CHANGES IN THE DISTRIBUTION OF CARBONIC ANHYDRASE ACTIVITY IN THE RAT MYOCARDIUM AND LIVER DURING ACUTE DICHLOROETHANE POISONING (HISTOPHOTOMETRIC INVESTIGATION)

S. N. Sergeev and R. V. Berezhnoi

UDC 615.917.547.412.4].07:
[616.127+616.36]-008.931-092.18

Histochemical changes in the distribution of carbonic anhydrase activity in the myocardium and liver of rats were studied after acute oral poisoning with dichloroethane. Kurate's method in Hausler's modification was used. For a semiquantitative assessment of the changes in enzyme activity a histophotometric method was used. A definite increase in carbonic anhydrase activity in the myocardium and liver was observed in most of the experimental animals and the index of correlation between these changes remained the same as in the intact rats.

KEY WORDS: carbonic anhydrase activity; dichloroethane; liver; myocardium.

Only a few investigations have been devoted to the histochemical study of the distribution of carbonic anhydrase activity in the organs and tissues. They have yielded data on the distribution of carbonic anhydrase while the functional state of the body remained unchanged: in the brain [8, 11], the tissues of the eye [5, 7], the uterus [6], and the kidney [1, 9, 10, 12]. Histochemical changes in carbonic anhydrase activity during a change in the physiological state of the body have been studied only a few times [2, 3].

Considering the important role of carbonic anhydrase as a regulator of the acid–base balance of the body, a histochemical study of the changes in activity of this enzyme in the liver and myocardium was made in experiments on animals with acute dichloroethane poisoning, for acute poisoning with this compound is known to lead to changes in the tissues and to the development of alkalosis.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature albino rats. Dichloroethane was introduced into the proximal part of the esophagus of the animals through a rigid tube by means of a syringe. In all the experiments the volume of the substance injected was constant, namely 1 ml. If the volume of dichloroethane was less than 1 ml, the total volume of the mixture injected was made up to 1 ml with warm tap water, which was added until a homogeneous emulsion was obtained. Two series of experiments were carried out, in which the dose of pure dichloroethane was 0.1-0.3 ml (20 animals) and 0.5-1 ml (14 animals), respectively. The basic assumption was that the lethal dose of dichloroethane for man is 30-100 ml (up to 1.4 g/kg body weight). To study the dynamics of the histochemical changes reflecting carbonic anhydrase activity, the animals were killed at various times after administration of the dichloroethane. To compare the changes in carbonic anhydrase activity, each experimental animal was accompanied by its own control, which was killed (by decapitation) at the time when the experimental animal died or was killed. To rule out the effect of thickness of the section or differences in quality of the batch of reagent and working solutions, the myocardium and liver from the experimental and control animals were mounted on the microtome stage in a single block and frozen with solid carbon dioxide; a single section was cut and placed on a slide. The sections were cut in a cryostat at

Central Forensic Medical Laboratory, Ministry of Defense of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 91-93, January, 1977. Original article submitted May 26, 1976.

−16°C. Activity of the enzyme was studied by Kurate's method in Hausler's modification.* Control sections were incubated in the presence of diamox (the Hungarian preparation "Fonurit" was used). The method of preparing sections form different organs of different animals on the same slide, used in these experiments, enabled the changes in carbonic anhydrase activity in the homonymous organs from the experimental and control animals to be compared and, at the same time, the correlation between these changes in different organs of the same animal to be determined. Considering that during the detection of carbonic anhydrase activity fine-grain deposits of cobalt sulfide are distributed relatively uniformly in the sarcoplasm of the heart muscle fiber and in the cytoplasm of the liver cells within the hepatic lobule, for a semiquantitative determination of the changes in enzyme activity the method of histophotometry suggested by the writers previously [4] was used. In the course of study of the preparations the indices of enzyme activity (optical density) were recorded from 10-12 fields of vision. The numerical results were subjected to statistical analysis. Correlation between changes in enzyme activity in tissue sections from the organs of the experimental and control animals was calculated by the equation:

$$K = \log \frac{T_o}{T_{exp}} / \log \frac{T_o}{T_c},$$

where K is the index of correlation between changes in enzyme activity; T_o the transmission of light in the zone of the slide and coverslip with a layer of glycerol and gelatin between them, away from the tissue section; T_{exp} the transmission of light in the zone of the tissue section through the organ of the experimental animal, and T_c the transmission of light in the region of the tissue section from the organ of the control animal.

