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CHARACTERISTICS OF HEPATOCYTES CONTAINING α-FETOPROTEIN IN REGENERATING MOUSE LIVER

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After poisoning SWR mice of different ages with single or repeated doses of CCl_4 vapor the synthesis of the embryo-specific protein α-fetoprotein (α-FP) was induced. The greatest rise in the α-FP level was observed in mice under 1 month old. In sections through the liver regenerating after CCl_4 poisoning, α-FP was found in hepatocytes indistinguishable from the main population: in small cells in young animals and in large, polyploid hepatocytes in the repeatedly poisoned mice. The only distinguishing feature of the α-FP-containing cells after poisoning of the mice with different doses of CCl_4 was that most of them were on the boundary with the necrotic zone. A similar localization of α-FP-containing hepatocytes was observed when two other hepatotoxins were used: paracetamol and allyl alcohol.

KEY WORDS: *α-fetoprotein; immunofluorescence; hepatocytes; regeneration of the liver.*

Synthesis of the embryonic serum protein α-fetoprotein (α-FP) is renewed in the regenerating mouse liver after carbon tetrachloride poisoning [1, 3]. The writers showed previously that under these conditions α-FP is contained in typical mature hepatocytes, which amount to not more than a few percent of the total number of undamaged cells and are located chiefly at the boundary with the necrotic zone [7].

The object of this investigation was to study the localization of α-FP in the regenerating mouse liver depending on the age of the animals, the degree of maturity of the hepatocytes, and the character of liver damage.

EXPERIMENTAL METHOD

Regeneration of the liver in SWR mice (male and female) of different ages (from 13 days to 7 months) was induced by poisoning with CCl_4 vapor in a concentration of 0.005 ml in 3 liters air [3] or 0.003 and 0.001 ml in 3 liters air. Allyl alcohol and paracetamol were injected intraperitoneally in physiological saline in doses of 5 and 40 mg/100 g body weight, respectively [10, 11]. On the 2nd or 3rd day after poisoning the mice were decapitated and the blood levels of α-FP determined by the precipitation reaction with a standard test system [4]. Repeated CCl_4 poisoning was carried out with intervals of not less than 18 days between doses. On the second to third day after each dose of the poison, blood samples were taken from the retro orbital sinus of the mice for determination of the α-FP concentration. The mice were killed after the 1st, 2nd, 3rd, 4th, 6th, 7th, and 9th doses of the poison. The localization of α-FP and γ-globulin was studied in paraffin sections (3 μ) of the liver by the indirect immunofluorescence method [12]. Pieces of liver were fixed in a mixture of ethanol with acetic acid at 4°C. The technique of processing of the material was described

