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Some Observations on the Fine Structure of Vacuolar Apparatus Affected by Dehydrocholic acid, Cholesterol and Phosphatidylcholine

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담즙산과 cholesterol 및 phosphatidylcholine이 vacuolar apparatus에 미치는 영향에 관한 미세구조적 관찰

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요 약

본 연구에서는 담즙산과 cholesterol 및 phospholipid가 흰쥐 간세포내 소기관에 미치는 영향을 조사하여 이들 담즙물의 수송기전을 알아 보고져 하였다.

정상군과 각 실험군에서 관찰된 Golgi장치는 거의가 cis side를 담세관으로 향하고 있었다.

각 실험군에서는 ER과 Golgi 장치 및 용해소체 등 소기관의 수적 증가, 소조의 팽대, budding 등이 관찰되었다.

Dehydrocholic acid 투여군에서는 cis Golgi cistern의 선상 팽대을 많이 볼 수 있었는데 공포(vacuole)들은 담세관과 Golgi 장치사이에서 관찰되었으며 ER과 담세관 사이에서도 관찰할 수 있었다.

Cholesterol 투여군과 Phosphotidylcholine 투여군에는 Golgi 장치의 모든 소조들이 팽대되어 있었으며 ER 유래의 공포와 용헤소체가 증가되어 있었는데 용해소체의 증가는 특히 cholesterol 투여군에서 현저하였다.

이상의 소견으로 미루어 담즙성분의 분비에 관여하는 주된 세포내 소기관은 ER과 Golgi 장치 및 용해소체로 추정된다. 그러나 dehydrocholic acid는 ER과 cis Golgi 소조 유래의 공포에 의해 분비되며 Cholesterol과 phosphatidylcholine은 ER에서 또는 trans Golgi 소조을 거친 후 용해소체를 통해서 배출될 것으로 추정되는데 특히 cholesterol은 용해소체를 통해서 빈번히 배출될 것으로 생각된다.

Key words : Golgi apparatus, Endoplasmic reticulum, Lysosome, Vacuole, Dehydrocholic acid, Cholesterol, Phosphatidylcholine

Introduction

Bile salts, phospholipids, cholesterols are produced in the hepatocytes and secreted into the bile canaliculi (Crawford *et al*., 1991; Crawford *et al*., 1994).

The mechanisms involved in the secretion of

primary organic solutes in bile may include aqueous diffusion, vacuole mediated transport, and transport via a soluble carrier protein or lipid (Liscum and Dahl, 1992). However, the morphological results provide no evidence either for the first two possibilities. A fundamental question is how the organic solutes affect the vacuolar apparatus such as endoplasmic reticulum (ER), Golgi apparatus, lysosomes and vacuoles and where they pass through in the hepatocytes.

The actual origin, pathway and fusion of vacuoles with the canalicular membrane may be observed in the morphological study, since the hepatocellular structure is maintained, with retention of the organelles in its normal pericanalicular location by electron microscopy.

In recent years, considerable progress has been made in the elucidation of the mechanisms of hepatocellular transport of bile constituents in association with vacuolar apparatus even in the field of biochemistry (Jones *et al.*, 1979; Dubin *et al.*, 1980; Suchy *et al.*, 1983; Simion *et al.*, 1984; Reuben and Allen, 1990; Erlinger, 1990; Boyer and Meier, 1990; Reynier *et al.*, 1992; LeSage *et al.*, 1993; Kast *et al.*, 1994; Crawford *et al.*, 1994). The constellation of vacuoles between the bile canaliculi and vacuolar apparatus may represent a pathway for the transcellular transport of bile constituents and multiple pathways within the vacuolar apparatus may exist (LeSage *et al.*, 1993). However, the mechanisms of transcellular transport of them have mainly been supported by biochemical analysis, but less well understood in the morphological aspect.

The purpose of the present study was to investigate the influence of dehydrocholic acid, phosphatidylcholine and cholesterol to the vacuolar apparatus to better understand the intracellular pathways involved in bile formation.

Materials and Methods

Albino rats (Wistar, male, 250~280 g) were used for this study. The animals were divided into two groups: normal and experimental. The experimental group was subdivided into three groups; dehydrocholic acid, phosphatidylcholine and cholesterol.

In the dehydrocholic acid group, the liver tissues were taken at 10, 20 and 40 min following the administration of dehydrocholic acid (0.05 mg/g bw).

In the phophatidylcholine group, the liver tissues were taken at 10, 20 and 30 min following the administration of phosphatidylcholine (0.25 mg/g bw).

In the cholesterol group, the liver tissues were taken at 20, 40 and 80 min following the administration of cholesterol (0.2 mg/g bw).

Liver tissues of all the animals were taken under the sodium pentobarbital (Nembutal, 0.015 mg/g body weight) anesthesia at 3 hr after the last feeding. The liver tissue was taken immediately after the 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. All the tissue pieces were postfixed with 1% osmium tetroxide in the same buffer for 2 hr at 4°C. The fixed tissues were dehydrated in a series of graded ethanol and embedded in Epon mixture. They were cut on an ultramicrotome (LKB 2088). The thin sections were stained with uranyl actate and lead citrate, and observed by using the transmission electron microscope (Hitachi H-600).

