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# INTRALOBULAR DISTRIBUTION OF ALBUMIN SYNTHESIS IN HEPATOCYTES OF THE NORMAL AND REGENERATING MOUSE LIVER

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Mammalian serum albumin is known to be synthesized in the liver. Immune electron-microscopic investigations carried out in recent years [8, 11, 14] on rat liver have shown that all hepatocytes synthesize albumin, and that immunohistochemical data on the existence of specialized albumin-producing cells in the liver, obtained previously, are artefacts. In the investigations cited, no heterogeneity of distribution of albumin among the liver lobules could be found, which is not in agreement with the presence of marked gradients in the lobule affecting many features such as glycogen content, enzyme activity, and ability to detoxicate organic and inorganic substances [5, 7, 12].

This paper describes an immune electron microscopic investigation of intralobular differences in albumin synthesis in the normal and regenerating adult mouse liver.

## EXPERIMENTAL METHOD

Noninbred mice and BALB/c/J mice weighing 20-30 g, obtained from the Nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used. The liver of normal mice and 70 h after poisoning with $CCl_4$ vapor [1] was studied.

An albumin preparation was obtained from adult mouse serum by double polyacrylamide gel electrophoresis [2]. No contamination with other serum proteins was found in the concentrated albumin preparation by precipitation in agar and electrophoresis. Antiserum against albumin was obtained by immunization of rabbits in the popliteal lymph nodes with a preparation of pure albumin [3]. Monospecific antibodies were obtained by means of an immunosorbent prepared from purified albumin, covalently bound with Sepharose CNBr-4B (from Pharmacia, Sweden) in accordance with the firm's instructions. Fab'-fragments of antibodies were obtained from the isolated antibodies [9]. The preparation of Fab'-fragments was conjugated with horseradish peroxidase type VI, RZ-2,9 (from Sigma, USA) [12].

The liver was fixed by our modified method of perfusion with saponin [10]. Under ether anesthesia, after application of a ligature a needle was introduced into the posterior pole of the spleen, and the organ perfused with 25 ml of a solution of 0.05% glutaraldehyde + 6% paraformaldehyde + 0.05% saponin in 0.15 M cacodylate buffer, pH 7.4. Perfusion was carried out with a peristaltic pump at the rate of 5 ml/min. After perfusion pieces of liver 1 $cm^3$ in volume were postfixed with a solution of 6% paraformaldehyde + 0.05% saponin for 3 h at 4°C. The pieces were then washed for 12-15 h with buffer with the addition of 3.5% sucrose and 0.05% saponin. The pieces were frozen with liquid nitrogen and cryostat sections cut to a thickness of 15 μ and treated with 0.1 M lysine solution for 30 min. Next, 20-30 freely floating sections were incubated in 0.3 ml of a solution of Fab + horseradish peroxidase con-

