with *Amoeba proteus*.[15] After feeding, such amebas discharge the debris remaining within the food vacuoles in the form of food balls into the surrounding medium. Such a pathway has not been established previously in mammalian cells. Nevertheless, a number of papers have appeared indicating this possibility, since images suggestive of lysosomal debris are found in normal and experimentally altered situations in both bile canaliculi and in renal tubules.[11-13,16]

These studies also may help explain the pronounced peribiliary localization of secondary lysosomes and Golgi apparatus that have been observed in the hepatic parenchymal cells. Since much available evidence indicates that the polarization of the Golgi apparatus is indicative of the direction of secretory activity, it would appear that in the liver cells the Golgi apparatus may be concerned principally with the lysosome system and also with the biliary excretory pathway. Since many of the materials secreted by the liver evidently are released into the space of Disse, it would follow that this pathway of secretion may be through pathways which do not include the Golgi apparatus.

The significance of ferritin in the cell sap is not known. At least some, if not all, of this could reflect movement of intralysosomal ferritin during processing. This will be discussed in detail in the next paper in this series. Our present studies, however, do not exclude the possibility of additional excretory pathways.

Summary

The present paper presents a study of a pathway by which iron leaves liver cells when these cells have been loaded previously with iron by dietary means. It was found that at least a portion of the iron which is concentrated as ferritin within secondary lysosomes probably is discharged by these lysosomes into the bile canaliculi. The mechanism of this biliary loss of iron is most likely reverse pinocytosis, and the evidence presented is fourfold: (1) Electron-dense aggregates devoid of

an investing membrane and resembling the content of peribiliary lysosomes are found within bile canaliculi in sections of liver. (2) When sections of bile pellets are studied, similar membrane-free, electron-dense deposits are seen. (3) Numerous particles of ferritin and hemosiderin are observed in preparations made by spraying bile on electron microscope grids. (4) The increased biliary content of iron measured chemically coincides with the appearance of ferritin in the bile on electron microscopy.

This pathway does not account for all the iron that leaves the liver cell. It does indicate that some iron is lost by discharge from secondary lysosomes into the bile by the process of reverse pinocytosis.

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[*Illustrations follow*]

Legends for Figures

Fig 1. Light micrograph from normal animal fed standard diet, showing normal hepatic architecture. A portal area is seen at lower right. Perls'. × 500.

Fig 2. Light micrograph from control animal fed corn grits-lard diet without added iron for 2 weeks. Cytoplasm of hepatic cells contain numerous lipid droplets. Occasional granular particles representing iron are seen near portal area at lower right. Perls'. × 500.

Fig 3. Portal area from animal on iron-loading diet for 3 weeks. The dense cytoplasmic accumulation of Perls-positive material was heaviest in portal region and consisted of dense granules within each hepatic cell which were localized in peribiliary region. Perls'. × 500.

Fig 4. Portal area of animal on iron-loading diet for 3 weeks and subsequently placed on standard diet for 3 days. Perls-positive material is less concentrated than in the fully iron-loaded animal. At this time, iron content of bile was elevated. Perls'. × 500.

Fig 5. Portal area of animal 1 week following change from iron-loading to standard diet. Particulate Perls-positive material here is less concentrated and less dense than in fully iron-loaded animal. Perls'. × 500.

Fig 6. Portal area of animal 2 weeks following change from iron-loading to standard diet. Perls-positive material is markedly decreased and less dense. Perls'. × 500.

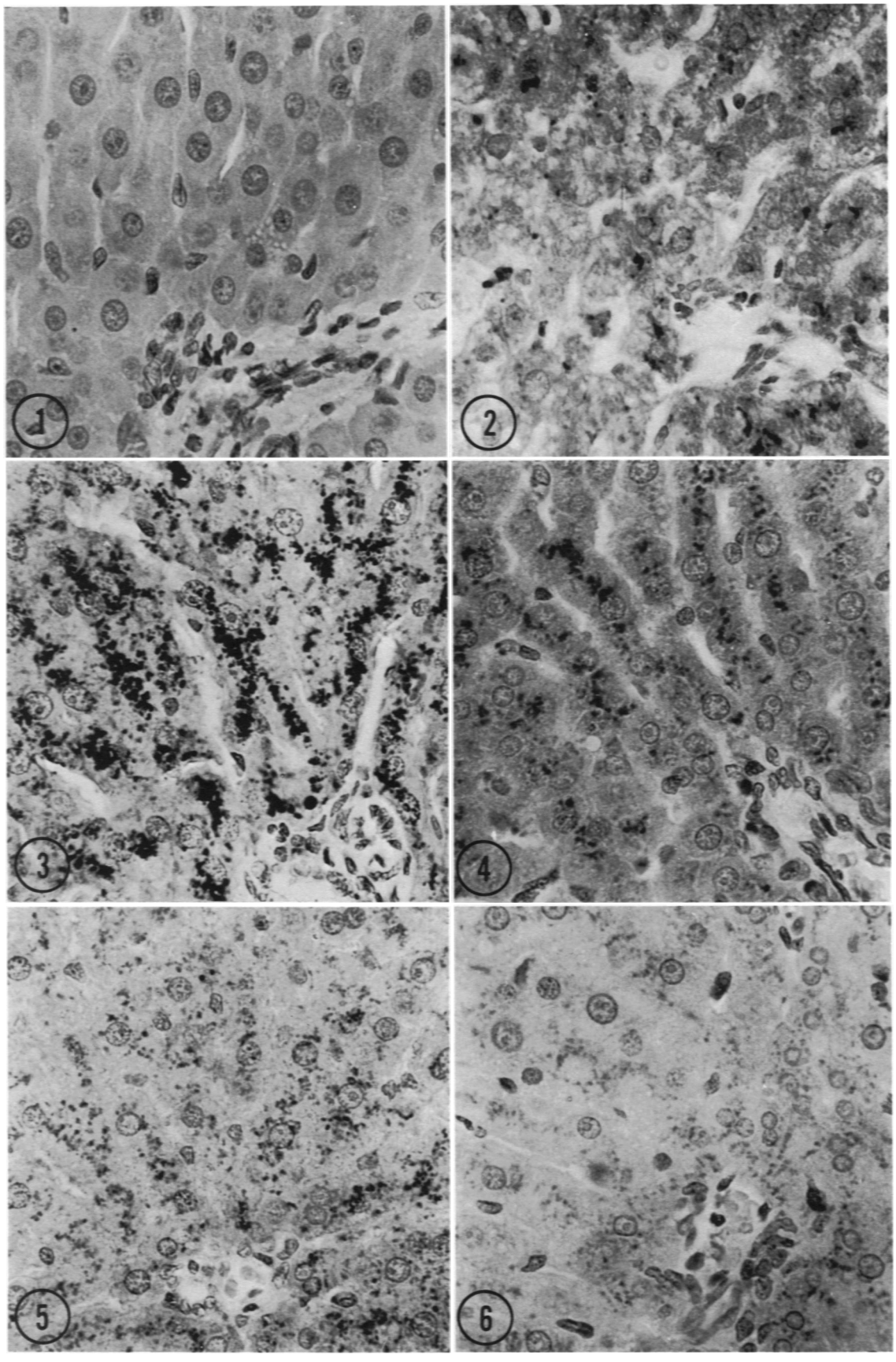
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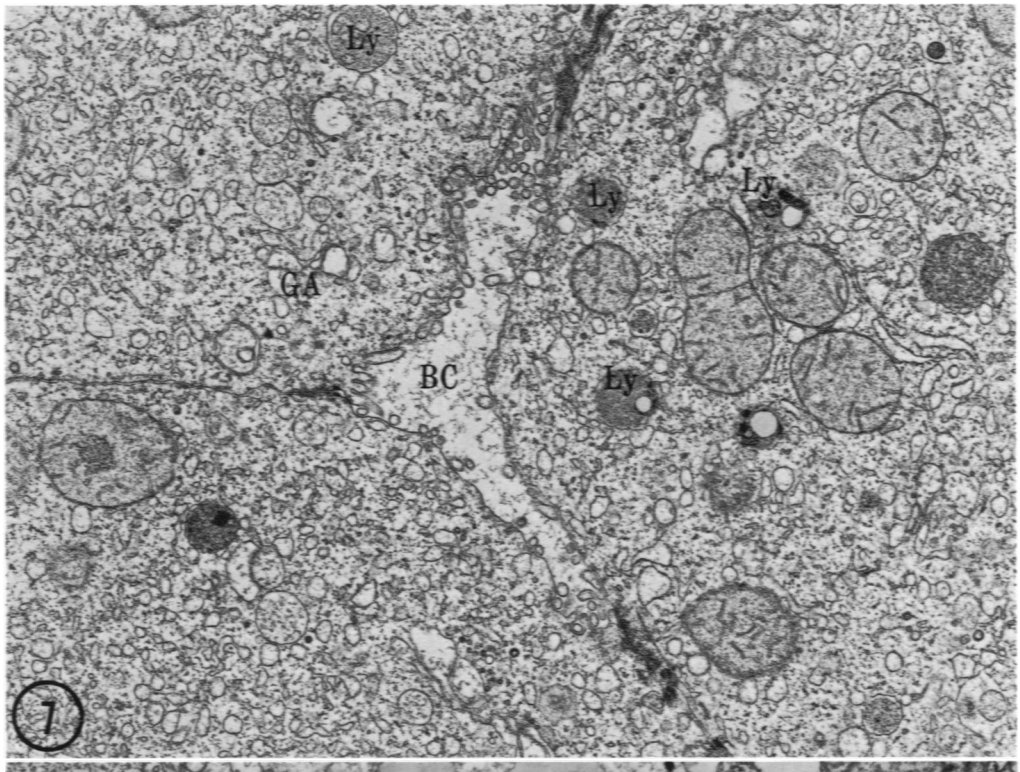
Fig 7. Electron micrograph of liver from normal animal on standard diet shows normal hepatic ultrastructure. Three hepatocytes abut on a bile canaliculus (*BC*). Golgi complex (*GA*) is small, and secondary lysosomes (*Ly*) in peribiliary region are inconspicuous. × 26,500.

