EXOCYTOSIS OF LYSOSOMAL ENZYMES BY HEPATOCYTES INTO BILE DURING REGRESSION OF CIRRHOSIS OF THE LIVER

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Lysosomes may play an important role in the formation of certain components of bile [5, 8, 9]. Biochemical investigations of bile have revealed the presence of lysosomal hydrolytic enzymes [1], and this finding, together with the characteristic arrangement of lysosomes at the biliary pole of the hepatocytes, led to formulation of the hypothesis that lysosomal content undergo exocytosis into the bile, as an important excretory pathway in the liver [2]. The presence of lysosomal enzymes in the bile has been confirmed by biochemical studies [4-7, 10], but no direct morphological proof of this excretory pathway has been obtained. The question of the mechanisms of entry of lysosomal enzymes into the bile thus remains open.

This paper describes morphological evidence of exocytosis of lysosomal enzymes by hepatocytes into the bile. This information was obtained during a study of mechanisms of resorption of fibrous tissue in the liver during involution of cirrhosis.

EXPERIMENTAL METHOD

Cirrhosis of the liver was induced by subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in olive oil into noninbred male albino rats once a week for 5 months. The injections of CCl_4 then ceased and animals with developed cirrhosis were divided into two groups. The animals of group 1 underwent resection of the left lobe of the liver 7 days after the last injection of CCl_4 in order to stimulate regeneration. The animals of group 2 did not undergo an operation. Material for investigation was taken during resection (animals of group 1) and also 10, 12, 14, 17, 22, 27, and 37 days after the last injection of CCl_4 simultaneously on the animals of both groups, and treated by histochemical methods to detect acid phosphatase (AP) at the ultrastructural level, using sodium β-glycerophosphate as the substrate [3]. Incubation in medium without substrate and incubation with the addition of the enzyme inhibitor, 0.01 M sodium fluoride, to the medium served as the control. Ultrathin sections were stained with uranyl acetate and examined in the ÉMV-100BR electron microscope.

EXPERIMENTAL RESULTS

On electron-histochemical investigation of the liver during involution of cirrhosis, the product of the reaction for AP was found in the lumen of individual small bile ducts on the membranes of various vesicles, and on microvilli and the cytolemma (on the luminal side) of the epithelial cells of the ducts (Fig. 1). The reaction product also was found in some biliary tubules, where it was in contact with the microvilli of hepatocytes (Fig. 2). These observations are evidence that the bile contained lysosomal enzymes. Departure of lysosomes containing the product of the reaction for AP from the hepatocytes into a biliary tubule also was observed; a lysosome could be seen at the periphery of the hepatocyte immediately by the cytolemma, but later it passed into the lumen of a biliary tubule, where destruction of its membrane began (Fig. 3). Lysosomes can evidently be discharged from hepatocytes into the bile together with their own membrane.

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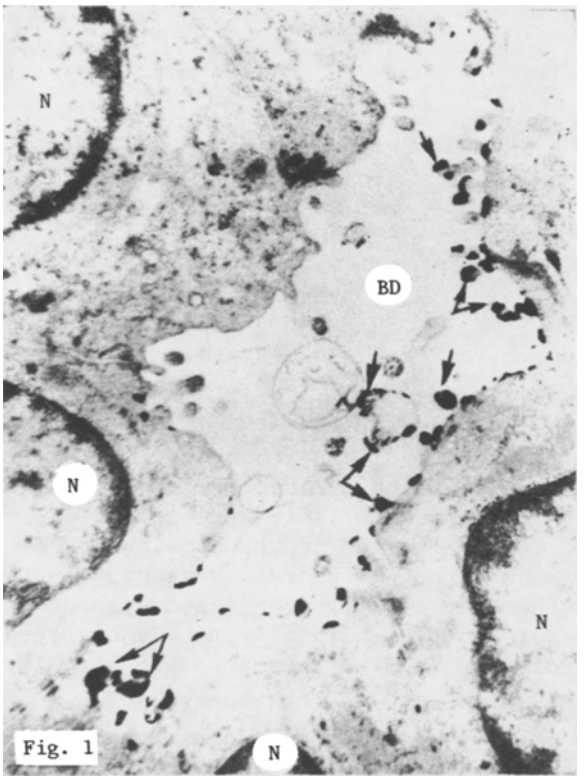
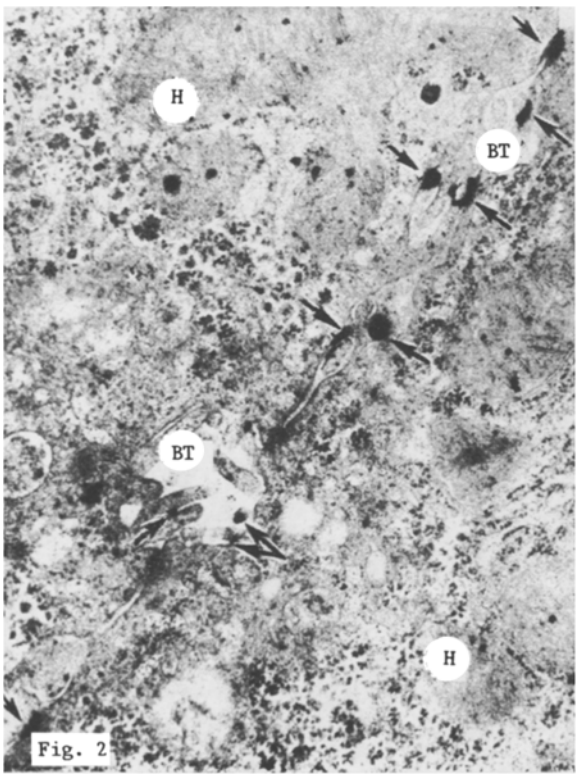


