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# Microsomal enzymes inducers and serum minerals in carbon-tetrachloride hepatotoxicity

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With 1 table

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Carbon tetrachloride in single or repeated doses produces characteristic hepatic lesions, including in turn in the liver further biochemical and pathological disturbance (38).

Early changes in calcium and potassium content of mitochondria after oral $CCl_4$ intoxication have been found by *Reynold* et al. (30).

*Tahani* et al. (35) reported an increase in serum zinc, copper, iron, calcium, potassium and sodium, while for magnesium no significant change was observed in acute liver damage, due to a single intraperitoneal injection of carbon tetrachloride.

Considerable evidence is now available suggesting that the production of liver damage is linked with an increased activity of the liver microsomal enzymes (22).

The effect of mircosomal enzymes inducers on hepatic function in mice and rats were studied when phenobarbital-Na was given before or after poisoning with carbon tetrachloride (15).

Phenobarbital pretreatment enhanced the rise in serum transaminase activity, hepatotoxicity and lethality induced by carbon tetrachloride poisoning (20, 24, 14).

However, we found that phenobarbital had a protective effect when given in repeated doses together with small doses of $CCl_4$. Protection was evident from reduced activity of plasma glutamic-pyruvic transaminase and reduced liver necrosis as demonstrated by histologic evaluations (11).

The purpose of the present work is to study the effect of phenobarbital which is known to be an inducer of microsomal enzymes (6) and propionyl promazine which is an inhibitor (41) on serum zinc, copper, iron, calcium, magnesium, potassium and sodium in carbon-tetrachloride intoxication.

## Material and methods

Male Sprague-Dawley rats, weighing 150–200 g, were used in all studies. The animals were maintained on an ad-libitum diet and water.

Ten animals were included in each tretment group. The animals were divided into six groups: Control group, Group treated with $CCl_4$ (0.1 ml/kg s.c.), dilut-

ed in the ratio of 1:1 with paraffin oil, was administered daily for ten days. Phenobarbitone group (60 mg/kg i.p.), propionyl-promazine group (2 mg/kg i.m.), and two other groups were administered either phenobarbitone plus $CCl_4$ or propionyl promazine plus $CCl_4$.

The animals were administered daily for a period of 10 days. The animals were then sacrificed by light ether anesthesia and blood samples were taken from the orbital plescus and blood was collected for serum minerals determination.

The method of *Sinaha* and *Gabrielli* (34) was used for determination of serum zinc and copper. Serum iron, potassium and sodium were estimated by the method published in *Beckman* Analytical method by Atomic Absorption Spectrophotometer. Serum Calcium and magnesium were determined using the method of *Willis* (39).

## Results and discussion

In the present work, carbon tetrachloride in ten repeated doses of (0.1 ml/kg s.c.) resulted in a significant increase in serum iron, copper, zinc, calcium, potassium and sodium. Also in a previous work we found that a single intraperitoneal injection of 2.5 ml/kg body weight has the same effect (35).

This confirms the previous finding of *Villela* (38) that repeated small doses of $CCl_4$ have an additive effect, also the onset of the pathological changes depends on the route of administration.

The results of the present work also show that propionyl promazine when administered alone or togehter with $CCl_4$ has no effect on serum minerals.

Phenobarbitone when administered alone increased serum minerals except sodium and magnesium, but to a lesser degree than $CCl_4$, while phenobarbitone when given repeatedly together with small doses of $CCl_4$ led to normalization of serum iron, potassium and calcium. Also serum zinc and copper were significantly lower than in case of $CCl_4$.

The initial lesion produced by $CCl_4$ seems to be cytoplasmic, mitochondrial damage comes later (36, 1). In early stages of intoxication, cytological lesions were more pronounced in the ergastoplasm as confirmed by electron micrographs (2), leakage of cytoplasmic enzymes results from this injury (37).

*Prasad* (25) showed that zinc is present in several metaloenzymes such as alkaline phosphatase and is important in their activities. Therefore, the increased serum zinc in $CCl_4$ phenobarbitone and phenobarbitone plus $CCl_4$ treated rats may be attributed to increased serum enzymes (11).