Fig 8. Two hepatocytes and bile canaliculus (*BC*) from animal fed corn grits-lard diet without added iron. Cells contain numerous lipid droplets and show dilatation of endoplasmic reticulum and of cisternae of Golgi apparatus (*GA*). Mitochondria are swollen and often contain paracrystalline densities within the matrix (*arrow*). × 46,000.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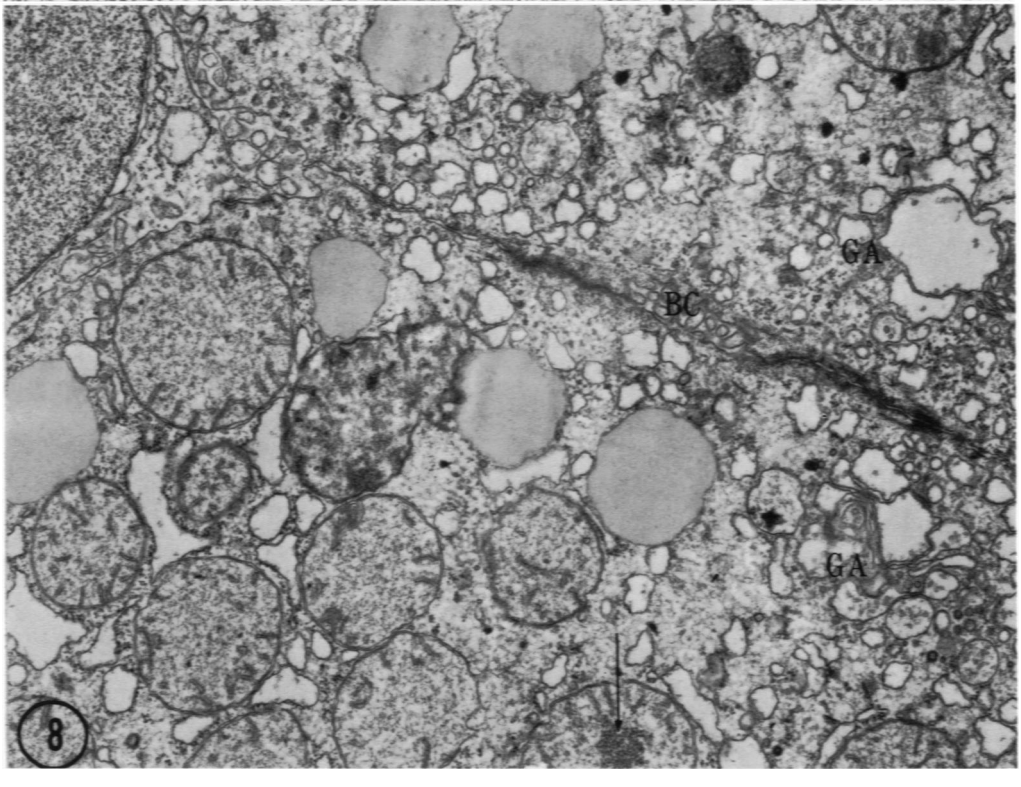
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Fig 9. Confluence of three hepatocytes at bile canaliculus (*BC*) from animal on iron-loading diet for 3 weeks and subsequently placed on standard diet for 24 hr. Secondary lysosomes in peribiliary region are large, numerous, and contain dense granular deposits probably representing ferritin and hemosiderin. × 23,000.

