PHARMAGOLOGY

EFFECT OF DICHLOROETHANE ON VITAMIN C METABOLISM

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(Received June 20, 1957. Presented by B. A. Lavrov, Member AMS USSR)

We have shown in a number of published papers [1] that the exercise of the protective function of vitamin C in response to toxic factors is associated with its increased utilization and expenditure in the animal organism. It is of importance that in organisms not capable of the biosynthesis of this vitamin, which has to be supplied with the food, its increased expenditure must lead to impoverishment of the ascorbic acid reserves of the tissues. In organisms able to synthesis ascorbic acid its increased expenditure leads to increased biosynthesis, and its concentration rises in the tissues.

We have examined the effect of the chlorinated hydrocarbon dichloroethane on the ascorbic acid content of the organs of experimental animals capable of its synthesis.

EXPERIMENTAL METHODS

The experimental animals were female albino rats, weighing from 180 to 210 g. The animals were maintained on a mixed diet, with addition of fish oil.

The first series of experiments was on 30 rats, divided into 3 equal groups. They were subjected to a single exposure to dichloroethane, lasting for two hours, in a special chamber. For 2 groups the dichloroethane concentration in the chamber was 20 mg/liter. The first group was killed by decapitation immediately after exposure, and the second was killed a day later. The third group was similarly exposed to a concentration of 10 mg/liter for 2 hours, and the rats were killed immediately after.

The organs were analyzed for ascorbic acid immediately after death, by our usual method [2]. Ascorbic acid was determined in the liver, the walls of the small intestine, the spleen, the brain, the kidneys, the adrenal glands, and the heart.

In the second series of experiments, on two groups of ten rats each, we studied the effects of repeated small doses of dichloroethane, over a long time. One group was exposed daily for two hours to a chamber concentration of 0.3 mg of dichloroethane per liter, and the second to a concentration of 0.6 mg per liter. The animals were killed at the end of a month, during which time they had suffered 25 exposures.

As controls we took a group of 15 rats, which were not subjected to dichloroethane inhalation.

EXPERIMENTAL RESULTS

Experiments on Single Acute Poisoning with Large Doses of Dichloroethane

After a single 2-hour exposure to a concentration of 20 mg of dichloroethane per liter the ascorbic acid content of the liver rose by 80% (T = 8.0) over the controls, and of the spleen by 36% (T = 4.1). A rise of 15% in brain ascorbic acid can be considered to be a very large one, since numerous researches have shown that

brain ascorbic acid varies normally within very narrow limits, and is far less variable than is the ascorbic acid content of the liver, spleen, and certain other organs. The considerable rise in the ascorbic acid concentration of cardiac tissue (30%) is of interest. The range of variation of the ascorbic acid content of the heart is also usually very narrow. The ascorbic acid content of the intestinal wall of the exposed animals was 20% higher than in the controls, but because of considerable individual variations (m = 2.6) the coefficient of significance does not attain the value of 3, although it is not far from it. There was no change in the ascorbic acid content of the adrenals. As for the kidneys, a survey of the results found will be deferred to the section devoted to discussion of results, below.

Statistically significant increases in ascorbic acid content of the organs of rats killed 24 hours after exposure for 2 hours to a concentration of 20 mg of dichloroethane per liter were found for the same organs as the first group (T greater than 3 in all cases), with the difference that the increase in the liver was now only 36%, as compared with 80% in the first group; in the other organs the increase remained the same, and there was still no change in the ascorbic acid content of the adrenals.

Statistically significant, and considerable rises were also found in the last group of rats, exposed to 10 mg of dichloroethane per liter, for the same organs as in the preceding groups, except in the intestine. The ascorbic acid contents of the adrenals and the kidneys remained unchanged.

Experiments on Chronic Poisoning

The ascorbic acid content of all the organs examined was at the normal level in the group of rats which had been exposed 25 times during a month to concentrations of 0.3 mg of dichloroethane per liter.

We were only able to analyze the liver and the brain of the rats of the group similarly given 25 exposures to concentrations of 0.6 mg of dichloroethane per liter. The ascorbic acid contents of these organs were very high, differing so much from the controls that T attained a value of 9.

The large increase in ascorbic acid content encountered in most of the organs of rats which had been subjected to inhalation of dichloroethane is undoubted evidence of a very considerable rise in the rate of synthesis of ascorbic acid. As has been shown earlier [3, 5, 6], such a rise in the rate of synthesis is a compensatory process, in response to an increased rate of expenditure of ascorbic acid, which participates in the defensive reaction of the organism to toxic factors. It hence follows that inhalation of dichloroethane considerably raises the body requirements for ascorbic acid.