Results

Normal group: Bile canaliculi appeared as spaces between adjacent hepatocytes sealed by zonulae occludentes and were provided with microvilli. The pericanalicular cytoplasms showed densely packed actin filaments. The actin filaments around the bile canaliculi extended into the microvilli to form a core of filaments.

The vacuolar apparatus such as ER, Golgi apparatus and lysosomes were located in the vicinity of bile canaliculi. The cisterns of ER were vesicular or tubular and contained fibrillar materials of low electron densities. Most of the Golgi apparatus showed cis cisterns facing toward the bile canaliculi. The contents within the Golgi cisterns were increased in density from the cis side to the trans side. Vacuoles were difficult to observe around the bile canaliculi, but lysosomes could be seen around the bile canaliculi.

Experimental groups: Bile canaliculi were well preserved with their auxiliary structures such as zonulae occludentes and microvilli. The actin filaments were also observed around the bile canaliculi as seen in the normal group.

Dehydrocholic acid group: The vacuolar apparatus were predominant around the bile canaliculi and some of them were fused to the luminal plasma membrane of bile canaliculi.

The findings were most extensive in the rats injected 20 min prior to the sampling. Thus it was concentrated on those affected by dehydrocholic acid.

The vesicular cisterns of ER were almost devoid of visible contents or contained only a few materials of low electron density, which appeared usually to lie on the inner surface of their limiting membranes.

Some of the vacuoles were seen between the ER and bile canaliculi. The Golgi apparatus were more frequently observed in the vicinity of bile canaliculi. Most of the Golgi apparatus showed the cis side facing toward the bile canaliculi (Figs. 2 and 3) as seen in the normal group. The cis Golgi cisterns were lacking in the visible contents and showed linear saccular fashions or small buds. The vacuoles, probably originating through a process of pinching off from the cis Golgi cistern. The vacuoles were also found between the Golgi apparatus and bile canaliculi and some of them fused to the canalicular membrane.

The trans Golgi cisterns and vacuoles showed contents of moderate electron densities. The vacuoles derived from the trans Golgi cisterns were not be seen orienting in the direction toward the bile canaliculi. The vacuoles seen in the vicinity of the trans side also showed moderate or high electron densities.

The lysosomes were usually round or oval and smooth or slightly irregular in contours. They were dense or less dense and homogeneous or mottled with materials showing various densities. They showed bud like protrusions or segments, which transformed to be vacuoles. However, the direct fusion between the lysosome and bile canaliculi were not observed (Figs. 2 and 3).

Cholesterol group: The findings were most extensive in the rats injected 30 min prior to the sampling. Thus it was concentrated on those affected by cholesterol at 30 min after the administration. Bile canaliculi were not dilated, but seemed to be filled with invisible contents as seen in the phosphatidylcholine group. ER and showed dilated cisterns, which were vesicular or short tubular. The cisterns contained fibrillar or relatively particulated materials of low electron

densities. These dilated cisterns appaered to occasionally be connected with not dilated RER, which were filled with fibrillar materials of low electron densities. Vacuoles were frequently seen in the immediate vicinity of bile canaliculi and were almost devoid of visible contents, or contained cloudy materials of low electron densities, which appeared to lie on the inner surface of the limiting membranes. The vacuoles seemed to be derived from the SER. Some of vacuoles were fused to the bile canaliculi.

The Golgi apparatus faced toward the bile canaliculi as seen in the normal and dehydrocholic acid groups. The contents within the Golgi cisterns increased in electron densities from the cis side to the trans side. The individual cisterns were distended, but the sacculation of cis Golgi cisterns were occasionally observed in the vinicity of bile canaliculi.

The lysosome appeared to be increased in the vicinity of bile canaliculi. They were usually round or oval and smooth or slightly irregular in contours. Some of them cotained homogeneous materials of low electron densities. Others were mottled with materials showing various degrees of electron densities. They were larger in size and number. However, the direct fusion between the lysosome and canalicular membrane was not observed (Figs. 4, 5 and 6).

Phosphotidylcholine group: The findings were most extensive in the rats injected 30 min prior to the sampling. Thus it was concentrated on those affected by phosphatidylcholine.

Bile canaliculi were not dilated, but seemed to be filled with invisible contents. The vesicular cisterns of ER, Golgi apparatus, lysosomes and vacuoles could be observed in the immediate vicinity of bile canaliculi. However, they were less than those in the cholesterol group.

The ER cisterns were dilated and showed vesicular or short tubular in appearances. They were almost devoid of visible contents or contained only a few materials of low electron density, which might appear to lie on the inner surface of their limiting membranes.

Vacuoles were seen in the immediate vicinity of bile canaliculi. They were almost devoid of visible contents, but contained only a few cloudy materials of low electron densities.

