EFFECT OF DDT ON DISTRIBUTION OF NICOTINAMIDE COENZYMES (NAD AND NADP) IN CELL STRUCTURES OF THE LIVER AND BRAIN

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Dichlorodiphenyltrichloroethane, when administered to rats in large doses, or when given for long periods in small doses, lowers the content of nicotinamide coenzymes in homogenenates and cell structures of the liver and brain. The fall in level of NAD and NADP in the nuclei, mitochondria, and cytoplasmic fluid of the liver and brain is extremely irregular. The most marked changes are found in the mitochondria.

After a long exposure of warm-blooded animals to small doses of dicholorodiphenyltrichloroethane (DDT), considerable changes were found [5] in the content of oxidized and reduced forms of nicotinamide-adenine nucleotides (NAD and NADP; NAD.H_2 and NADP.H_2) in the heart, liver, and brain tissues.

Investigations of nicotinamide-adenine nucleotides undertaken on subcellular structures in cases of experimental myocarditis, denervation of muscles, carcinogenesis, and thyrotoxicosis have shed light on some of the problems in this field of pathology [3, 4, 9, 11].

Toxicological aspects of this problem have received inadequate study. The only work so far published is by Christie and Le Page [8], Galagher and Koch [12], and Galagher [13], who studied the state of the nicotinamide coenzymes in cell structures of the liver after poisoning with heliotrine, sporidesmin, and pyrrolidine.

To supplement the writers' earlier observations, changes in the content of NAD and NADP in cell structures of the liver and brain under the influence of different doses of DDT, acting for different periods, were studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats. DDT (70 mg/kg) was given in vegetable oil by mouth on three occasions (acute poisoning), and also in a dose of 3.5 mg/kg daily for 5 months (chronic poisoning). The animals were sacrificed by decapitation. Cell fractions of the liver and brain were isolated by differential centrifugation. The isolation medium was 0.25 M sucrose with the addition of 50 mmoles nicotinamide to prevent enzymic breakdown of the nicotinamide-adenine nucleotides. The nuclei were sedimented at 1500 rpm. The fraction of mitochondria was obtained by centrifugation of the supernatant for 20 min at 15,000 rpm. The cytoplasmic fluid was used in the experiments after sedimentation of the nuclear and mitochondrial fractions. The mitochondria were washed in 0.25 M sucrose solution with 50 mmoles nicotinamide and resuspended in cold 0.25 M sucrose solution. The content of nicotinamide coenzymes in the homogenates and cellular structures of the liver and brain was determined fluorometrically [14]. The content of NAD and NADP was expressed in μg/g fresh weight of tissues.

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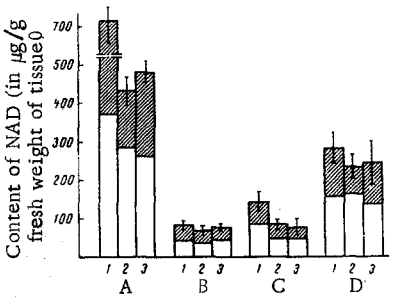

Fig. 1

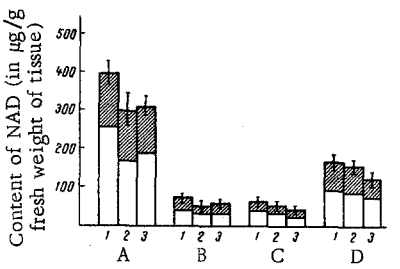

Fig. 2

Fig. 1. Content of nicotinamide nucleotides in subcellular structures of the liver: A) homogenate; B) nuclei; C) mitochondria; D) supernatant; 1) control; 2) acute DDT poisoning; 3) chronic DDT poisoning. Unshaded part of columns represents oxidized forms, shaded part reduced forms.

Fig. 2. Content of nicotinamide nucleotides in subcellular structures of the brain. Legend as in Fig. 1.

EXPERIMENTAL RESULTS

The results given in Fig. 1 show that in acute DDT poisoning and after chronic exposure to small doses the content of nicotinamide-adenine nucleotides in liver homogenate was lowered by 40 and 37%, respectively. Marked changes were observed in some cell structures: nuclei, mitochondria, and cytoplasmic fluid. However, the NAD and NADP levels fell by different amounts in the different fractions. The content of nicotinamide-adenine nucleotides in the liver mitochondria, for example, fell almost by half in both acute and chronic poisoning with the pesticide. The level of the nicotinamide coenzymes in the cytoplasmic fluid fell in these two cases by 15 and 13%, respectively. The content of nucleotides in the nuclei was practically unchanged after administration of DDT.

