한국전자현미경학회지 제 28 권 제 2 호 1998
Korean J. Electron Microscopy 28(2), 171~180(1998)

# A Cytochemical Study on the Vacuolar Apparatus Participating in the Transport of Bile Acids in the Rat Hepatocytes (Cytochemical Study on the Vacuolar Apparatus for Bile Acid Transport)

Young-Chul Shin
Department of Anatomy, Korea University College of Medicine, Seoul, Korea

# 담즙산 분비과정에 관여하는 흰쥐 간세포내 소기관에 관한 세포화학적 연구

신 영 철
고려대학교 의과대학 해부학교실
(Received February 9, 1998)

## 요 약

본 연구에서는 dehydrocholic acid를 투여한 흰쥐 간세포의 vacuolar apparatus를 세포화학적으로 관찰하고자 하였다.

박절편에서 Golgi 장치의 형성면 수조는 소낭상으로 팽대된 부분이 열지어 있었으며 어떤것은 돌출되어 있었다. 이러한 소낭 모양의 돌출부는 용해소체 표면에서도 관찰되었다. 소포들은 Golgi 장치의 형성면 수조와 용해소체 및 담세관 주위에서 볼 수 있었으며 소포가 담세관에 융합되어 있는 것도 관찰되었다. 이러한 소견들은 dehydrocholic acid 투여 후 20분이 경과된 흰쥐에서 현저하였다. 정상군이나 실험군에서 거의 모든 Golgi 장치의 형성면은 담세관을 향하고 있었다.

박절편에서 Golgi 장치의 형성면 수조와 소낭 모양의 돌출부 및 소포는 가시물질을 거의 함유하고 있지 않았다. Osmium은 이들구조에서 심하게 침착되어 있었다. 용해소체와 그 주위에 있는 소포에서는 acid phosphatase 활성이 나타났다. 그러나 담세관 주위에 있는 소포에서는 osmium의 침착이나 acid phosphatase 활성이 경하게 나타나거나 관찰되지 않았다. 이러한 증거들로 미루어 소포는 Golgi 장치의 형성면과 용해소체에서 유래되며 이들소포가 담세관에 융합됨으로서 담즙산이 분비될 것으로 생각된다. 그러나 소포가 담세관에 접근해 감에 따라 소포의 효소활성은 저하되는 것 같이 보인다.

**Key words** : Vacuolar apparatus, Golgi apparatus, Endoplasmic reticulum, Lysosome, Vesicle, hepatocyte, Dehydrocholic acid

## INTRODUCTION

The Golgi apparatus was regarded not to be concerned at sometime in the past, in spite of its intimate topographical relation to the bile canaliculi (Cramer and Ludford, 1926; McIndoe, 1928; Kirkman and Severinghous, 1937; Graffin,

1947; Elias, 1949; Ashworth and Saunders, 1960; Karrer, 1961; Essner and Novikoff, 1962; Reese and Rimington, 1964). Although some studies suggested the presence of vesicles participating in the process of bile secretion (Reese, 1960; Ohkita *et al.*, 1961; Biava, 1964), the exact nature of the vesicles has not been determined.

Recent studies, however, suggested that the vacuolar apparatus are involved in the intracellular transport of bile acid in the hepatocytes (Grogory *et al.*, 1978; Johnes, *et al.*, 1979; Simion *et al.*, 1984a, b; Reuben and Allen, 1986; Marzolo *et al.*, 1990; Alves *et al.*, 1993).

Immunocytochemistry (Lamri *et al.*, 1988) and EM autoradiography (Goldsmith *et al.*, 1983; Suchy *et al.*, 1983) showed that bile acids were transported through the ER and Golgi apparatus before being secreted into the bile canaliculi. Some other studies have suggested that bile acids or salts appeared to have an affinity for ER and Golgi apparatus (Suchy *et al.*, 1983; Erlinger, 1990; Reuben and Allen, 1990; Reynier *et al.*, 1992; Kast *et al.*, 1994; Crawford *et al.*, 1994), and may stimulate the movement of vesicles towards the bile canaliculi (Dubin *et al.*, 1980; Hayakawa *et al.*, 1990; Boyer and Meier, 1990; Crawford *et al.*, 1991; Reynier *et al.*, 1992; LeSage *et al.*, 1993). It has also been suggested that the transport in vesicles from the lysosome (Johnson *et al.*, 1990; Lesage *et al.*, 1993).

