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TIME COURSE OF LIVER ACID PHOSPHATASE ACTIVITY DURING INVOLUTION OF CIRRHOSIS

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KEY WORDS: involution of cirrhosis of the liver; partial hepatectomy; acid phosphatase; hepatocytes; connective-tissue cells.

Collagenolytic activity of lysosomal enzymes has been found in the normal and cirrhotic liver in a number of biochemical investigations [2, 6-10, 12, 13, 15]. Changes in enzyme activity in the liver during cirrhosis also have been observed in the few histochemical studies which have been undertaken of this problem [3, 4, 11, 14].

To determine the link between lysosomal activity of various intracellular enzymes of the liver and the process of collagen resorption, changes in acid phosphatase (AP) activity were investigated by quantitative histochemical methods in the cirrhotically changed liver after cessation of exposure to the pathogenic factor causing cirrhosis.

EXPERIMENTAL METHOD

Cirrhosis of the liver was induced by injection of 0.2 ml of a 40% solution of CCl_4 in olive oil subcutaneously into noninbred male albino mice once a week for 5 months. The injections of CCl_4 were then stopped and animals developing cirrhosis were divided into two groups. The animals of group 1 underwent resection of the left lobe of the liver 5 days after the last injection of CCl_4. Animals of group 2 were not subjected to operation. Material for investigation was taken during resection and 5, 10, 20, 30, and 60 days after resection, concurrently in animals of both groups. Experiments were carried out on 30 animals (15 in each group).

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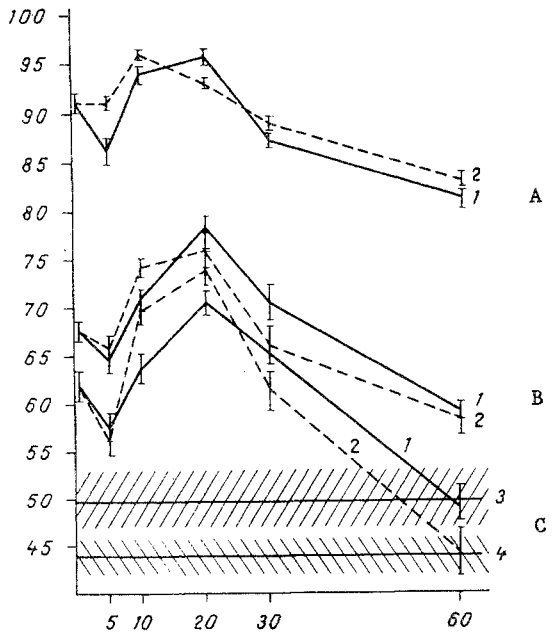

