

Effect of Chlorocamphene on the Isoenzyme Spectrum of Lactate Dehydrogenase in Rat Serum and Liver

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Rats were used to study the general activity and the isoenzyme spectrum of lactate dehydrogenase (LDH) during single-instance and long-term introduction of polychlorocamphene.

Total lactate dehydrogenase activity decreases in the liver during the single-instance introduction of half the LD₅₀ (120 mg/kg). The isoenzyme spectrum of LDH is characterized by an increase in the quantity of LDH₁, LDH₂, and LDH₃ and by a decrease in the amount of LDH₄. The overall LDH activity does not change in blood serum. The isoform ratio changes insignificantly and LDH₁ falls, but normalizes 15 days after the introduction of the compound.

Long-term introduction of polychlorocamphene at levels 1/100 the LD₅₀ dose over 1.3 and 6 months causes a reduction in the overall LDH activity, both in the liver and in the serum. A decrease in the activity of the basic LDH isoenzyme of the liver (LDH₂) and a sharp increase in LDH₃ are characteristic for the isoenzyme spectrum of the liver. LDH₁ and LDH₄ decrease and LDH₂ and LDH₃ increase in blood serum. Beginning with the third month of polychlorocamphene introduction, LDH₁ tends to return to normal levels. LDH₁, LDH₂, and LDH₃ do return to normal levels, while LDH₃ increases regularly. This results in a reduction of the number of H subunits and an increase of M subunits. This is characteristic of hypoxic states.

On comparing the changes in the LDH enzymes of the liver and blood serum, it can be considered that the introduction of polychlorocamphene does not result in an increase in the permeability of the cellular membranes of the liver for LDH isoenzymes, while the observed isoenzyme spectrum shifts in blood serum are either the result of the biosynthesis of the isoforms of this enzyme changed by the compound or the result of the permeability for them of cells of other tissues.

The wide use in agriculture, industry and daily life of various chemical substances makes study of their effect on warm-blooded organisms and man an urgent task.

An important role in the toxic effect of foreign chemical substances is played by the intensity of their penetration into cells, accumulation in in-

tracellular structures, association with basic chemical constituents of cells, and consequently the degree of disturbance of biochemical processes supporting vital activity.

Because of the importance of enzymes in the normal metabolism of an organism it is not surprising that a large number of studies directed toward explanation of the mechanism of the toxic effect of chemical substances are devoted to study of their effect on activity of enzymes and enzyme systems. Specific inhibition of cholinesterase by

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phosphoroorganic compounds, of monoaminoxidase by hydrazines, of sulphydryl enzymes by alkylating agents, oxidizers and substances forming mercaptides, and of iron-containing enzymes by cyanides has been established.

In recent years, related to successes of theoretical and clinical study of enzymes, it has become possible to study in greater detail the effect of chemical substances on metabolic processes in organisms. In the present paper we discuss study of the effect of unfavorable factors on an isoenzyme spectrum of tissue and blood serum.

Isoenzymes as molecular models have found broad application in the solution of many problems in enzymology, protein chemistry, the regulation of organism functions under normal conditions, and during change of some factors in the internal and external media. A study of the isoenzyme spectrum of tissues and blood serum of various animals and man under normal conditions has made it possible to establish a number of practically important principles, and primarily its species, age, and organ specificity. As a result, a definite character of relationship among isoenzymes in blood serum has been revealed in pathology. For example, myocardial infarction in man causes a characteristic change in lactate dehydrogenase (LDH) of blood serum: an increase of LDH₁, a decrease in LDH₂ and LDH₃, and nearly complete disappearance of LDH₄ and LDH₅ (1-3) in liver diseases (infectious hepatitis and cirrhosis), there is a decrease in LDH₁ and LDH₂ and an increase in LDH₃, LDH₄, and LDH₅ (4-7).

Data are available on the change of the isoenzyme spectrum of lactate dehydrogenase in blood serum of animals affected by toxic substances. It is shown that during the acute exposure of rabbits to arsenic and cobalt, the LDH₄ and LDH₅ content increases in blood serum (8). A decrease of LDH₁ and LDH₃ has been found with exposure to mercury (9, 10), and an increase of LDH₅ during CCl₄ exposure (11). In previous studies (12) we established both the common nature and non monotypic nature of changes of an isoenzyme spectrum of LDH in blood serum of rats receiving DDT, the γ -isomer of hexachlorocyclohexane, and Sevin. All the pesticides studied produced different increases in values of subunits of M type, which is confirmed by their hepatropic effect. The nonmonotypic nature applied to changes of hybrid forms of LDH₂ and LDH₄. LDH₂ was reduced with introduction of DDT and increased under influence of γ -hexa-

chlorocyclohexane and Sevin, and changes of LDH₄ were reversed.