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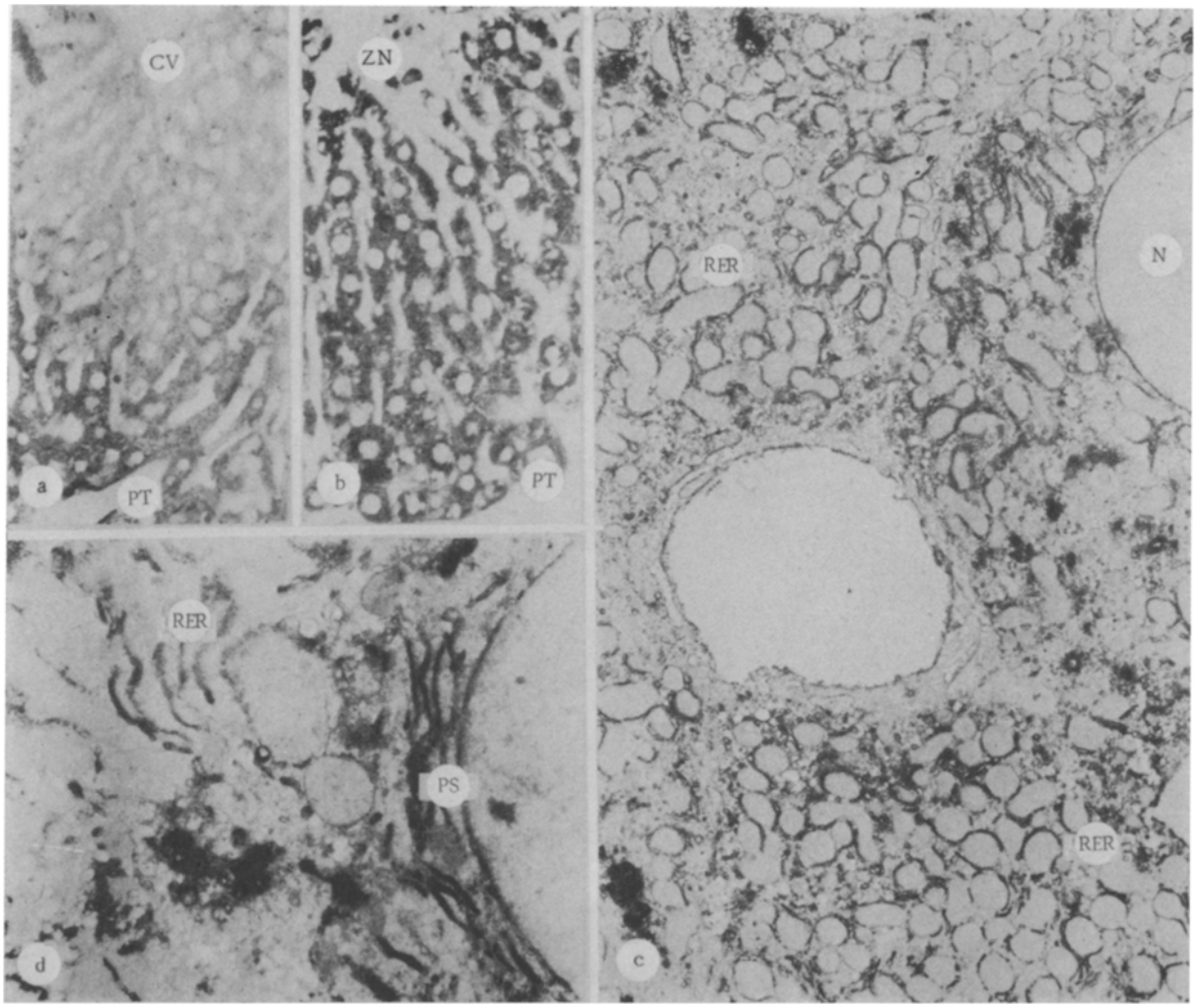


Fig. 1. Immunocytochemical localization of albumin synthesis in mouse hepatocytes: *a*) Gradual weakening of hepatocyte staining from portal tract (PT) toward central vein (CV). Semithin section of normal liver. 50 ×. b) All hepatocytes have high intensity of staining. ZN) Zone of necrosis. Semithin section through regenerating liver. 50 ×. c) Fragments of hepatocyte in periportal zone. Lamellar apparatus (LA) has most intense staining. N) Nucleus, RER) rough endoplasmic reticulum. 5200 ×. d) Hepatocyte fragment from periportal zone. Albumin present in perinuclear space (PS), in RER, and in LA. 17,000 ×.

jugate with the addition of 0.05% saponin for 2 h at room temperature. The sections were then washed with buffered physiological saline for 1 h, and peroxidase activity was revealed by means of 3,3-diaminobenzidine. The sections were fixed in 1.33% $OsO_4$ solution and quickly dehydrated in acetones of increasing concentration, and embedded in a mixture of Epon and Araldite. For this purpose, the sections were transferred in a drop of mounting medium to the flat surface of prepolymerized blocks and covered with pieces of glass slide, coated with a 1% formvar film. After polymerization at 60°C for 12 h, the slide could easily be separated from the surface of the block. Semithin and ultrathin sections were cut on the LKB III Ultrotome. Ultrathin sections were examined without additional staining in the IEM-100C electron microscope.

## EXPERIMENTAL RESULTS

Positive staining for albumin was revealed in semithin sections of normal liver in the form of two types of staining of hepatocytes: dark brown granular cytoplasmic deposits and pale brown diffuse staining of the cytoplasm itself. Albumin was revealed with the highest intensity in hepatocytes of the periportal zones of the hepatic lobule, which had both types of staining. Nearer to the central vein the intensity of hepatocyte staining gradually de-