Fig. 1

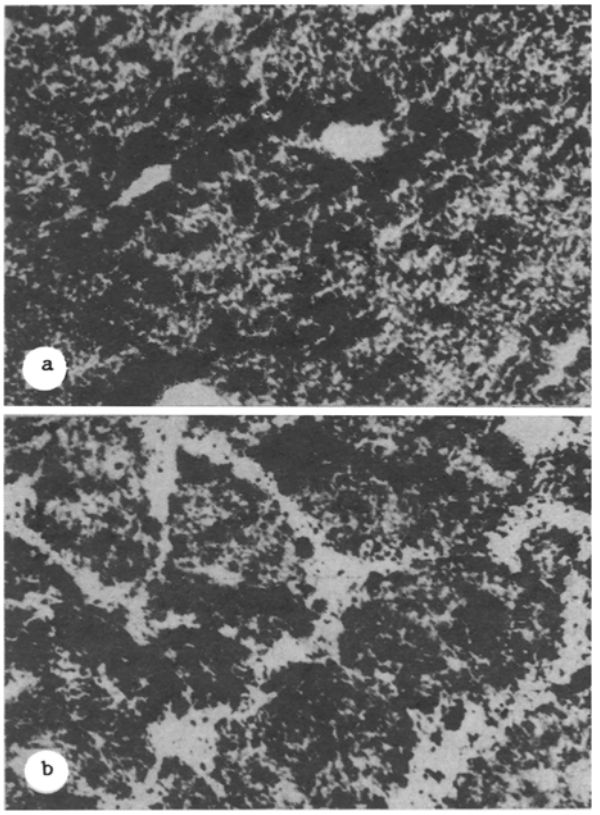

Fig. 2

Fig. 1. Time course of AP activity in liver during involution of cirrhosis. Ordinate, absorption of light (in %); abscissa, time after resection (in days). 1) Resection; 2) without resection; 3) control of total AP activity; 4) control of AP activity in hepatocytes. A) AP activity in connective-tissue cells; B) total AP activity; C) AP activity in hepatocytes.

Fig. 2. Reaction for AP in liver in cirrhosis: a) liver of intact mouse (control); b) cirrhosis. Here and in Fig. 3: magnification 80 x.

At each time of the investigation three animals from the first group and three from the second were studied. Material for histological investigation was fixed in 10% neutral formalin and sections were stained with hematoxylin and eosin and by Van Gieson's method. AP was demonstrated histochemically by the simultaneous azocoupling with naphthol phosphate AS-MX method [5]. Three preparations were obtained from each sample of liver, each containing three sections (27 sections altogether in a group at each time of investigation). In all cases the reaction was carried out simultaneously in experimental and control (liver of intact mice) material. AP activity was determined quantitatively with the aid of an MPV microscope-photometer (Leotz, West Germany). Measurements (27 in each case) were made in transmitted light with a wavelength of 540 nm [1]. In each section the quantity of light absorbed by the test zone was determined as a percentage by the equation:

$$A = \frac{C_0 - C_1}{C_0} \cdot 100,$$

where A is the absorption of light, C_0 the intensity of light above a region of the preparation not involved in the section (background intensity), and C_1 the intensity of light above the test zone.

EXPERIMENTAL RESULTS

AP activity in the hepatocytes at the height of development of cirrhosis was 41% higher ($p<0.001$) than in the control (Figs. 1 and 2), but 5 days after resection, AP activity in the groups investigated was lower ($p<0.05$) than at the height of development of cirrhosis. There was no difference between the groups. On the 10th day after resection AP activity was sharply increased ($p<0.01$ for both groups); much more so ($p<0.01$), moreover, in animals not subjected

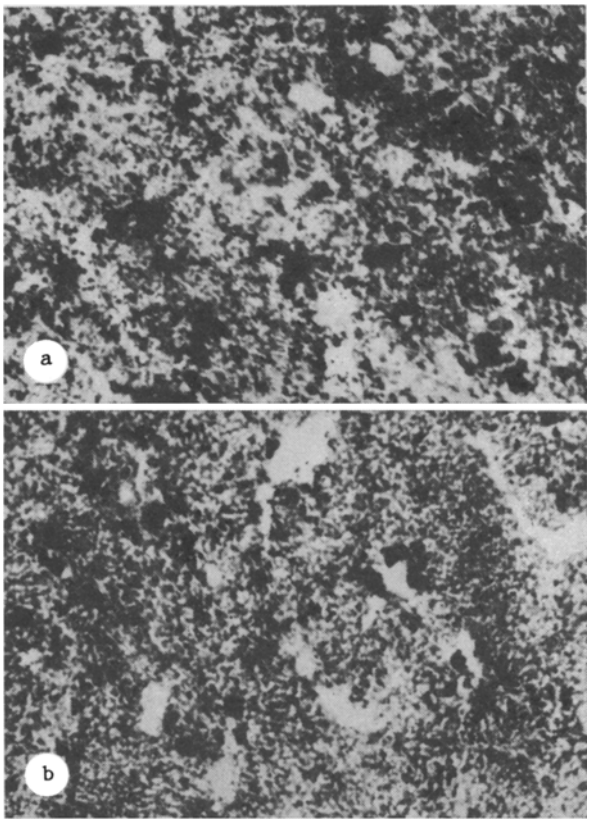

Fig. 3. Reaction for AP in liver 10 (a) and 20 (b) days after resection. Many concentrations of connective-tissue cells with high enzyme activity (black masses); b) high enzyme activity in hepatocytes. Few concentrations of connective-tissue cells with high AP activity.