The present study was designed to investigate the ER, Golgi apparatus, lysosomes and vesicles to define the site of the organelles, where the vesicles are derived from.

## MATERIALS AND METHODS

Albino rats (Sprague Dawley, male, 250~280 g) were used in the present study. The animals were divided into two groups: normal control and experimental. The experimental group was subdivided into three groups to which 10% dehydrocholic acid (DA, 0.05 mg/g body weight) was treated at 10, 20 and 40 min prior to the sampling. The liver tissues were taken under sodium pentobarbital (Nembutal, 0.015 mg/g body weight) anesthesia at 3 hr after the last feeding. And then liver tissues were taken immediately after laparotomy.

For the thin section, the liver tissues were cut into small pieces and immediately immersed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.3) for 2 hr at room temperature. The tissue pieces were postfixed with 1% osmium tetroxide in the same buffer for 2 hr at 4°C. Some tissue pieces were stained *en bloc* with 0.5% aquaous uranyl acetate for 2 hr at room temperature after postfixation. The fixed tissues were dehydrated in a series of graded ethanol and embedded in Epon mixture.

For the cytochemical study, the perfusion was carried out through the portal vein with ice cold 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.3) under sodium pentobarbital (Nembutal, 0.015 mg/g body weight) anesthesia. The liver tissues were immersed for 1 hr in the same fixative as used in the perfusion. After an overnight rinse in 0.1 M sodium cacodylate buffer containing 10% DMSO and 7.5% sucrose, the frozen tissues were cut on a cryostat (Leika, Jung CM 3000) at 75 μm in thickness and incubated in thiamine pyrophosphatase (TTPase) media for 70 min at 37°C, and incubated in acid phosphatase (APase) for 40 min at 37°C respectively. And then they were postfixed in 1% osmium tetroxide for 1 hr and processed for the electron microscopy. For the prolonged osmification, some of the liver tissues were immersed in

2% aqueous osmium tetroxide and incubated at 4 °C for 48 hr. The osmificated specimens were processed for electron microscopy.

The thin sections were cut on an ultramicrotome (LKB 2088), and stained with uranyl acetate and lead citrate, and were observed by using transmission electron microscope (TEM, Hitachi-H 600).

## RESULTS

Thin sections: Both in normal and experimental groups, the vacuolar apparatus such as ER, Golgi apparatus and lysosomes were observed in the vicinity of bile canaliculi. The cis Golgi cisterns were almost devoid of visible contents, and usually faced toward the bile canaliculi (Figs. 1, 2, 3 and 4). The trans Golgi cisterns showed contents with moderate densities as well as the vesicles located near by (Fig. 1). The vesicles were dense and were not seen orienting in the direction toward the bile canaliculi. The lysosomes in the vicinity of bile canaliculi were round or oval in shape, smooth or slightly irregular in contour, and dense or mottled with electron dense materials. In the normal group, however, the vesicles were difficult to observed near the cis Golgi cistern and bile canaliculi. In the experimental group, the vesicles were usually round, oval or short tubular in shape and were frequently observed near the cis Golgi cisterns, lysosomes and bile canaliculi, especially in the rats, 20 min after the administration of DA (Figs. 1, 2 and 5). The cis Golgi cisterns were sacculated in line.

The saccule occasionally occurd by elongation and attenuated neck (Figs. 1, 2, 3 and 4). The lysosomes and multivesicular bodies were frequently observed in the vicinity of bile canaliculi, and showed protrudent saccule (Fig. 7). The vesicles near the lysosomes and multivesicular bodies were orienting in the direction toward the bile canaliculi. However, the direct fusion between lysosomes and bile canaliculi was not observed.