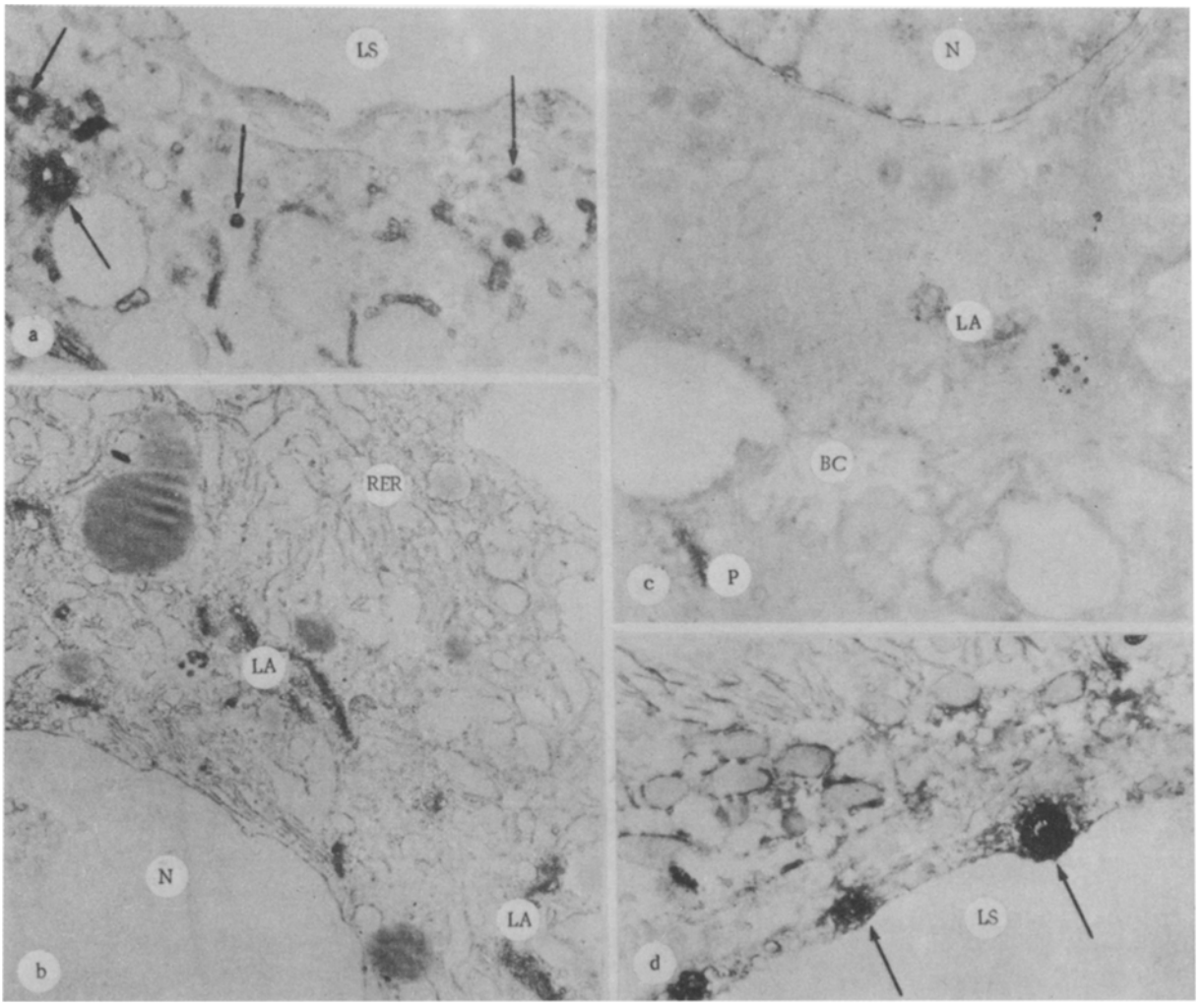


Fig. 2. Ultrastructural localization of albumin in normal mouse liver: *a*) Positively stained vesicles (arrows) near sinusoidal part of hepatocyte plasmalemma. LS) Lumen of sinus. 18,000 ×; b) Hepatocyte fragment from intermediate zone of hepatic lobule. Traces of staining of membranes (RER) and intense staining of LA. 14,000 ×; c) Fragments of hepatocytes of centrilobular zone. Traces of staining of LA in region of bile capillary (BC). 15,000 ×; d) Albumin present in vacuoles (arrows) in endothelium of venous sinus. 10,000 ×. (P) not identified in the Russian; possibly LA — Editor.

creased, initially due to weakening and disappearance of the diffuse staining of the cytoplasm. Finally, hepatocytes adjacent to the central veins contained single small cytoplasmic granules, which were hard to distinguish from the general background (Figs. 1a-3a).

Thus with the light microscope there were already grounds for the suggestion that albumin synthesis in hepatocytes in lobules of the normal adult mouse liver is distributed along a gradient falling from the portal veins toward the central veins.