to the operation (group 2) than in mice undergoing resection (group 1) (Fig. 1, Fig. 3a). AP activity reached a maximum 20 days after resection ($p<0.0001$) when it was 68% higher than in the control, and again there was no difference between the groups (Fig. 1, Fig. 3b). Later, until the 30th day enzyme activity in the animals of group 1 fell gradually ($p<0.01$), after which it returned toward normal 60 days after resection. Enzyme activity in animals not undergoing the operation fell sharply, and by the 30th day after resection it was significantly lower than in group 1 ($p<0.05$), and reached control values after 60 days.

The connective-tissue cells of the fibrous septa in both groups of animals showed high AP activity at all times of the experiment, despite a progressive decline in the number of these cells after 10-20 days of involution of cirrhosis. The difference between maximal and minimal activity throughout the period of regression of the process was only 5.5% ($p<0.001$; Figs. 1-3). In the animals of group 1, AP activity 5 days after resection was reduced ($p<0.01$) The number of connective-tissue cells also was reduced compared with that in the animals of group 2. By the 10th day, AP activity had risen sharply ($p<0.001$), although it was lower than in group 2 ($p<0.05$) at this same time, but reached a maximum 20 days after resection although the difference between the 10th and 20th days after the operation was not significant. AP activity then fell gradually ($p<0.001$). In the animals of group 2 AP activity, which did not fall in the period until the 5th day, reached a maximum (the level observed in the animals of group 1 20 days after the operation) 10 days after resection ($p<0.001$). After 20 days AP activity fell considerably compared with that observed at the previous time ($p<0.001$) and it was much lower than in the animals of group 1 ($p<0.05$). Later during the investigation the same trend was observed as in the animals of group 1, and there was no difference between the groups.

It was impossible in this investigation to measure AP activity separately in the stellate reticuloendotheliocytes because of the absence of any reliable criteria for distinguishing them from the other numerous connective-tissue cells found in the liver during cirrhosis. Activity of the enzyme in the stellate reticuloendotheliocytes was included in the total AP activity in the liver.

Total AP activity in the liver in cirrhosis was 36% higher ($p<0.001$) than normally (Figs. 1 and 2). It was virtually unchanged in both groups of animals 5 days after resection, and then rose sharply in both groups until the 10th day ($p<0.001$); this parameter was higher, moreover, in mice not undergoing resection than in animals undergoing the operation ($p<0.01$; Figs. 1 and 3a). AP activity in both groups of animals 20 days after resection reached a maximum (56% above the normal), but it increased significantly ($p<0.001$) compared with the previous period of investigation only in the animals of group 1 (Figs. 1 and 3b). Total AP activity in the liver 1 month after the operation showed a considerable decrease compared with that at the previous time in the animals both of group 1 ($p<0.01$) and of group 2 ($p<0,001$), but 2 months later it was reduced even more compared with the previous time ($p<0.001$ for both groups of mice), although remaining higher in all the experimental animals than in the control ($p<0.05$). At the last two times of the experiment there was no difference between activity of the enzyme in the groups.

AP activity both in the hepatocytes and in the connective-tissue cells thus increased earlier in the process of evolution of cirrhosis in animals not subjected to resection than in mice undergoing the operation, but to judge by the total AP activity, and also its activity in the hepatocytes, this delay in the animals of group 1 is apparently compensated by the slower decline in enzyme activity in the hepatocytes (Fig. 1).

