STRUCTURE OF THE LIVER AS A POSSIBLE FACTOR IN THE REGULATION OF α-FETOPROTEIN SYNTHESIS

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During regeneration of the mouse liver after poisoning with CCl_4 and paracetamol, hepatocytes containing α-fetoprotein (αFP) lose their membrane antigen that served as marker for biliary capillaries. Both during regeneration and in early postnatal development, cessation of αFP synthesis by the cells coincides with the appearance of an antigen marking the biliary capillaries on their surface. It is suggested that cessation of αFP synthesis is the result of establishment of a system of contacts characteristic of the definitive hepatic column.

KEY WORDS: α-fetoprotein; membrane antigens; system of contacts; regeneration of the liver; ontogeny.

It is well known that synthesis of α-fetoprotein (αFP) – a carcinoembryonic antigen of the liver – is renewed during regeneration of this organ [1]. It was shown previously that αFP synthesis is renewed in mature differentiated hepatocytes even before they embark upon the cycle of DNA synthesis [9]. During the formation of extensive zones of necrosis as a result of poisoning with CCl_4, paracetamol, or allyl alcohol, most αFP-containing hepatocytes are found in the perinecrotic zone – where histological structural changes connected with destruction and subsequent repair of the hepatic columns are most intensive [4, 9]. The question arises whether the structure of the liver itself is a factor responsible for the regulation of αFP synthesis.

Changes in histological structure are always connected with changes in the established system of cell contacts. These changes are reflected in changes in the distribution of membrane antigens on the cell surface. Antibodies against two liver membrane antigens (Iag and IIag) are available [5, 6]. With the aid of these antibodies the state of the plasma membranes of αFP-containing cells was investigated during regeneration of the liver and in early postnatal ontogeny.

EXPERIMENTAL METHOD

The liver of adult C3HA mice was studied immunohistochemically on the 2nd-5th days after poisoning with CCl_4 vapor [2] or by intraperitoneal injection of paracetamol [4], and the liver of young SWR and AKR mice was similarly studied between the 1st and 14th days after birth. Pieces of liver 2-3 mm thick were fixed in an acetone–formalin–phosphate buffer mixture and embedded in paraffin as described previously [3]. Serial sections 3μ thick were treated by the indirect immunofluorescence method [8], using preparations of monospecific rabbit antibodies against αFP and Iag and monospecific rabbit antisera against mouse γ globulin and IIag, and also a fluorescent monkey antiserum against rabbit globulins produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology. The Iag and IIag were obtained by lysis of a suspension of liver cells by the nonpolar detergent Triton X-100. The presence of antigens in the extracts was tested by antisera against liver cell ghosts [6]. Purification was carried out in several stages: 1) Gel-filtration of the Triton extract on Sephadex G-200. Iag was found in fractions containing proteins with mol. wt. of about 160,000, including γ globulin, IIag in samples containing proteins with mol. wt. of about 60,000, including albumin. 2) Ion-exchange column chromatography of samples containing the antigens. Iag and IIag were adsorbed on DEAE-Sephadex in 0.005M Na-phosphate buffer, pH 7.0, and eluted from the column with a 2.5M NaCl salt gradient. 3) Contamination with serum proteins was removed with the aid of glutaraldehyde immunosorbents containing antibodies against pure preparations of the corresponding serum proteins. The purity of the resulting samples of antigens was verified by electrophoresis in polyacrylamide gel. Preparations