Available literature and our own data concerning sensitivity of LDH isoenzymes to the effect of toxic substances, particularly of pesticides, and the informative nature of this method were the basis of the present research.

The purpose of the research was to study the effect of a chloroorganic pesticide of polychlorocamphene (toxaphene) on the activity of LDH isoenzymes in blood serum and liver in white rats.

Polychlorocamphene (PCC) is a complex mixture of chlorinated terpenes. The substance manifests a generally toxic effect, but in the clinical picture of acute and chronic exposure, symptoms predominate which indicate primary damage to the liver and nervous system. This may be related to our determination (13) of the presence of the pesticide in the liver and to its specific accumulation in phospholipids and free cholesterol in the central nervous system.

Methodology

The experiments were conducted with male white rats weighing from 200 to 250 g. Polychlorocamphene in the form of a water emulsion was introduced P.O. into the animals in doses 1/2 LD₅₀ (120 mg/kg) once and 1/100 LD₅₀ (2.4 mg/kg) daily for 1, 3, and 6 months. With the single dose of the preparation, measurements were made 1, 5 and 15 days after its introduction. Determination of total activity of LDH was by the method of Sevela and Tovarek (14), expressed in micromoles of pyruvic acid per milliliter of serum or per gram of tissue. The LDH isoenzyme was determined by using the method of Helm (1) as modified by Korovkina (15). The BIAN-2 densitometer was used for reading the electrophorograms. The activity of the individual isoenzymes was expressed as percentages with respect to their sum.

Results and Discussion

In liver and blood serum of control rats we found LDH isoenzymes, activity of which was distributed as follows: in liver, LDH₁ 0.8%, LDH₂ 1.6%, LDH₃ 3.8%, LDH₄ 21.2%, LDH₅ 72.4%; in serum, LDH₁ 8.8%, LDH₂ 3.8%, LDH₃ 8.1%, LDH₄ 15.6%, LDH₅ 63.7%.

With a single introduction of PCC at a level of 1/2 LD₅₀ (120 mg/kg), the activity of total LDH in

liver declined sharply (26% after the first day, 39% after 5 days, and 45% after 15 days) (Fig. 1). The observed changes are evaluated as stable, since the activity does not normalize 15 days after introduction, but falls even further in comparison to the day 1 and day 5. Significant changes are noted in relation to the LDH isoforms. One day after the single introduction of the preparation the isoenzyme spectrum of LDH of the liver is characterized by a

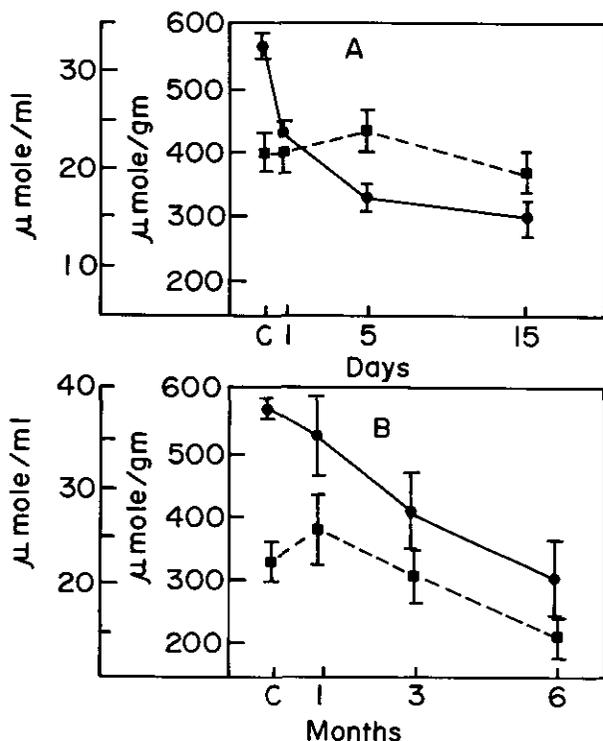


FIGURE 1. Effect of PCC on the LDH activity of (●) the liver and (□) blood serum: (A) LDH activity at various times after the single-instance introduction of PCC in a 120 mg/kg dose; (B) LDH activity during the long-term introduction of PCC at a 2.4 mg/kg dose (compared to the control group).