Cytochemical studies: Both in normal and experimental groups, the osmium deposits were present on the cis Golgi cisterns by the prolonged osmification (Figs. 3 and 4). The cis Golgi cisterns showed osmium deposits and many of them faced toward the bile canaliculi (Figs. 3 and 4). TPPase were apparant on the trans Golgi cisterns which faced toward the opposit side of bile canaliculi (Fig. 2). In the experimental groups, the osmium deposits were heavy on the cis Golgi cisterns, on the protrudent saccule and on the vesicles isolated near by (Figs. 3 and 4). The osmium deposits, however, were light or not observed on the ER or vesicles in the immediate vicinity of bile canaliculi. The vesicles near the lysosomes showed acid phosphatase (APase) activities as well as the lysosomes (Figs. 5 and 6). However, the APase activities were not observed on the vesicles as approaching closer to the bile canaliculi (Figs. 5 and 6).

## DISCUSSION

In the present study, the ER, Golgi apparatus, lysosomes and vesicles were frequently observed in the vicinity of bile canaliculi after the administration of DA. This suggests that the vacuolar apparatus may be involved in the transport of DA in the hepatocytes.

After the administration of DA, the cis Golgi cisterns were sacculated in line. The saccule was occasionally protruded with attenuated neck. It suggests that the vesicles may be derived from the cis Golgi cisterns as specific granu-

les in the neutrophil as reported by Bainton and Farquhar (1966). The vesicles were found near the bile canaliculi, and some of them were fused to them. Sometimes they appeared to be streaming in a row toward the bile canaliculi. The vesicles were almost devoid of visible contents as seen on the cis Golgi cistern, protrudent saccule and bile canaliculi. Similar finding was noted by Stein and Carruthers (1961) in the bile canaliculi. It seems useful to consider the transport process of bile constituents as water secretion and the location of this cellular phase may appear to be watery vacuolation as suggested by Brauer (1959), since most of the bile acids would be secreted in the conjugated form, which is water soluble. Lake *et al.* (1985) using fluid phase markers suggest that water may be released from vesicles into bile. Thus, the finding of the vesicles seems to be in agreement with the biochemical property of primary bile. However, the trans Golgi cisterns were dense as well as vesicles near by, and the vesicles were not orienting in the direction toward the bile canaliculi. This may imply that the vesicles derived from the trans Golgi cisterns are not involved in the transport of bile acids.

In the cytochemical observations, the trans Golgi cisterns exhibited reaction products for TPPase (Goldfischer *et al.*, 1971; Pavelka and Ellinger, 1983; Krstic, 1986) and faced toward the opposit side of bile canaliculi. The cis Golgi cisterns exhibited osmium deposits by the prolonged osmification (Pavelka and Ellinger, 1983). The osmium deposits were also observed on the protrudent saccule of the cis Golgi cisterns and vesicles near by. However, the vesicles did not show osmium deposits in the immediate vicinity of bile canaliculi. This suggests that the vesicles are derived from the cis Golgi cistern and that the properties of vesicles are changed as approaching closer to the bile canaliculi.

After the administration of DA, however, the protrudent saccule was also observed on the lysosome and multivesicular bodies. The vesicles were seen orienting in the direction toward the bile canaliculi. Some of the vesicles appeared to be fused to bile canaliculi, but no direct fusion between lysosomes and bile canaliculi was observed. The protrudent saccule on or vesicles near the lysosomes showed APase activity, but the activity in the vesicles is decreasing as approach to the bile canaliculi. It seems like the activity in the immediate vicinity of bile canaliculi is diminishing as the osmium deposits are diminishing as vesicles approach closer to the bile canaliculi.

Some investigators have reported that ER and Golgi complex have been implicated in the transcellular movement of high bile acid loads (Suchy *et al.*, 1983; Lamri *et al.*, 1988; Crawford *et al.*, 1991). However, the protrudent saccule on the cis Golgi cisterns and fusion between the vesicles and bile canaliculi were encountered in the normal rats, even if they were rarely seen. Thus the transcellular transport via vacuolar apparatus may not be in the high loads of bile acids.

