INDUCTION OF α-FETOPROTEIN SYNTHESIS IN NONPROLIFERATING HEPATOCYTES

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Synthesis of α-fetoprotein (α-FP) has regularly been found in populations of proliferating cells; in the yolk sac, in the embryonic and early postnatal liver, in hepatocellular tumors, and also during regeneration of the liver after extensive hepatectomy or poisoning with various hepatotoxins [1]. Data on α-FP synthesis in the resting hepatocyte population would be of fundamental importance because they would prove unambigously whether or not α-FP synthesis is connected with cell proliferation.

Meanwhile, data on the characteristic localization of α-FP in the liver regenerating after poisoning by hepatotoxins [3, 4, 8] suggested that induction of α-FP synthesis takes place through a disturbance of specific intercellular contacts in the hepatic column [1, 3]. To test this hypothesis investigations on models with different types of arbitrary damage to the structures of the columns were necessary.

The object of the present investigation was to study whether α-FP is synthesized in the mouse liver after minor injuries to the organs, for it was shown previously that after removal of a small quantity of liver tissue mitotic activity of the liver cells was extremely low and virtually indistinguishable from that in the normal liver [5].

EXPERIMENTAL METHOD

Experiments were carried out on male and female mice of the SWR, CC57BR, and BALB/c/J lines aged 2-3 months. The animals were anesthetized with hexobarbital and small pieces were removed from the left lobe of the liver (from 10 to 80 mg), or superficial injuries were inflicted with the blunt rounded end of a glass rod.

SWR and CC57BR mice were decapitated 1, 3, 4, 5, 6, 7, and 10 days after the operation; mice of the BALB/c/J line were decapitated three days after the operation. Pieces of liver adjacent to the zone of injury and pieces from undamaged lobes were fixed in a mixture of acetone, formalin, and phosphate buffer (in the ratio 9:5:6) [2].

α-FP was demonstrated in sections through the liver by the indirect peroxidase reaction using a complex of rabbit antibodies with peroxidase (PAP, from Dako, Denmark) [10]. Isolation of monospecific antibodies against α-FP and verification of their specificity was described previously [7]. Treatment of the next serial section with antiserum against mouse γ-globulin served as the control for nonspecific penetration of α-FP from the blood into the injured cells [8].* The α-FP concentration in the sera was determined by the agar diffusion method with a standard test system [6].

Proliferative activity of the hepatocytes was studied on three SWR mice receiving saturation labeling with ^{3}H-thymidine (specific activity 21.4 Ci/mmole in a dose of 0.2 μCi/g body weight hourly for 3 or 6 days. A uniform supply of ^{3}H-thymidine was ensured by means of a special miniature osmotic pump (the Alzet TM, from Alza Corp., USA), which was introduced into the animal's peritoneal cavity during the operation. In mice receiving saturation labeling, pieces of small intestine also were fixed to monitor incorporation of ^{3}H-thymidine into dividing cells. To detect proliferating cells, after peroxidase staining the sections were

*The antiserum against mouse α-FP was generously provided by A. K. Yazova and antiserum against mouse γ-globulin by O. M. Lezhneva.

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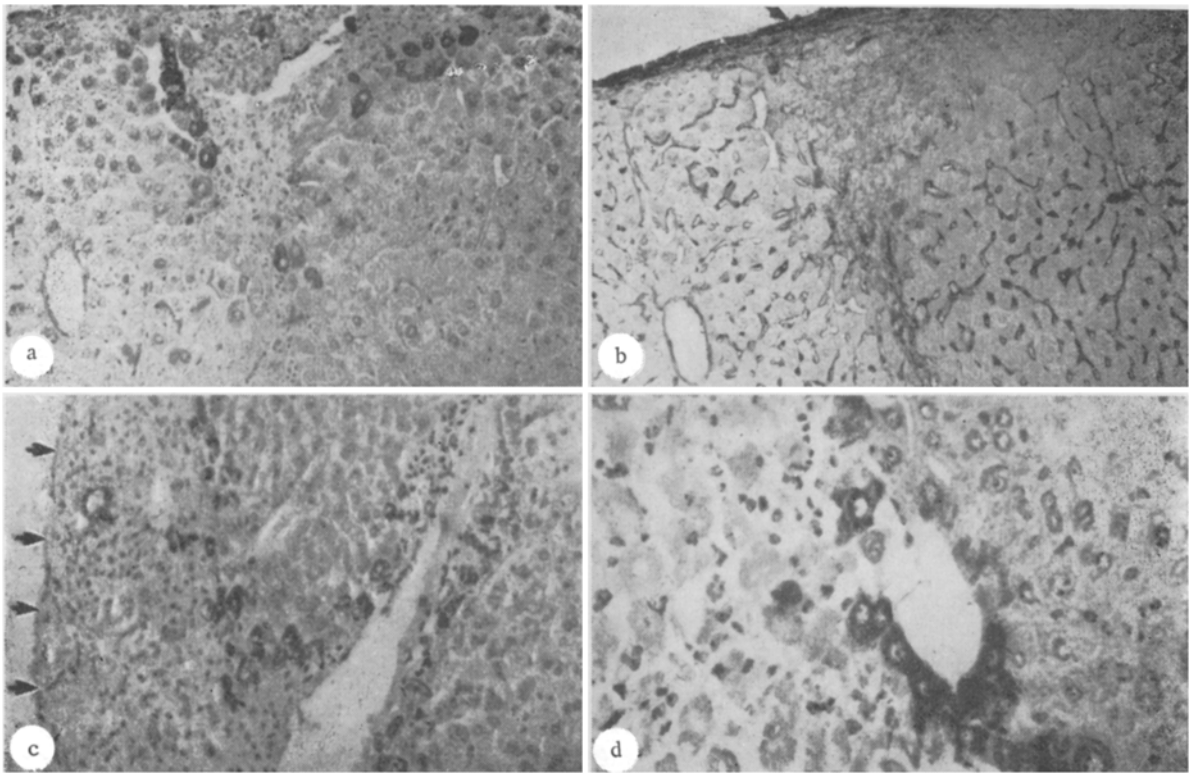

