LIVER SINUSOIDAL CELL ULTRASTRUCTURE IN PARTIALLY HEPATECTOMIZED RATS

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Two-thirds of the liver was removed from male Wistar rats weighing 160-200 g. In response to the operation phasic changes occurred in the structure of the lysosomal apparatus in the Kupffer cells. The number of primary lysosomes in the Kupffer cells was increased 2.5 h after partial hepatectomy, and the size and polymorphism of the lysosomes were increased after 9 h. At the peak of mitotic activity of the hepatocytes (30 h after partial hepatectomy) mainly secondary lysosomes were identified in the Kupffer cells, but 48 h after the operation, on the other hand, their number was reduced, although "young" forms of primary lysosomes appeared. Manifestations of fatty infiltration predominated in the endothelial cells and reached a maximum at the peak of mitosis of the hepatocytes. The results are evidence that ultrastructural changes in the sinusoidal cells depend on the phases of reparative regeneration of the liver.

KEY WORDS: regeneration of the liver; Kupffer cells; lysosomes; mitosis.

Reparative regeneration of the hepatocytes after partial hepatectomy (PH) has been studied in adequate detail. Meanwhile, little is known about the particular features of morphological and functional adaptation of the hepatic stroma after PH. This must be regarded as a significant gap in our knowledge, for inducing influences controlling morphogenetic changes in embryogenesis [3] and reparative regeneration of parenchymatous organs in pathology [4, 6] originate from the stroma of the organ. It is probable that morphogenetic feedback also exists from the parenchyma to the stromal cells. The Kupffer cells and endothelial cells are the principal components of the hepatic stroma. The study of Kupffer cells during reparative regeneration of the liver is of special interest, because they are a unique depot of lysosomal enzymes, to which at the present time the role of universal regulators of intercellular interaction in the connective-tissue system [8] and of modifiers of growth and differentiation of many cellular systems in vitro [7] is now ascribed.

EXPERIMENTAL METHOD

Male Wistar rats weighing 160-200 g were used. The left and middle lobes of the liver were removed under ether anesthesia by the method of Higgins and Anderson. The animals were decapitated 5-6 at a time 2.5, 9, 30, and 48 h after hepatectomy. The course of regeneration was monitored by calculating the mitotic index of the hepatocytes. For electron microscopy the liver was perfused with 1.5% glutaraldehyde solution in phosphate buffer (pH 7.4) by Wisse's method [9] in the modification of Alekseev and Prokhorov [1]. The material was embedded in Araldite. Sections 1-1.5 μ thick were stained with methylene blue. Ultrathin sections, stained with lead citrate, were examined under the IEM-100B microscope. The morphological criteria of Wisse [9] were used to identify sinusoidal cells.

EXPERIMENTAL RESULTS

Normal endothelial cells (Fig. 1a) are flat and form the canal of the hepatic sinusoid. In thinner areas of the cytoplasm, fenestrae are regularly found – oval pores up to 0.1 μ in diameter. Openings exceeding 0.1 μ in diameter are much less common. The granular cytoplasmic reticulum (GCR) consists of cisterns running parallel to the cell membrane. The concentration of mitochondria and elements of GCR in the cytoplasm of