## ABSTRACT

In the present study, the vacuolar apparatus were investigated in the hepatocytes of rats treated with DA by transmission electron microscopy of conventional and cytochemical thin sections.

In the rats after 20 min of dehydrocholic acid treatment, the cis Golgi cisterns were sacculated in line. The saccule occasionally occured by elongation and attenuated neck. The lysosomes also showed protrudent saccule. The vesicles

were observed near the cis Golgi cisterns, lysosome and bile canaliculi. Some of the vesicles appeared to be fused to bile canaliculi. The cis Golgi cisterns usually faced toward the bile canaliculi both in normal and experimental groups. The cis Golgi cisterns, protrudent saccule and vesicles were almost devoid of visible contents. The osmium deposits were heavy on the protrudent saccule as well as on the cis Golgi cisterns or on the vesicles isolated near by, but they were light or not observed on the vesicles in the immediate vicinity of bile canaliculi. The acid phosphatase activities appeared on the lysosome and vesicles located near by, but did not appear on the vesicles as approaching closer to the bile canaliculi.

The evidence suggests that the vesicles are derived from the cis Gogi cistern and lysosomes and fuse to bile canaliculi for exocytosis, and that the activity in the vesicles is diminished as approaching closer to the bile canaliculi.

## ACKNOWLEDGMENT

This study was supported by a grant from the Research Institute of Life Science, Korea University. My appreciation is to Mr. Chang-Hyun Park for his technical assistance.

## REFERENCES

Ashworth CT, Sanders E, 1960. Anatomic pathway of bile formation, Am. J. Path. 37, 343-355

Alves C, Dippe PV, Amoui M, Levy D, 1993. Bile acid transport into hepatocyte smooth endoplasmic reticulum vesicles is mediated by microsomal epoxide hydrolase, a membrane protein exhibiting two distinct cytological orientations, J. Biol. Chem. 268(27), 20148-20155

Bainton DF, Farquhar MG, 1966. Origin of granules in polymorphonuclear leukocytes, J. Cell Biol. 28, 277-301

Biava CG, 1964 Studies on cholestasis. A re-evaluation of the fine structure of normal human bile canaliculi, Lab. Invest. 13, 830-864

Boyer JL, Meier PJ, 1990. Hepatic bile transport; (33) Characterizing Mechanisms of Hepatic Bile Acid Transport Utilizing Isolated Membrane Vesicles, Methods in enzymology, 192, 517-534

Brauer RW, 1952 Mechanisms of bile secretion, J. Am. med. Ass. 169, 1462-1466, 1959

Cramer W, Ludford RJ, 1926. On the cellular mechanism of bile secretion and its relation to the Golgi apparatus of the liver cell, J. Physiol. Lond. 62, 74-80

Crawford JM, Barnes S, Stearns RC, Hastings CL, Godleski J, 1994. Ultrastructural Localization of a Fluorinated Bile Salt in Hepatocytes, Lab. Invest. 71, 42-51

Crawford JM, Gollan JL, 1991. Transcellular transport of organic anions in hepatocytes: still a long way to go, Hepatology 14, 192-197

Crawford JM, Vinter DW, Gollan JL, 1991. Taurocholate induces pericanalicular localization of C6-NBD-ceramide in isolated hepatocyte couplets, Am. Physiol. Soc. G120-132

Dubin M, Maurice M, Feldmann G, Erlinger S, 1980. Influence of colchicine and phalloidin on bile secretion and hepatic ultrastructure in the rat: possible interaction between microtybules and microfilaments, Gastroenterology 79, 646-654

Elias H, 1949. A reexamination of the structure of the mammalian liver: II. The hepatic lobule and its relation to the vascular and biliary systems, Am. J. Anat. 85, 379-456

Erlinger S, 1990. Role of intracellular organelles in the hepatic transport of bile acids, Biomed. Pharmacother. 44, 409-416

Essner E, Novikoff AB, 1962. Cytochemical studies on two functional hepatomas, Interrelations of endoplasmic reticulum, Golgi apparatus and

lysosomes, J. Cell Biol. 15, 289-312

Goldfisher S, Essner E, Schiller B, 1971 Nucleoside diphosphatase and thiamine pyrophosphatase activities in the endoplasmic reticulum and Golgi apparatus, J. Histochem. Cytochem. 19, 349-360