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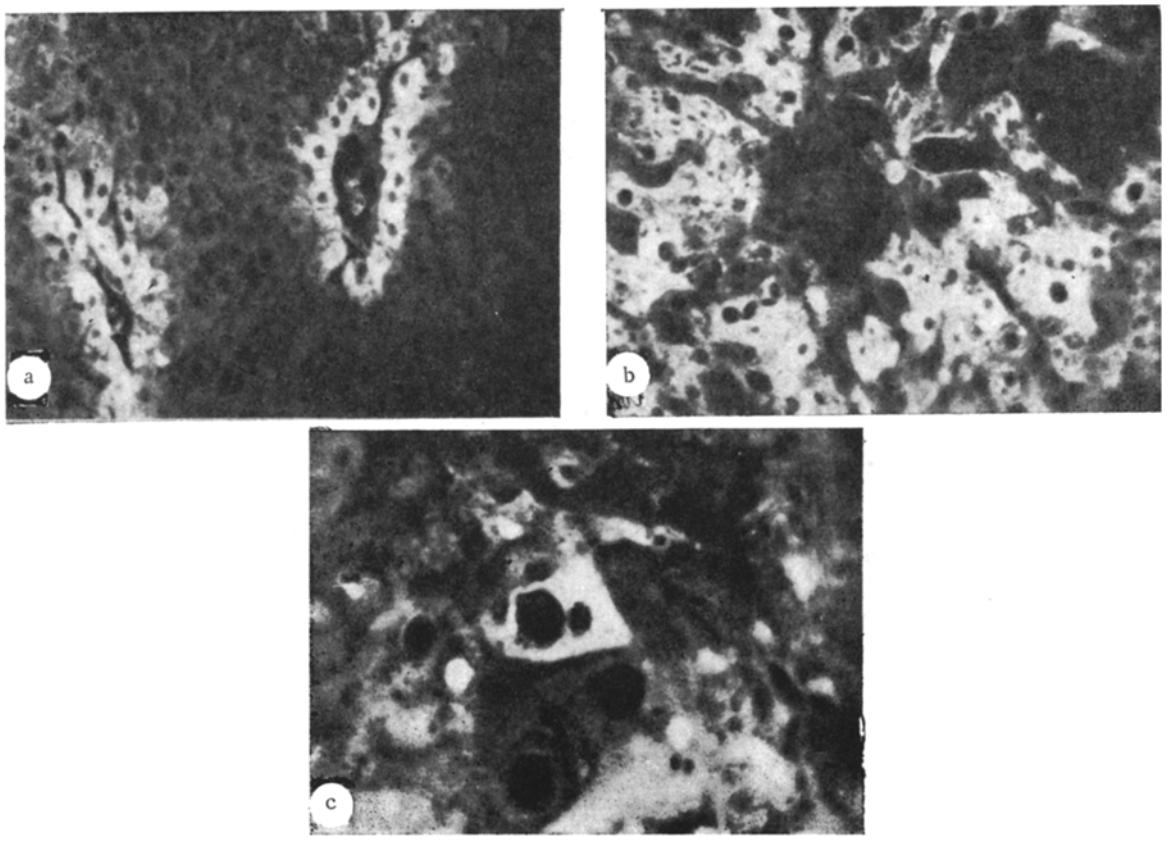

Fig. 1. Detection of α-FP in hepatocytes of different ploidy in mouse liver regenerating after CCl_4 poisoning. a, b) Sections through liver of 13-day-old mice of one litter before and 2 days after CCl_4 poisoning; c) section through liver of 6-month-old mouse on third day after seventh dose of CCl_4. All sections treated with antibodies against mouse α-FP. Objective 40×, homal 3×.

previously [6]. The preparation of mouse α-FP, purified by electrophoresis in polyacrylamide gel, and the antiserum against it [12] were generously provided by A. K. Yazova. The methods of isolating monospecific antibodies against α-FP and of testing their specificity by neutralization of α-FP were described previously [6]. Monospecific antiserum against γ-globulin was obtained by O. M. Lezhneva by immunization of rabbits by injection into the lymph nodes. Serial sections of the liver were stained for α-FP and for γ-globulin, the serum marker. The presence of α-FP in the cells not containing γ-globulin was regarded as specific [6, 7]. Sections stained for α-FP, after examination and photography, were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The central lobules of the mouse liver and the most highly differentiated cells, able to metabolize carbon tetrachloride, are damaged in CCl_4 poisoning. Regeneration of the liver is complete 12-15 days after poisoning, and repeated poisoning during that period does not induce the formation of zones of necrosis [9]. After CCl_4 poisoning regeneration of the liver accompanied by induction of α-FP synthesis was observed in SWR mice of different ages. In the youngest mice, aged 13 days, postnatal α-FP synthesis still continued and a high level of α-FP was found in the blood, and it rose even higher after poisoning. In sections through the liver of normal mice of this age, as described previously [5], α-FP-containing hepatocytes were located only around the central veins (Fig. 1a). On the second day after CCl_4 administration, foci of centrilobular necrosis in the poisoned mice occupied the region where these cells were situated, and α-FP was found in approximately 30% of the undamaged hepatocytes located chiefly in the center of the lobules (Fig. 1b). α-FP synthesis usually ceases three weeks after birth [5]. Poisoning of mice aged three weeks with CCl_4 also was accompanied by the appearance of high α-FP concentrations in their blood. On the second to third day after poisoning α-FP-containing cells accounted for 15-20% of their total number of undamaged parenchymatous cells, compared with not more than 5-7% in mice aged 2-3 months.