In the regenerating mouse liver the region of the central veins of the majority of hepatic lobules was occupied by foci of necrosis. Residual hepatocytes had a uniform and high intensity of staining of their cytoplasm similar in type to that of the periportal hepatocytes of normal liver, without an intensity gradient (Fig. 1b).

Electron-microscopic investigation of ultrathin sections of the normal liver revealed albumin in the perinuclear space, the rough and smooth endoplasmic reticulum, the lamellar apparatus, and cytoplasmic vesicles of the hepatocytes. The intensity and extent of staining of these organelles varied depending on the position of the hepatocyte in the lobule. In periportal hepatocytes the reaction product stained all the above-mentioned organelles intensely, but the most intense staining was observed in numerous groups of the lamellar apparatus (Fig. 1c). In the endoplasmic reticulum albumin was present on membranes of the cisterns, in their lumen, and on bound ribosomes. The reaction product stained elements of the lamellar apparatus

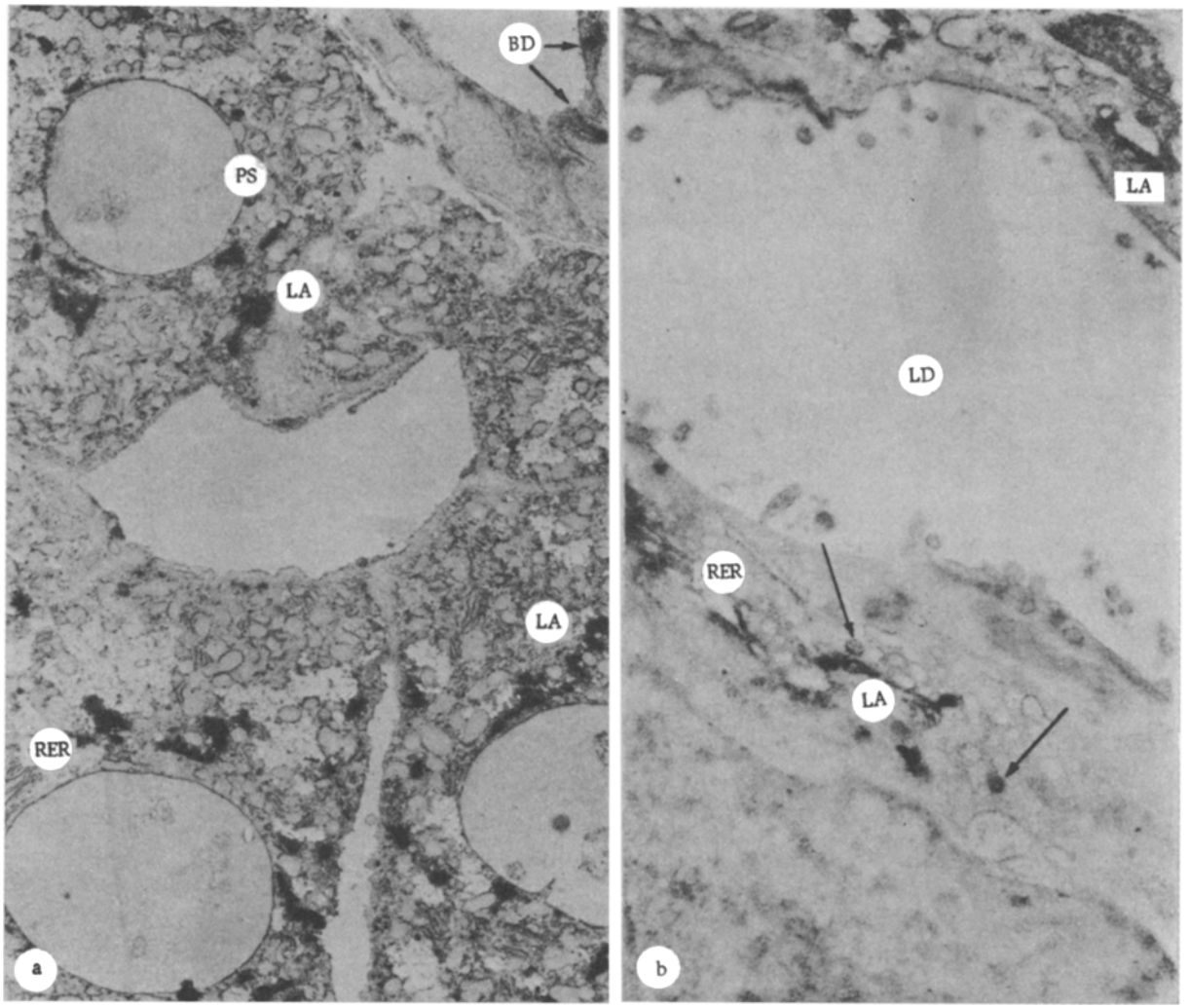


Fig. 3. Ultrastructural localization of albumin in regenerating mouse liver. *a*) Hepatocyte fragments of periportal zone and region of portal bile duct (BD). Albumin present in PS, RER, and LA of hepatocytes, and also in cytoplasm of epithelium of bile ducts (arrows). 3300 ×; b) Fragments of epithelial cells of portal BD. Staining of solitary cisterns of RER, LA, and cytoplasmic vesicles (arrows). LD) Lumen of duct. 35,000 ×.

and the adjacent vesicles partially or completely (Fig. 1d). In some groups of the lamellar apparatus massive deposition of reaction product prevented determination of its exact localization. Small cytoplasmic vesicles containing reaction product were often found near the sinusoidal part of the plasmalemma of the hepatocytes, and some of the vesicles fused with it (Fig. 2a). In hepatocytes of the intermediate zone and the peripheral part of the central zone of the hepatic lobule the intensity and extent of staining of the system of the endoplasmic reticulum were less than in the periportal hepatocytes (Fig. 2b). Besides stained cisterns of the reticulum, others which were unstained or segmentally stained were found. The number of stained groups of the lamellar apparatus in the hepatocytes was visibly reduced, but the intensity of their staining remained quite high. In hepatocytes forming a ring of one or two layers around the central veins, deposition of reaction product was almost absent (Fig. 2c). Only traces of staining of individual cisterns could be seen in the endoplasmic reticulum. Single weakly stained groups of the lamellar apparatus were found only in the region of the bile capillaries. In the normal mouse, liver albumin was found also in the endothelium of the venous sinuses, where the reaction product sometimes stained single large vacuoles (Fig. 2d).