These results are evidence that partial resection in cirrhosis has no effect in principle on the time course and level of total activity of lysosomal hydrolytic enzymes in the liver during involution of the process.

The greatest reduction in the thickness of the fibrous septa was observed in histological preparations during the first 3 weeks of regression of cirrhosis. AP activity, reflecting activity of lysosomal hydrolytic enzymes taking part in collagen resorption in the liver also increased and reached a maximum 3 weeks after resection. This is evidence of close interaction between lysosomal activity of the hepatocytes and of the connective-tissue cells of the liver and collagen resorption during the involution of cirrhosis, and also that the most intensive lysis of collagen takes place during the first 3 weeks of regression of the process. This applies equally to both groups of experimental animals.

To judge by the trend and levels of total AP activity and also by the histological picture, no differences in principle could be found between spontaneous regression of cirrhosis after discontinuation of CCl_4 administration and regression preceded by partial resection of the liver.

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EFFECT OF PLANT GROWTH REGULATORS — HYDRAZINE DERIVATIVES — ON MICROSOMAL SYSTEMS OF THE LIVER

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KEY WORDS: p-hydroxylation of aniline; N-demethylation of aminopyrine; superoxide dismutase; lipid peroxidation; hydrazine derivatives.

Among the plant growth regulators used in agriculture, the hydrazine derivatives are attracting increasing interest. However, the mechanism of their toxic action on the body has not been adequately studied. In particular, there are no data on their effect on microsomal mono-oxygenase systems of the liver. However, these systems are known to perform the function of chemical protection of the organism.

Hydrazines belong to the class of inhibitors of microsomal cytochrome P-450, and they act indirectly through their metabolites [11]. It has been shown that the toxic effect of hydrazine is realized through the formation of intermediate compounds of free-radical nature in the course of oxidative metabolism [9]. During microsomal oxidation of hydrazine derivatives, oxygen radicals are formed [4]. The effect of several plant growth regulators which are hydrazine derivatives was studied on hydroxylase reactions of microsomes, on their superoxide dismutase (SOD) activity, and on lipid peroxidation (LPO).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200 ± 20 g. Animals of the experimental groups were given Gidrel (hydrazinium 2-chloroethyl-bis-phosphonic acid), LD_{50} 2200 mg/kg body weight, Digidrel (dimethylhydrazinium 2-chloroethyl-bis-phosphonic acid), LD_{50} 3350 mg/kg, DSA (Daminozide; N,N'-dimethylhydrazide of succinic acid), LD_{50} 10,000 mg/kg, and MH (maleic hydrazide sodium salt), LD_{50} 15,000 mg/kg, in doses corresponding to 0.1 and 0.05 LD_{50}, perorally, once only.

The animals were decapitated 24 h after administration of the compounds. The microsomal fraction was obtained from liver homogenate by differential centrifugation [2]. The velocity of p-hydroxylation of aniline in the microsomes was determined from the quantity of p-aminophenol formed [10]. Cumyl hydroperoxide (CHP) was used as the cosubstrate [2]. The velocity of N-demethylation of aminopyrine in a CHP-dependent system was estimated from the accumulation of formaldehyde in the incubation medium, using the color reaction in [6]. SOD activity was determined by the method in [9], based on the ability of microsomes to inhibit the color reaction of reduction of nitroblue tetrazolium with the formation of formazan. The state of LPO was determined by the level of malonic dialdehyde (MDA) [1]. The protein concentration in the microsomal suspension was determined by Lowry's method [5], using bovine serum albumin as the standard. Binding of substrates with the oxidized form of cytochrome P-450 was determined by measuring the differential absorption spectra of the microsomes.

EXPERIMENTAL RESULTS

The hydrazine derivatives of phosphonic acid, Gidrel and Digidrel, in a dose of 0.1 LD_{50} slowed the velocity of aniline hydroxylation by more than half and demethylation of aminopyrine by 1.5-2 times. At the same time, SOD activity of the microsomes was inhibited and LPO decreased.

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