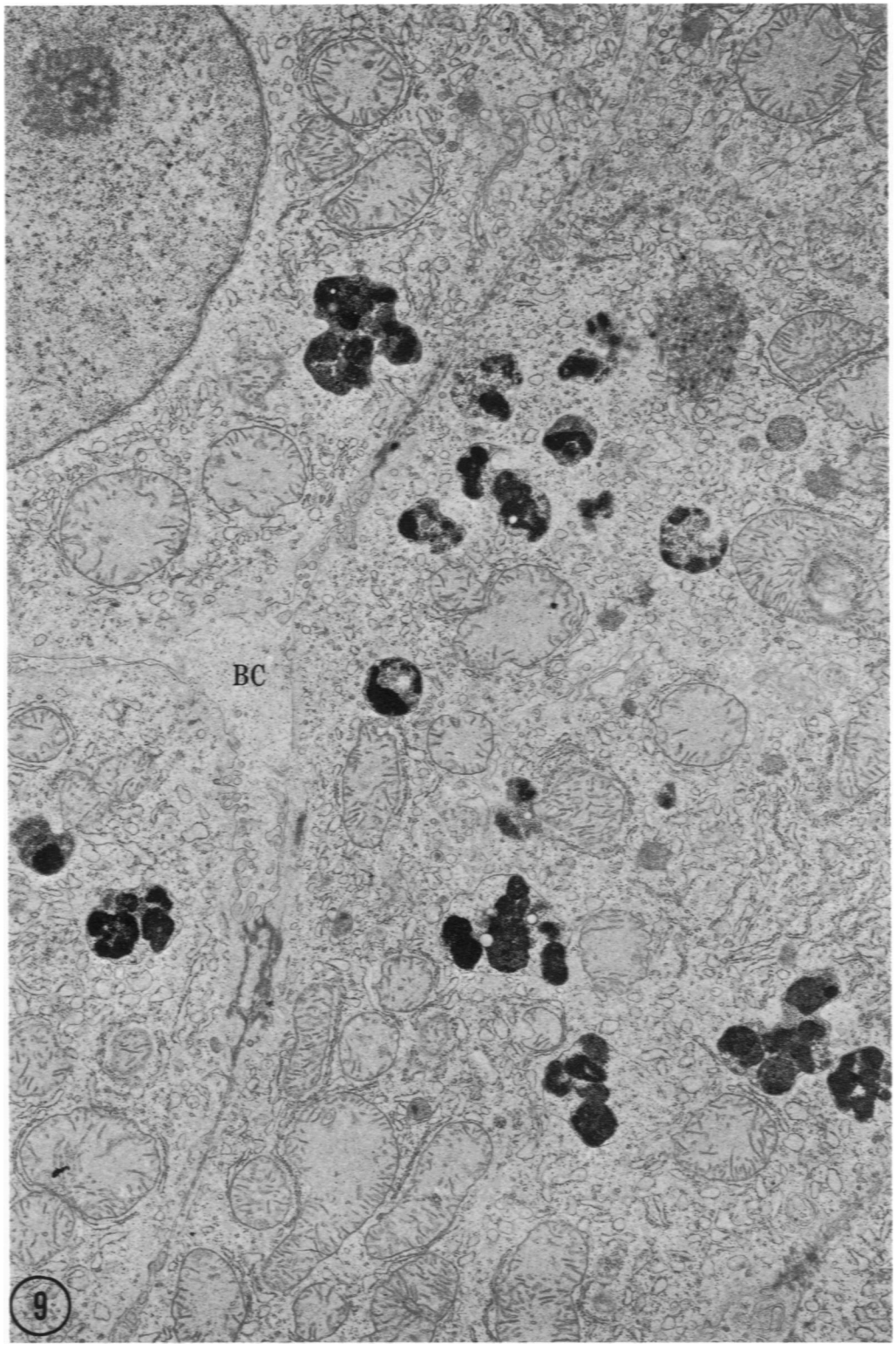
BC
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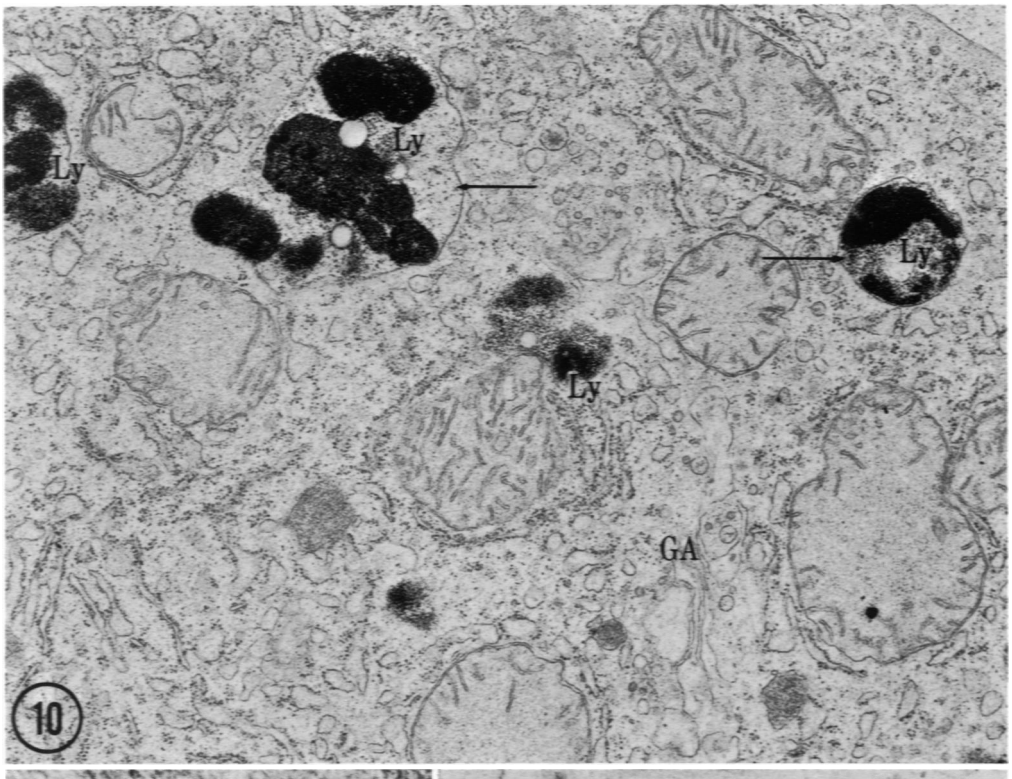
Fig 10. Higher magnification of secondary lysosomes (*Ly*) in peribiliary region of Fig 9. Electron-dense material is particulate and enveloped by a single membrane. At least a portion of this electron-dense material represents ferritin (*arrows*). Ferritin is not present within Golgi apparatus (*GA*) or mitochondria, but scattered particles occur in the cell sap. × 39,000.