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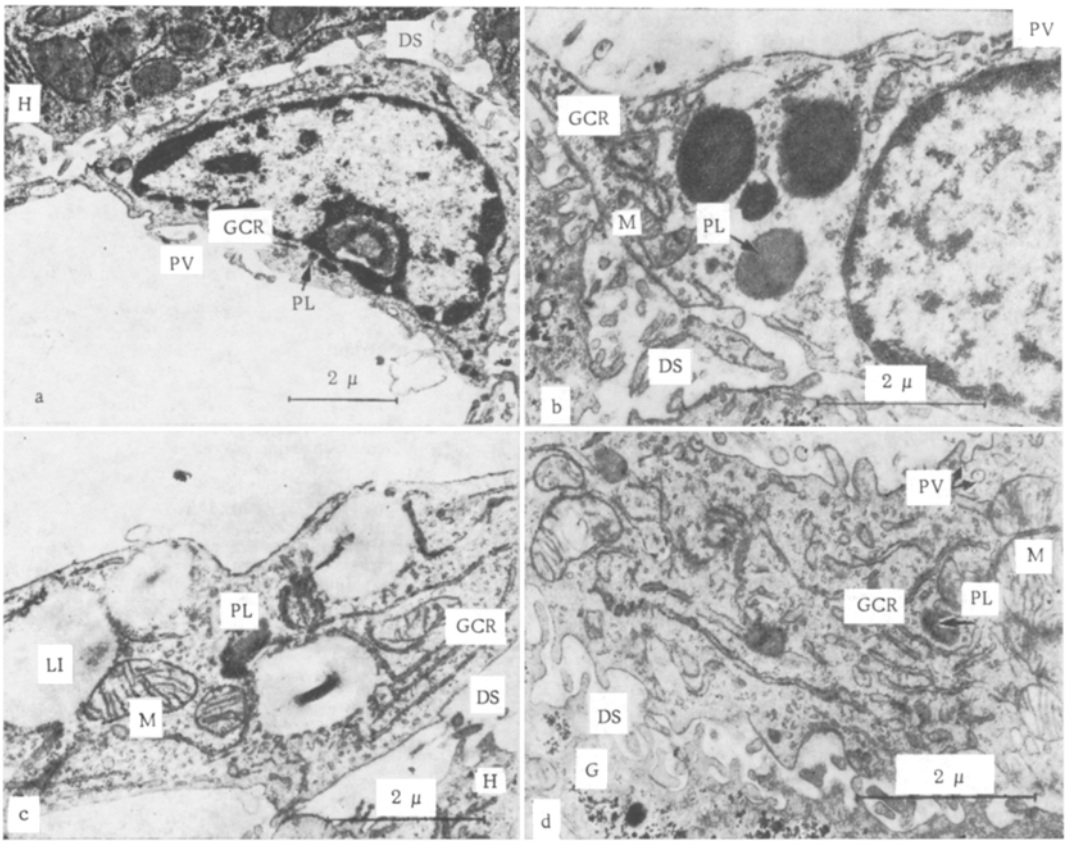

Fig. 1. Changes in ultrastructure of endothelial cell: a) endothelial cell of hepatic sinusoid of intact rat; b) endothelial cell 9 h after PH; c and d) fragment of endothelial cell 30 and 48 h respectively after PH. PL) Primary lysosomes; M) mitochondria; PV) pinocytotic vacuoles; GCR) granular cytoplasmic reticulum; DS) Desse's space; LI) lipid inclusions; H) hepatocyte; a), 10,000×, b and d) 16,700×, c) 15,000×.

the endothelial cells is greater than in the Kupffer cells. The Kupffer cells (Fig. 2a) contain a more strongly developed lysosomal apparatus and unique structural adaptation for endocytosis: vermiform structures, a glycocalyx, phagocytic pseudopodia, and so on. Their cytoplasm usually contains many pinocytotic vacuoles and phagosomes.

The lumen of the sinusoids and the Desse's spaces were considerably widened 2.5 h after PH. Multiple lipid droplets, similar in size to the mitochondria and located, as a rule, in the perisinusoidal zones of the cytoplasm, appeared in most hepatocytes. There were few glycogen granules in the lipid-containing cells. The number of primary lysosomes in the peribiliary regions was increased. There were fewer microvilli on the plasmalemma facing Desse's space. The openings in the endothelium were more irregularly arranged than normally, whereas fewer fenestrae were seen. The structure of the organelles of the endothelial cells at this time was close to normal. Only the number of secondary lysosomes and residual bodies in their cytoplasm was increased appreciably. The GCR and lamellar complex were more clearly defined than normally in the Kupffer cells. The number of primary lysosomes was increased but they were near normal in size.

