Relationship between Serum Enzymes and Histological Changes in Liver after Administration of Heptachlor in the Rat

by V. KRAMPL
Institute of Industrial Hygiene and Occupational Medicine
Bratislava, Czechoslovakia

Investigations described previously /5/ showed that after a single dose as well as during the long-time administration of heptachlor, a chlorinated cyclodiene insecticide, increased glutamic-pyruvic transaminase and aldolase in the serum of rats. The conclusion was drawn that an increase of activity in these enzymes may represent a suitable criterion for the evaluation of acute and subacute effects of heptachlor.

It has also been shown /1,4/ that some chlorinated insecticides cause pathologic alterations in the liver of experimental animals. Therefore the main objective of this study was to determine the relationship between increase in the serum enzyme activity, described above, and histological changes in liver following administration of heptachlor. Specifically, and from a therapeutic and preventive aspect, information is needed concerning the time course of these alterations.

Materials and Methods

Female Wistar rats, 200-300 g maintained on Larsen diet were used in these experiments and were fasted overnight prior to killing.

There were three experimental groups. Two groups for subacute treatment received daily 7 and 12 mg/kg body weight of heptachlor /Purity 98 %/. The dosage for the acute group was 60 mg/kg of body weight /approximately 2/3 LD_{50}/. Doses were administered in pure vegetable oil by means of oral tube. Control animals received only oil.

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Subgroups of both experimental and control animals were sacrificed by decapitation at different times over a 4 week period. In the acute group animals the times were 2, 24 and 72 hours after a single dose of heptachlor and in the subacute groups on the third, seventh, fourteenth and twenty-eight day of the experiment. Blood was drawn for serum enzyme analysis and the livers were excised for histological examination and hepatic enzyme analysis.

Glutamic-pyruvic transaminase /GPT/ was determined colorimetrically by the method of Reitman and Frankel /6/. Aldolase /ALD/ was assayed also colorimetrically by a modification of the Sibley and Lehninger procedure /7/. Activity of GPT and ALD is expressed as Karmen units and micromoles respectively per milliliter of serum /or milligram fresh tissue/ under standard conditions.

Results and Conclusions

Analysis of comparisons between the control subgroups in each main subacute group yielded no statistically significant differences and therefore were combined. The mean values and S.D. of serum and liver enzyme activity of GPT and ALD are shown in Table 1. In all figures the values for 0 day represent the base-line of normal enzyme activity using the values in Table 1. A t-test was applied to the differences between the mean values of the base-line and those obtained from the several experimental treatments. See Figures 1-4.

At 2 hours after a single dose of heptachlor /Figure 1/ activity of GPT in liver significantly increased but then slightly decreased and at 72 hours was below normal. Serum GPT increased continuously until a maximum was reached at 72 hours.

The time course of the effect of heptachlor in both subacute experiments is depicted in Figure 2. With repeated doses of heptachlor the activity of hepatic GPT decreases below normal. The decreases are statistically significant on the seventh day for 7 mg dosage and on the seventh and fourteenth day for 12 mg dosage.

On the other hand activity of serum GPT increases with repeated doses and the differences were statistically significant on the third, seventh and fourteenth day for both subacute groups.

However, the terminal values for both hepatic and serum GPT tend to approximate the base value for both groups by the twenty-eight day.

The changes in ALD activity in both liver and serum for both the acute group and subacute groups show similar effects over time.

TABLE 1

Normal control serum and liver enzyme activity
/Mean value and S.D./

Control	Enzyme	Activity Units ± S.D.	Activity Micromoles ± S.D.
For Acute group /N=6/	Serum GPT	43±8	
	Liver GPT	52-13	
	Serum ALD		6.6±1.5
	Liver ALD		4.6-0.9
For Subacute group 7 mg /N=24/	Serum GPT	50±10	
	Liver GPT	66-7	
	Serum ALD		5.4±0.6
	Liver ALD		4.4-0.4
For Subacute group 12 mg /N=24/	Serum GPT	51±10	
	Liver GPT	56-6	
	Serum ALD		5.2±0.5
	Liver ALD		4.2-0.4

In the acute group /Figure 3/ the hepatic ALD activity shows significant increase at 2 hours followed by a decrease which is significantly below normal at 72 hours. In the case of serum ALD activity there is elevation throughout the 72 hours and the values at 24 and 72 hours were significantly different from normal.