In the residual hepatocytes of the periportal, intermediate and, to some extent also, the central zones of the hepatic lobules of the regenerating liver, intensive and extensive staining of the perinuclear space of the endoplasmic reticulum and lamellar apparatus could be seen, without any visible decrease in its intensity from the periphery toward the center of the lob-

ules (Fig. 3a). Moreover, weak staining of cisterns of the rough endoplasmic reticulum, the lamellar apparatus, and cytoplasmic vesicles in the epithelial cells of the periportal bile ducts was found in the regenerating liver (Fig. 3a, b).

Immune electron microscopy of the normal mouse liver thus showed that all hepatocytes contain albumin, but the amount of it visible varied considerably depending on the position of the hepatocyte in the lobule. It was also found that the reaction product is localized in the hepatocytes only in organelles connected with synthesis and secretion of "export" proteins [9]. Consequently, when the intensity and extent of staining of these organelles are compared in hepatocytes in different zones of the hepatic lobule it can be concluded that in the normal mouse liver albumin synthesis is distributed in the hepatocytes along a gradient falling from the periphery toward the center of the lobule. The reason why our data do not agree with the results of some immunocytochemical investigations, which found no gradient of albumin synthesis in the rat liver [5, 10, 13], is not yet understood. Most probably it is connected with the technical difficulties of immune electron microscopy.

It was shown previously that synthesis of some proteins and enzymes is carried on to an equal degree by all hepatocytes in the liver of newborn animals [4]. The development of heterogeneity of the hepatocytes in relation to their morphology and function takes place in the postnatal period and is linked with the formation of the definitive lobular structure of the liver [4]. Consequently, the discovery of intralobular differences in the distribution of synthesis of "adult" proteins, including albumin, can be used as a morphological criterion when the various levels of postnatal morphogenetic differentiation of the liver are to be evaluated and its final stage determined.

Our data show that absence of intralobular differences in albumin synthesis is characteristic of the regenerating adult mouse liver, imitating its similarity with the neonatal liver [4]. Possibly the "uncoupling" of the hepatocytes during regeneration of the liver [7] leads to loss of the gradient of synthesis of the secreted proteins.

When the results of this investigation were analyzed the answer to yet another very important question in hepatology was sought: Can albumin synthesis be used as a marker of hepatocytes? For the normal mouse liver this question can be answered in the affirmative. However, during proliferative processes in the adult liver and, in particular, during regeneration, albumin synthesis is not a distinguishing feature of the hepatocyte, as is shown by expression of this synthesis in the epithelial cells of the periportal bile duct.

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