Fig 11. Electron micrograph of portion of peribiliary lysosome (*arrow*), limited by a single trilaminar membrane and containing typical particles of ferritin as shown in the inset at higher magnification (*arrows*). Scattered particles of ferritin are seen in cell sap. × 67,500.



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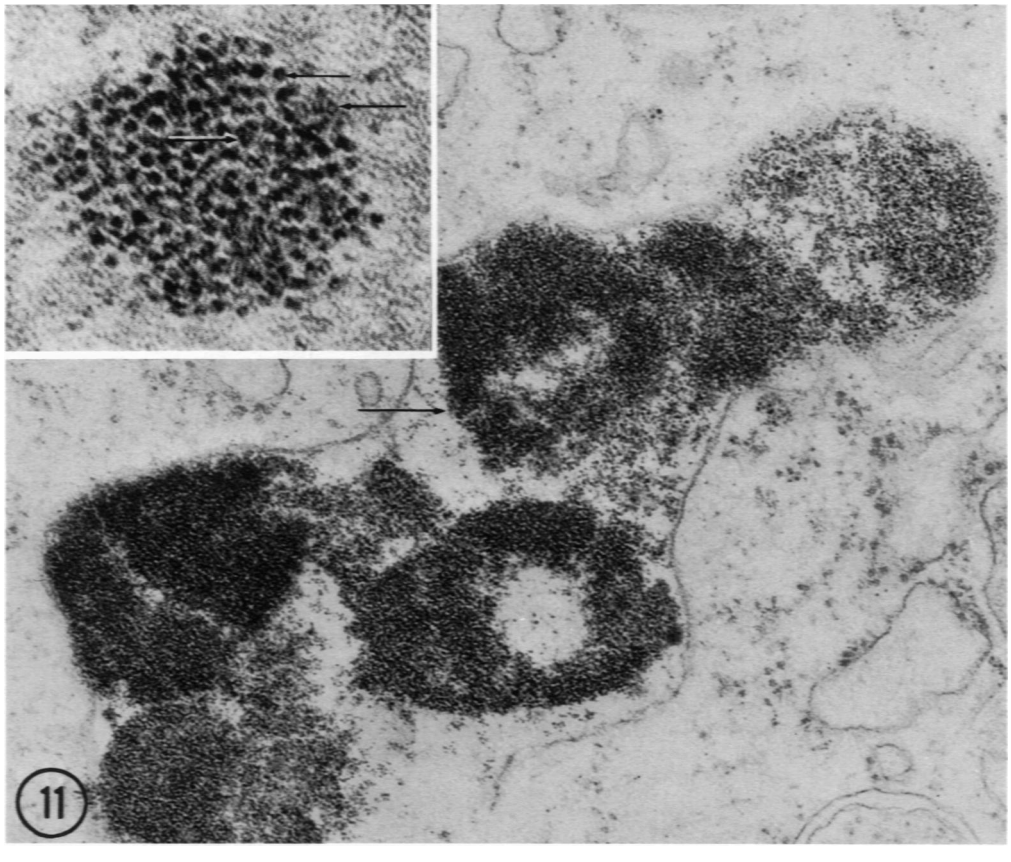

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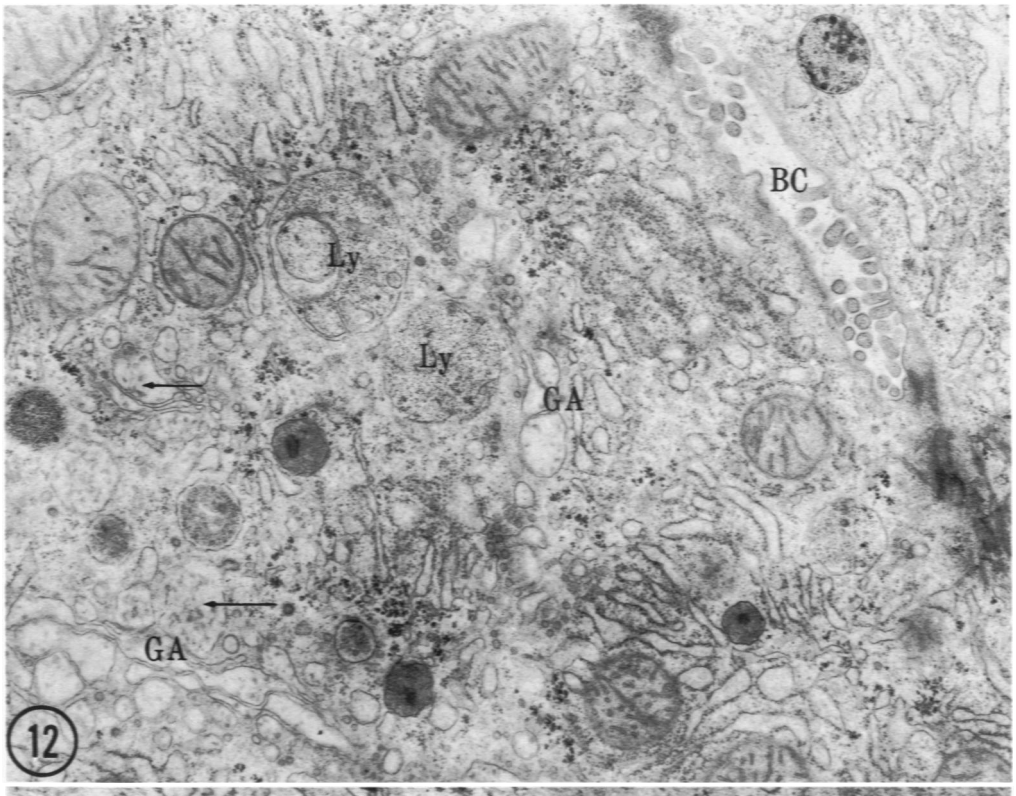
Fig 12. Three days following change from iron-loading to standard diet, the number and density of lysosomes (*Ly*) in peribiliary region is decreased. Numerous densities representing triglyceride or lipoprotein are present within Golgi (*GA*) cisternae (*arrows*). Bile canaliculus (*BC*). × 16,000.