*Kazimierz* (17) found that in liver damage by $CCl_4$, the accumulation of radioactive zinc was highest in the mitochondria accompanied by a decrease in nuclear fraction radioactivity. This suggested displacement of zinc from nuclei to the cytoplasm.

The level of blood zinc was also raised by the administration of adrenaline (9). *Calvert* and *Brody* (4) suggested that a predominant factor in the hepatoxicity of $CCl_4$ is an anoxia produced through the mediation of the sympathetic nervous system. Release of adrenaline from the adrenal medulla under sympathetic stimulation is also suggested.

Table 1.

| Item | | Zinc μg% | Copper μg% | Iron μg% | Calcium mg% | Magnesium mg% | Potassium mg% | Sodium mg% |
|---|---|---|---|---|---|---|---|---|
| Control | | 133.4 | 118.9 | 135.2 | 8.57 | 4.00 | 22.8 | 228.7 |
| | S.E. ± | 4.27 | 5.12 | 6.13 | 0.37 | 0.13 | 0.36 | 3.19 |
| $CCl_4$ | | 213.3 | 199.5 | 218.5 | 9.95 | 3.96 | 26.6 | 271.0 |
| | S.E. ± | 7.27 | 9.69 | 10.0 | 0.22 | 0.34 | 0.78 | 7.49 |
| P:C vs $CCl_4$ | | 0.005 | 0.005 | 0.005 | 0.025 | n.s. | 0.05 | 0.005 |
| Phenobarbitone | | 174.0 | 169.0 | 187.2 | 9.76 | 3.72 | 25.1 | 248.5 |
| | S.E. ± | 11.29 | 9.49 | 12.21 | 0.39 | 0.30 | 1.12 | 11.57 |
| P:C vs Phen. | | 0.05 | 0.025 | 0.05 | 0.025 | n.s. | 0.05 | n.s. |
| Pheno + $CCl_4$ | | 164.1 | 154.7 | 149.3 | 8.49 | 3.57 | 22.6 | 291.0 |
| | S.E. ± | 9.22 | 4.88 | 6.54 | 0.59 | 0.38 | 0.52 | 4.91 |
| P:C vs Ph. + $CCl_4$ | | 0.005 | 0.005 | n.s. | n.s. | n.s. | n.s. | 0.005 |
| $CCl_4$ vs Ph. + $CCl_4$ | | 0.005 | 0.005 | 0.005 | 0.005 | n.s. | 0.05 | 0.05 |
| Propionyl-Prom | | 120.4 | 134.5 | 153.8 | 8.37 | 3.88 | 22.2 | 226.5 |
| | S.E. ± | 9.45 | 8.74 | 8.47 | 0.17 | 0.11 | 0.47 | 3.57 |
| P:C vs prop. | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Prop. + $CCl_4$ | | 232.8 | 200.5 | 213.5 | 9.81 | 3.19 | 25.2 | 283.5 |
| | S.E. ± | 11.57 | 14.53 | 8.13 | 0.21 | 0.42 | 0.82 | 12.17 |
| P:C vs Prop. + $CCl_4$ | | 0.005 | 0.005 | 0.005 | 0.005 | n.s. | 0.05 | 0.005 |
| $CCl_4$ vs Prop. + $CCl_4$ | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

On the other hand, liver disease was found to affect zinc in blood (13). The high level of serum zinc was related to hepatic damage. A portion of this increase was due to hemolysis of erythrocytes (40).

In carbon tetrachloride treated rats, the serum copper was higher than that of the control. Phenobarbitone, phenobarbitone plus $CCl_4$ have the same effect (table 1).

*Recknagel* and *Litteria* (27) found that carbon tetrachloride diffuses rapidly and is found in the liver to a maximum level 1½ hr after its administration. *Christie* and *Judah* (5) suggested that the change in the liver indicate a direct physical attack of the drug on liver mitochondria, degeneration and death of the cells results from the disorganization of mitochondrial structure.

Clearly then carbon tetrachloride increase the permeability of the mitochondrial membrane and high level of serum copper is attributed to this hepatic damage (28).

