ENZYME TESTS FOR MONITORING LIVER FUNCTION DURING TREATMENT OF CARBON TETRACHLORIDE POISONING

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An attempt was made to diagnose the character of the liver lesion by determining the activity of the enzymes fructose-1-phosphate adolase, pseudocholinesterase, and phosphomonoesterase-1. The diagnostic value of these enzymes was tested in experiments after administration of poisons and ligation of the bile duct. These same enzyme tests were used to assess the efficacy of chemotherapy in carbon tetrachloride (CCl_4) poisoning. By means of these enzymes it is possible to differentiate between various types of liver lesion arising through the action of hepatotoxic poisons and after ligation of the bile duct. By studying changes in the activity of these enzymes in the blood of animals receiving CCl_4 after preliminary treatment with phenobarbital and SKF-525A, the authors showed that fructose-1-phosphate aldolase reflects the severity of liver damage in CCl_4 poisoning.

Enzyme tests are widely used in the diagnosis of liver diseases [1]. However, many of the tests used in clinical practice (determination of alanine- and aspartate-aminotransferase, lactate dehydrogenase, etc.) are nonspecific and, in addition, a change in the activity of the enzymes used in the blood does not reflect the state of the liver function. More recently, attempts have been made to use enzyme tests to assess the functional state of the liver and to determine the character of its injury [2].

The writers have used three enzymes for this purpose: fructose-1-phosphate aldolase (F-1-P aldolase), pseudocholinesterase, and phosphomonoesterase-1. Normally F-1-P aldolase is absent from the blood and enters it only in acute liver necrosis [3]. Pseudocholinesterase is synthesized by the liver, and its activity in the blood falls sharply when necrotic and degenerative changes occur in the cell and the protein-synthetic function of the liver is disturbed.

An increase in phosphomonoesterase-1 activity in the blood of animals is evidence of a disturbance of the formation of bile and its excretion via the biliary tract.

To study whether the character of liver damage can be determined on the basis of changes in enzyme activity, experimental models of liver lesions were created: necrotic (by means of thioacetamide), mixed necrotic and degenerative (with CCl_4), fatty degeneration (with ethionine), and occlusion of the biliary tract (by ligation of the bile duct). These same enzyme tests were used to assess the efficacy of chemotherapy in CCl_4 poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats weighing 180-200 g divided into four groups: (one for each experimental model). Each group contained from five to eight rats. The animals of group 1 received CCl_4 by subcutaneous injection in a dose of 0.6 ml/100 g body weight, and the animals of group 2 received thioacetamide by intraperitoneal injection of the 2% aqueous solution in a dose of 15 ml/100 g body

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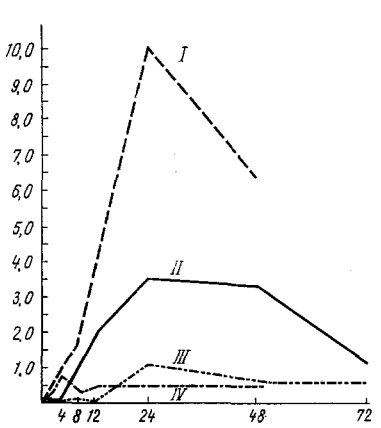

Fig. 1

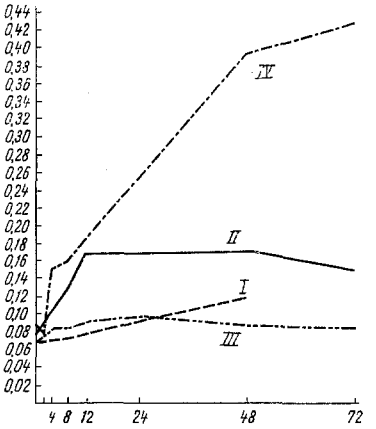

Fig. 2

Fig. 1. Change in F-1-P aldolase activity in blood serum of rats poisoned with CCl_4 (I), thioacetamide (II), and ethionine (III) and after ligation of bile duct (IV). Here and in Figs. 2 and 3: abscissa, time (in h); ordinate, activity (in moles/min/ml).

Fig. 2. Change in phosphomonoesterase-1 activity in blood serum of rats poisoned with CCl_4 (I), thioacetaminde (II), and ethionine (III) and after ligation of bile duct (IV).

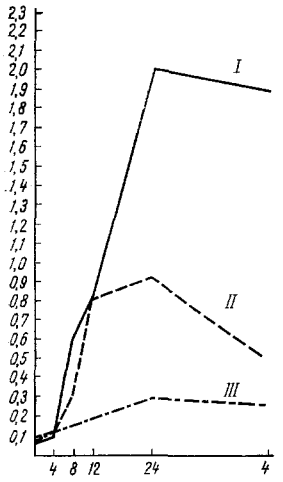

Fig. 3. Change in F-1-P aldolase activity in blood serum of rats poisoned with CCl_4 (II) and in rats poisoned with CCl_4 after preliminary treatment with phenobarbital (I) and SKF-525A (III).

weight. The rats of group 3 received ethionine by intraperitoneal injection of the 3% aqueous solution in a dose of 100 mg/100 g body weight divided into two portions given at intervals of 1 h. (Because of selective sensitivity to this poison, only females were used in this series of experiments.) In the animals of group 4 the bile duct was ligated under general ether anesthesia.