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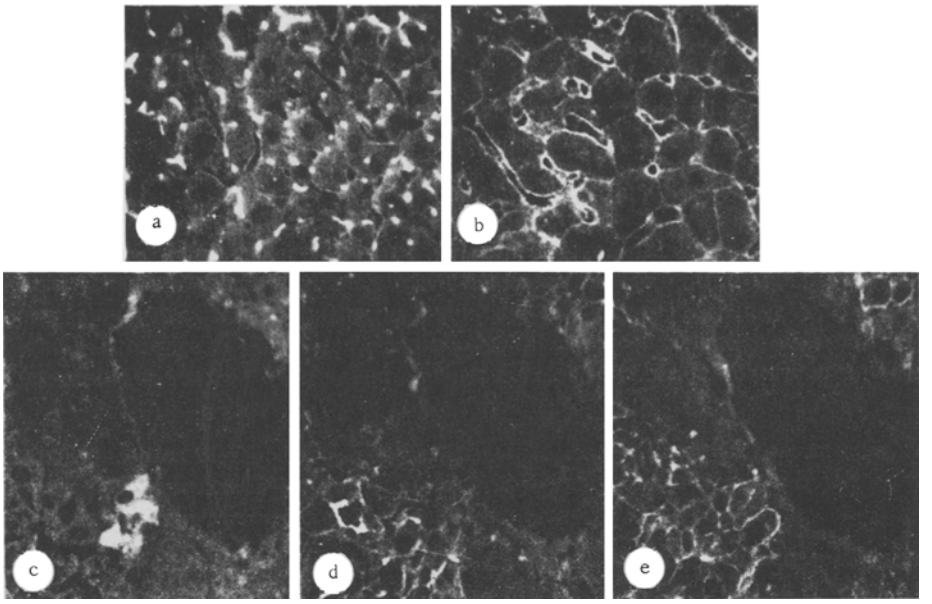

Fig. 1. Adult mouse liver: a, b) normal; a) Iag (160×), b) IIag (192×); c, d, e) liver regenerating after CCl_4 poisoning, 72 h, 3 consecutive serial sections (160×): c) αFP, d) Iag, e) IIag.

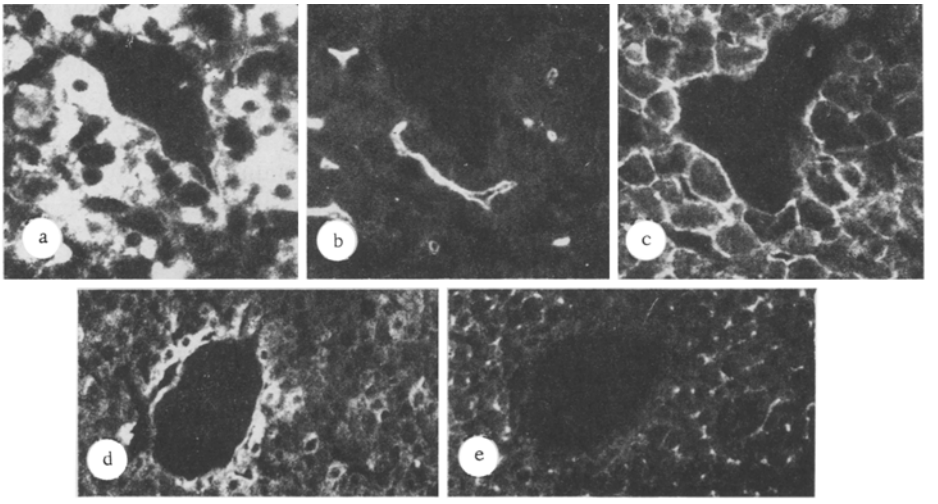

Fig. 2. Mouse liver during early postnatal development: a, b, c) first day after birth, three consecutive serial sections (320×): a) αFP, b) Iag, c) IIag, d, e) 11th day after birth, two consecutive serial sections (160×); d) αFP, e) Iag.

of the pure antigens were used to obtain monospecific antisera. Samples of monospecific antibodies were obtained on glutaraldehyde and Sepharose immunosorbents.* Treatment of one of the serial sections with antiserum against mouse γ globulin was used as the control for uptake of plasma proteins by the hepatocytes [10]. αFP-containing cells in which γ globulin was found were disregarded in the subsequent analysis. The specificity of fluorescence was verified by the use of monospecific antibodies and antisera neutralized with equivalent quantities of antigens.

*Antibodies against αFP were generously provided by N. V. Éngel'gardt and V. S. Poltoranina, and the antiserum against mouse γ globulin by O. M. Lezhneva.