Fig 13. Higher magnification of lysosomes (*Ly*) from a rat, 3 days following return to standard diet. Lysosomes are less concentrated than in lysosomes from rats studied at 1 day following dietary change (Fig 9). Amount of ferritin and hemosiderin in any given lysosome appears reduced. Double-membrane-limited autophagic vacuole (*AV*) contains ferritin particles within its matrix and in spaces between membranes (*arrow*). No ferritin is seen within cisternae of rough endoplasmic reticulum or in the microbody (*Mb*). × 37,000.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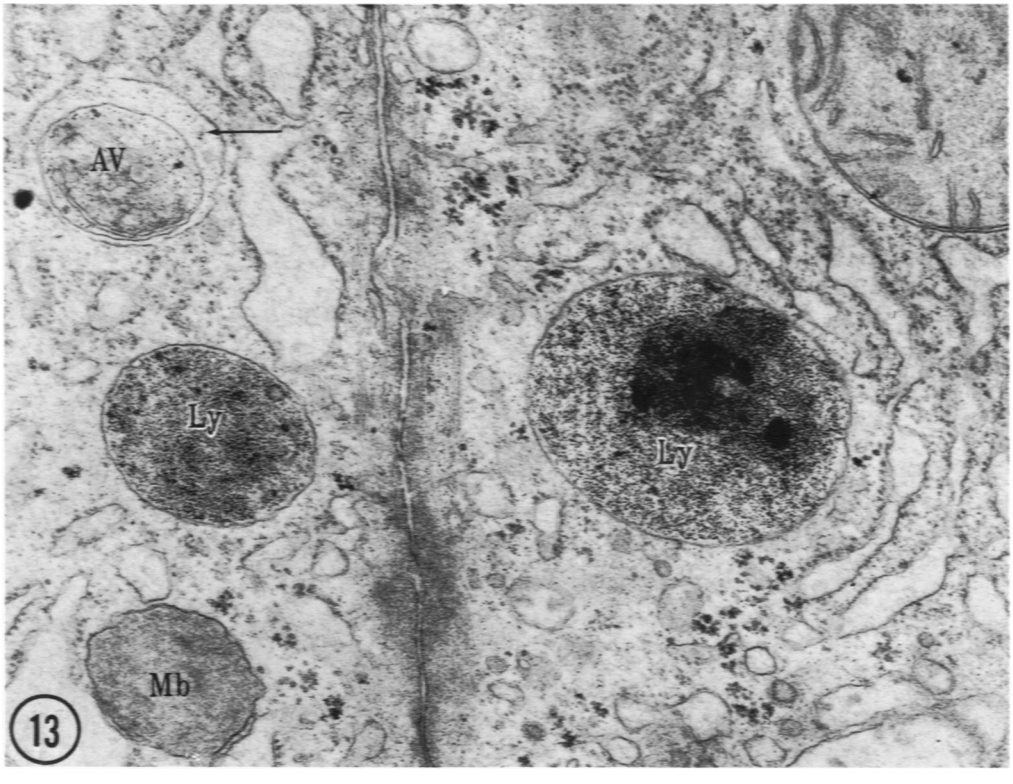
AV
Ly
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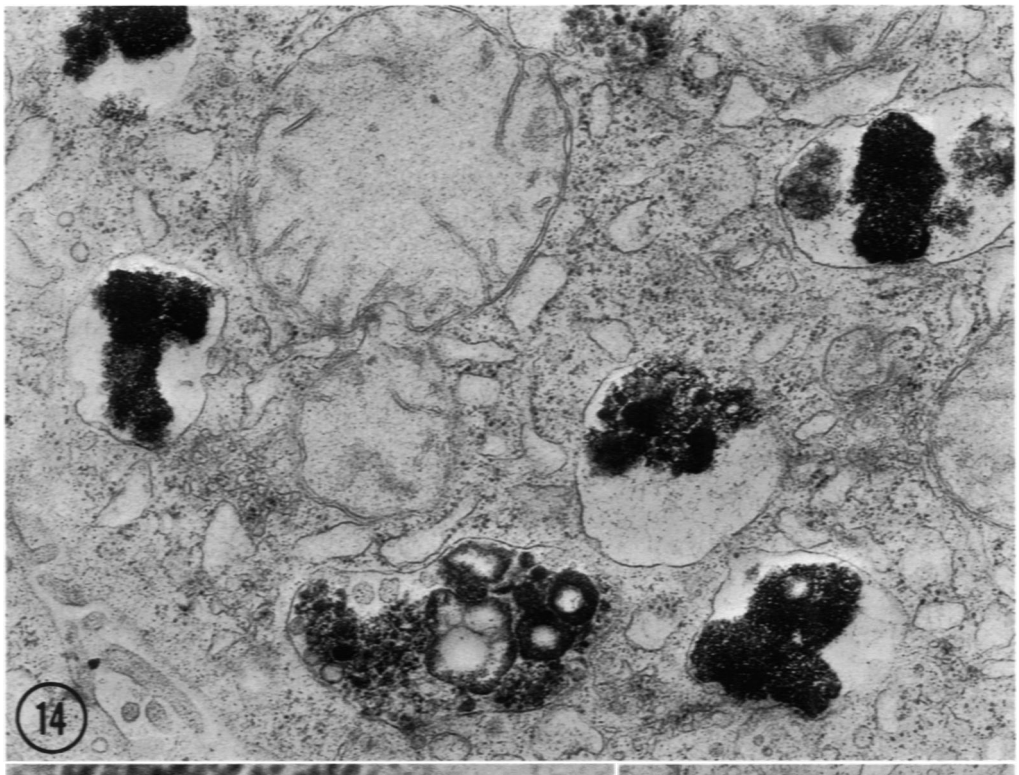
Fig 14. Four days following return to standard diet, peribiliary lysosomes show only a few ferritin particles in a pale matrix and irregular clumps of dense material presumed to represent hemosiderin. × 80,000.

Fig 15. High magnification of lysosome after 3 weeks of iron loading. Note irregular particles which do not show typical appearance of ferritin (cf Fig 11 inset). This material may represent hemosiderin. × 310,000.

Fig 16. High magnification of lysosome after 5 days unloading, showing very dense, irregular clumps of material presumed to represent hemosiderin. × 330,000.