For both subacute groups /Figure 4/ the hepatic ALD activity decrease to the seventh day when it is significantly different from normal then rises again to approximately the normal control level. In contrast the serum ALD activity rises significantly over time followed by a decrease. For the 7 mg group the mean values were significantly different from base values on the third, seventh and fourteenth days, returning to approximately normal on the twenty-eight day. The differences between base-line and the 12 mg group were significant on the seventh, fourteenth and twenty-eighth days. However, one should note that the value for the twenty-eight day falls below the value for the fourteenth day.

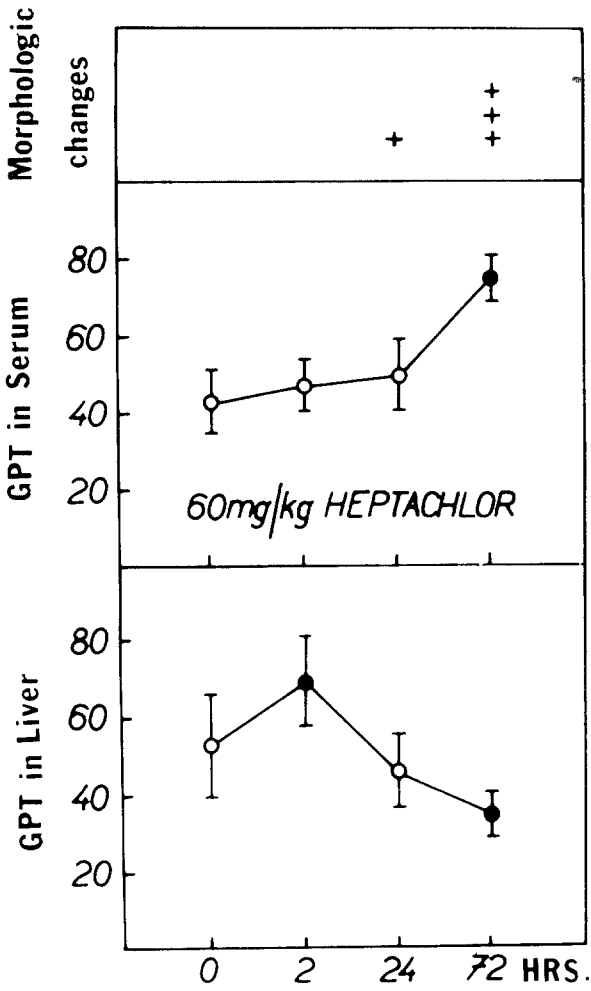

Figure 1. Relationship between the activity of glutamic-pyruvic transaminase /GPT/ in liver /Karmen units per mg fresh tissue/ and serum /Karmen units per ml serum/ and histological changes in liver after a single dose /60 mg/kg B.W./ of heptachlor. Each point represents the mean value and S.D. of 6 animals. Base-line /N =6 control animals/ given at zero time. Significance levels: ● = $P < 0.05 - 0.001$; ○ = N.S. Morphologic changes: + = slight; ++ = moderate; +++ = elevated; ++++ = advanced degree of damage.