quantitative increase of the first three isoforms (LDH₁ is increased by 325%, LDH₂ by 256%, and LDH₃ by 84% with respect to the control group) and some decline of the LDH₄ (by 32%). In subsequent days these relationships are basically maintained (Table 1). It should be noted that with a single introduction of PCC, the greatest changes noted are in LDH₁, the relative activity of which increases sharply in one day (by 325%), increasing still more in 5 days (by 825%) and being maintained at a high level after 15 days (600% and

above in comparison to the control group). Considering literature data on the adaptive significance of isoenzymes and data on the greater activity of LDH₁ at low pyruvic acid concentration in tissue (16, 17); it can be assumed that a single introduction of PCC results in a disturbance of metabolism which in turn leads to a pyruvic acid deficit, while an increase of LDH₁ under the given conditions is a manifestation of the adaptive reaction of tissue which is directed towards the maintenance of cellular homeostasis.

In blood serum, the total LDH activity practically remains unchanged under these conditions (Fig. 1), and the relationship of isoforms also changes insignificantly (Table 1). The most characteristic displacement may be considered to be decline of LDH₁ beginning with the first days after introduction, attaining a maximum in 5 days and displaying a tendency to normalization in 15 days. The activity of this isoform fell with respect to that of the control group 77% after the first day; 82% after 5 days, and 38% after 15 days. As a result of the displacements occurring, the quantity of H subunits decreases, which is ordinarily related to a developing hypoxic condition (18-20).

With extended exposure to PCC in dosage of 1/100 LD₅₀ (2.4 mg/kg) in liver, the total LDH activity declines. The rate of decline is directly related to duration of introduction of the preparation; LDH activity declines by 6% after 1 months, 25% after 3 months, and 48% after 6 months, in comparison to the control group (Fig. 1).

Reduction in the activity of the primary LDH isoenzyme, LDH of the liver and sharp increase of LDH₃ are characteristic for an isoenzyme spectrum. The relative activity of LDH was reduced by 10% after a 3-6 month daily introduction of polychlorocamphene, LDH₃ activity increased by 31% after 1 month, 76% after 3 months and 118% after 6 months. LDH₁ increases the same as with single introduction of a large dose, but it is reliable only for 3 months of daily introduction (187% in comparison to the control group) (Table 2).

Most characteristic of extended exposure to PCC is the decline of relative activity of LDH₅ which is the principal LDH isoenzyme in liver, and increase of LDH₃. The relative activity of the latter isoform increases 31% at 1 month, 76% at 3 months, and 118% in 6 months (Table 1).

On the basis of the obtained data it may be assumed that extended introduction of the preparation in small dosages produces metabolic

Table 1. LDH isoenzyme spectrum in the liver and serum of rats at various times after single introduction of PCC at a dose of 120 mg/kg.

Time after PCC dose, days	No. of animals N	Isoenzyme activity, %									
		Liver					Blood serum				
		LDH ₁	LDH ₂	LDH ₃	LDH ₄	LDH ₅	LDH ₁	LDH ₂	LDH ₃	LDH ₄	LDH ₅
Control	10	0.8 ± 0.4	1.6 ± 0.5	3.8 ± 0.6	21.2 ± 1.1	72.4 ± 2.2	8.8 ± 0.6	3.8 ± 0.3	8.1 ± 0.8	15.6 ± 1.1	63.7 ± 1.5
1	6	3.4 ± 0.6 ^a	5.7 ± 0.5 ^a	7.0 ± 1.2 ^b	14.5 ± 0.5 ^a	69.4 ± 4.6	2.1 ± 0.6 ^a	3.6 ± 0.9	11.6 ± 1.3 ^b	14.5 ± 2.2	68.1 ± 1.2 ^b
5	6	7.4 ± 0.6 ^a	2.0 ± 0.2	5.2 ± 0.3 ^c	23.5 ± 1.6	61.9 ± 5.2	1.6 ± 0.5 ^a	5.9 ± 1.5	11.9 ± 2.0	12.9 ± 1.7	67.5 ± 3.5
15	6	5.6 ± 0.2 ^a	2.0 ± 0.6	4.0 ± 0.3	17.4 ± 1.2 ^c	71.0 ± 3.2	5.5 ± 0.3 ^a	3.9 ± 0.8	9.7 ± 0.9	13.4 ± 2.7	67.8 ± 2.5

^ap < 0.001.

^bp < 0.02.

^cp < 0.05.

Table 2. Isoenzyme spectrum of LDH in the liver and serum of rats during long-term introduction of total PPC dose of 2.4 mg/kg.

