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Serum enzyme changes associated with carbon disulfide hepatotoxicity in experimental animals

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With 2 figures and 1 table

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The study of hepatic response to chemical injury is of prime importance to the toxicologist. The liver is specifically affected by a large number of chemical agents. Serum enzyme changes associated with chemical hepatotoxicity indicate liver-cell alteration (6).

In carbon-disulfide-intoxicated rabbits vacuolization and parenchymatous degeneration of the liver occurred (2). Centrilobular congestion and mild fatty degeneration was recorded by *Cohen* et al. (4).

There is evidence that in tissue damage cellular enzyme activity actually increases (5, 20) simultaneously with serum enzyme increase.

In case of carbon disulfide, *Andri* and *Pozzi* (1) found that glutamic oxalacetic transaminase diminished in liver mitochondrial preparations of rabbits chronically poisoned by carbon disulfide, while glutamic pyruvic transaminase was not influenced.

However, no change in glutamic oxalacetic transaminase whether in blood or liver was reported by *Minden* (16). Also, *Vertin* (25) observed no change in serum glutamic oxalacetic or serum glutamic pyruvic transaminases in a group of fifteen people exposed to carbon disulfide.

Alkaline phosphatase was found to be inhibited in serum and tissues of rabbits exposed to carbon disulfide (4).

Lactic dehydrogenase activity was found to be increased by the influence of carbon disulfide (16).

Due to this controversy it is felt that it is interesting to evaluate the activities of serum glutamic pyruvic, glutamic oxalacetic transaminases, alkaline phosphatases and lactic dehydrogenase enzymes in carbon-disulfide-intoxicated rats by six different doses over a period of 60 days.

Material and methods

The material of this study comprised 60 rats of both sexes weighing 120–150 g. Rats were categorized into six groups each of ten. They were injected intramuscularly with a dose of 0.05 ml carbon disulfide in 0.2 ml olive oil daily over a period of 60 days. During experimentation, rats were fed laboratory diet ad libitum (17). Every 10 days, rats of one group were killed by decapitation and blood was collected.

Twenty-four rats of similar weight were included, fed on the same diet and injected with 0.2 ml olive oil alone to serve as controls. Four of them were killed with each of the injected groups.

The method of *Reitman* and *Frankel* (18) was used for determination of serum glutamic pyruvic and glutamic oxalacetic transaminases. Serum-alkaline phosphatase was estimated by the method of *King* and *Armstrong* (13). The method of *Wroblewski* (26) was used to determine serum lactic dehydrogenase activity.

Results and discussion

Pathological changes in the liver due to carbon disulfide has been shown in a previous work to depend on the degree of intoxication. Liver showed diffuse fatty infiltration in all intoxicated groups (fig. 1). With increasing the doses in group 5 and 6, inflammatory manifestation in the form of hepatitis was noted. The liver cells were necrotic particularly in the peripheral zones associated with fibrosis and infiltration by mononuclear cells, as shown in figure 2 (7).

In the present work certain derangements in the activities of both serum glutamic pyruvic and glutamic oxalacetic transaminases could be demonstrated.

After 10 or 20 carbon disulfide injections, no significant changes of serum transaminase from the value obtained for the control could be shown.

With increasing the number of carbon-disulfide doses from 30 up to 60 injections, the values of both serum glutamic pyruvic and glutamic oxalacetic transaminases were significantly higher than that of the control, as shown in table 1.

The results are in concord with the previous results of *Favero* et al. (10) who found an increase of serum glutamic oxalacetic and glutamic pyruvic transaminases after intramuscular injection of carbon disulfide to rabbits.