Fig. 1. Product of reaction for AP (arrows) in lumen of small bile duct (37th day after the last injection of CCl_4). BD) Bile duct; N) nuclei of epithelial cells of BD. 28,000 ×.



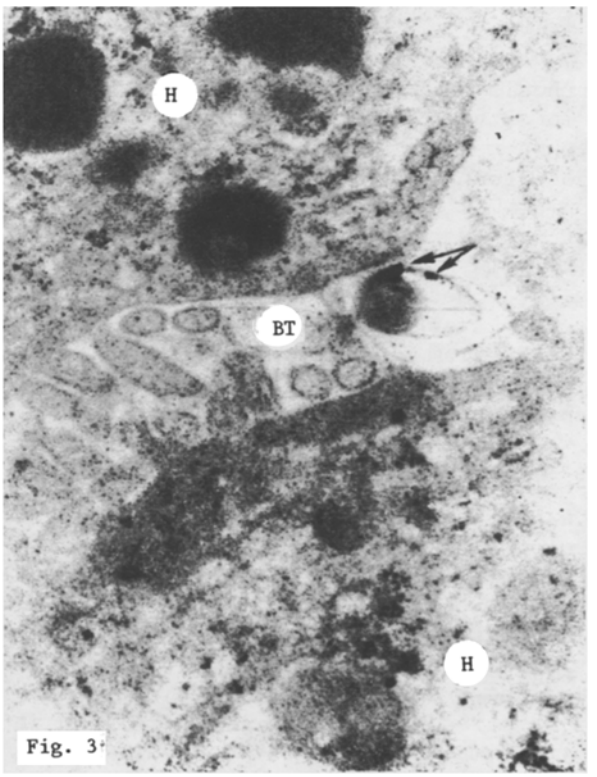


Fig. 2 Fig. 3

Fig. 2. Product of reaction for AP (arrows) in lumen of biliary tubules (37th day after last injection of CCl_4). BT) Biliary tubules; H) hepatocytes. 40,000 ×.

Fig. 3. Lysosome with disrupted membrane containing product of reaction for AP (arrows) in lumen of BT (22nd day after last injection of CCl_4). 80,000 ×.

The phenomenon described above took place in animals of both groups. No reaction product was found in the lysosomes, biliary tubules, or bile ducts during examination of control preparations.

The results thus indicate that during involution of cirrhosis exocytosis of lysosomal enzymes by hepatocytes into the bile takes place in the liver. Since the process described above was observed 5 days after the last injection of CCl_4, when most fibrous tissue had already undergone resorption and the liver structure was largely restored, there is every reason to suppose that exocytosis of lysosomal enzymes by hepatocytes into the bile is not the result of the pathological process in the liver, but is a mechanism of bile secretion. This hypothesis is supported by the discovery of lysosomal enzymes in the bile of intact animals in a number of biochemical investigations [1, 2, 4-7, 10].

The physiological role of lysosomal "unloading" of hepatocytes into the bile is unknown. Some workers consider that it is responsible for removing indigestible particles from hepatocytes, through their accumulation in secondary lysosomes [1, 2]. It has also been suggested that some substances from hepatocytes, especially those transported by vesicles, can be associated with primary lysosomes before they are secreted into the bile by exocytosis. Lysosomal enzymes can degrade, or metabolize somehow or other, certain substances for their excretion into the bile [5].

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SPECIFIC FEATURES OF HEALING OF VENTRAL SKIN WOUNDS IN RATS

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Most experimental studies of skin wounds have been undertaken on the dorsal region of rats and mice [1, 2]. Meanwhile no investigations of the healing of skin wounds on the ventral aspect of the trunk of laboratory animals could be found in the literature. The skin on the ventral aspect is thinner than in the dorsal region, it has a well-developed elastic skeleton, and contains fewer hairs. These differences suggested that the healing of wound detects on the ventral aspect of the trunk in animals would have certain characteristic distinguishing features.

The aim of this investigation was to compare the healing of full-thickness skin wounds on the ventral and dorsal aspects of the trunk in rats. During analysis of the results attention was concentrated on contraction of the wounds and completeness of repair of the skin.

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