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# Morphological Evidence for the Transport of Dehydrocholic Acid in the Hepatocyte as Revealed by Freeze Fracture Replica

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# 급속동결할단법에 의한 간세포내 Dehydrocholic Acid 수송에 관한 형태학적 관찰

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## 요 약

본 연구에서는 박절편과 동결할단복제법을 이용하여 흰쥐 간세포에서 dehydrocholic acid가 수송되는 경로를 전자현미경적으로 조사하고자 하였다. 정상군이나 dehydrocholic acid 투여군에서 대부분의 Golgi 장치는 형성면을 담세관으로 향하고 있었다. Dehydrocholic acid 투여 20분 후에 세포질내세망과 Golgi 장치 및 소포 등이 담세관 주위에 증가되어 있었는데 특히 Golgi 장치 형성면에서는 소포가 될 것으로 추정되는 싹이 돌출되어 있었으며 소포들은 담세관에 융합된 것들도 관찰되었다.

이러한 소견으로 미루어 담즙산의 분비는 Golgi 장치 형성면의 싹이 유리되어 형성된 소포가 담세관막에 융합되므로서 이루어질 것으로 추정된다.

**Key words** : Golgi apparatus, Endoplasmic reticulum, Vesicle, Hepatocyte, Liver, Taurocholic acid

## INTRODUCTION

It is generally known that the mechanisms of intracellular transport of bile components include aqueous diffusion, transport via a soluble carrier protein (Sugiyama *et al.*, 1983; Stolz *et al.*, 1986; Henderson *et al.*, 1986), and a transport mediated with vesicles (Kitamura *et al.*, 1990; Crawford and Gollan, 1991; Berr *et al.*, 1993).

Previous studies have shown that bile salts localize to the Golgi apparatus under physiological conditions (Lamri *et al.*, 1988), while it has been described that most intracellular bile acids are bound to specific cytosolic proteins at physiologic concentrations (Stolz *et al.*, 1989; Erlinger, 1985). Some others have suggested 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). Recent studies, however, have suggested that bile acids or salts appeared to have an affinity for ER and Golgi apparatus (Reuben and Allen, 1990; Kast *et al.*, 1994; Suchy *et al.*, 1983; Reynier *et al.*, 1992; Crawford *et al.*, 1994; Erlinger, 1990; Jones *et al.*, 1979; Simion *et al.*, 1984; Simion *et al.*, 1984), and that they may stimulate the movement of vesicles towards the bile canaliculi (LeSage *et al.*, 1993; Reynier *et al.*, 1992; Dubin *et al.*, 1980; Boyer and Meier, 1990). It may be supported by the fact that the Golgi apparatus has a major role in vesicular secretory processes (Griffiths and Simons, 1986) and is particularly abundant in the pericanalicular area (Taatjes and Roth, 1986), and that ER is the site, in which the synthesis of bile acids occurs in the hepatocytes (Johnson *et al.*, 1990). In addition, most of the bile constituents may be transported by the bounded membrane since bile is soluble in water and the cell membrane does not permit the penetration to the materials soluble in water or macromolecules. It may be the key, therefore, to define the mode of transport by vacuolar apparatus in the elucidation of the mechanism of bile secretion in the hepatocyte.

The present study was designed to investigate intracellular pathways of vesicles with their origin and destination.

## 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 and experimental. The experimental group was subdivided into three groups to which dehydrocholic acid (0.003 mg/g body weight) was injected 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. The liver tissues were taken immediately after laparotomy.

For thin sections, 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. The thin sections were cut on an ultramicrotome (LKB 2088), and stained with uranyl actate and lead citrate.

For freeze fracture replicas, the liver tissues were cut into small pieces and immediately immersed in the same fixative used for the thin sections for 2 hr at 4°C. The fixed tissues were equilibrated with 35% glycerol dissolved in the same buffer for 6 hr at 4°C. The tissues were quickly frozen in liquid nitrogen. The frozen tissues were brought into the freeze etching device (Eiko Engineering Co., FD-3), cleaved at −120°C and shadowed on the fractured surfaces of the tissue immediately with platinum-pladium followed by carbon coating at a vaccum of $10^{-10}$ torr in a freeze etching device. The replicas were detached from the tissues in the detergent and rinsed three times in distilled water.

All the specimens were observed by using transmission electron microscope (TEM, Hitachi H-600).