Fig 17. After 3 days of unloading, small clusters of electron-dense material (*arrow*) resembling lysosome content are seen within bile canaliculi. Note that peribiliary lysosomes (*Ly*) contain similar appearing dense material. Bile canaliculus (*BC*). × 40,000.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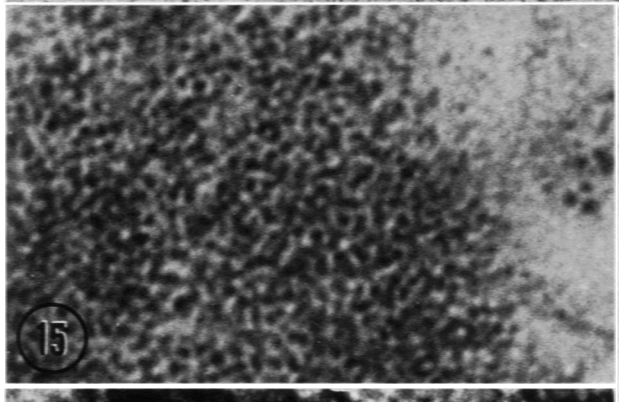
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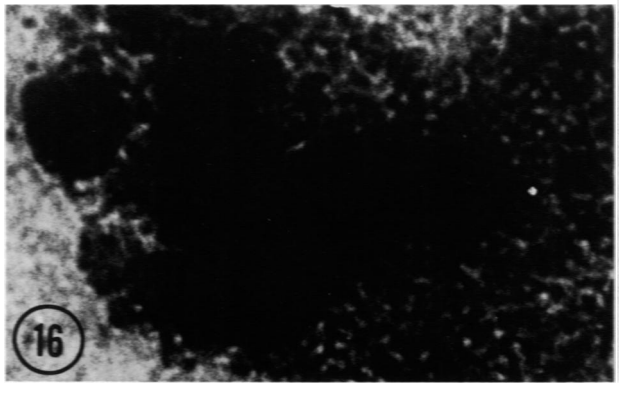
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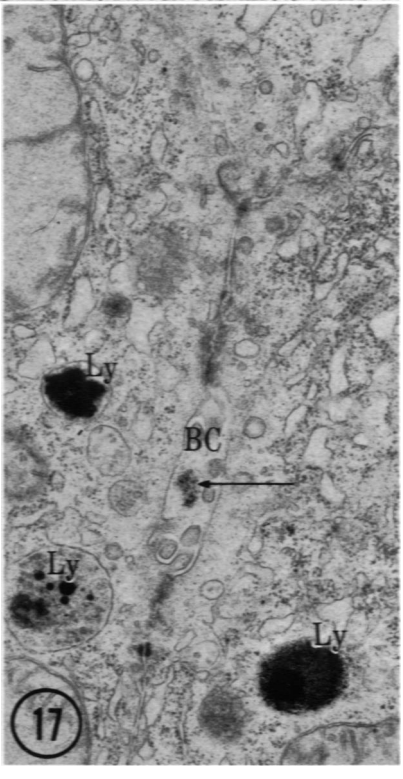
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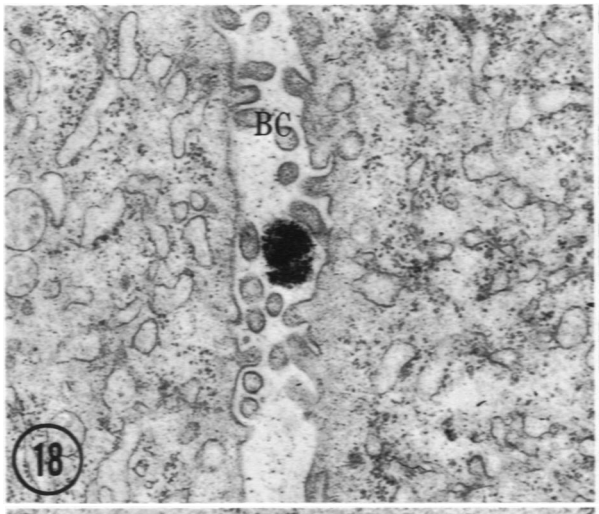
Fig 18 and 19. Bile canaliculi (*BC*) from rats after 4 and 5 days unloading, respectively, contain clusters of electron-dense particles. These clusters are free of a limiting membrane and are indistinguishable from lysosomal contents. Fig 18 × 46,000; Fig 19 × 65,500.

