CHANGES IN THE ASCORBIC ACID CONTENT OF THE INTERNAL ORGANS OF WHITE RATS IN RESPONSE TO THE ACTION OF CHLORINATED HYDROCARBONS

COMMUNICATION II. THE EFFECT OF METHYLENE CHLORIDE

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It was shown in the previous communication [3] that the acute and repeated action of dichloroethane vapor causes severe changes in the rate of biosynthesis of ascorbic acid in rats and that under these circumstances characteristic changes develop in the ascorbic acid concentration in the animal's organs.

Information in the literature on the toxicology of methylene chloride is comparatively scanty and indicates its marked narcotic action [2, 4, 12] and degenerative changes which it produces in the parenchymatous organs.

Gross [8], for instance, after repeated poisoning of rats by exposures of 8 hours to the vapor of methylene chloride in concentration of 4-6 mg/l, found morphological changes only after 75 days, consisting of slight atrophy and central lobar fatty degeneration of the liver. In other research [10], by the action of methylene chloride in a concentration of 34 mg/l for 4 hours per week over a period of $7\frac{1}{2}$ weeks only a weak or moderate degree of fatty degeneration of the liver was observed in a proportion of the experimental animals.

Morphological investigations carried out on the organs of mice exposed to a single inhalation of methylene chloride in a concentration of 45 mg/l, and dying during the exposure, showed well-marked congestion of all the internal organs. In the liver swelling of the reticulo-endothelial cells was observed, and in some cases proliferation of these cells and their infiltration with tiny droplets of fat. In the kidneys slight signs of parenchymatous degeneration of the epithelium of the convoluted tubules were seen, and in the heart, proliferation of histiocytic cells and interstitial tissue. In the brain there was, besides congestion, capillary stasis, signs of perivascular and pericellular edema, acute swelling of the protoplasm of the nerve cells of the cortex, the subcortical ganglia, the brain stem and the other divisions of the brain. In animals dying a few days after poisoning, the changes in the organs were more pronounced than in the animals which died during exposure.

There are isolated reports of toxicoses in man from the action of methylene chloride.

Klotzbücher [9] reported poisoning of 10 persons, one of whom died. These patients developed loss of consciousness with, in some cases, tonic and clonic convulsions, absence of pupillary reactions and quickening of the pulse and respiration. Moskowitz and Shapiro [11] described the acute poisoning of 4 workmen from inhalation of methylene chloride vapor under industrial conditions.

During examination of workmen using methylene chloride, disturbances were found both in the nervous system and in the internal organs. Their main complaints were dizziness, headaches, pains in the region of the

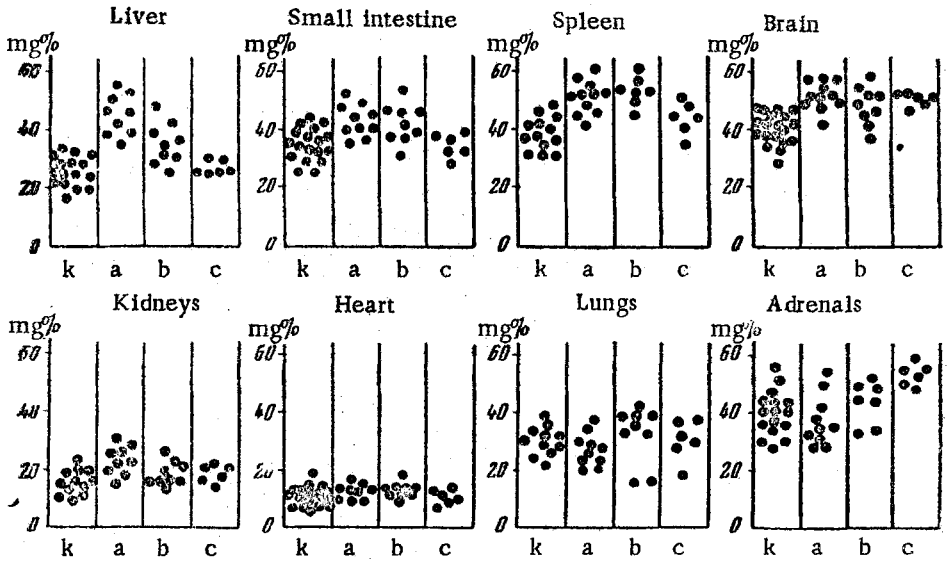
heart and a pricking sensation in the eyes. From their medical histories, in acute methylene chloride poisoning, patients had a sensation of intoxication, with severe dizziness, vomiting and general weakness.

In the present communication we give data on the action of this member of the group of chlorinated hydrocarbons — methylene chloride — used in several industrial processes and, in particular, in the manufacture of cinefilm.

We investigated the changes in the ascorbic acid concentration in the organs of rats weighing 180-200 g subjected to acute and repeated exposure to methylene chloride.

