PLASMA ENZYME ACTIVITY IN RATS WITH VARIOUS TYPES OF ACUTE TOXIC HEPATITIS

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UDC 616.36-099-092.9-07 : 616.153.1-074

Necrosis of liver cells caused by administration of thioacetamide and CCl_4 is accompanied by a marked increase in activity of alanine- and aspartate-aminotransferases, fructose-1-monophosphate- and fructose-1,6-diphosphate-aldolases, and glutamate dehydrogenase. Fatty infiltration of the liver caused by ethionine did not produce a marked increase in enzyme activity. The results indicate that the necrotic component plays a major role in the increase in blood enzyme activity.

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The mechanism by which tissue enzymes pass into the blood during the action of various toxic substances continues to attract the attention of investigators [1, 3, 5, 8, 13]. Poisons causing morphologically different types of lesions in the cells of the same organ are of special interest in this connection.

In the present investigation hepatotropic poisons were used for this purpose, for their action on the liver causes fatty degeneration (ethionine), a mixed type of lesion with fatty degeneration and necrosis (CCl_4), or predominantly necrotic changes (thioacetamide). As indicator enzymes of liver cell damage, enzymes located mainly in the matrix of the liver cells were chosen: alanine- and aspartate-aminotransferases (ALA, ASP; I.C. E. 2.6.1.2 and 2.6.1.1), fructose-1,6-diphosphate and fructose-1-monophosphate aldolases (FDP, FMP; I. C. E. 4.1.2.7), and glutamate dehydrogenase, a mitochondrial enzyme (GDH; I. C. E. 1.4.1.2).

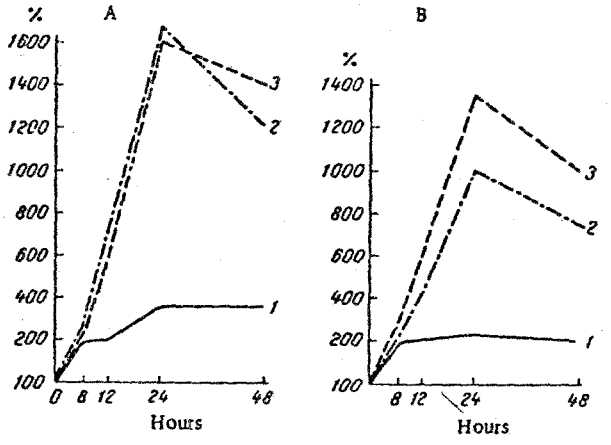

Fig. 1. Changes in activity of alanine-aminotransferase (A) and aspartate-aminotransferase (B) in blood plasma of rats after injection of ethionine, thioacetamide, and CCl_4. 1) Ethionine; 2) thioacetamide; 3) CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 220-250 g. All the animals were fasted for 12 h before the beginning of the experiment. The rats were divided into three groups depending on the poison investigated. Ethionine was injected intraperitoneally as a 3% aqueous solution in a dose of 100 mg/100 g body weight in two injections at an interval of 1h. Because of selective sensitivity to this poison, in this series of experiments only females were used. The animals of group 2 received thioacetamide by subcutaneous injection of a 2% aqueous solution in a dose of 15 mg/100 g body weight. The rats of group 3 received CCl_4 subcutaneously in a dose of 0.6 mg/100 g body weight. Citrated plasma was obtained from blood taken from the rats' caudal vein in a volume of 0.2 ml 8, 12, 24, 36, and 48 h after injection of the poison. The enzyme activity was determined by microexpress methods developed in our laboratory [2].

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Department of Biochemistry, N. I. Pirogov Second Moscow Medical Institute (Presented by Corresponding Member of the Academy of Medical Sciences of the USSR A. A. Pokrovskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 5, pp. 60-63, May, 1968. Original article submitted February 4, 1967.

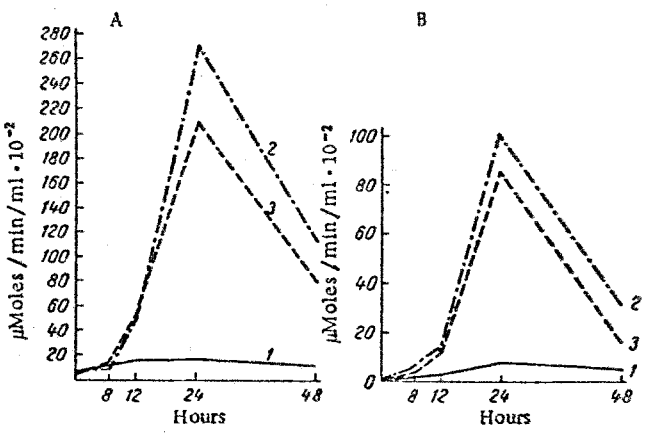

Fig. 2. Changes in activity of fructose-1,6-diphosphate aldolase (A) and fructose-1-monophosphate aldolase (B) in blood plasma of rats after injection of ethionine, thioacetamide, and CCl_4. Legend as in Fig. 1.