Fig 20, 21, and 22. Electron micrographs of fixed bile sediment collected 4 days following dietary change. Electron-dense material similar to that present within bile canaliculi in liver sections (Fig 18 and 19) is present. This material resembles content of peribiliary lysosomes. These clusters are not limited by a membrane. Diffuse particles and membranous debris are also present. Fig 20 × 216,000; Fig 21 × 117,000; Fig 22 × 88,000.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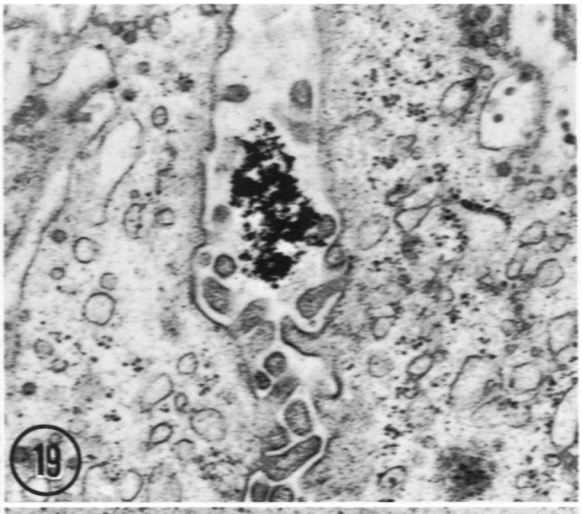
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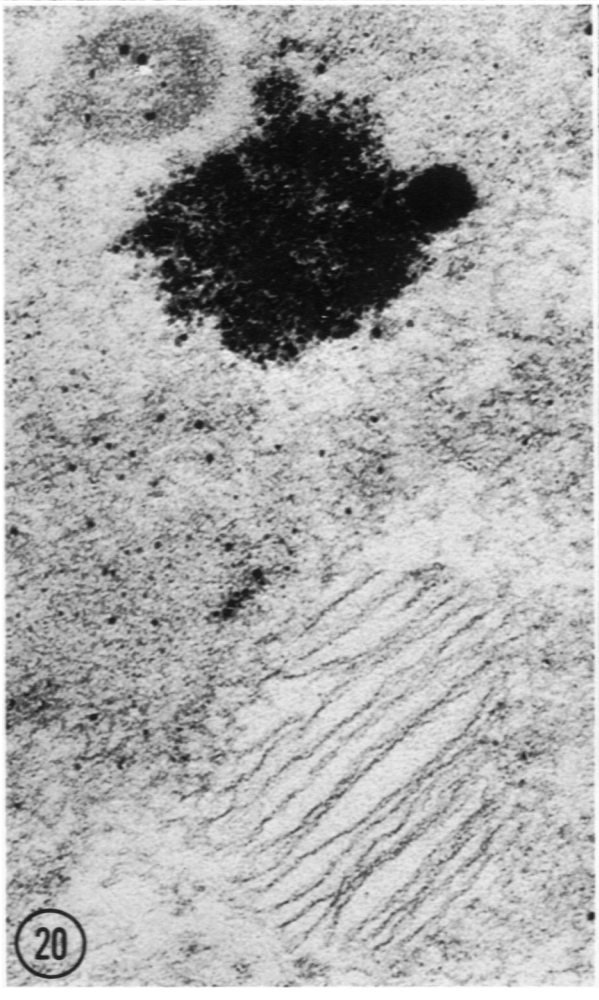
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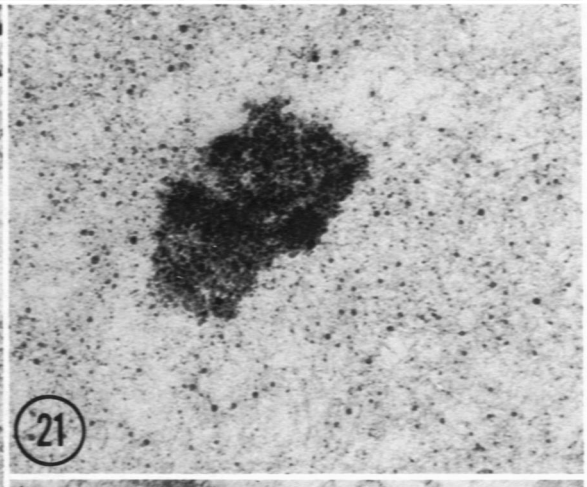
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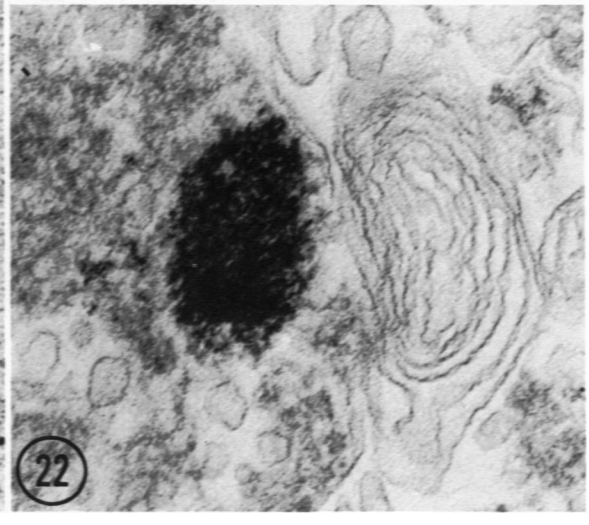
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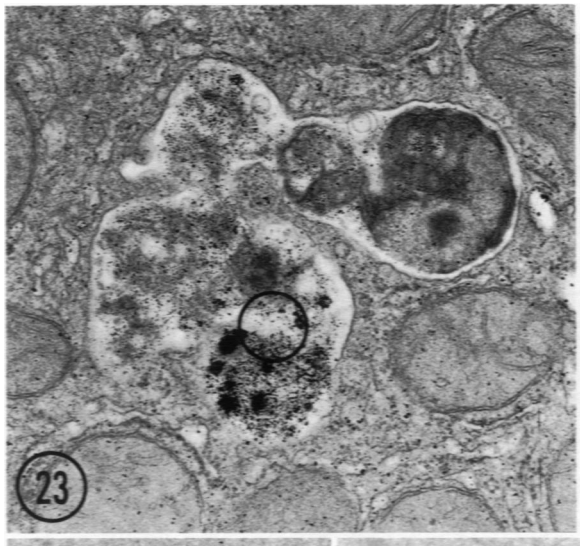
Fig 23. Electron micrograph of liver from animal on iron-loading diet for 3 weeks. Lysosome contains both large, electron-dense lead phosphate deposits as well as small, scattered, less-electron-dense particles which represent iron hydroxide micelles. Unstained section. × 41,000.