The vacuoles seemed to be derived from the SER and some of them were fused to the bile canaliculi.

The Golgi apparatus showed cis cisterns facing toward the bile canaliculi as seen in the normal and dehydrocholic acid groups. The individual cisterns of Golgi apparatus were distended, but sacculation of cis Golgi cisterns were dificult to observe. The Golgi cisterns showed contents of moderate electron densities, although they were more dense in the trans side.

The lysosomes appeared to be less increased in size and number in comparison with those seen in the cholesterol group. The direct fusion between the lysosome and canalicular membrane was not observed (Figs. 7 and 8).

Discussion

The major findings of the present study relate to the influence of bile acid, phospholipid and cholesterol to the fine structures of ER, Golgi apparatus and lysosomes with vacuoles and their fusion with the bile canaliculi. These primary solutes in bile are metabolically related with one another. Bile acids are major products of cholesterol metabolism (Boyer and Meier, 1990), and stimulate the biliary excretion of phospholipids and cholesterol (Schersten *et al.*, 1971; Reuben and Allen, 1986; Crawford, *et al.*, 1988; Marzolo, 1990; Crawford *et al.*, 1991; Cohen *et*

al., 1994).

The intracellular transport of bile acids in the hepatocytes may involve several pathways (Reynier *et al.*, 1992). The previous studies reported that the Golgi apparatus appeared to have a particular affinity for bile salts (Reuben *et al.*, 1990; Simion *et al.*, 1984; Simion *et al.*, 1984; Schubert *et al.*, 1984). The presence of radioactive bile acids has been demonstrated in the Golgi apparatus by EM autoradiography (Goldsmith *et al.*, 1983; Suchy *et al.*, 1983; Reynier *et al.*, 1992). Gregory *et al.*(1978) suggested, however, that the bile acids have high-affinity binding sites in the SER and Golgi apparatus. It has been demonstrated by immunocytochemistry (Lamri *et al.*, 1988) and EM autoradiography (Suchy *et al.*, 1983) that bile acids are transported through SER and Golgi apparatus before secretion into the bile. It has been suggested that the vacuolar structures increased in the pericanalicular region after stimulated bile acid secretion (Jones *et al.*, 1979; Hayakawa *et al.*, 1990; Crawford *et al.*, 1991; Reynier *et al.*, 1992), and that bile acids are apparently transported in vesicles (Dubin *et al.*, 1980) and modulate movement of intracellular vesicles towards the canaliculi (LeSage *et al.*, 1993).

From the above studies, it is assumed that the ER and/or Golgi apparatus involve in the intracellular transport of bile acids. This may be reasonable, since 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 ER is the site, in which the synthesis of bile acids occurs in the hepatocytes (Johnson *et al.*, 1990). Although the previous investigators have not described on the presence of vacuoles derived from the cis Golgi cisterns, it needs to define the site where the vacuoles are derived from, if the bile acids are transported via the vacuoles to the bile canaliculi.

In the present study, the Golgi apparatus appeared to be predominant in the vicinity of bile canaliculi following the administration of dehydrocholic acid, although ER and lysosomes were also increased. The vacuoles were frequently buding off from the cis Golgi cisterns facing toward the bile canaliculi and fused to the bile canaliculi, but less frequently from the ER. This suggests that if the cis Golgi cistern plays a main role in the secretion of dehydrocholic acid. This observation was different from the previous studies suggesting that the intracellular transport of bile acids proceeds via the trans Golgi cisterns. The vacuoles in the vicinity of the trans side were different from those near the cis Golgi cisterns in appearance, and the vacuoles derived from the trans Golgi cisterns could not be seen orienting in the direction toward the bile canaliculi. These types of vacuoles derived from trans and cis Golgi cisterns can readily be identified by their characteristic features, accompanied by their origin, distribution and appearance. Vacuoles were also budding off from the ER cisterns in the vicinity of bile canaliculi, even if they were less frequent in comparison with those of Golgi apparatus. The vacuoles derived from the ER cisterns may contain lipids stimulated by dehydrocholic acids.

The lysosomes appeared to slightly be increased around the bile canaliculi in the dehydrocholic acid group. They showed bud like protrusions or segments, which may be transformed to be vacuoles. This may be the feature showing pathway of some dehydrocholic acid passed the lysosomes via the trans Golgi cisterns, since the bile acid modulate the movement of lysosomes toward the bile canaliculi (LeSage *et al.*, 1993).