## RESULTS

Thin sections: In the normal group, vacuolar

apparatus such as ER and Golgi apparatus were observed in the vicinity of bile canaliculi. The Golgi apparatus usually showed cis sides facing toward the bile canaliculi. In the experimental groups, the vacuolar apparatus including vesicles appeared to be increased in the immediate vicinity of bile canaliculi, especially in the rats, after 20 min of dehydrocholic acid treatment. Many of cis Golgi cisterns faced toward the bile canaliculi and showed linearly arranged saccules or buds that assumed to be vesicles (Fig. 1). The vesicles were usually round, oval or short tubular in shape. The cis Golgi cisterns and vesicles were almost devoid of visible contents, Vesicles could be seen in the immediate vicinity of bile canaliculi and were fused to them (Fig. 3).

Freeze fracture replicas: In the normal group, the ER and Golgi apparatus were located in the vicinity of bile canaliculi. The cis Golgi cisterns usually appeared to face toward the bile canaliculi. In the experimental groups, however, especially rats, after 20 min of dehydrocholic acid treatment, the membranous organelles including vesicles were frequently observed. The Golgi apparatus were usually located near the bile canaliculi and their cis sides faced toward the bile canaliculi. The buds with narrow neck were present on the cis Golgi cisterns (Fig. 2). The vesicles were increased in the vicinity of bile canalicului. The fusion between vesicles and luminal plasma membrane of bile canaliculi were frequently observed (Figs. 4, 5).

## DISCUSSION

Since an electron microscopy showed increase of the Golgi apparatus after administration of bile acid (Johnes, *et al.*, 1979), it has been reported that the vesicular traffic from the Golgi apparatus to the pericanalicular region is enhanced by bile acids (Hayakawa *et al.*, 1990; Crawford *et al.*, 1991). Some have shown that bile salts were transported to the bile canaliculi by a vesicular pathway, possibly in part via the Golgi apparatus (Reynier *et al.*, 1992), some another have suggested that the taurocholate is transported in the ER (Reuben and Allen, 1986; Alves *et al.*, 1993; Kast *et al.*, 1994). Marzolo *et al.* (1990) have described that vesicular material could be recruited by a fraction of bile acids fluxing through the SER, in which bile acids are synthesized and transported to the Golgi apparatus. Immunocytochemistry (Lamri *et al.*, 1988) and EM autoradiography (Goldsmith *et al.*, 1983; Suchy *et al.*, 1983; Reynier *et al.*, 1992) showed that bile acids were transported through the ER and Golgi apparatus before secretion into the bile canaliculi. From the previous studies, it is assumed that the ER and Golgi apparatus are important participants in the intracellular transport of bile acids in the hepatocytes. However, it may be dificult to identify whether the cisterns belong to ER or Golgi apparatus and what cisterns the vesicles originate from, if ER is present in the Golgi apparatus as an integral part as described by Morre *et al.* (1974).

In the present study, the ER, Golgi apparatus, lysosomes and vesicles appeared to be increased in the vicinity of bile canaliculi after dehydrocholic acid treatments. This also suggests that the vacuolar apparatus are involved in the intracellular transport of dehydrocholic acids in the hepatocytes as described by previous investigators (Johnes, *et al.*, 1979; Marzolo *et al.*, 1990; Hayakawa *et al.*, 1990; Crawford *et al.*, 1991). It may need to detect, however, the site of the organelle, where the vesicles are originate from. Both in normal and experiemntal groups, the cis Golgi cisterns usually faced

toward the bile canaliculi as described by Shin (1978), Shin and Yamada (1986), and Shin (1988). The origin and fusion of vesicles are easily identified in the rats treated with dehydrocholic acid. The vesicles were frequently budding off from the cis Golgi cisterns, and they were particularly well shown in the freeze fracture replica. The buds were connected to the cis Golgi cisterns with narrow neck. This suggests that the buds on the cis Golgi cisterns were pinching off rather than fusing. The evidence led to the assumption that the buds separate from the cis Golgi cisterns to be vesicles, which release into the bile canaliculi after fusion with their luminal plasma membranes. Since many of the cis Golgi cisterns showed linear saccular fashions or buds, and vesicles were orienting in the direction toward the bile canaliculi and some of them fused to the bile canaliculi, the vesicles may be derived from the cis Golgi cisterns in association with bile formation as specific granules derived from the cis Golgi cisterns in the neutrophil as reported by Bainton and Farquhar (1966). Grogory *et al.* (1978) suggested that the bile acids have high-affinity binding sites in the SER and Golgi apparatus. However, since the buds on the cis Golgi cisterns and the vesicles fused to bile canaliculi were encountered even in the normal rats, the trancellular transport via vesicles would not be the mechanism for high loads of bile acids.