EXPERIMENTAL RESULTS

The plasma membranes of all hepatocytes in the normal adult mouse liver contained Iag and IIag. Neither antigen is strictly specific for the liver, for they are also found in the intestine and kidney, but in the liver they are located only in the hepatocytes and not in the epithelium of the bile ducts nor in the endothelium or the Kupffer cells. Iag marks regions of the membranes of contacting hepatocytes forming a biliary capillary (Fig. 1a). In other words, it marks the highly specialized membrane structures specific for contacts between hepatocytes within each column. IIag is distributed over the whole membrane of hepatocytes. There is substantially more of it in the region facing the blood sinuses (Fig. 1b).

Poisoning with CCl_4 and also with paracetamol led to the formation of local zones of necrosis. When CCl_4 was used they were always strictly centrolobular and affected 40-45% of the hepatocytes in the lobule [11], but when paracetamol was used they were more extensive and not so regular [4]. Everything stated below applies equally to both types of regeneration, the only difference being that after paracetamol poisoning the course of regeneration was rather longer.

Synthesis of αFP after CCl_4 poisoning began soon after the foci of necrosis were completely formed, i.e., toward the end of the 1st day after poisoning. At that time it was sometimes possible to find single αFP-containing cells. A substantial number of them appeared toward the end of the 2nd day [9].

As a rule they were all located in the perinecrotic zone and, like all the remaining hepatocytes, they carried Iag and IIag on their surface. Toward the end of the 3rd day after CCl_4 poisoning the number of αFP-containing cells reached a maximum (5-7%) [9]. Meanwhile the necrotic areas were freed from remains of dying cells by structures of the reticuloendothelial system, and active replacement of the injured areas by hepatocytes began. All αFP-containing cells at that time were distributed among the cells replacing the damaged regions (Fig. 1c). Under these circumstances they lost their Iag but preserved their IIag (Fig. 1d, e). IIag was uniformly distributed over the membrane, but sometimes the quantity of it fell sharply, although it evidently did not disappear completely from the membrane. Later, when the foci of necrosis were entirely filled with hepatocytes, αFP disappeared and the normal distribution of Iag was restored. The same events took place, although rather more slowly, during paracetamol poisoning. It must be emphasized that not all hepatocytes that have lost Iag contain αFP but, starting from a definite moment, all αFP-containing cells lose their Iag. The larger the zone of cells without Iag, under these circumstances, the more numerous the αFP-containing cells in them.

A similar connection between the distribution of membrane antigens and the presence of αFP in the cell also was found in the first 2 days of life of the mice.

During the first days after birth, all hepatocytes in mice are known to contain αFP. The number of αFP-containing hepatocytes then falls, and by the end of the second week only one layer of these cells can be found near the central veins [7].

Examination of the sections in the present experiments showed that on the first day after birth all or nearly all hepatocytes contained αFP (Fig. 2a). IIag was uniformly distributed over the surface of all hepatocytes (Fig. 2c). The location of Iag was not exactly the same as in the adult liver. It outlined structures of glandular type formed by rosettes of cells, a unique kind of "biliary acini" (Fig. 2b). Later, after 3 days the number of αFP-containing cells decreased, a redistribution of Iag began, but the character of distribution of IIag remained unchanged. On the 6th-8th day marked attraction of the αFP-containing cells toward the central veins was observed, whereas Iag in the zones of greatest accumulation of αFP-containing cells was either absent or present in much smaller amounts. By the 11th-13th days the number of αFP-containing cells decreased. Only one layer of cells remained near the central veins (Fig. 2d). Iag was absent in this layer (Fig. 2e). Later αFP disappeared and Iag appeared. Disappearance of αFP and appearance of Iag coincided in time. They were followed by establishment of the characteristic distribution of IIag for adult cells.

The data described above show that the formation of specific contacts during construction of the columns of the liver, whether during ontogeny or during regeneration, coincides in time and topography with the "switching off" of αFP synthesis. This result is not surprising when regeneration is examined: the localization of the αFP-containing cells determines their unusual position in the system of contacts and their irregular, "sliding" form during the replacement of necrotic areas points to high membrane activity. The formation of the columnar structure of the liver in postnatal ontogeny involves all the hepatocytes. Changes here are less local. However, the differentiation here also of a pericentral zone containing αFP, and deficient in Iag, is evidence that the "switching off" of αFP synthesis in ontogeny may take place by a mechanism identical with that of regeneration, and very possibly connected with the formation of a definite system of contacts.

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