Fig 24. Higher magnification of region circled in Fig 23, showing ferritin particles (*single arrows*) and larger, denser, and more irregular deposits of lead phosphate (*double arrows*). × 300,000.

Fig 25. Sinusoidal border of hepatocyte from animal 1 day following dietary change. Occasional small single-membrane-limited bodies containing iron (*arrow*) are seen near microvilli lining the space of Disse. × 79,000.

Fig 26. Dispersed preparation of commercial horse spleen ferritin sprayed on carbon-coated grids and examined fresh illustrates more clearly the typical appearance of ferritin. × 67,000.

Fig 27 and 28. Dispersed preparation of bile collection of animals 4 and 5 days, respectively, following change from iron-loading to standard diet. Numerous particles identifiable as ferritin are present (*arrows*). Fig 27 × 770,000; Fig 28 × 770,000.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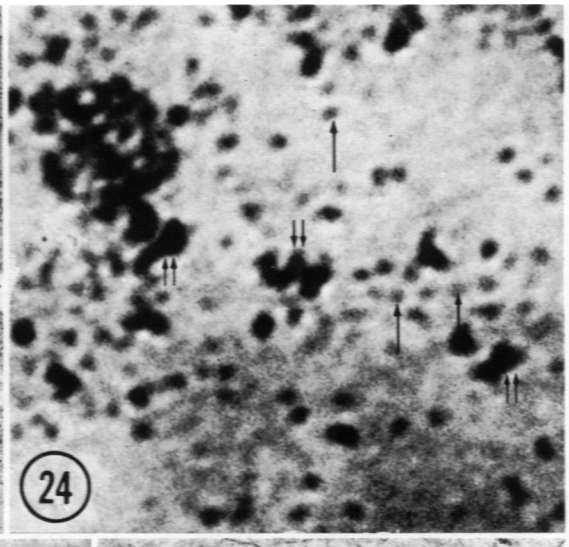
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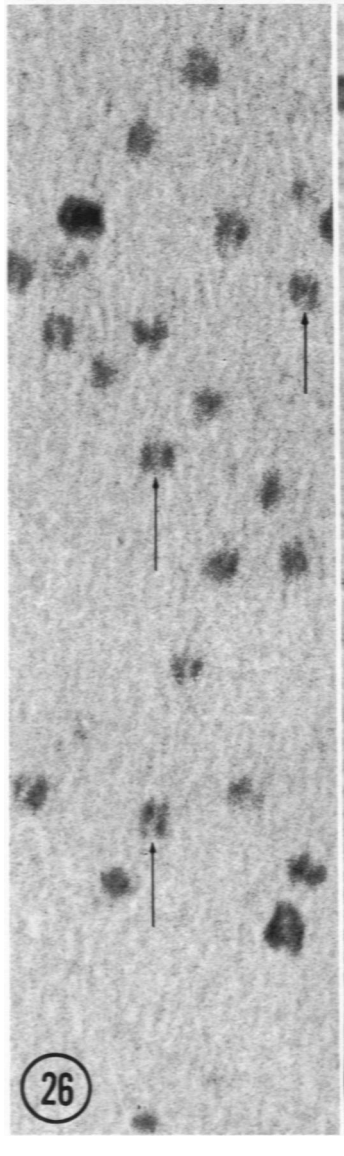
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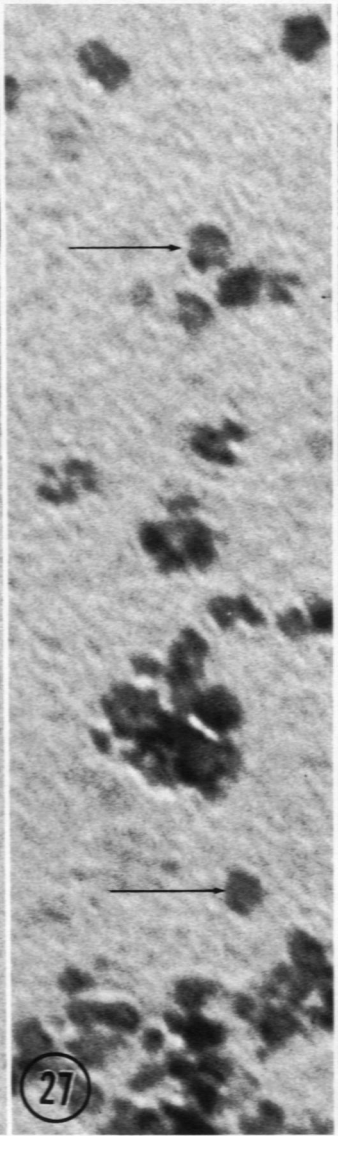
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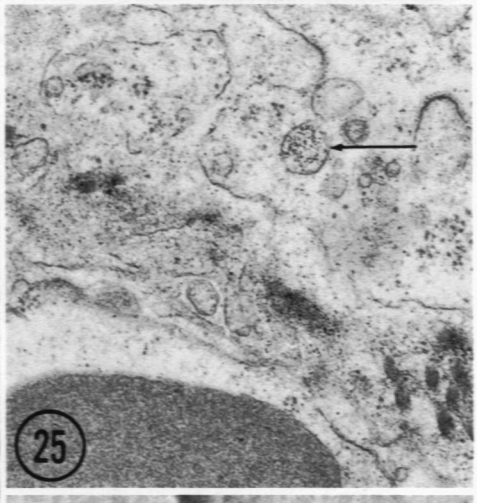
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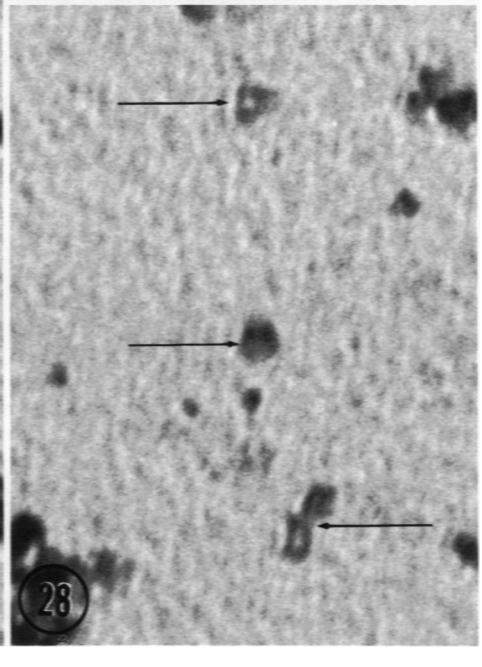
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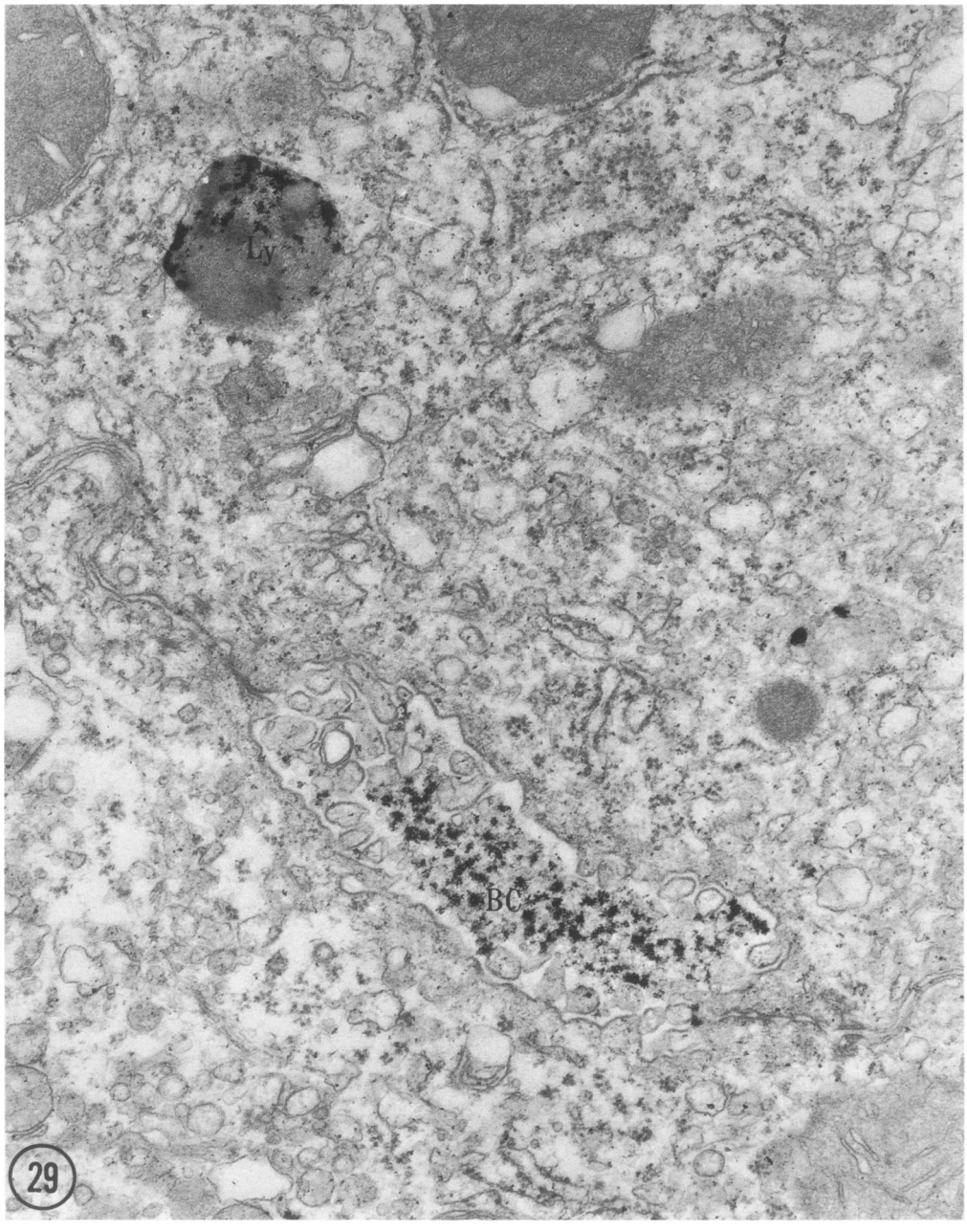

Fig 29. Electron micrograph of liver from normal animal on standard diet; tissue incubated for demonstration of acid phosphatase activity. Lead phosphate reaction product is seen within debris of a bile canaliculus (*BC*) and lysosome (*Ly*). Within the lysosome the dense lead phosphate reaction product is readily distinguished from the finer, more discrete particles of ferritin. Unstained section. × 31,000.