Goldsmith M, Huling AS, Jones AL, 1983. Hepatic handling of bile salts and protein in the rat during intrahepatic cholestasis, Gastroenterology 84, 978-986

Graffin AL, 1947. The excretion of fluorescein by the liver under normal and abnormal conditions, observed in vivo with the fluorescence microscope, Am. J. Anat. 81, 63-116

Gregory DH, Vlahcevic ZR, Prugh MF, Swell L, 1978. Mechanisms of secretion of biliary lipids: role of a microtubular system in hepatocellular transport of biliary lipids in the rat, Gastroenterology 74, 93-100

Hayakawa T, Cheng O, Ma A, Boyer JL, 1990. Taurocholate stimulates transcytotic vesicular pathways labeled by horseradish peroxidase in the isolated, Gastroenterology 99, 216-228

Jones AL, Schmucker PL, Mooney JS, 1979 Alterations in hepatic pericanalicular cytoplasm during enhanced bile secretory activity, Lab. Invest. 40, 512-517

Johnson WJ, Chacko GK, Phillips MC, Rothblat GH, 1990. The Efflux of Lysosomal Cholesterol from Cells, J. Biol. Chem. 265, 5546-5553

Karrer HE, 1961 Electron microscope observations on chick embryo liver glycogen, bile canaliculi, inclusion bodies and hematopoiesis J. Ultrastruct. Res. 5, 116-141

Kast C, Stieger B, Winterhalter KH, Meier PJ, 1994. Hepatocellular Transport of Bile Acids, J. Biol. Chem. 269, 5179-5186

Kirkman H, Severinghous AE, 1937. A review of the Golgi apparatus, Anat. Rec. 70, 413-431

Krstic R, 1986. Ultracytochemical evidence for the presence of GERL in pinealocytes of the Mongolian gerbil (Merions unguiculatus), Cell Tissue Res. 246, 583-588

Marzolo MP, Rigotti A, Nervi F, 1990. Secretion of Biliary Lipids from the Hepatocyte, Hepatology 12, 134S-142S

Lake JR, Licko V, Van Dyke RW, Scharschmidt BF, 1985. Biliary secretion of fluid-phase markersby the isolated perfused rat liver, J. Clin. Invest. 76, 676-684

Lamri Y, Roda A, Dumont M, Feldmann G, Erlinger S, 1988. Immunoperoxidase localization of bile salts in rat liver cells, J. Clin. Invest. 82, 1173-1182

LeSage GD, Robertson WE, Baumgart MA, 1993. Bile acid-dependent vesicular transport of lysosomal enzymes into bile in the rat, Gastroenterology 105, 889-900

McIndoe AH, 1928. The structure and arrangement of the bile canaliculi, Arch. Path. 6, 598-614

Ohkita H, Iwasaki R, Takabayashi, H, Kondo S, Nango M, Shinji S, Donomae T, Nakajima I, Tatsumi S and Kitani T, 1961. Morphological studies on bile secretion, Med. J. Osaka Univ. 12, 75-93

Pavelka M, Ellinger A, 1983. Effect of colchicine on the Golgi complex of rat pancreatic acinar cells, J. Cell Biol. 97, 737-748

Reese AJM, 1960. The effect of hypoxia on liver secretion study by intrvital fluorescence microscopy, Br. J. exp. Path. 41, 527-535

Reese AJM, Rimington C, 1964. Biliary and urinary excretion of porphyrins in the rat studied by intravital fluorescence microscopy, Br. J. exp. Path. 45, 30-36

Reuben A, Allen RM, 1986. Intrahepatic sources of biliary-like micelles, Biochim. Biophys. Acta, 876, 1-12

Reuben A, Allen RM, 1990. Taurocholate Transport by rat liver Golgi vesicles (Abstract), Gastroenterology 98, A624