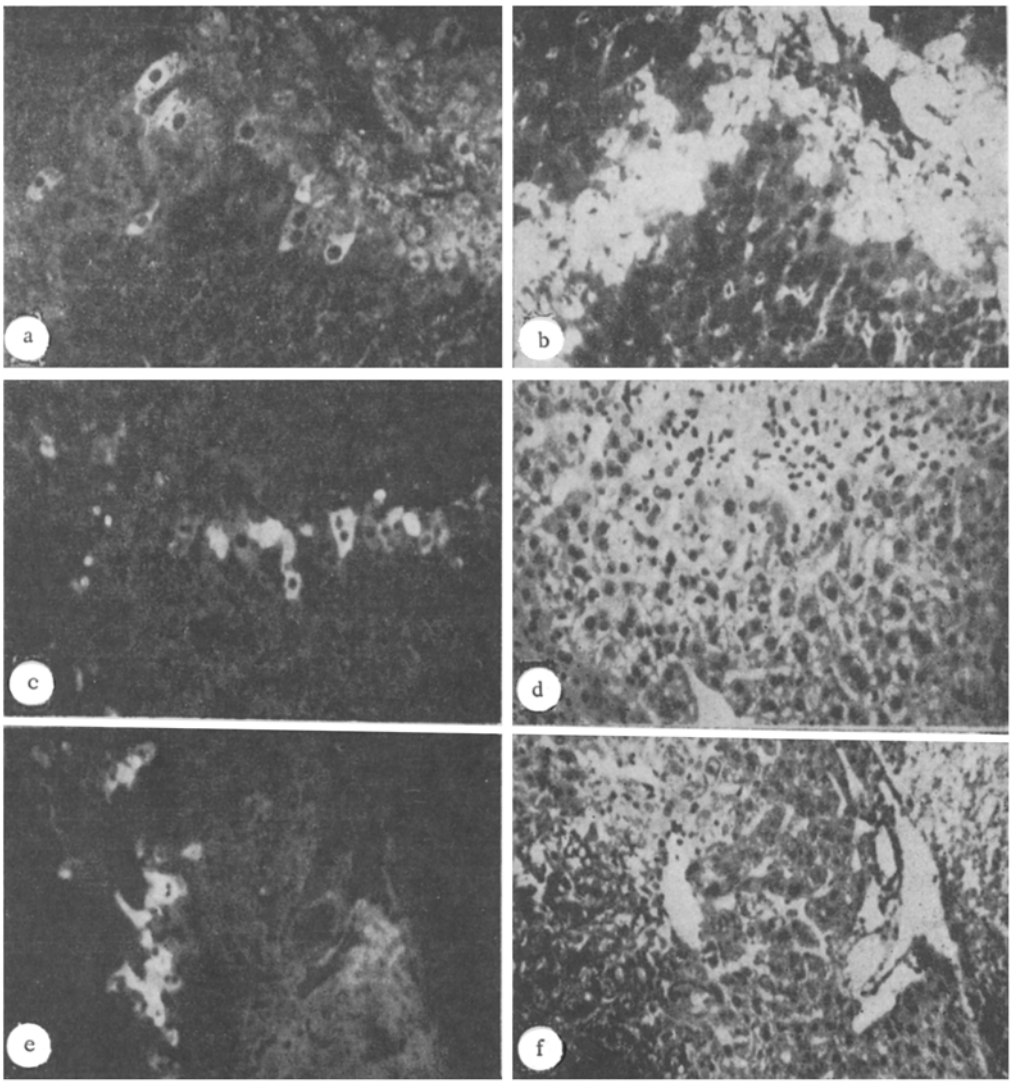

Fig. 2. Location of α-FP-containing hepatocytes in liver of mice poisoned with CCl_4 (a, b), allyl alcohol (c, d), and paracetamol (e, f). a, c, e) Sections through liver on second day after poisoning; incubated with antibodies against α-FP; b) neighboring section to section a, treated with antibodies against γ-globulin; d, f) sections c and e stained with hematoxylin—eosin; a, b, c, e, f) objective 20×, homal 3×; d) objective 25×, ocular 10×.

In some animals α-FP-containing cells were found only in the periportal region. In most mice in all the experiments the main site of α-FP-containing hepatocytes was the zone immediately adjacent to the zone of centrilobular necrosis (Fig. 2a, b). With a reduction in the poisoning dose of CCl_4 from 0.005 to 0.003 ml/3 liters air the area of the zones of necrosis was reduced, as was also the quantity and intensity of fluorescence of the α-FP-containing cells, but the α-FP-containing hepatocytes revealed were also located on the boundary of the growing foci of necrosis. In mice receiving 0.001 ml CCl_4/3 liters air, no α-FP was detected in the blood on the second day after poisoning, no foci of necrosis were found in sections through the liver, and only foci of weak infiltration were observed around the central veins.

The foci of necrosis were removed and replaced by intact hepatocytes. Toward the end of regeneration the zone of cells bordering on the zone of necrosis lies in the center of the lobules [8]. To discover whether during repeated poisoning of mice with CCl_4 cells capable of producing α-FP are eliminated, the animals were exposed to the repeated action of CCl_4. Sixty mice were used. CCl_4 was given at long intervals between doses so that all sessions of poisoning were followed by the formation of necrosis. In most animals induction of α-FP synthesis was observed after each dose of the poison. However, in six mice α-FP ceased to be detectable by the precipitation test after the 3rd, 8th, 9th, and 10th doses, and in two mice