Period of introduction of PCC, months	No. of animals N	Isoenzyme activity, %									
		Liver					Blood serum				
		LDH ₁	LDH ₂	LDH ₃	LDH ₄	LDH ₅	LDH ₁	LDH ₂	LDH ₃	LDH ₄	LDH ₅
Control	10	0.8 ± 0.4	1.6 ± 0.5	3.8 ± 0.6	21.2 ± 1.1	72.4 ± 2.2	8.8 ± 0.6	3.8 ± 0.3	8.1 ± 0.8	15.6 ± 1.1	63.7 ± 1.5
1	6	2.0 ± 0.6	3.0 ± 0.8	5.0 ± 0.6	20.0 ± 1.5	70.0 ± 1.5	3.3 ± 0.7 ^a	5.5 ± 0.5 ^b	12.9 ± 0.9 ^a	12.7 ± 1.1 ^c	65.6 ± 2.3
3	6	2.3 ± 0.5 ^d	1.7 ± 0.1	6.7 ± 1.1 ^d	23.5 ± 1.6	65.9 ± 2.3 ^c	5.9 ± 0.8 ^b	2.7 ± 0.7	8.3 ± 0.7	15.7 ± 1.5	67.4 ± 1.0
6	6	1.1 ± 0.5	2.0 ± 0.4	8.3 ± 0.6 ^a	23.1 ± 0.6	65.6 ± 1.9 ^c	5.9 ± 0.4 ^a	2.2 ± 0.5 ^b	7.7 ± 1.4	13.3 ± 0.7	70.7 ± 1.6 ^a

^ap < 0.001.

^bp < 0.01.

^cp < 0.05.

^dp < 0.02.

changes in liver generally similar to changes observed during the introduction of a single large dose. However, extended introduction of small quantities of PCC produces (especially in longer periods such as 3 and 6 months) a condition wherein not only the quantity of pyruvic acid declines in liver tissue, but the transformation into lactic acid of isoform LDH₅ is also retarded. This is suggested by the increase of LDH₁. The latter possibly determines the low level of activity of total LDH after introduction of PCC for 3 and 6 months. Moreover, the long-term introduction of small quantities of the preparation apparently also acts as a stimulating factor, which intensifies the proliferation processes in liver tissue. This can be assumed on the basis of the acute increase in LDH₃ as a function of introduction time (21).

With blood serum, extended introduction of PCC produces a decrease in activity of total LDH only after 6 months of daily introduction (Fig. 1).

Changes in relationship of isoforms are noted much earlier. A reduction in LDH₁ (63%) and LDH₄ (19%) and an increase in the LDH₂ and LDH₃ (by 45 and 59%, respectively) are observed in blood serum after 1 month. Beginning with the third month of PCC introduction, LDH₁ tends to return to normal levels; LDH₂, LDH₃, and LDH₄ return to normal, while LDH₅ increases uniformly during this period (5% increase after 3 months, 10% increase after 6 months) (Table 2). This results in the reduction in the number of H subunits and in the increase of M subunits. This, as was mentioned earlier is characteristic for hypoxic states.

Thus, the conclusion indicated in this study is that introduction of PCC into an animal both as a single large dose and as repeated small doses produces conditions of hypoxia in the organism and disturbs the normal course of energetic metabolism in liver tissue. In the case of a single large dose, the chief factor apparently is the developing deficiency of pyruvate, and therefore the observed increase of LDH₁ may be considered to be a manifestation of adaptational reaction of tissue responding to the changing substrate level. With extended introduction of small quantities of PCC, deficiency of pyruvate also apparently occurs, but with time, adaptational-compensatory possibilities of tissue weaken (after 6 months of PCC exposure, the activity of LDH₁ is half that after 1 or 3 months), while the simultaneously decreasing activity of LDH₅ still further reduces the capability of liver tissue to form lactate from pyruvate, i.e., it inhibits one of the

energetic mechanisms of tissue, namely, the process of glycolysis.

A comparison of changes of LDH isoenzymes in liver and serum, suggests introduction of PCC does not produce increased permeability of membranes of liver cells for LDH isoenzymes, and observed displacements in the isoenzyme structure in blood serum are the result of modification the process of biosynthesis of isoforms of this enzyme or their permeability to cells of other tissues.

A study of the effect of pesticides on the condition of multiple molecular forms of enzymes makes it possible to judge not only the effect of the preparation on a given enzyme but also to make assumptions concerning its effect on biochemical processes which determine the causal-evidential relationships of molecular mechanisms which serve as the basis of the toxic action of pesticides.

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