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CHALONES OF THE LIVER

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Injection of liver extract and blood serum of healthy intact mice and also of the blood serum from clinically healthy persons into CBA × C57BL hybrid mice sharply inhibits mitotic activity of hepatocytes in the liver regenerating after partial hepatectomy. Extracts of regenerating liver and blood serum of animals with a regenerating liver do not inhibit mitosis in hepatocytes. Blood serum from a patient with postnecrotic active cirrhosis of the liver not only did not inhibit mitoses in the hepatocytes but actually increased their number. It is suggested that the concentration of chalones is reduced in the cirrhotic liver.

KEY WORDS: chalones; regeneration of the liver; cirrhosis of the liver; blood serum.

Recent work has shown that cells contain, and probably produce, substances inhibiting mitotic activity in the same tissues. Bullough [4] has called these substances chalones. They have been shown to be tissue specific but not species specific.

By using liver extracts from adult intact animals some workers have induced inhibition of mitotic activity of hepatocytes in the regenerating liver [9-13]. Blood serum of adult intact animals has also been shown to have a chalone-like action. When injected into animals after partial hepatectomy, it inhibited the mitotic activity of the hepatocytes [3, 6, 8, 10].

The object of this investigation was to study the action of mouse liver extract and also of mouse and human blood sera on the regenerating liver.

EXPERIMENTAL METHOD

Liver extract was prepared by the method of Verly et al. [11]. The mice were decapitated and the livers removed and homogenized with water (in the ratio of 1:4) in a Potter's homogenizer. The resulting homogenate was centrifuged on the VAC-601 ultracentrifuge at 20,000 rpm for 30 min and the supernatant was drawn off and centrifuged again at 40,000 rpm for 100 min. All operations were carried out at 4°C.

To obtain serum, the blood was centrifuged at 3000 rpm for 10 min at 4°C.

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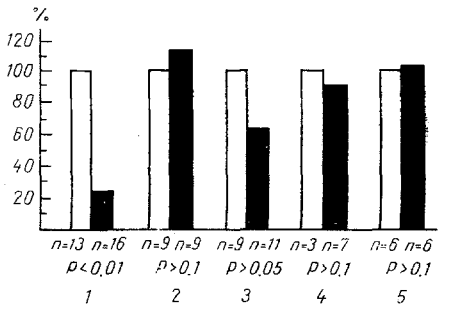

Fig. 1. Effect of liver extract on mitotic activity of regenerating mouse liver. Ordinate, mitotic index (shaded columns), in % of control (unshaded columns, 100%); abscissa: 1) liver homogenate from intact mice, 2) physiological saline, 3) homogenate of intact liver kept at −20°C for 12 days, 4) homogenate of intact liver heated to 60°C for 30 min, 5) homogenate of regenerating liver.

Tests were carried out on 224 male CBA × C57BL hybrid mice weighing 16-20 g. Two-thirds of the liver was resected by the method of Higgins and Anderson [7]. The operations were performed on all the animals at the same time of day (noon to 1 p.m.). Liver extract and blood serum were injected intraperitoneally into mice in a dose of 1 ml/100 g body weight 27-28 h after the operation. This time corresponded approximately to the ends of the presynthetic period and the beginning of DNA synthesis in mice after partial hepatectomy [1, 2]. The animals were killed 44-45 h after the operation, when mitotic activity had risen to its maximum.

Partially hepatectomized mice were used as the control for each experiment.

The liver was fixed in formalin–ethanol–acetic acid fixative. Paraffin sections 5 μ thick were stained with hematoxylin–eosin. The number of mitoses was counted in 3000 cells.

The significance of differences between the control and experimental results was determined by the U test.

EXPERIMENTAL RESULTS

Liver extract from intact adult mice (Fig. 1, 1) was found to inhibit mitotic activity of the hepatocytes in the regenerating liver sharply (by 77.2%).

To make sure that the effect of inhibition of mitotic activity was not simply the result of intraperitoneal injection of the fluid, 0.2 ml of physiological saline was injected into each of the groups of animals. No inhibition of mitotic activity was found in the regenerating livers of these animals (Fig. 1, 2). These results confirm that the livers of adult intact animals contain a factor which inhibits mitosis in hepatocytes.

Liver extract kept in a refrigerator at −20°C for 12 days inhibited mitotic activity by only 27% (Fig. 1, 3), whereas extract heated for 30 min to 60°C had no effect whatever on mitotic activity (Fig. 1, 4). It can be concluded that, like the epidermal chalone [5], the hepatic loses its antimitotic activity if kept for a long time and is completely inactivated by heat.

Unlike extract of intact liver, extract of regenerating liver (44 h after the operation) had no inhibitory action on mitosis (Fig. 1, 5); this is evidence that chalones are present in reduced amounts in the regenerating liver or are completely absent.

Blood serum of intact adult mice inhibited mitotic activity in the regenerating liver by 53.6% (Fig. 2, 1). Meanwhile, the blood serum of mice with regenerating livers had no effect (Fig. 2, 2).

Since chalones are species nonspecific, the action of human blood serum on the regenerating mouse liver was studied. Blood serum from three clinically healthy persons (Fig. 2, 3-4) sharply inhibited mitosis (by 87.8, 67.6, and 82% respectively) in the hepatocytes of mice after partial hepatectomy. The presence of a chalone-like factor in the blood serum of a person with a healthy liver was thus confirmed.

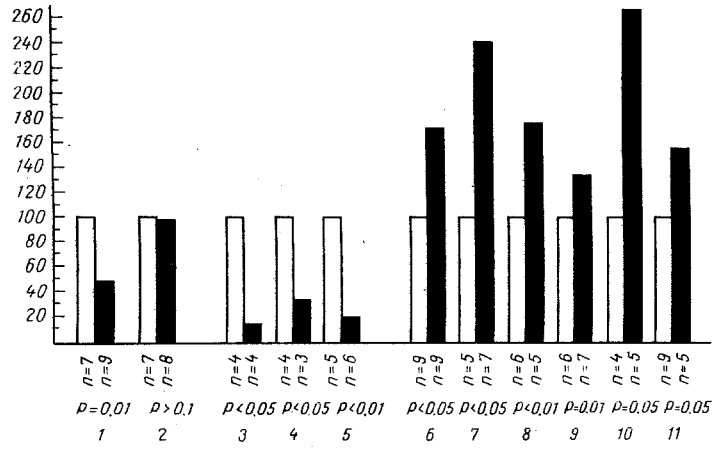

Fig. 2. Effect of blood serum on mitotic activity of regenerating mouse liver. Ordinate, as in Fig. 1; abscissa: 1) blood serum of intact mice, 2) serum of mice with regenerating livers, 3-5) healthy human blood serum, 6-11) blood serum of patients with cirrhosis of the liver.

One of the principal signs of cirrhosis of the liver is the formation of nodules of regeneration of the parenchyma. Accordingly, a decrease in the chalone content in the cirrhotic liver and absence of inhibition of mitosis in animals after injection of the patients' serum into them can be postulated. The action of blood serum obtained from six patients with postnecrotic active cirrhosis of the liver was studied. In no case did their serum inhibit mitosis but, on the contrary, after its administration the number of mitoses increased considerably (Fig. 2, 6-11).

Consequently, blood serum from patients with postnecrotic active cirrhosis of the liver had no antimitotic action, a result which confirms the view that the chalone content is reduced in the cirrhotic liver.

Besides a decrease in the chalone concentration, stimulation of mitosis may, perhaps, also be connected with certain humoral factors contained in patients' blood serum.

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