EFFECT OF SOME PESTICIDES ON ACTIVITY OF ISOENZYMES OF SERUM LACTATE DEHYDROGENASE

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The pesticides DDT, lindane, and sevine, when injected into rats in a dose of 0.2 LD_{50} caused changes in activity of the total serum lactate dehydrogenase and of its isoenzyme spectrum. The most characteristic finding is an increase in subunits of the M-type.

In various pathological states the isoenzyme spectrum of serum lactate dehydrogenase (LDH) is sharply modified. Investigations [2, 4, 9] have revealed differences in behavior of isoenzymes in some diseases of the liver, heart, and blood. Recent work has demonstrated changes in isoenzyme activity in the blood serum under the influence of industrial poisons [4, 6, 8]. In connection with the extensive use of pesticides and their harmful action on the animal and human body, the obtaining of data reflecting the mechanism of action of these chemical compounds is of great importance at the present time.

In the investigation described below changes in activity of isoenzymes of serum LDH were studied in warm-blooded animals exposed to certain pesticides: dichlorodiphenyltrichloroethane (DDT), the γ-isomer of hexachlorocyclohexane (lindane), and n-methylnaphthylcarbamate (sevine).

EXPERIMENTAL METHOD

Experiments were carried out on 30 male albino rats. The pesticides for study were given by mouth daily for 3 days. The dose used was 0.2 LD_{50}: DDT 70 mg/kg, lindane 34 mg/kg, sevine 144 mg/kg. Activity of serum LDH isoenzymes of the rats was determined by Helm's method [2] of electrophoresis on agar, as modified by Korovkin [1], using veronal-medinal buffer (pH 8.6, ionic strength 0.05). The thickness of the agar plate for electrophoresis was 3 mm and the conditions were as follows: potential gradient 10 V/cm, current 7-8 mA, duration 60 min. Electrophoresis was carried out with cooling. The resulting agar plates were incubated at 37° in darkness for 120 min in medium of the following composition: 3 ml phosphate buffer, pH 7.4; 1 ml 1 M sodium lactate solution, 1 ml 1% nitro-BT solution, 1 ml 0.005 M $MgCl_2$ solution, 1 ml 1% NAD solution, 0.25 ml 1% phenazine methosulfate solution. At the end of incubation the plates were rinsed with water and immersed in fixing solution for 1 h (5 parts by volume glacial acetic acid, 70 parts by volume ethanol, and 25 parts by volume water). The plates were then removed, covered with filter paper, and dried. The results were read on a densitometer. Activity of each fraction was calculated by Kaplan's method [3].

Total serum LDH activity of the rats was determined by the method of Sevela and Tovarek [7]. Activity of the enzyme was expressed in μmoles pyruvate/mg protein/h.

RESULTS

Five LDH isoenzymes were found in the blood serum of the control rats, and their activity was distributed as follows: LDH_1 28%, LDH_2 8%, LDH_3 11%, LDH_4 8%, and LDH_5 45% (Fig. 1). These results are in

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TABLE 1. Effect of Pesticides on Isoenzyme Spectrum and Activity of LDH from Rats' Blood Serum

Experimental conditions	Activity of LDH isoenzymes, %					Total LDH activity, μmoles pyruvate/mg protein/h	H-type, %	M-type, %
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5			
Control	28	8	11	8	45	0,46±0,03	41,5	58,5
DDT	23	5	8	10	54	0,47±0,06 $P>0,5$	33,5	66,5
Lindane	23	10	7	6	54	0,17±0,01 $P<0,001$	33,5	64,5
Sevine	22	9	5	3	61	0,25±0,02 $P<0,001$	32,3	67,7

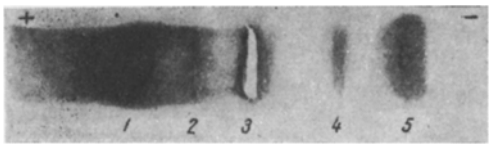

Fig. 1. Electrophoresis of LDH isoenzymes from blood serum of albino rat: 1) LDH_1; 2) LDH_2; 3) LDH_3; 4) LDH_4; 5) LDH_5.

agreement with those obtained by Ramsey et al. [5], who showed that the M-type is predominant in rats. The results are given in Table 1.

After administration of DDT, the total serum LDH activity was unchanged, but the isoenzyme spectrum showed substantial changes (Table 1). Administration of lindane in an isotoxic dose caused a sharp decrease in total LDH activity (by 63% compared with the control) and changes in the isoenzyme spectrum: activity of LDH_1, LDH_3, and LDH_4 was reduced while activity of LDH_2 and LDH_5 was increased.

After administration of sevine the changes were less marked than after lindane: a decrease in total serum LDH activity (by 43% relative to the control), a decrease in activity of LDH_1, LDH_3, and LDH_4, and an increase in activity of LDH_2 and LDH_5.

Analysis of the results shows that whereas lindane had the greatest effect on total LDH activity, changes in the isoenzyme spectrum were most marked after administration of sevine, in which case the changes in activity of LDH_3, LDH_4, and LDH_5 were particularly marked (decreases of 55 and 62% and an increase of 35% respectively). It must also be noted that whereas activity of LDH_1, LDH_3, and LDH_5 generally speaking changed in a similar manner following administration of all three pesticides, LDH_2 and LDH_4 behaved differently during administration of the different pesticides: after DDT, LDH_2 activity was reduced, while after sevine and lindane it was increased; activity of LDH_4 was increased after DDT, but decreased after sevine and lindane. The dissimilar effect of different pesticides on the isoenzyme spectrum of serum LDH of rats discovered by these experiments is evidently due not only to differences in the nature of the substances used, but also differences in the mechanism of their action, as reflected primarily by their effect on structural components of the enzyme protein molecule.

However, besides these differences, some common features in their action will also be noted: all investigated pesticides caused an increase, which varied in each case, in the number of subunits of the M-type, confirming their hepatotropic action, and also a decrease in the number of subunits of H-type, suggesting that in subacute poisoning with these pesticides disturbances exist not only in the liver, but also in the myocardium.

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