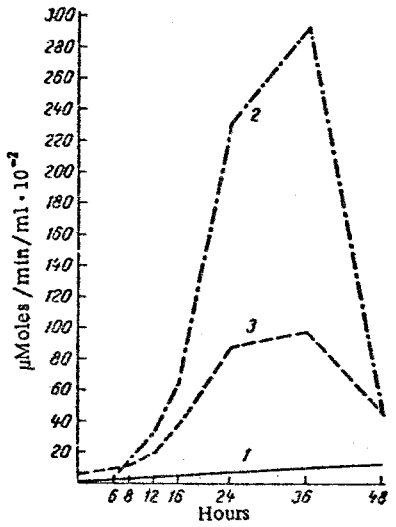

Fig. 3. Changes in plasma glutamate dehydrogenase activity of rats after injection of ethionine, thioacetamide, and CCl_4. Legend as in Fig. 1.

EXPERIMENTAL RESULTS

As Fig. 1A shows, plasma ALA activity after injection of ethionine increased slightly (to approximately 200% of the initial level 8 and 12 h after injection of the poison and to 330% after 24 and 48 h). In the case of thioacetamide, however, ALA activity reached 700% of the initial level 12 h after injection, 1600% 24 h after, and 1300% 48 h after injection. The changes in ALA activity after administration of CCl_4 were approximately the same. Changes in activity of the blood enzymes after administration of poison of a similar character were also found during the study of ASP (Fig. 1B), FDP (Fig. 2A), and FMP (Fig. 2B). There was a noticeable difference in the character of the curves reflecting the changes in activity in the aldolase and transaminase groups. Curves showing changes in activity formed a much more distinct maximum, occuring at 24 h.

The reasons for this difference are not completely clear, but they may be connected with differences in the rate of elimination of enzymes from the blood.

The data given in Fig. 3 show changes in activity of GDH, a mitochondrial enzyme. It is clear that after administration of poisons producing necrosis, a sharp rise in blood dehydrogenase activity took place within 6 h after injection. The maximal increase in GDH activity (16,200%) was found 36 h after injection of thioacetamide. When CCl_4 was used, the activity of the enzyme showed a smaller increase by this time (5400%). In rats poisoned with ethionine, no significant increase in the blood glutamate dehydrogenase activity was found at any time of the investigation. Only a slight tendency was noted for the GDH activity to increase 48 h after injection of the poison. The increase in activity of this enzyme was much greater after administration of the poison with the more marked necrotic action.

It follows from these facts that acute ethionine fatty infiltration of the liver does not produce liberation of enzymes into the blood, whereas necrotic injury to the liver produces a marked increase in activity of the investigated enzymes.

In this connection results described in the literature [12] and obtained in our laboratory [3] concerning the ability of certain phenothiazine derivatives to modify the increased blood activity of enzymes produced by administration of CCl_4 and thioacetamide are interesting. Administration of phenothiazines to animals poisoned with these substances prevents the development of necrotic changes in the liver without preventing the accumulation of fat (dissociation of the necrotic and degenerative components). Under these circumstances the blood enzyme activity of the experimental animals falls significantly. As the results of a preliminary histological investigation showed, administration of thioacetamide and CCl_4 leads to necrosis of a large number of liver cells 24 h after injection of the poison, whereas ethionine causes necrosis only of individual hepatocytes.

Many investigations have shown that the increase in blood enzyme activity is a sensitive test of liver damage. The slight increase in blood enzyme activity after administration of so powerful a hepatotropic poison as ethionine is a little unexpected. However, since fatty infiltration of the liver developing in rats kept on a high-fat, choline-free diet does not lead to an increase in serum enzyme activity [4, 14], it may be considered that fatty degeneration of the liver cells does not itself give rise to such an increase in the blood enzyme level. It is therefore interesting to note that in acute alcoholic fatty infiltration of the liver, the blood enzyme activity rises very slightly [7].

It is well known that exposure to "respiratory" poison [8] and to hypoxia [11], like administration of necrotic poisons to animals, leads to liberation of enzymes from the cell. The view was thus confirmed that the permeability of the cell membrane for protein is regulated by the ATP concentration, and that a lowering of the ATP level is responsible for the liberation of enzyme into the extracellular space both during cell "asphyxia" and during necrosis [1, 5, 8].

At the same time, the results of this investigation show that administration of ethionine, causing an early and sharp increase in the ATP content of the liver [10], is not accompanied by any marked degree of increase in blood enzyme activity. These results suggest that the liberation of enzyme protein from the cell as it undergoes necrosis, while dependent on the energy supply of the cell, cannot be attributed to a decrease in the general ATP level. This interpretation of the problem is supported by the work of Blume and co-workers [6] on the redistribution of ATP in the cell under the influence of CCl_4.

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