In the experiments to study the chemotherapeutic action of phenobarbital and SKF-525A, all animals were given a subcutaneous injection of CCl_4 in a dose of 0.1 ml/100 g body weight. Phenobarbital (in a dose of 100 mg/kg body weight) was injected intraperitoneally for the three days before administration of CCl_4, while SKF-525A (in the same dose) was also injected intraperitoneally 30 min before the CCl_4. Blood was taken from the caudal vein 4, 8, 12, 24, 48, and 72 h after the injection of CCl_4. The blood of all the animals was tested before the experiment began. Activity of the enzymes was determined by express micromethods developed in Pokrovskii's laboratory [4] and expressed as the number of micromoles substrate hydrolyzed by 1 ml serum per minute.

EXPERIMENTAL RESULTS

The changes in F-1-P aldolase activity in the animals receiving CCl_4, thioacetamide, and ethionine and in the animals with a ligated bile duct are shown in Fig. 1. The greatest increase in activity of the enzyme was found in rats receiving CCl_4 and thioacetamide (groups 1 and 2). F-1-P aldolase activity reached its maximum 24 h after administration of CCl_4 and 24-48 h after injection of thioacetamide. Activity of this enzyme was virtually unchanged in in the animals of groups 3 and 4.

The greatest changes in pseudocholinesterase activity were found in the rats of groups 1 and 3: 24 h after CCl_4 administration it was 225% higher than normal, while after injection of ethionine its activity was 43% of normal. No significant changes in pseudocholinesterase activity were found in groups 2 and 4.

Activity of phosphomonoesterase-1 (Fig. 2) in the animals of group 4 measured 72 h after ligation of the bile duct was 466% of its initial value. A slight increase in the activity of this enzyme was observed in the rats of group 2, but in the animals of groups 1 and 3 it was not significantly changed.

In the separate series of experiments in which CCl_4 poisoning was carried out after preliminary administration of phenobarbital and SKF-525A, the following results were obtained. F-1-P adolase activity was clearly increased (Fig. 3, II) in the rats treated with CCl_4, but by a much smaller degree than in the rats in the experiments described above, for in that case a rather smaller dose of CCl_4 was used (0.1 ml/kg body weight). In the animals receiving CCl_4 in the same reduced dose, but after preliminary injection of phenobarbital, F-1-P aldolase activity was much higher than that in the animals receiving CCl_4 only (Fig. 3, I). In the rats receiving SKF-525A before CCl_4 poisoning, the change in F-1-P aldolase activity was not significant (Fig. 3, III).

The pseudocholinesterase activity was somewhat reduced in all the animals of this series after 24-48 h. Phosphomonoesterase-1 activity was increased, also in all the animals but, in particular, in the rats receiving prophylactic phenobarbital; the increase in activity of the enzyme was smaller in animals receiving CCl_4 only; in rats receiving CCl_4 after SKF-525A the changes in activity of this enzyme were not significant.

Characteristic morphological changes in the liver produced by the action of the poisons used have been fully described in the literature [5, 6]. When the liver tissue is damaged, the blood enzyme spectrum is modified. Some enzymes enter the blood stream, chiefly from the cytoplasm. Changes in F-1-P aldolase pseudocholinesterase, and phosphomonoesterase-1 activity, in the writers' view, characterize the processes taking place in the liver most clearly.

In necrosis of the liver produced by the action of thioacetamide and in the mixed necrotic and degenerative lesion produced by CCl_4 considerable outpouring of aldolase into the blood stream was found; in the liver lesion produced by ethionine and ligation of the bile duct, no increase in F-1-P aldolase activity was observed, for in both cases the necrotic component was absent. The level of activity of pseudocholinesterase in the blood depends on its synthesis by the liver. Since ethionine lowers the ATP level in the liver and thus contributes to the disturbance of its synthetic function, pseudocholinesterase activity fell sharply in the blood of the animals receiving ethionine. In rats poisoned with CCl_4 the pseudocholinesterase activity rose a little to begin with: in the first stages, with the dose of CCl_4 used, the necrotic component was evidently predominant and the enzyme was delivered mechanically in the liver of animals receiving the reduced dose of CCl_4, and for that reason the pseudocholinesterase activity in the blood was lowered.

Ligation of the bile duct led to the sharpest increase in phosphomonoesterase-1 activity in the blood.

Changes in the activity of these enzymes evidently reflect the degree of damage to the organ caused by poisoning. Since the action of CCl_4 depends on the rate of its metabolism in the liver [7], administration of compounds increasing the rate of metabolism of the poison increases its toxicity, while administration of inhibitors of the enzyme systems of CCl_4 metabolism reduces its toxicity. Phenobarbital, DDT, etc., are usually used as activators of CCl_4 while SKF-525A is used, in particular, as an inhibitor.

The dynamics of changes in F-1-P aldolase satisfactorily reflects both the activating and inhibitory action of these substances. In experiments in which the animals received phenobarbital as well as CCl_4, the aldolase activity was sharply increased, while in the experiments in which the inhibitor SKF-525A was given this increase was negligible. The dynamics of pseudocholinesterase activity, as was shown above, with the dose of CCl_4 given does not reflect changes in the toxicity of the poison due to administration of the chemotherapeutic agents. Phosphomonoesterase-1 was rather more demonstrative in this case, for the character of the change in its activity differs only a little from that of aldolase, but the changes are much less marked.

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