EXPERIMENTAL METHOD

Two series of experiments were carried out to study methylene chloride poisoning under static conditions. In the first series of experiments 20 rats were subjected to acute poisoning with methylene chloride in a concentration of 40 mg/l by exposure for 2 hours. The ascorbic acid content of the organs of animals, killed by decapitation, was investigated one and 24 hours after poisoning. In the second series of experiments we studied



Ascorbic acid content in the organs of rats after inhalation of methylene chloride. k) Control; a) methylene chloride, 40 mg/l, one exposure, killed after 1 hour; b) methylene chloride, 40 mg/l, one exposure, killed after 24 hours; c) methylene chloride, 0.7 mg/l, repeated exposure, killed after one month.

the action of low concentrations of methylene chloride (0.6-0.7 mg/l) after repeated poisoning for 2 hours a day, 6 times a week, for one month. Experimental animals were killed one hour after the end of the last exposure.

As controls we used 20 rats of the same sex and weight, kept under identical conditions, but not exposed to the action of methylene chloride. The ascorbic acid concentration in the liver, small intestine, spleen, cerebral hemispheres, kidneys, adrenals, heart and lungs of these animals was determined immediately after death.

The control animals were examined at the same times as the experimental. The results obtained were subjected to statistical treatment.

EXPERIMENTAL RESULTS

In response to the action of methylene chloride vapor in a concentration of 40 mg/l, the animals developed a clear picture of poisoning, with disturbances of the coordination of movements, dragging of the limbs, lying on the side and, finally, narcosis. After the conclusion of the exposure to the poison the animals quickly recovered, and 40-50 minutes later they appeared outwardly healthy.

In the second series of experiments, in response to repeated poisoning the behavior of the animals in the poison chamber was normal, and no clinical signs of methylene chloride poisoning were present.

The results obtained showing the ascorbic acid concentration in the organs are given in the figure.

In the animals poisoned by one exposure to methylene chloride in a concentration of 40 mg/l, after one hour a sharp increase in the ascorbic acid concentration was observed in the very great majority of organs examined, with a very high value for the significance of the difference from the control figures. In the liver, for instance, the ascorbic acid concentration increased by 70% (from 26.1 to 44.4 mg%), in the small intestine by 25% (from 34.9 to 43.8 mg%), in the spleen by 40% (from 39.3 to 57.7 mg%), in the cerebral hemispheres by 29% (from 40.5 to 52.1 mg%), in the heart by 30% (from 9.5 to 12.4 mg%) and in the kidneys by 20% (from 17.4 to 21.1 mg%).

The increase in the ascorbic acid concentration in the majority of organs indicated a general increase in the biosynthesis of ascorbic acid in response to the action of this compound.

Just as in guinea pigs, which do not synthesize ascorbic acid, in analogous conditions the converse occurred — a decrease in the ascorbic acid content of the organs [6, 7]; the increase in the biosynthesis of ascorbic acid in animals which do synthesize this vitamin was evidence of the increased expenditure of ascorbic acid in the poisoned animals, in compensation for which a controlled increase in the rate of biosynthesis of ascorbic acid took place.

The logical deduction from this fact is that, in persons exposed to the action of methylene chloride vapor in industrial conditions, there is increased expenditure of ascorbic acid by the tissues and it is essential to increase the supply of this vitamin.

The increase in the concentration of ascorbic acid in the barrier organs — the liver and kidneys — was evidence of the toxic action of this factor [1, 5]. Its influence on the blood system was reflected by an increase in the ascorbic acid concentration in the spleen. Its severe effect on the central nervous system was shown by the significant increase in the ascorbic acid concentration in the cerebral hemispheres, etc.

The increased concentration of ascorbic acid in all the organs enumerated was still present 24 hours after the single exposure to the poison. In the liver, for instance, by comparison with the controls there was an increase of 26%, i.e., 44% less than one hour after poisoning, and this difference was significant ($T = 4.1$). In the brain and kidneys the increase in the ascorbic acid concentration after 24 hours was also less than in the previous group; in the remaining organs — the intestine, spleen and heart — the ascorbic acid concentration remained at a high level; the action of methylene chloride was also shown by an increase in the ascorbic acid concentration in the lungs.

The changes observed after repeated inhalations of small doses (0.6-0.7 mg/l) of methylene chloride differed from those taking place after a single exposure to a large dose. As may be seen from the figure, this type of poisoning had an effect on the central nervous system (brain) and the adrenal-sympathetic system (adrenals). The increase in the concentration of ascorbic acid in the cardiac muscle was close to significant ($T = 2.5$).

With small doses no marked spread of the toxic action was observed, since no changes were found in the ascorbic acid content of the liver, kidneys, spleen or intestine.

SUMMARY

Acute single action (inhalation) of a high concentration (40 mg/l) of methylene chloride is associated with the rise of ascorbic acid biosynthesis with a sharp increase of its concentration in a number of organs (liver, small intestine, spleen, brain, kidneys, heart, lungs). This reflects the involvement of the corresponding systems of the body into the protective reaction against the toxic effect of methylene chloride. In 24 hours after the inhalation this action is still pronounced, although slightly weakened.

Repeated daily inhalations of methylene chloride (concentration — 0.6-0.7 mg/l) for the period of one month causes a pronounced effect on the central nervous and adrenal sympathetic systems, judging by the dynamics of ascorbic acid content in the organs.

The data obtained demonstrate the increased utilization of ascorbic acid during the action of methylene chloride on the organism and give grounds for concluding that persons subjected to the effect of methylene chloride vapors require supplementary vitamin C administration.

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