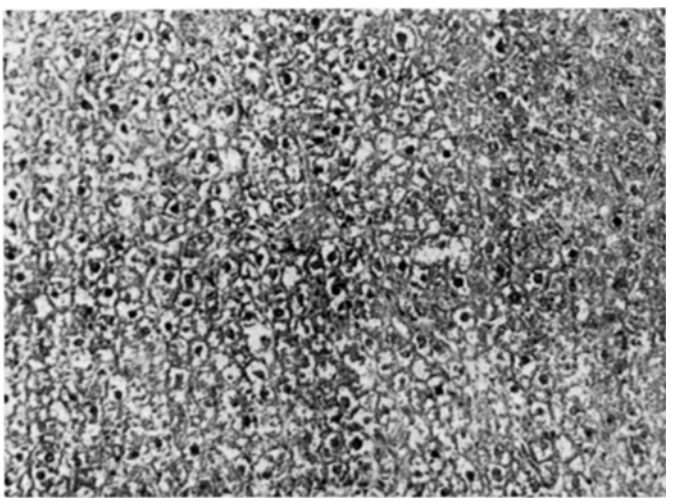

Fig. 1. A section in the liver stained with H and E showing fatty infiltration.

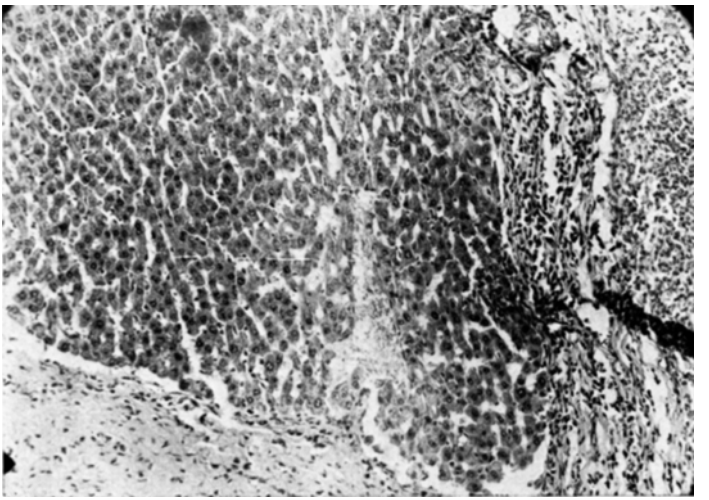

Fig. 2. A section in the liver stained with H and E showing liver cells fibrosis.

The liver may play a role in causing such enzymatic abnormalities. The increased activity of serum glutamic pyruvic and glutamic oxalacetic transaminases may be explained due to increased cell membrane permeability under the effect of carbon-disulfide intoxication either through the direct toxic action of carbon disulfide on liver (15) or shrinkage of hepatic vasculare with resultant hypoxia of the hepatic cells (7) and with lozing of the protein materials including enzymes (9).

Intramuscular administration of carbon disulfide caused also an increase in the activity of serum-alkaline phosphatase and such increase was significant with prolongation of intoxication, as shown in table 1.

These results are contradictory with the finding of *Cohen* et al. (4) and *Teresa* and *Barbara* (23).

Alkaline phosphatase was found to be inhibited in serum and tissues of rabbits exposed to carbon disulfide by inhalation (4). The inhibition of alkaline phosphatase by carbon disulfide was presumably due to the formation of zinc and magnesium dithiocarbamate in the course of carbon-disulfide metabolism (19).

Carbon disulfide in the blood exists in two forms, a free and a chemically bound form. Carbon disulfide has been shown to combine in blood with amino acids and proteins giving dithiocarbamates and thiozolidone (21).

Cohen et al. (3) presented the hypothesis that chelation of polyvalent inorganic ions by thiocarbamates and thiozolidone, produced during the biotransformation of carbon disulfide in the body, disturbs cellular metabolism and leads to cell injury. The distribution of copper and zinc in the tissues of exposed animals differed greatly from that in the control (19). Also a significant decrease was observed in the levels of serum zinc, iron, calcium and magnesium in carbon-disulfide-intoxicated rats (8).

Table 1. Range mean ± S.E. For serum glutamic oxalacetic (GOT), glutamic pyruvic (GPT) alkaline phosphatase and lactate-dehydrogenase activities in control and carbon-disulfide-intoxicated rats.