It has also been studied on the lipids in associ-

ation with translocation in the hepatocytes. Crawford *et al.*(1991) have described that the transport pathways of lipid involve the Golgi apparatus. Liscum (1990) and Bretscher and Munro (1993) have described that the Golgi may play a role in the transport of exogenously derived cholesterol. However, other investigations are in contradiction to the above studies suggesting that the intracellular lipid transport proceeds via Golgi apparatus. Several investigators (Gregory *et al.*, 1975; Nemchausky *et al.*, 1977; Gregory *et al.*, 1978; Jones *et al.*, 1979; Robins and Brunengraber, 1982; Nervi *et al.*, 1984) have described that the precursors of biliary lipids are normally transported in vesicles from their site of origin in the ER. It has also been suggested that the cholesterol is released from the ER in vesicles (Urbani and Simoni, 1990; Johnson, *et al.*, 1990). Liscum and Dahl (1992) have proposed the evidence that cholesterol bypasses the Golgi apparatus en route from the ER to the plasma membrane. Johnson *et al.* (1990) noted that the kinetics of exogenous cholesterol movement were consistent with vesicular trafficking. Moreau *et al.*(1991) have reported that the ER appeared to have a particular affinity for phosphotidylcholine as well as cholesterol. Berr *et al.*(1993) have described that phosphatidylcholine is carried in vesicles in the primary hepatic bile.

As shown in the previous studies, the intracellular pathways of cholesterol and phospholipid in the hepatocytes were still incompletely understood. In the hepatocytes from rats treated with cholesterol and phosphatidylcholine observed in the present study, the vacuoles derived from the ER appeared in the vicinity of bile canaliculi and individual cisterns of the Golgi apparatus appeared to be dilated with the increase of lysosomes, although the sacculation of cis Golgi cisterns were occasionally observed. From this findings, it is assumed that the intracellular transport of cholesterol and phosphatidylcholine to the bile canaliculi involve three transport processes. The first is to trnasport the cholesterol and phosphatidylcholine in the vacuoles from their sites of origin in the ER to the bile canaliculi and the second is to pass them lysosomes via the trans Golgi cisterns en route from the ER to the bile canaliculi. The cholesterol, however, may be transported much more pass the lysosomes via the trans Golgi cisterns in comparison with phosphaticylcholine, since the lysosomes appeared to be predominant in the vicinity of bile canaliculi in the rat treated with cholesterol. The third is to transport them via the vacuoles derived from the cis Golgi cisterns. This may be the pathway for lipids such as sphingolipids which are systhesized in the cis Golgi cistern with the increase of cholesterol concentration as suggested by Jeckel *et al.* (1990).

Abstract

The influence of dehydrocholic acid, cholesterol and phosphstidylcholie to the fine structure of vacuolar apparatus was investigated to better understand the mechanism of intracellular transport of bile constituents in the hepatocytes of rats.

The cis Golgi cisterns faced toward the bile canaliculi both in normal and experimental groups.

In the hepatocytes from the rats of experimental groups, the primary organic solutes in bile influence the Gogi apparatus, ER and lysosome in the way of increase, cisternal dilation or budding to form the vacuoles. In the dehydrocholic acid group, the cis Golgi cisterns

appeared to be sacculated and showed buds, which were probably separated to be vacuoles. Some of the vacuoles appeared to be fused to the bile canaliculi. In the cholesterol and phosphatidylcholine groups, the Golgi cisterns appeared to be dilated and lysosomes were increased in the vicinity of bile canaliculi. The cis Golgi cisterns showing linear saccular fashions were occasonally observed. The increase of lysosomes were more predominant in the cholesterol group.

The evidence suggests that dehydrocholic acid is mainly transported through the ER and cis Golgi cisterns, and cholesterol and phosphatidylcholine are mainly transported through the ER and lysosomes via the trans Golgi cisterns, but the cholesterols are frequently transported via the lysosomes.

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FIGURES LEGENDS

Fig. 1. Micrograph illustrates a bile canaliculus surrounded (BC) by two adjacent hepatocytes from a normal rat. The Golgi apparatus (G) and lysosomes (arrow) are located in the vicinity of bile canaliculus. The cis Golgi cistern faces toward the bile canaliculus. ×24000

Fig. 2. Micrograph illustrates a transversely sectioned bile canaliculus (BC) surrounded by two adjacent hepatocytes from a rat 20 min after administration of dehydrocholic acid. Two Golgi apparatus (G) are located in the vicinity of bile canaliculus (BC). The cis Golgi cisterns are sacculated and face toward the bile canaliculus. A vacuole (arrowhead) is fused to the luminal plasma membrane of bile canaliculus. Arrow indicates the lysosome. ×20000

Fig. 3. Micrograph illustrates a transversely sectioned bile canaliculus (BC) surrounded by two adjacent hepatocytes from a rat 20 min after administration of dehydrocholic acid. The cis Golgi cistern faces toward the bile canaliculus. Vacuoles are seen in the vicnity of the bile canaliculus. A vacuole (arrowhead) is fused to the canaliculuar membrane. ×22000