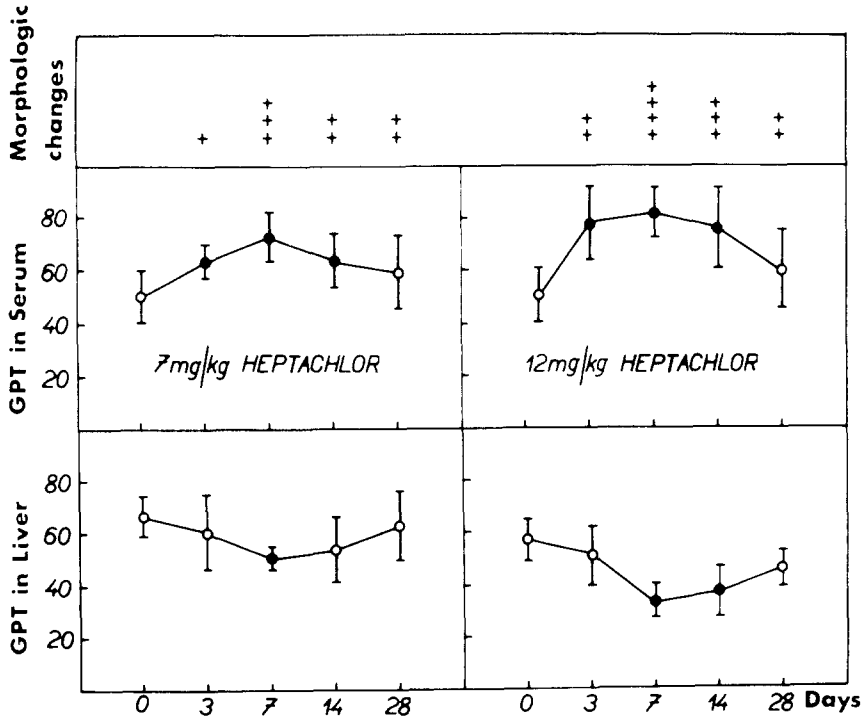

Figure 2. Relationship between the liver and serum GPT and histological changes in liver during administration of both 7 and 12 mg/kg B.W./ day of heptachlor. Base-line /N =24 control animals/ given at zero time.

Histologic examination of the liver revealed only slight morphologic changes. However, the maximum alterations coincided with days on which hepatic and serum enzyme activity was significantly different from normal. Also the time course of morphologic changes parallels the time course of the enzyme activity.

At 72 hours with hematoxylin-and-eosin section vacuolated cells and pycnotic nuclei were observed and the cytoplasma was stained more darkly. In other areas, hepatocellular structure was intact and there was minimal evidence of single monocellular necrosis with inflammatory reaction.

On the seventh day with 7 mg doses, there was more marked monocellular necrosis with inflammatory reaction. The other areas showed significant vacuolar dystrophy.

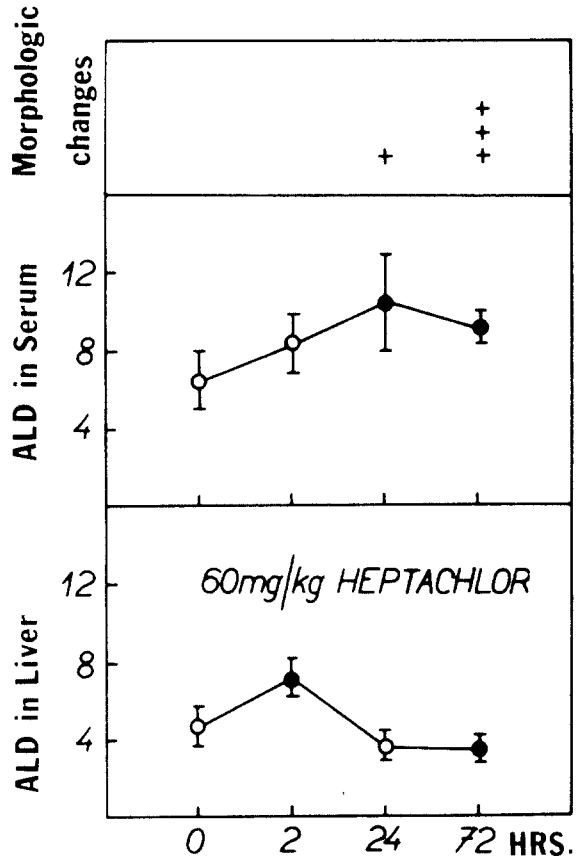

Figure 3. Relationship between the activity of aldolase /ALD/ in liver and serum and histological changes in liver after 60 mg/kg B.W. of heptachlor. The ALD activity is expressed in micromoles.