Reynier MO, Hashieh IA, Crotte C, Carbuccia N, Richard B, Gerolami A, 1992. Monensin action on the Golgi complex in perfused rat liver : Evidence against bile salt vesicular transport,

Gastroenterology 102, 2024-2032

Stein JW, Carruther JS, 1961. Studies on the fine structure of the biliary tree, Am. J. Pathol. 38, 639-661

Suchy FJ, Balistieri WF, Hung J, Miller P, Garfield SA, 1983 Intracellular bile acid transport in rat liver as visualized by electron microscope autoradiography using a bile acid analogue, Am. J. Physiol. 245, G681-G689

## FIGURE LEGENDS

**Fig. 1.** Portions of hepatocytes from a rat, 20 min after administration of dehydrocholic acid. The Golgi apparatus (G) is located in the vicinity of bile canaliculus (BC). The cis Golgi cistern (double arrows) is sacculated and faces toward the bile canaliculus. Vesicles (arrow) are seen near the bile canaliculus. Vesicles (small arrow) near the trans Golgi cistern show moderate electron densities. L: lysosomes. Bar=0.5 μm

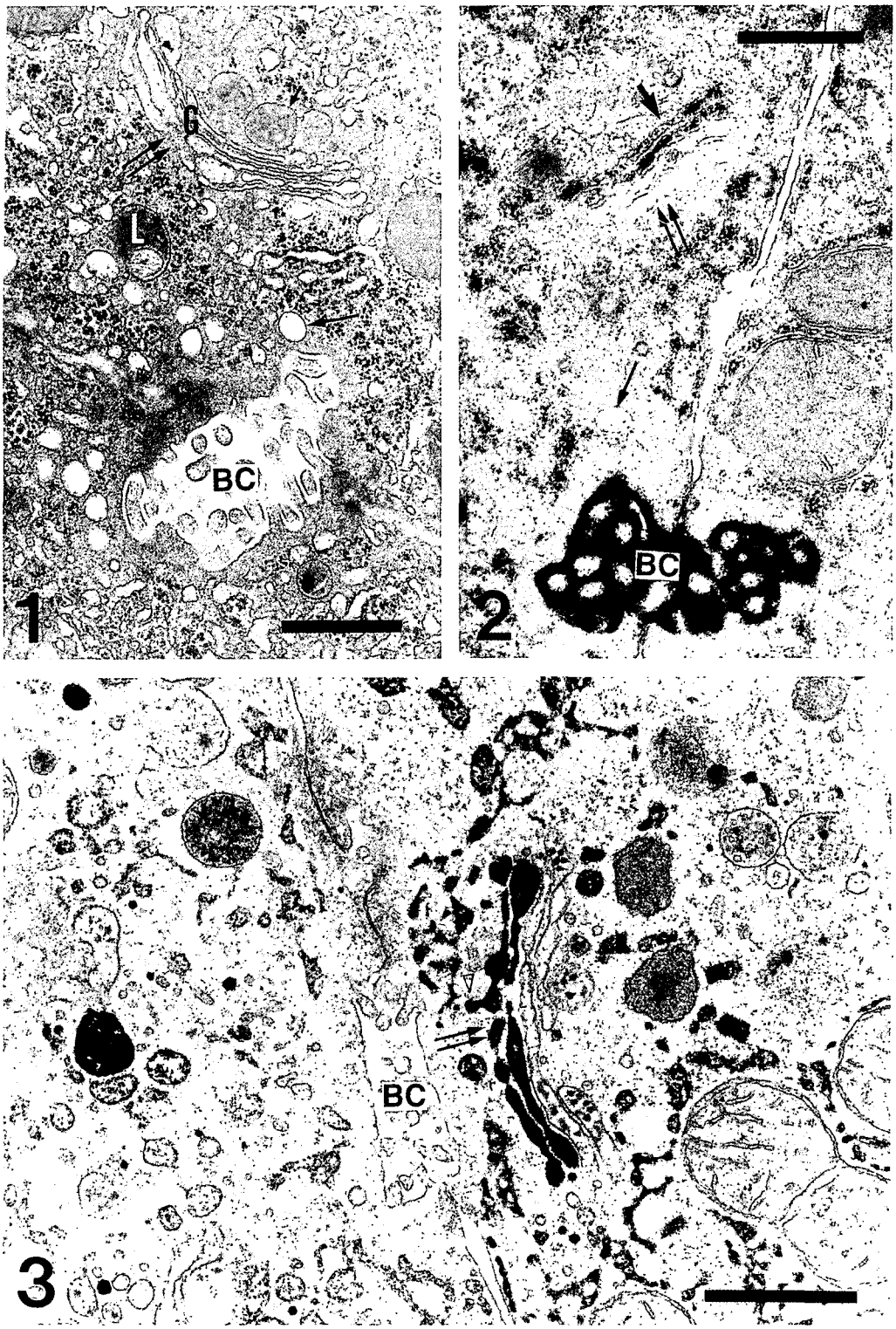
**Fig. 2.** Portions of hepatocytes from a rat, 20 min after administration of dehydrocholic acid. The reaction product of thiamine pyrophosphatase is apparant on the trans Golgi cisterns (thick arrow) which faces toward the opposit side of bile canaliculi (BC). The cis Golgi cistern (double arrows) is sacculated. A vesicle (arrow) is seen near the bile canaliculus. Bar=0.5 μm