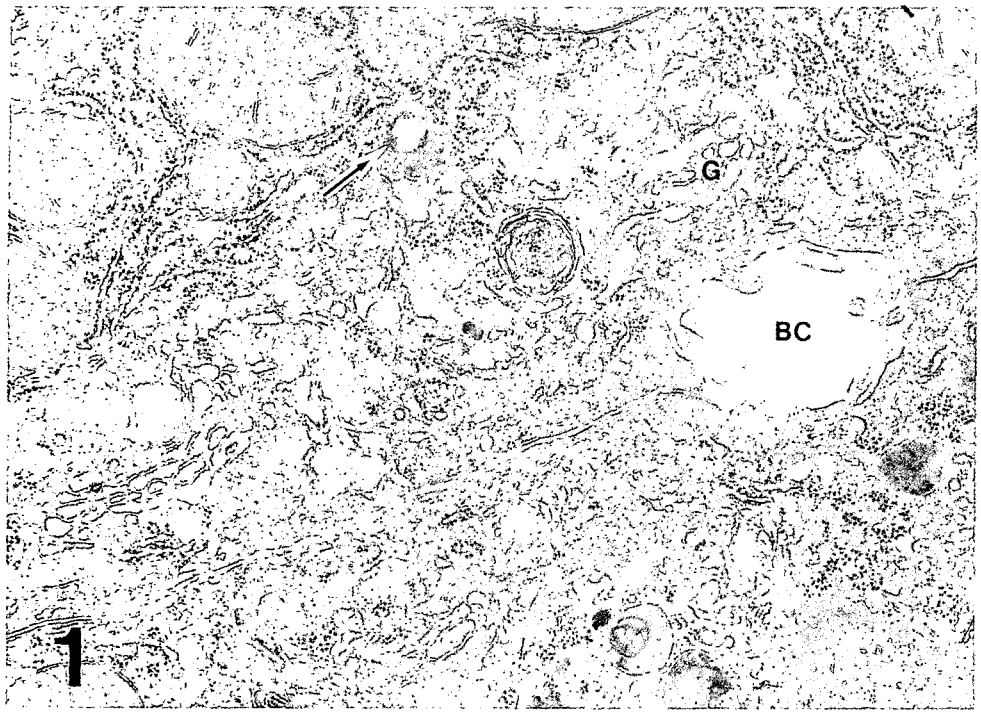
Fig. 4. Micrograph illustrates a longitudinally sectioned bile canaliculus (BC) surrounded by two adjacent hepatocytes from a rat treated with cholesterol. Vacuoles (arrowhead) are fused to the luminal plasma membrane of bile canaliculus. Arrow indicates the lysosome. ×10000

Fig. 5. Micrograph illustrates a transversely sectioned bile canaliculus (BC) surrounded by two adjacent hepatocytes from a rat treated with cholesterol. A Golgi apparatus (G) is seen near the bile canaliculus. The cis Golgi cistern is sacculated and faces toward the bile canaliculus. Lysosomes (arrow) are located near the bile canaliculus. Vacuoles (arrowhead) are fused to the luminal plasma membrane of the bile canaliculus. ×22000

Fig. 6. Micrograph illustrates an obliqually sectioned bile canaliculus (BC) surrounded by two adjacent hepatocytes from a rat treated with cholesterol. The Golgi apparatus showing dilated cisterns (G) lysosomes (arrow) appear to be crowded in the vicinity of bile canaliculus. Arrowhead indicates vacuoles. ×15000

Fig. 7. Micrograph illustrates a bile canaliculus (BC) surrounded by adjacent hepatocytes from a rat treated with phosphatidylcholine. The Golgi apparatus (G) showing short dilated cisterns in the vicinity of bile canaliculus. Two vacuoles (arrowhead) are fused to the luminal plasma membrane of bile canaliculus. Arrow indicates the lysosome. ×22000