Fig. 1. α-FP and γ-globulin in mouse liver sections three days after operation. a,c, d) α-FP; b) γ-globulin. a, b) mechanical injury (160 ×); c, d) distal removal of part of left lobe (magnification 144 and 225 ×, respectively). Arrows indicate surface of injury.

coated with type M photographic emulsion. The autoradiographs after development were stained with Mayer's hematoxylin and mounted in balsam.

EXPERIMENTAL RESULTS

No α-FP could be found by the gel diffusion method in the blood serum of any of the experimental animals.

Morphological investigation of the liver in the postoperative period revealed signs of inflammation in the zone of injury. No cells containing α-FP could be found either in the injured or in the intact lobes of the liver 24 h after the operation. However, on the 3rd-4th day α-FP-containing hepatocytes were found in the liver of all the animals studied, in areas immediately adjacent to the foci of inflammation (Fig. 1). The intensity of staining varied, but α-FP-containing hepatocytes with low or average intensity of staining were predominant. The most brightly stained cells as a rule were concentrated near vessels lying close to the zone of inflammation. Sometimes α-FP containing hepatocytes were arranged along the walls of these vessels, sinking into areas with intact parenchyma (Fig. 1c). In zones remote from the injured surface and also in intact lobes, solitary palely stained cells with α-FP were found near some vessels.

The character of distribution of α-FP-containing cells was the same for mice of all three lines studied. Differences were found in the number and intensity of staining of these cells. The clearest pattern of α-FP distribution was observed in BALB/c/J mice, which had the highest serum α-FP level both under normal conditions (1 μg/ml) and during regeneration of the liver (1 mg/ml) [9].

On the 5th day the number of α-FP-containing hepatocytes decreased, and in some animals at this time no α-FP-containing cells were seen. On the 6th day only solitary palely stained hepatocytes could be seen, adjacent to the zone of inflammation (Fig. 2b). On the 7th-10th day, no α-FP-containing cells were found.