The observed decrease in the content of nicotinamide-adenine nucleotides in the mitochondria and homogenate of the liver of animals receiving DDT was evidently due to an increase in activity of NAD-hydrolyzing enzymes and to disturbance of the permeability of the mitochondrial membranes, with subsequent loss of coenzymes into the cytoplasmic fluid.

This hypothesis was confirmed by preliminary experiments carried out on subcellular structures of the liver and brain, in which an increase in the activity of enzymes participating in hydrolysis of the nicotinamide nucleotides was found.

Other evidence of a disturbance of the permeability of the mitochondria is given by the change in contractile properties of the mitochondria observable under these conditions [6].

As the results (Fig. 1) show, not only the total content of nicotinamide-adenine nucleotides in the liver homogenate was changed, but also the content of their separate forms. In acute DDT poisoning there was a greater decrease in the content of the reduced forms than of the oxidized. This may have been due to their increased oxidation in the respiratory cycle under the conditions of NAD deficiency. The content of oxidized and reduced forms of the coenzymes fell equally in the mitochondria.

Against the background of a marked decrease in the level of nicotinamide coenzymes in the brain homogenate, the study of the content of nicotinamide-adenine nucleotides in the subcellular structures of the brain revealed a relatively uniform decrease in all these structures (Fig. 2). The most marked effect was produced by chronic poisoning with small doses of DDT on the content of NAD and NADP in the brain mitochondria, where their level fell by one-third, principally on account of the oxidized forms.

The considerable decrease in content of NAD and NADP in the subcellular structures of the liver and brain observed in chronic DDT poisoning was evidently due to the cumulative property of the pesticide.

The decrease in content of nicotinamide nucleotides in the homogenate and subcellular fractions of the liver and brain thus indicates a reduction in the energy-producing capacity of the tissues in DDT poisoning. Investigations have in fact shown that clinical manifestations of DDT poisoning reflect a disturbance of nervous activity and profound changes in liver function and morphology [1, 2, 10]. It must be emphasized

that the decrease in content of NAD and NADP in the subcellular structures of tissues can influence not only the energy metabolism of the cells, but also the state of the systems of microsomal enzymes of the liver, responsible for the detoxication of foreign substances in the body [7, 15].

LITERATURE CITED

1. É. I. Makovs'ka and S. G. Serebryana, Fiziol. Zh. (Ukr.), No. 2, 251 (1961).
2. L. I. Medved' et al., in: General Problems in Industrial Toxicology [in Russian], Moscow (1967), p. 11.
3. S. E. Severin, L. A. Tseitlin, and T. N. Druzhinina, Biokhimiya, No. 1, 145 (1963).
4. V. I. Telepneva and N. A. Cherkashina, Vopr. Med. Khimii, No. 4, 393 (1966).
5. B. I. Khaikina and U. A. Kuz'minskaya, in: Hygiene and Toxicology of Pesticides and the Clinical Picture of Poisoning [in Russian], No. 5, Kiev (1967), p. 217.
6. B. I. Khaikina and A. E. Ostrovskaya, in: Hygiene and Toxicology of Pesticides and the Clinical Picture of Poisoning [in Russian], No. 5, Kiev (1967), p. 240.
7. J. Axelrod, in: Drugs and Enzymes, Prague (1965), p. 309.
8. G. Christie and K. Le Page, Biochem. J., 84, 25 (1962).
9. H. Coper, J. Helge, and H. Herken, Arch. Exp. Path. Pharmak., 243, 99 (1962).
10. W. Dale, Science, 142, 1472 (1963).
11. P. Emmelot and C. Bos, Exp. Cell Res., 14, 132 (1958).
12. C. Galagher and J. Koch, Biochem. Pharmacol., 3, 231 (1960).
13. C. Galagher, Biochem. Pharmacol., 13, 1017 (1964).
14. J. Huff and W. Perlzweig, J. Biol. Chem., 167, 157 (1947).
15. A. Morello, Canad. J. Biochem., 43, 1289 (1965).