Fig. 8. Micrograph illustrates two bile canaliculi (BC) surrounded by adjacent hepatocytes from a rat treated with phosphatidylcholine. The Golgi apparatus (G) showing dilated short cisterns and vacuoles (arrowhead) are seen in the vicinity of bile canaliculus. Arrow indicates the lysosome. ×10000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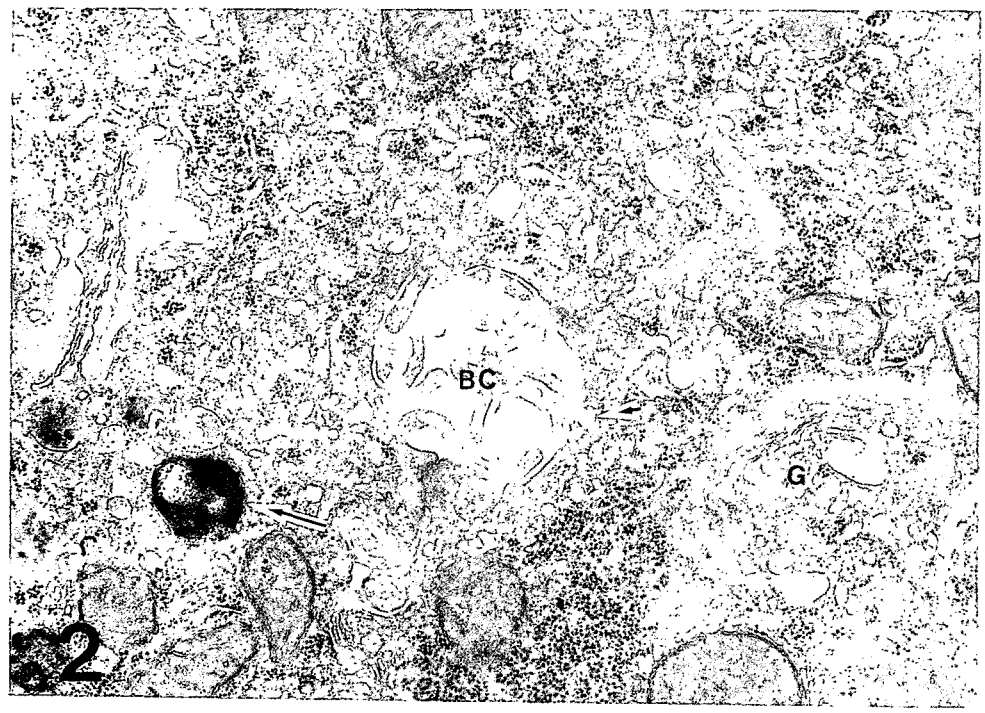
BC
1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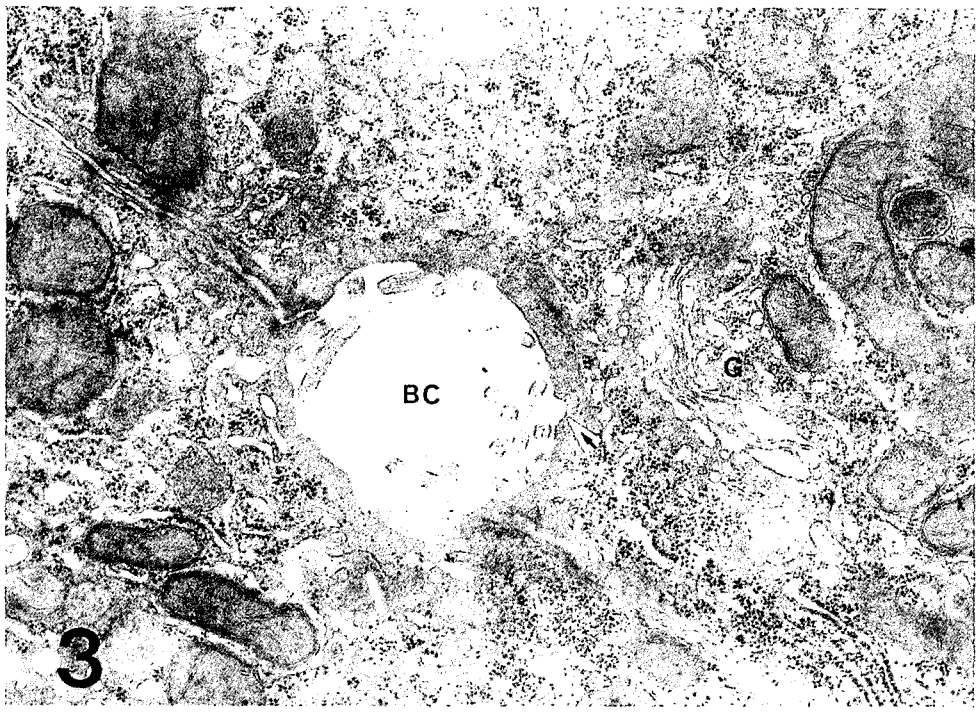
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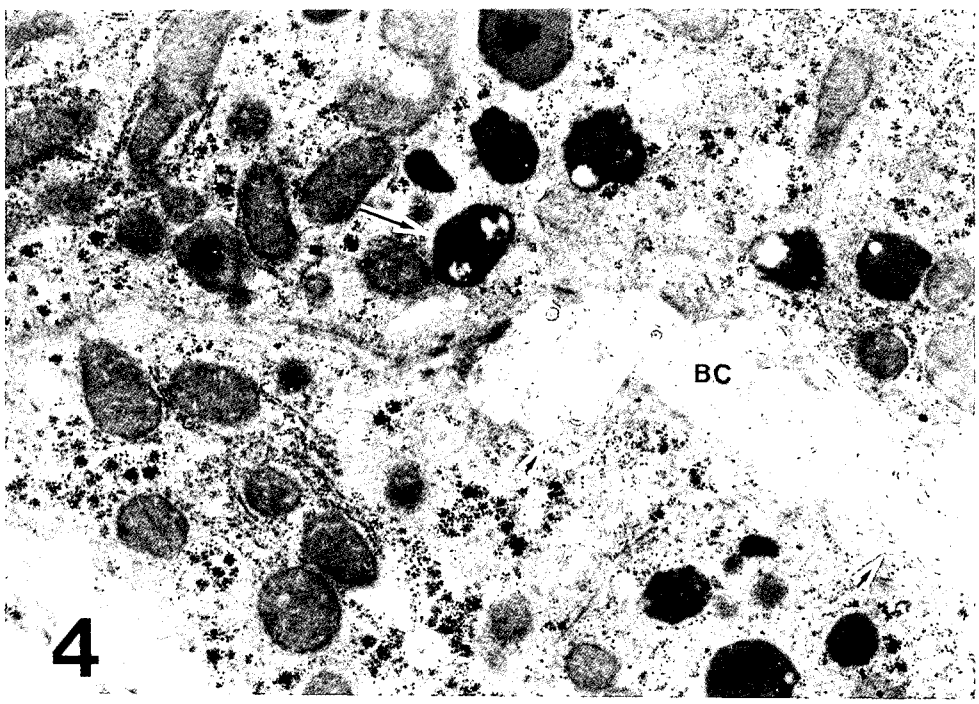
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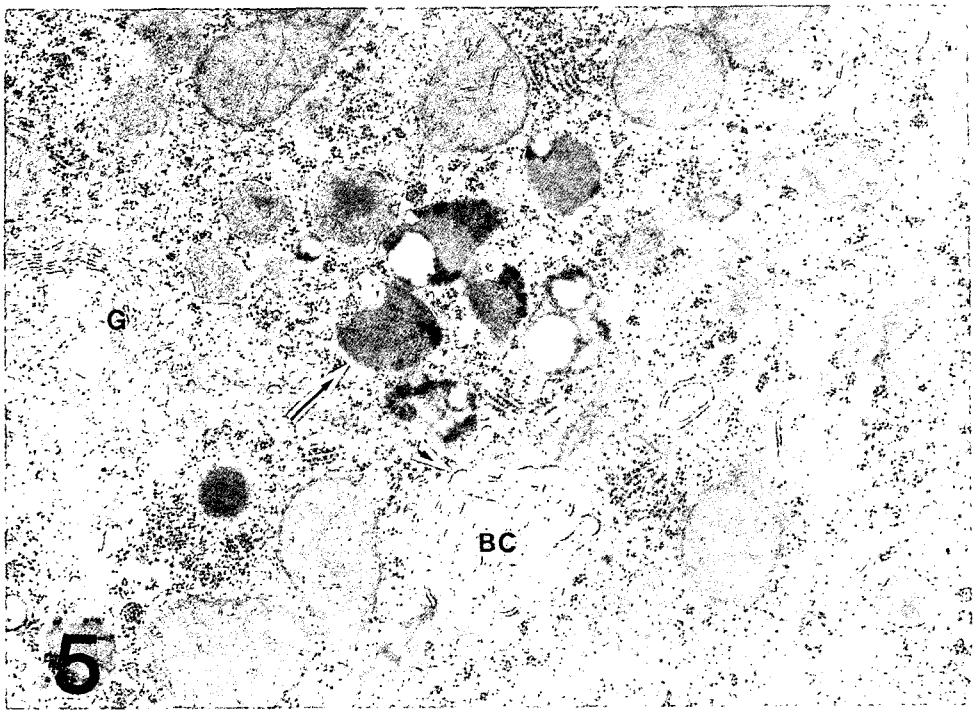