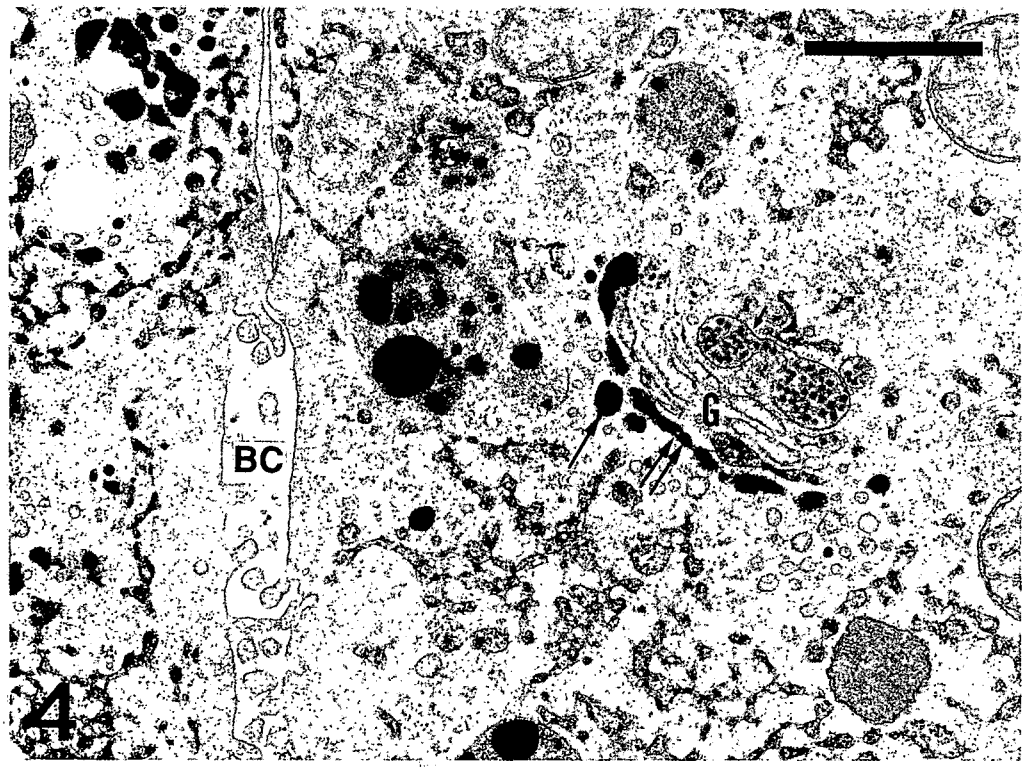
**Fig. 3.** Portions of hepatocytes from a rat, 20 min after administarion of dehydrocholic acid. The osmium deposit is seen on the cis Golgi cistern (double arrows) and bud (vacant arrowhead). The bud occurs by elongation and attenuated neck, which is connected to the cis Golgi cisterns. BC: Bile canaliculus. Bar=1 μm