## ABSTRACT

The pathway of intracellular transport of dehydrocholic acid was investigated in the hepatocytes of rats by transmission electron microscopy with conventional and freeze fracture methods.

Both in normal and experimental groups, the cis Golgi cisterns were sacculated and faces toward the bile canaliculus. In the experimental group, however, the cis Golgi cisterns showed buds, which were probably separated to be vesicles. Some of the buds were connected to the cisterns with the narrow neck. The vesicles were increased in the vicinity of bile canaliculi. The fusion between vesicles and bile canaliculus were frequently observed in the experimental group. This was particularly well shown in the freeze fracture replica. In the thin section, the vesicles were devoid of visible contents as seen in the bile canaliculli.

The evidence suggests that the vesicles are derived from the cis Gogi cistern in the way that buds pinch off, serve as vehicles to transport dehydrocholic acids and fuse to bile canaliculi for exocytosis.

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## FIGURE LEGENDS

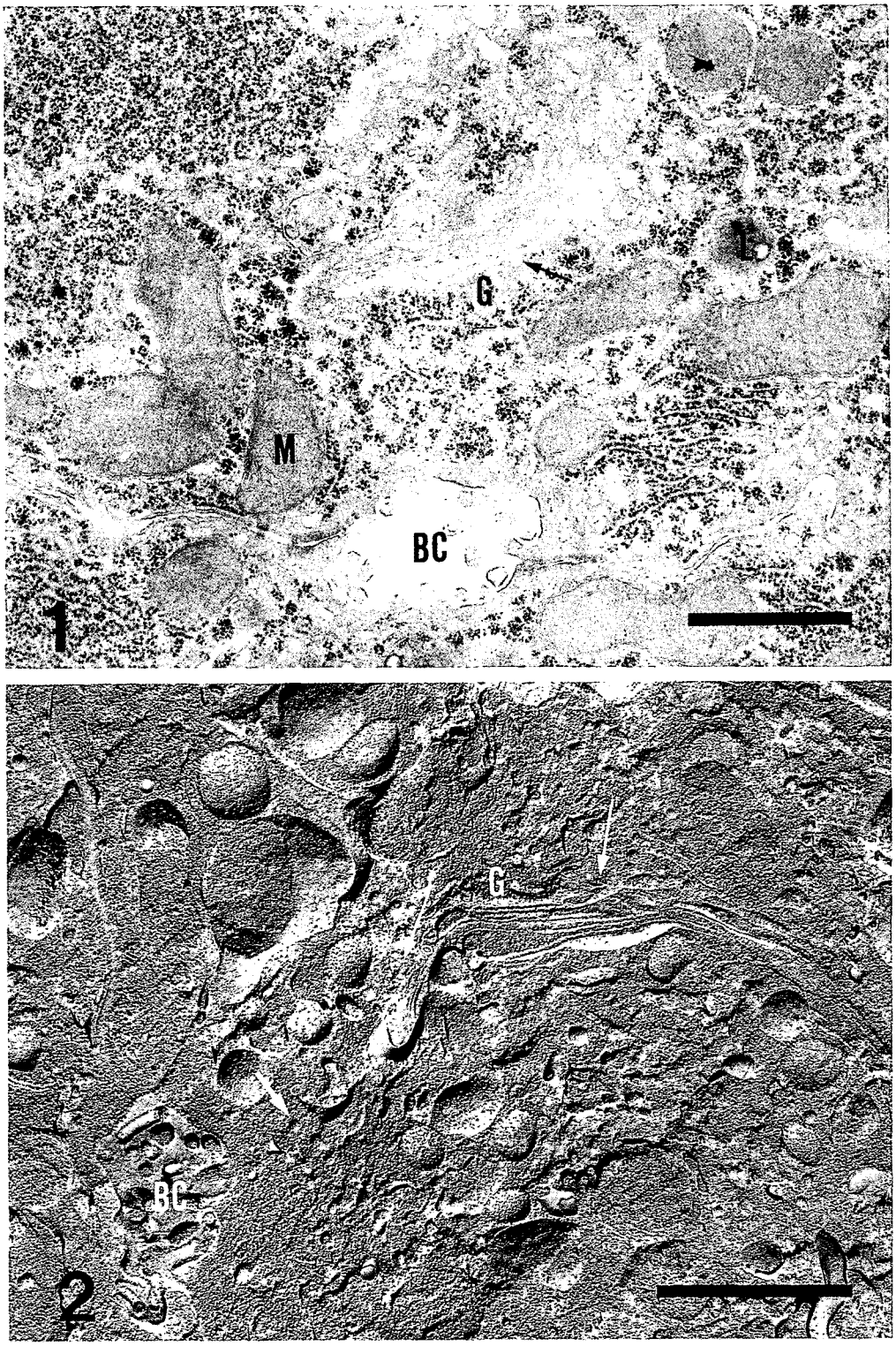
**Fig. 1.** Portions of the hepatocyte from a rat after 20 min of dehydrocholic acid treatment. The cis Golgi cistern (G) shows linearly arranged saccules or bud (arrowhead), and faces toward the bile canaliculus (BC). M: mitochondria, L: lysosome. Ba=1 μm