Mitoses were virtually absent in the hepatocytes 9 h after PH. As before, there were few microvilli in the cells. The number of glycogen granules in the cytoplasm was sharply reduced but the volume of lipid inclusions was increased. Primary lysosomes were more numerous, more widely distributed in the cytoplasm, and frequently concentrated near lipid droplets. Single mitochondria were in contact with lipid inclusions. Hyperplasia of the protein-synthesizing apparatus was observed. The number of primary lysosomes in the endothelial cells was significantly increased, and the largest of them were located near the nucleus (Fig. 1b), whereas groups of small lysosomes were concentrated in the peripheral zone. GCR was hypertrophied. Many micropinocytotic vesicles and small lipid inclusions were present in the cytoplasm. The Kupffer cells were larger than at the previous time of investigation or in the intact liver. The cytoplasm of most cells was highly saturated with organelles, but large primary lysosomes predominated (Fig. 2b). They were 1.5-2 times larger

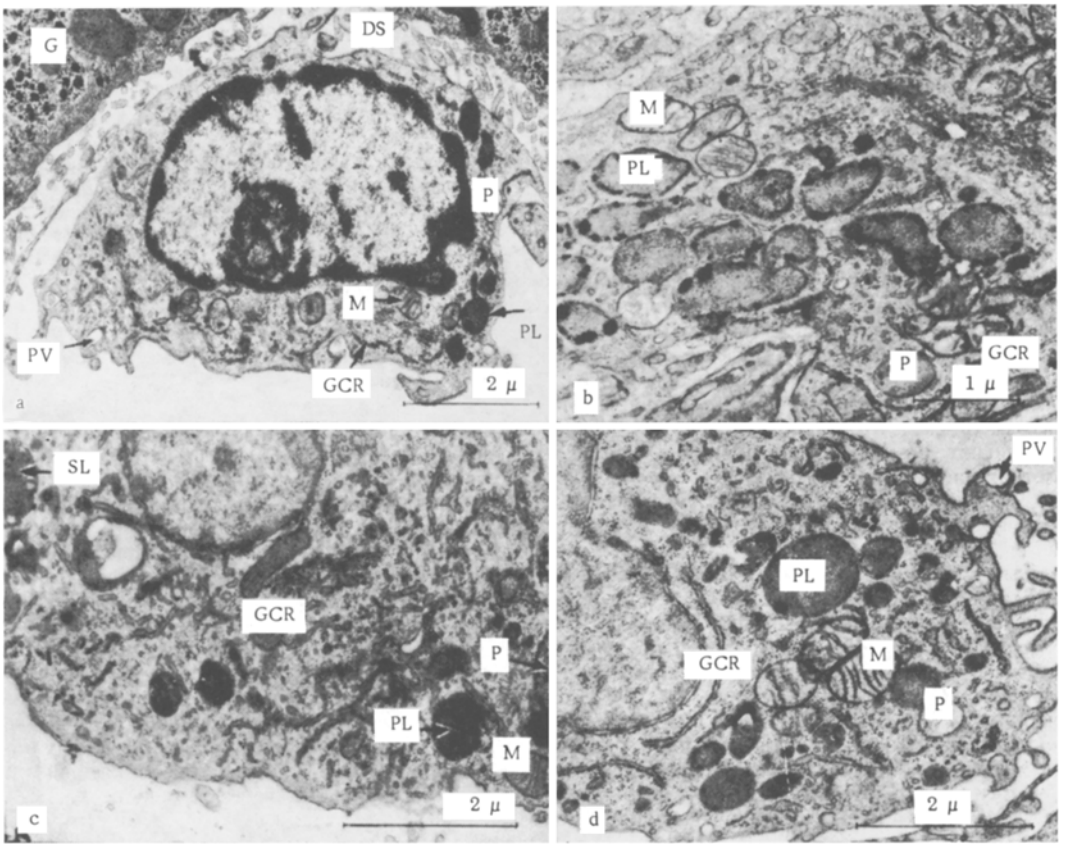

Fig. 2. Changes in ultrastructure of Kupffer cell. a) Kupffer cell from liver sinusoid of intact rat; b, c, d) fragment of Kupffer cell 9, 30, and 48 h respectively after PH. SL) Secondary lysosomes, P) phagosomes; remainder of legend as in Fig. 1. a) 13,000 ×, b) 20,000×, c and d) 17,000×.

than the analogous structures in the cells of intact animals. Small electron-dense granules were frequently seen in the matrix of the lysosomes, close to the membrane. Conjugation of lysosomes and also union of lysosomes with phagosomes were observed. In the Kupffer cells lipid inclusions were present, but fewer than in the endothelial cells.