EXPERIMENTAL RESULTS

The animals of both series of experiments died (or were killed) during the first 2 days after administration of the compound. All the animals receiving the maximal dose of the compound (1 ml) died during or within a few minutes after injection of the dichloroethane. During the reaction for carbonic anhydrase in the myocardium of the control animals, pale gray deposits of cobalt sulfide were distributed relatively uniformly and only in very small amounts in the sarcoplasm of the muscle fibers. In most experimental animals there was a small relative increase in the intensity of the cobalt sulfide deposits, which also were uniformly distributed in all parts of the section through the organ and within the muscle fiber. Only in a few cases were denser deposits found in the bundles of muscle fibers lying nearer to the endocardium of the left ventricle. Occasionally the density of the deposits in the myocardium of the experimental animal was less than in the control. The correlation between changes in carbonic anhydrase activity in sections through the myocardial tissue of the experimental and control rats was 1.07 (σ = 0.38). In the reaction for carbonic anhydrase in the liver of the control animals, deposits of cobalt sulfide were distributed uniformly in the cytoplasm of the parenchymatous cells, the nuclei of which contained no deposits and were clearly outlined (Fig. 1). As a rule the deposits were uniformly distributed in the liver cells in all parts of the liver lobule. Only in a few cases was a denser deposition of cobalt sulfide found in the parenchymatous cells at the periphery of the lobule, close to the septal veins. In most experimental animals a definite and uniform increase in the density of the deposits was observed in all fields of vision and within the liver lobule. Correlation between the changes in carbonic anhydrase activity in sections through the liver tissue of the experimental and control rats was 1.02 (σ = 0.23). Consequently, in both the myocardium and the liver of the experimental animals a small relative increase in carbonic anhydrase activity was observed.

Comparison of the carbonic anhydrase activity of the myocardium and liver showed that the index of correlation for the experimental animals was 0.62 (σ = 0.205), and for the control animals 0.65 (σ = 0.086). The results indicate that there was a relatively uniform increase in carbonic anhydrase both in the myocardium and in the liver of the experimental animals, and that this correlation remained virtually the same as in the control rats.

Experimental acute oral poisoning of rats with dichloroethane thus causes changes in the distribution of carbonic anhydrase activity in the myocardium and liver of the experimental animals. Statistical analysis of the results of the histophotometric investigation of these changes in the myocardium and liver showed a definite tendency for the activity of the enzyme to increase. Correlation between these changes in the myocardium and liver of the experimental animals remained the same as in the intact rats. It must be emphasized that

*For details of the method of investigation see the monograph by A. G. E. Pearce, Histochemistry, London (1968).

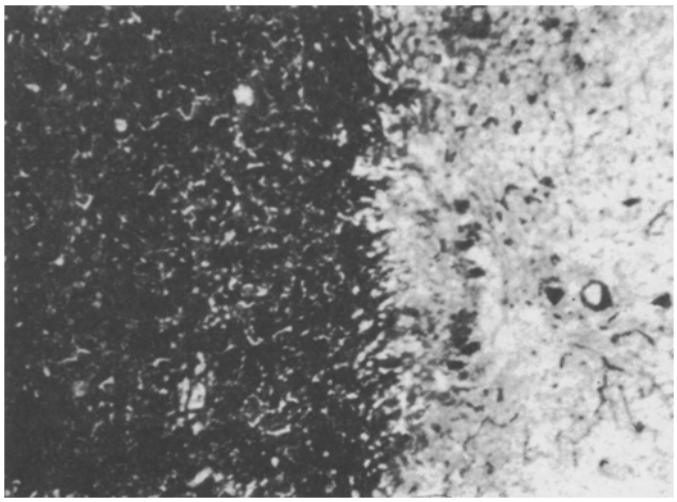

Fig. 1. Photomicrograph. Increase in density of deposits of cobalt sulfide in reaction for carbonic anhydrase in parenchymatous cells of liver of experimental animal (left) compared with control (right); 80×.

with the method used (when each experimental animal had its own control), individual variations in enzyme activity in the organs of the control animals due to changes in their physiological state cannot be taken into account.

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