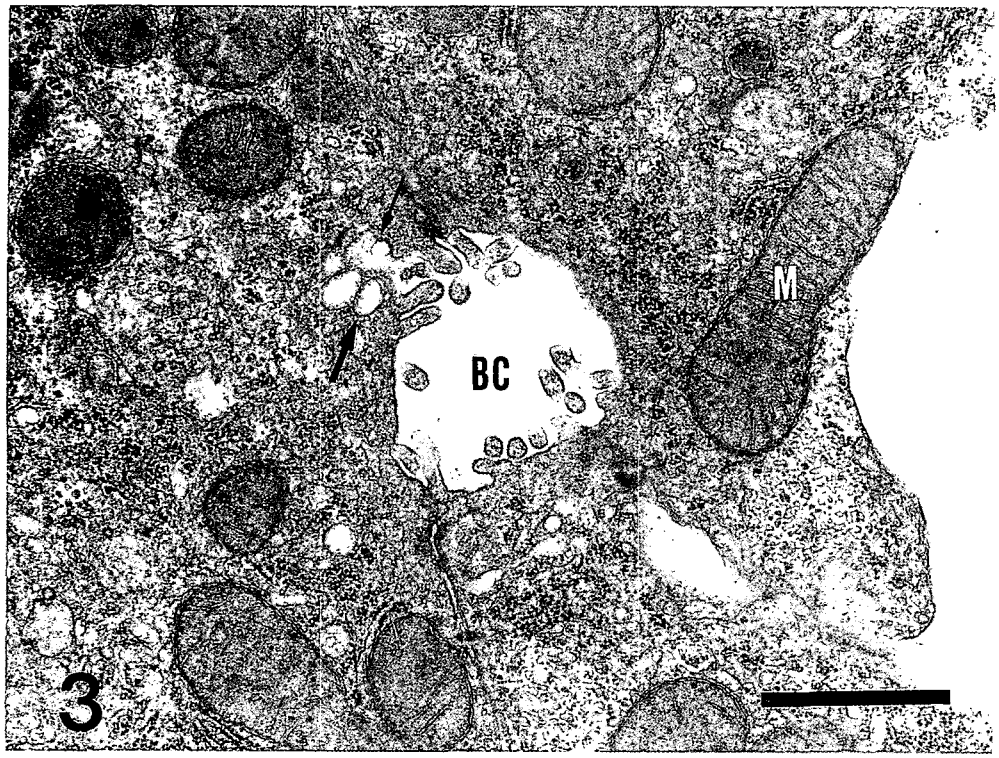
**Fig. 2.** Freeze fracture replica of hepatocytes from a rat after 20 min of dehydrocholic acid treatment. The vesicle (thick arrow) is seen in the vicinity of bile canaliculus (BC). The buds (arrow) are still attached to the cis Golgi cistern (G). Bar=1 μm

**Fig. 3.** Portions of the hepatocyte from a rat after 20 min of dehydrocholic acid treatment. Two vesicles (thick arrow) are seen in the immediate vicinity of bile canaliculus (BC), and a elongated vesicle (arrow) is fused to it. M: mitochondria. Bar=1 μm

**Fig. 4.** Freeze fracture replica of hepatocytes from a rat after 20 min of dehydrocholic acid treatment. A vesicle (arrow) is fused to the bile canaliculus (BC). Vesicles (thick arrow) are seen in the vicinity of bile canaliculus. Bar=0.5 μm

**Fig. 5.** Freeze fracture replica of hepatocytes from a rat after 40 min of dehydrocholic acid treatment. Two elongated vesicle (arrow) are fused to the bile canaliculus (BC). One of the vesicle (arrow) shows dilated end. Bar=0.5 μm


G
M
BC
1
G
BC
2


BC
M
3


BC
4


BC
5