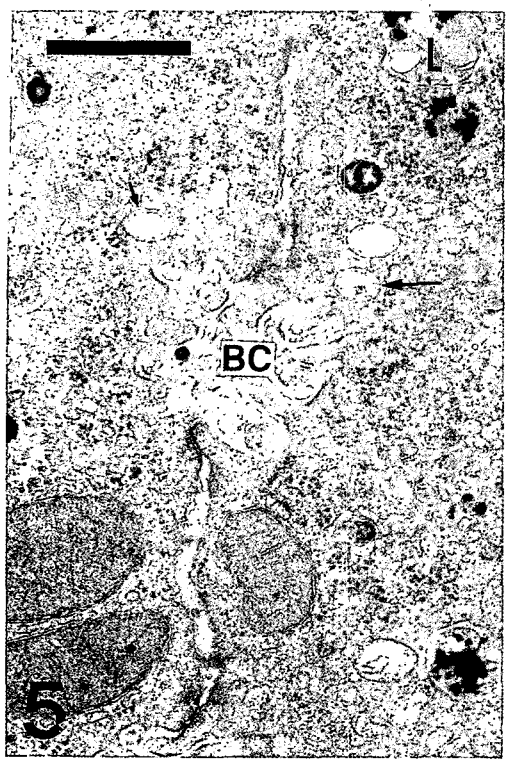
**Fig. 4.** Portions of hepatocytes from a rat, 20 min after administartion of dehydrocholic acid. The Golgi apparatus (G) is located in the vicinity of bile canaliculus (BC). Vesicles (small arrow) are seen near the trans Golgi cistern. The osmium deposits are heavy on the cis Golgi cistern (double arrows) and vesicles (arrow) isolated near by. Bar=1 μm

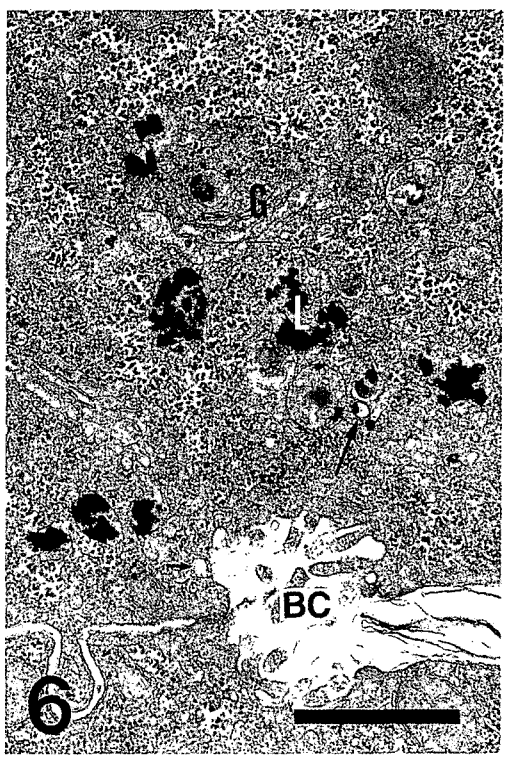
**Fig. 5.** Portions of hepatocytes from a rat, 20 min after administarion of dehydrocholic acid. Lysosomes (L) show acid phosphatase reaction product. Vesicles (arrow) showing acid phosphatase activities are seen near the bile canaliculus (BC). Vesicles (small arrow) devoid of acid phosphatase reaction products are also seen. Bar=0.5 μm

**Fig. 6.** Portions of hepatocytes from a rat, 20 min after administarion of dehydrocholic acid. Lysosomes (L) and vesicle (arrow) show acid phosphatase reactions. A vesicle (small arrow) is localized at the porobable site where it will be fused to bile canaliculus (BC). Bar=1 μm


G
L
BC
1
BC
2
BC
3


BC
G
4


L
BC
5


G
L
BC
6