We consider that important features of the reaction discovered by us are the selectivity and the dynamics of the changes in ascorbic acid encountered in the various organs, which are characteristic of each toxic factor, in the given case of dichloroethane, under the conditions applied by us. If we consider the results obtained by us from this point of view, we see that an exposure of 2 hours to concentrations of 20 mg of dichloroethane per liter evokes a pronounced response from a barrier organ, such as is the liver, from the central nervous system (the brain), from the blood system (the spleen), and from the myocardium. The rise in ascorbic acid concentration in the walls of the small intestine shows marked individual variations (m = 2.6), so that we can speak of a strong probability that this organ is immediately involved in the defensive reaction of the organism to the action of dichloroethane. We could observe no reaction from the adrenal glands.

The reactions found in the various organs 24 hours after exposure to dichloroethane were of the same kind as immediately after exposure, but were of a different intensity. Thus the intensity of the reaction found in the liver was smaller. An examination of the significance of the difference between the effect of dichloroethane on liver ascorbic acid content immediately after exposure, and 24 hours after, shows that T = 3.6. It is of considerable interest that 24 hours after exposure there is a significant rise in the ascorbic acid content of the walls of the small intestine, with a far smaller range of individual variations (m = 1.4), as compared with the reaction found immediately after exposure. This reaction of the thin intestine suggests the possibility that dichloroethane or its transformation products may be excreted into the intestine.

The reaction of the kidneys is of interest, from this point of view. We could find no significant difference either, immediately after exposure, or 24 hours later, between the ascorbic acid contents of the kidneys of the test animals and of the control group; comparison of the results found immediately after exposure with those found 24 hours revealed, however, a significant difference (T = 3.5). It might hence be supposed that the dynamics of the reaction are such that the maximum change in ascorbic acid concentration takes place at some time

less than 24 hours, for which reason we were unable to detect the corresponding reaction in the kidneys.

The reaction of the blood and central nervous systems, as well as of the myocardium, remained at the same high level at the end of the 24 hour interval.

The reaction found in rats exposed to a concentration of 10 mg of dichloroethane, and killed immediately after, was the same as for the higher dosage level of 20 mg per liter, as regards the organs involved, but the intensity of the reactions was smaller, approaching those found in rats killed 24 hours after exposure to the higher dosage level.

Repeated exposure to low concentrations of dichloroethane (0.3 mg per liter) did not cause any changes in the ascorbic acid content of the organs examined. A very pronounced rise in ascorbic acid content was, however, found in the group of rats subjected to repeated expsoure to concentrations of 0.6 mg of dichloroethane per liter, pointing to the toxic action of this concentration.

A comparison of our findings with published experimental data on the pathological changes produced by dichloroethane affords support to our views.

Thus Heppel, Neal et al., [7] found that rats poisoned with dichloroethane exhibited macroscopically evident hepatic hyperemia, and hyperemia with pin-point hermorrhages in the gastrointestinal mucosa; histological examination revealed degenerative changes in the kidneys, liver, and myocardium. Spenser, Rowe, et al., [8] showed that the most pronounced histological changes were in the kidneys, with smaller ones in the liver and lungs; they also reported biochemical changes in the blood. Larionov [4] found severe degenerative changes in the liver, kidneys, spleen, and heart of mice.

Thus our conclusions, based on a study of the dynamics of changes in distribution of ascorbic acid in various organs of rats poisoned with dichloroethane, are in agreement with published pathomorphological observations and studies of blood composition of rats subjected to the action of dichloroethane.

SUMMARY

The rate of biosynthesis of ascorbic acid and the changes of its concentration in the organs of rats was studied. It was demonstrated that dichloroethane causes increased expenditure of ascorbic acid in the organism of these animals. Dichloroethane, likewise, causes the immediate reaction of the liver, the central nervous system, the blood and the cardiac muscles. Intestines and kidneys respond later. Their reaction is manifested by increased content of ascorbic acid in these organs. This effect is still present 24 hours after a single inhalation of dichloroethane during 2 hours.

LITERATURE CITED

[1] L. M. Bremener, and B. A. Klyachko, et al., Farmakol. i Toksikol., No. 12 (6), p. 13 (1949).

[2] B. A. Lavrov, et al., Farmakol. i Toksikol., 12, No. 6, 13 (1949).

[3] B. A. Lavrov and B. I. Yanovskaya, Proceedings, VIII Congress of the Association of Physiologists, Biochemists, and Pharmacologists,* 1955.

[4] L.F. Larionov, Experimental Studies on Industrial Poisons, Vol. 25, Leningrad, 1936.

[5] B. I. Yanovskaya, Voprosy Pitaniya, Trudy Akad. Med. Nauk SSSR. 13 (1), 46 (1951).

[6] B. I. Yanovskaya, Biokhimiya, 17, No. 4 (1952).

[7] L. A. Heppel, P. A. Neal, F. S. Daft and other Journ. Industr. Hyg. and Tox., 1945, 27, 15; Pharmac. and Exper. Therap., 1945, 84, 53.

[8] H. C. Spenser, V. K. Rowe, E. M. Adams and other Arch. Industr. Hyg. and Occup. Med., 1951, 4, No. 5, 469.

* In Russian.