After repeated 12 mg doses of heptachlor, there was qualitative similarity to the 7 mg group in the histological picture but changes were quantitatively more extensive.

On the basis of these observations, one can conclude that the relationship between serum activities of GPT and ALD and damage of the liver is more complicated than simply mechanical leak of enzymes from

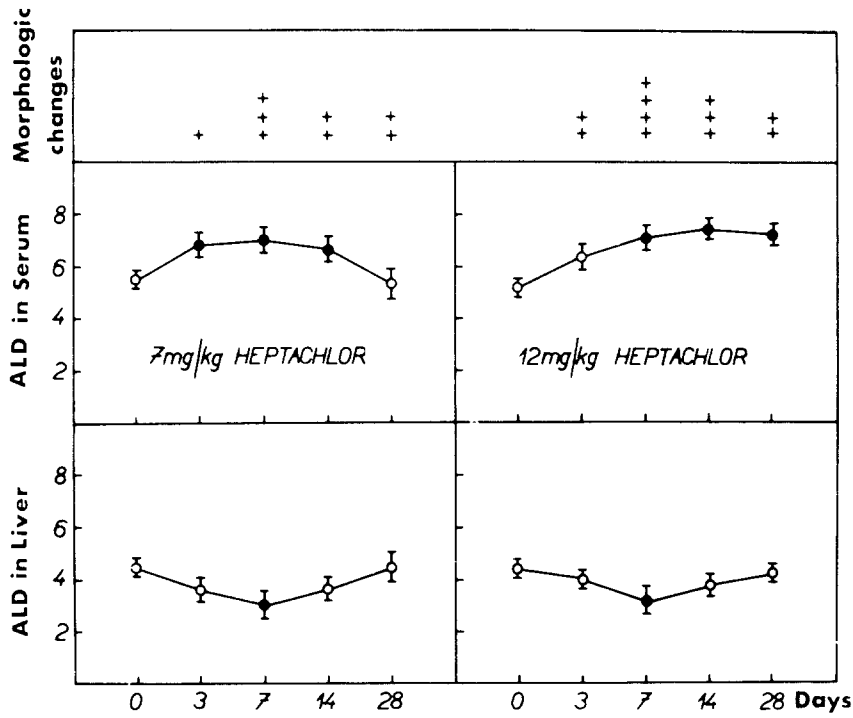

Figure 4. Relationship between the liver and serum ALD and histological changes in liver during repeated doses both of 7 and 12 mg/kg B.W./ day of heptachlor.

necrotic tissue to the blood. It is difficult, therefore, to attribute increase in serum activity as solely due to necrosis. Probably increase in activity is related to altered membrane permeability which allows intracellular enzymes to pass out of damage, but not necrotic, cells /2,3/. Furthermore, the increased efflux of these enzymes from liver cells was observed before the appearence of the maximum morphologic changes. Therefore, other mechanisms must be sought, not solely necrosis.

The data of serum and liver enzyme analysis indicated that activities of GPT and ALD were not altered significantly on the twenty-eighth day of

administration with the exception of ALD at 12 mg doses. Similarly, morphologic changes in the liver appeared to be smaller after the maximum of the seventh day. These results suggest, therefore, that adaptive compensatory mechanisms occurred in response to intoxication by heptachlor during the fourth week.

Thus from a therapeutic and preventive medical point of view it seems that activity of GPT and ALD may be used as an indicator of the effects of acute and subacute intoxication with heptachlor.

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