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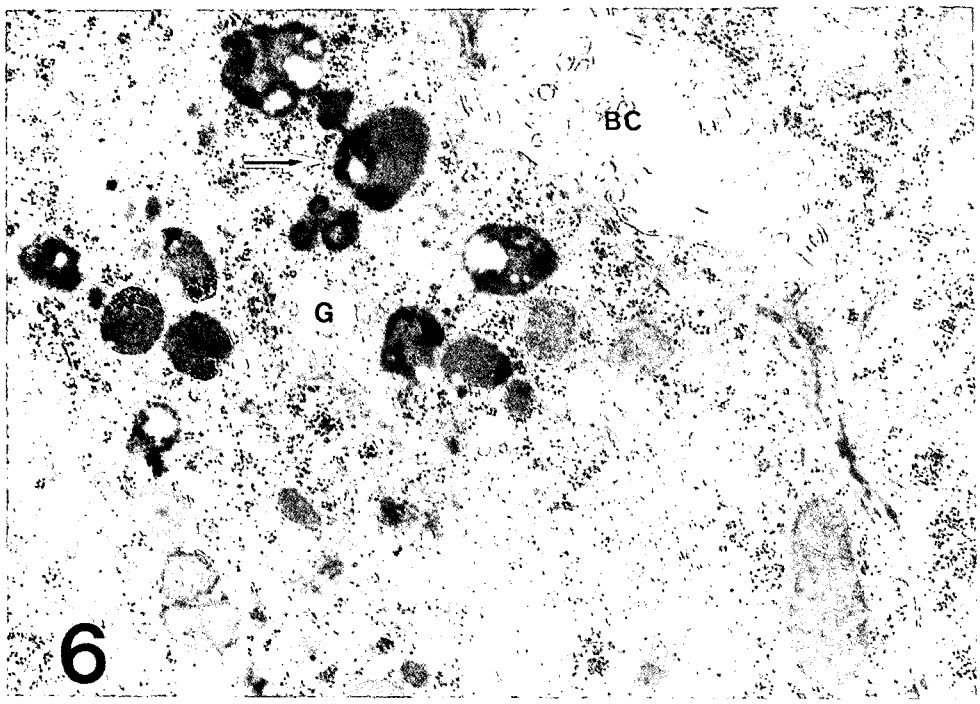
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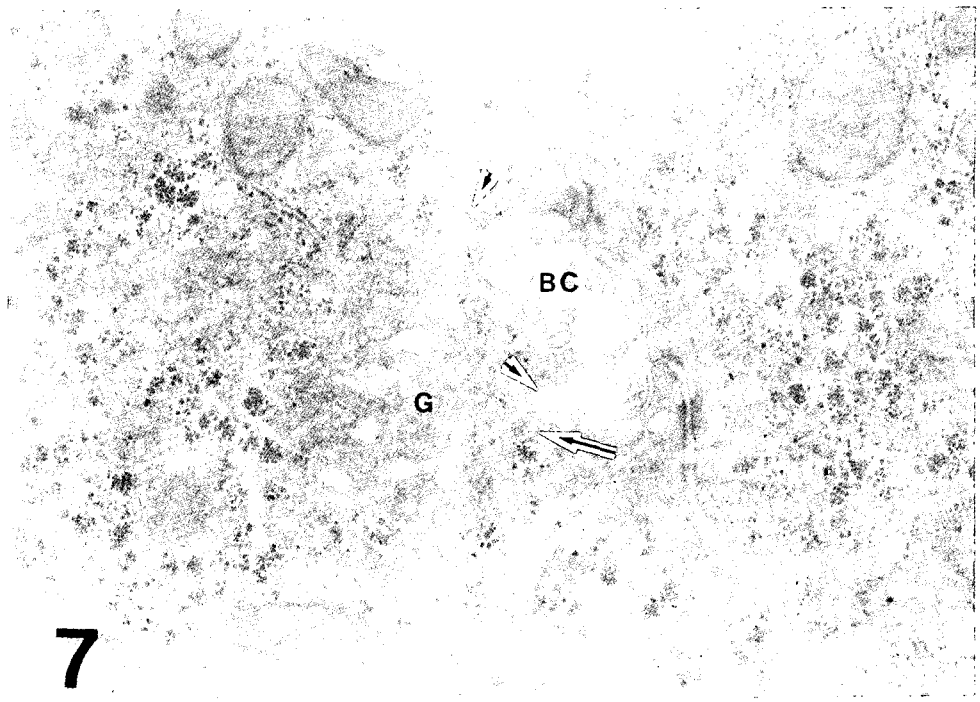
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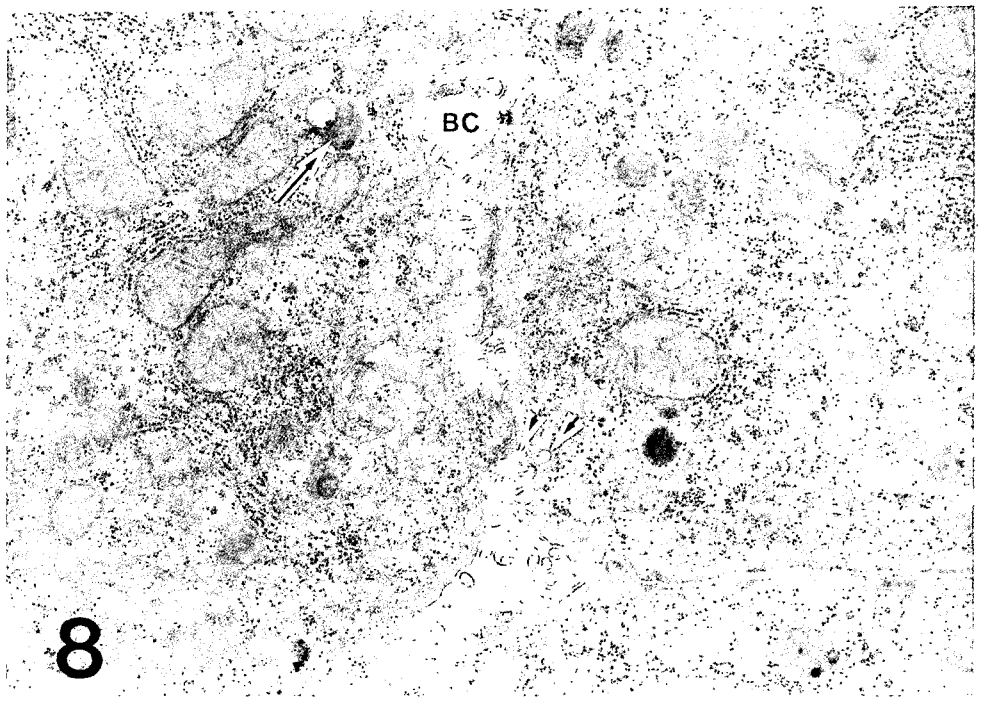
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