Const.		Control	Carbon-disulfide doses					
			10 inj.	20 inj.	30 inj.	40 inj.	50 inj.	60 inj.
SGOT	R	25–48	27–47	30–50	34–48	44–64	54–88	88–124
I.U./litre	M	35.9	36.8	41.0	44.8	63.4	80.7	107.4
	S.E.	±1.72	±1.99	±2.38	±2.35	±3.57	±4.43	±5.33
	P	–	0.15	0.10	0.05	0.005	0.005	0.005
SGPT	R	18–35	18–38	20–36	21–37	30–48	42–50	47–56
I.U./litre	M	24.9	26.7	28.3	30.3	36.3	44.3	50.1
	S.E.	±2.43	±1.88	±1.95	±2.10	±1.91	±1.06	±1.01
	P	–	0.15	0.15	0.10	0.025	0.005	0.005
SAlK.p.	R	6–22	12–25	14–24	18–25	23–35	33–46	38–54
K.A.U.	M	17.7	19.3	20.2	22.4	31.5	42.9	45.5
	S.E.	±1.54	±1.45	±1.47	±1.20	±1.45	±2.09	±1.31
	P	–	0.15	0.10	0.05	0.005	0.005	0.005
SLDH	R	54–130	48–136	58–146	54–154	58–169	52–172	54–176
I.U./litre	M	75.6	79.6	82.8	86.4	110.2	122.6	138.4
	S.E.	±5.43	±4.16	±7.16	±6.96	±7.32	±5.04	±8.76
	P	–	0.15	0.10	0.05	0.005	0.005	0.005

I.U./litre = International Unit/litre
K.A.U. = King Armstrong Units.

Alkaline phosphatase is an enzyme needing zinc as a prosthetic group and magnesium as activator (12). The data obtained by *Cohen* et al. (4) suggested that the concentration of activating ions in the serum and tissues of exposed animalis was low enough to limit the alkaline-phoshatase activity in the serum as well as tissue. Addition of magnesium resulted in restoration of most of the enzymatic activity. Thus it is clear that these contradictory results may be due to nutritional variation.

Tuba and *Madsen* (24) *Sukumara* et al. (22) found that there is a positive correlation between alkaline phosphatase and the food consumed.

Serum-alkaline phosphatase is known to increase due to overproduction or release of the enzyme by the liver in response to diverse stimuli as hepatocellular injury, increased intraductile pressure, inflammatory diseases of the ducts and expanding lesions compressing parenchyma and ducts of the biliary system (11).

The suggestion that the liver is the source of such enzymatic changes in the serum receives evidence from the finding of elevated serum transaminases in combination with increased alkaline phosphatase in these groups of intoxicated rats. Again, the increase was more pronounced with prolongation of carbon-disulfide intoxication.

Serum-lactate-dehydrogenase levels showed slight to moderate rise in groups administrated 10, 20 and 30 carbon-disulfide doses. Hyperactivation was observed in rats receiving aggressive doses of carbon disulfide.

Liver and skeletal muscle in particular contain large amounts of lactate-dehydrogenase enzyme and damage of these tissues is accompanied by a rise in serum-lactate dehydrogenase, as close as serum glutamic oxalacetic transaminase (14). Also, such an increase in lactate dehydrogenase is most probably due to interference of carbon disulfide with muscle-cell membrane leading to the enzyme release into the blood circulation.

It is concluded that the contradictory data found in the literature may be due to environmental and nutritional variation, of extent and duration of exposure or due to variations in the route of administration.

Summary

Investigations were performed to evaluate the activities of serum glutamic oxalacetic and glutamic pyruvic transaminases, alkaline phosphatase and lactate-dehydrogenase enzymes in rats intoxicated by different doses of carbon disulfide.

Serum GOT and GPT activities were elevated which may be due to CS_2 effect on cell membrane permeability.

Serum-alkaline-phosphatase activity showed also increment, which was again attributed to the liver affection.

A significant rise in serum-lactate-dehydrogenase activity which was referred to be as a result of muscle-lactate dehydrogenase release into the blood circulation.

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