*David* et al. (8) showed that the hepatic and brain copper concentration increased significantly as a result of phenobarbital treatment, while blood serum copper increased and muscle copper decreased.

In the present work, the mean serum iron in $CCl_4$ treated rats when compared with control rats, there is a statistically significant increase. Our finding agreed with that obtained by many investigators (23, 21, 16).

The increase of serum iron was explained due to a significant decrease of utilization of iron for the hemoglobin synthesis in intoxication with carbon tetrachloride (18).

*Okawara* (23) found that incorporation of iron into the hemopoietic organs and iron storage organs were retarded by $CCl_4$ poisoning.

Acute liver damage resulted in an increase in plasma iron (29), while in various degrees of chronic liver damage the plasma iron was not elevated in rats (10).

Therefore, the increase in serum iron results from the disintegration of hepatic cells.

Our data indicated that the serum iron was still high under the effect of phenobarbitone and phenobarbitone plus $CCl_4$.

The mean serum calcium was significantly high in $CCl_4$ and phenobarbitone treated rats. In animals treated with phenobarbitone plus $CCl_4$ no significant change in serum calcium was found.

*Ruichi* (31) showed that two hours after oral $CCl_4$ intoxication, calcium content of the mitochondria increased concomitantly with a decrease in potassium ion.

The time course of mitochondrial degeneration in $CCl_4$ poisoning appears to follow the increase in concentration of calcium in liver mitochondria (32).

*Ernesto* et al. (12) showed that in $CCl_4$ intoxication the balance between ADP phosphorylation and calcium uptake is shifted in favour of the later, thus contributing to the accumulation of calcium in mitochondria. On the other hand, *Kovacs* (19) found that $CCl_4$ did not change blood calcium.

*David* et al. (8) showed that phenobarbital injection did not significantly change the concentration of magnesium and calcium in liver.

No significant change in serum magnesium in our treated groups was observed. A slight decrease in serum magnesium was found in patients with liver disease (31).

Carbon tetrachloride administration induced a significant increase in the level of serum potassium and sodium. The serum potassium is still high under the influence of phenobarbitone, while serum sodium is not significantly higher than normal. Phenobarbitone administered together with $CCl_4$ normalised serum potassium.

Our finding agreed with that obtained by *Boda* et al. (3) who found that serum sodium increased while tissue sodium decreased due to carbon tetrachloride.

Carbon tetrachloride intoxication caused a kidney injury (33). When signs of intracellular dehydration occur, the plasma potassium may be somewhat higher than normal (7). The increase in serum potassium may also be from the red cell hemolysis.

That severe mitochondrial damage occurs in the liver after carbon tetrachloride poisoning has been abundantly confirmed. Liver mitochondria of rats poisoned with carbon tetrachloride are swollen. *Recknagel* and *Malamed* (26) showed that the mitochondrial swelling is due to an increase in mitochondrial membrane permeability. As a result, the mitochondria release into the surrounding medium cytochrome C and potassium (28).

It is concluded that a portion of the rise in serum minerals was due to increased permeability in damaged mitochondrial membrane as a result of the action of carbon tetrachloride. Also the decrease in serum minerals when $CCl_4$ was given together with phenobarbitone may be due to some protective effect of phenobarbitone on liver mitochondria.

*Summary*

The effect of ten repeated doses of carbon tetrachloride, phenobarbitone and propionyl promazine when administered alone or simultaneously with $CCl_4$ on serum minerals was investigated.

Carbon tetrachloride resulted in a significant increase in serum iron, copper, zinc, calcium, potassium and sodium. A portion of this rise was due to increased permeability in damaged mitochondrial membrane as a result of the action of $CCl_4$.

Propionyl promazine when administered alone or together with $CCl_4$ has no effect on serum minerals.

Phenobarbitone when administered alone increased serum minerals except sodium, but to a lesser degree than $CCl_4$, while phenobarbitone when given repeatedly together with small doses of $CCl_4$ led to a normalization of serum iron, calcium and potassium. Also serum zinc and copper were lower than in case of $CCl_4$. This may be due to some protective effect of phenobarbitone on liver mitochondria.

Serum magnesium was not affected in all the experimental groups.

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