after the 11th dose of the poison. No hepatomas were found at autopsy in any of the animals. Repeated poisoning followed by regeneration of the liver led to polyploidization of the hepatocytes, which were considerably larger than the normal cells characteristic of the liver of adult mice. α-FP was found in a few cells in the repeatedly poisoned animals, in polyploid hepatocytes (Fig. 1c).

In all the animals the α-FP-containing hepatocytes were morphologically indistinguishable from the general population: In the young mice they were small cells, whereas in repeatedly poisoned animals they were large hepatocytes with artificially increased ploidy. Location on the boundary with the zone of necrosis was the only feature distinguishing most the α-FP-containing hepatocytes from cells not containing α-FP. This arrangement of the α-FP-containing cells is presumably explained by some specific feature of the action of CCl_4. However, in mice poisoned with two other hepatotoxins, namely paracetamol and allyl alcohol, a high level of α-FP was found in the blood on the second to third day after poisoning, and α-FP-containing hepatocytes were arranged in the liver sections in bands along the extensive areas of necrosis (Fig. 2c-f). After poisoning with paracetamol, discrete α-FP-containing cells with pale fluorescence also were found along vessels, evidently central veins. In the writer's view, the localization of α-FP in the regenerating mouse liver corresponds to the sites of its synthesis, for correlation is found between the number and intensity of fluorescence of the α-FP-containing cells and the dynamics of the blood α-FP level in the poisoned animals. The characteristic perinecrotic locations of α-FP-containing cells suggests that the induction of α-FP synthesis in the regenerating mouse liver takes place either because of sublethal cell damage or because of disturbance of specific intercellular contacts in the regions of the trabecula destined to undergo necrosis [2]. Experimental verification of these hypotheses is now in progress.

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