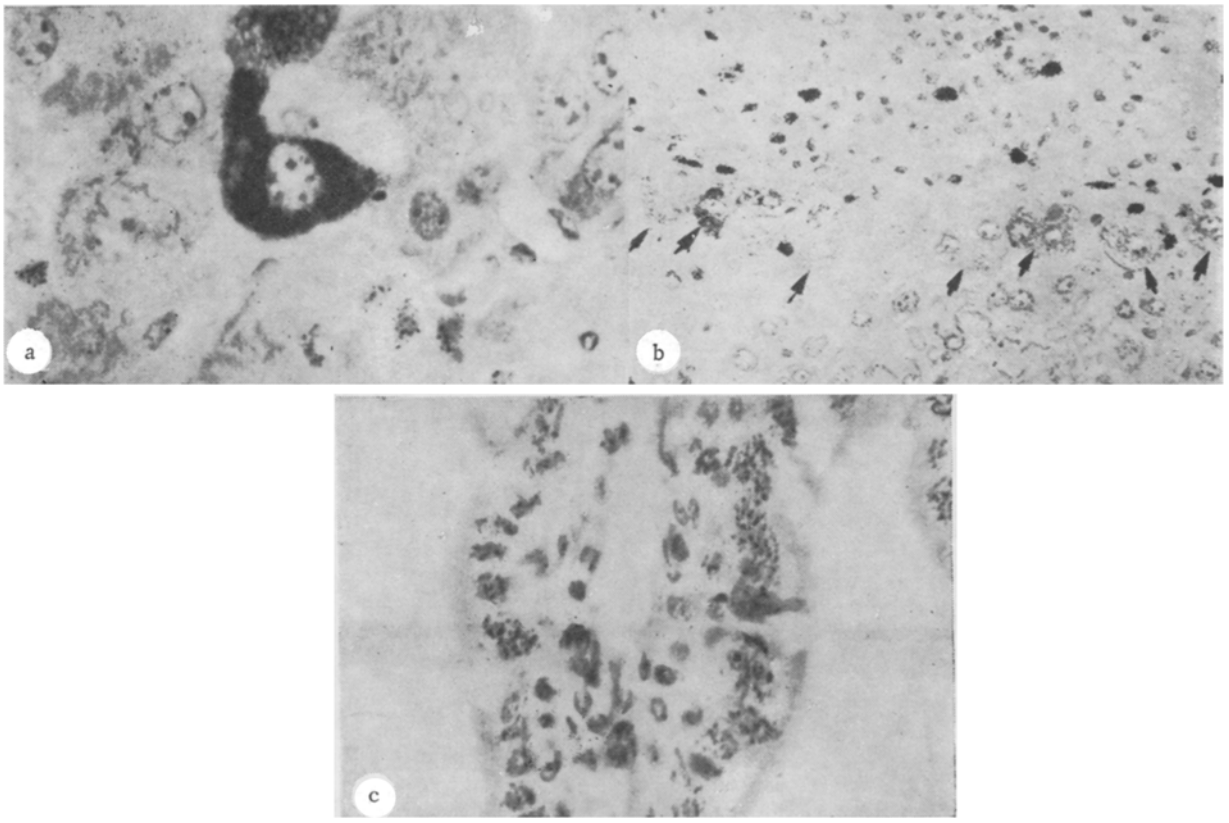

Fig. 2. Distribution of α-FP and ^{3}H-thymidine in sections through liver and small intestine after distal removal of parts of left lobe. a) Section through liver 3 days after operation (540 ×); b) liver, 6 days after operation. Arrows indicate α-FP-containing hepatocytes (225 ×); c) section through villus of small intestine (540 ×).

Analysis of the autoradiographs showed that cells of the small intestine and cells of the reticuloendothelial system of the liver, especially in zones of inflammation, incorporated ^{3}H-thymidine intensively during saturation labeling (Fig. 2). The overwhelming majority of hepatocytes contained no label (Table 1). Solitary hepatocytes which incorporated ^{3}H-thymidine were distributed near the liver capsule, in regions adjacent to the zones of injury, and near the portal vein. The number of labeled hepatocytes in both injured and intact lobes was approximately the same (Table 1). On the 3rd day after the operation labeled nuclei were more frequently seen in the population of α-FP-containing hepatocytes than in the general mass of hepatocytes (Table 1). However, the very small percentage (about 1) of hepatocytes which incorporated ^{3}H-thymidine among the total number of α-FP-containing cells suggests that α-FP synthesis is not connected with hepatocyte proliferation. This was revealed more clearly still when the distribution of labeled nuclei was studied 6 days after the operation. None of the hepatocytes in which α-FP was found contained ^{3}H-thymidine, and in the area adjacent to the zone of injury the percentage of labeled hepatocyte nuclei was no higher than on the third day (Table 1).

The results thus showed that α-FP synthesis is unconnected with hepatocyte proliferation and can take place in mature resting cells. The patterns of dynamics of α-FP-containing cells thus revealed, together with their clearly defined localization on the boundary with the zone of inflammation, are convincing evidence that the same factors cause α-FP synthesis both in the model used in this investigation and during regeneration of the mouse liver after poisoning with various hepatotoxins. One cause of the resumption of α-FP synthesis in hepatocytes is evidently a disturbance of the trabecular structure of the liver [1, 3].

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TABLE 1. Proliferative Activity of Hepatocytes of the General and α-FP-Containing Population

Index	Time after operation				
	3 days				6 days
	distal removal of part (50 mg) of left lobe of liver		mechanical injury		distal removal of part (80 mg) of left lobe of liver
	injured lobe	intact lobe	injured lobe	intact lobe	injured lobe
No. of heptatocyte nuclei in general population*:					
total	11 500	8000	7000	5000	10 000
with ^{3}H-thymidine	5	6	4	4	7
No. of hepatocyte nuclei in α-FP-containing population†:					
total	276	—	249	—	28
with ^{3}H-thymidine	2	—	3	—	0

*Total number of hepatocyte nuclei counted in one or two nonserial sections through the liver.

†Total number of nuclei of α-FP-containing hepatocytes counted in 5-8 nonserial sections through the liver.

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