The structure of the Desse's space was not yet back to normal 30 h after PH. The frequency of mitoses in the hepatocytes increased to 25.7 ± 2.1%. Their cytoplasm contained little glycogen. Lipid inclusions were large, sometimes with "moth eaten" borders. The lipid droplets were now distributed throughout the cytoplasm. Intercellular contacts were loose. There were fewer lysosomes than at the previous time of investigation.

Lipid inclusions were very numerous in the cytoplasm of the endothelial cells. They were distributed mainly in the perinuclear region (Fig. 1c). Meanwhile there were considerably fewer primary lysosomes. The GCR was more clearly defined than normally.

The Kupffer cells at this time contained far fewer primary lysosomes. By contrast, secondary lysosomes and residual bodies were now more numerous (Fig. 2c). The lamellar complex and GCR were more strongly developed. Lipid inclusions were seen, but fewer of them than in the endothelial cells.

The mitotic index 48 h after PH was down to 10.0 ± 1.5%. The glycogen content in the cytoplasm of the hepatocytes was increased. Lipid inclusions were numerous. Large droplets the same size as the cell nucleus were predominant. The GCR of the hepatocytes was very well developed. Intercellular contacts were often loose.

Lipid inclusions were rare in the endothelial cells. There were fewer primary and secondary lysosomes than at the previous time (Fig. 1d). The GCR was less well developed than 30 h after PH. There was a change in the character of the perforation of the endothelium, which had fewer wide openings and more frequent fenestrae.

Lipid inclusions were very rare in the Kupffer cells. The number of organelles in the cytoplasm at this period was almost normal. The lysosomal apparatus consisted of small primary lysosomes with a moderately dense matrix (Fig. 2d). There were fewer secondary lysosomes 48 h after PH. The GCR was well developed.

In response to PH characteristic ultrastructural transformations thus arise not only in the hepatocytes, but also in the cells of the hepatic sinusoids. The principal changes in the sinusoidal cells can be summarized as follows: first, infiltration of the Kupffer cells and, in particular, the endothelial cells by lipids. This process can be linked with the decrease in the ingestive capacity of the Kupffer cells in the regenerating liver described by the writers previously [2]. Lipid inclusions were more numerous in the endothelial cells, for these cells carry out endocytosis through pinocytosis [9]. Second, the area of the GCR in the endothelial cells and Kupffer macrophages of the regenerating liver was increased. Third, phasic changes took place in the structure of the lysosomal apparatus. They were more clearly defined in the Kupffer cells: in the early prereplicative period of regeneration, 2.5 h after PH, primary lysosomes accumulated in the Kupffer macrophages, and 9 h after the operation they were larger and showed a tendency to merge (conjugate) with the phagosomes. At the peak of mitotic activity of the hepatocytes, i.e., 30 h after PH, mainly secondary lysosomes could be identified in the Kupffer cells, but 48 h after the operation, on the other hand, there were fewer secondary lysosomes and new batches of primary lysosomes were appearing. Hence it can be concluded that during reparative regeneration of the liver the lysosome pool in the Kupffer cells is evidently renewed during the first 48 h after PH.

Variations in the structure of the lysosomal apparatus thus revealed correlated with phasic changes described previously in the activity of lysosomal enzymes in the Kupffer cell fraction of regenerating liver [5]. For instance, activity of acid DNase, acid RNase, cathepsin D, and β-glucosidase in the Kupffer cells rose sharply at a time when the number of primary lysosomes in the cells was increased in the prereplicative period of regeneration. All types of activity were reduced at the peak of mitosis of the hepatocytes (30-32 h after PH), when the number of primary lysosomes in the Kupffer cells became minimal, and increased again on recovery of the primary lysosome population toward 48 h after PH. The ultrastructural transformations of the sinusoidal cells of the liver thus depended essentially on the phases of its regeneration. The role of these changes in the process of reparative regeneration of the liver will be studied in future research.

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