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Effect of AMP on serum minerals in carbon-tetrachloride hepatotoxicity

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With 1 table

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Carbon-tetrachloride is well known to produce acute liver damage if given in single doses and cirrhosis if given repeatedly (13, 15). In acute liver damage an increase in plasma iron has been reported (21). However, plasma iron was not elevated in rats with various degrees of chronic liver damage (5). Liver disease was found to affect zinc in blood (6). Plasma copper was found to be raised in cases of hepatic fibrosis (7).

Early changes in calcium and potassium content of mitochondria, after oral CCl_4 intoxication, have been reported by *Reynolds* et al. (22). This indicates that a disturbance in minerals metabolism may occur in CCl_4 poisoning.

Adenosine monophosphate can form complexes with ions as copper and magnesium (2). The chelation of metal ions by adenosine monophosphate can affect serum minerals.

In another study we found that prior treatment with adenosine monophosphate ameliorated liver function in carbon-tetrachloride hepatoxicity (27).

The purpose of the present work is to study the effect of AMP on serum iron, copper, zinc, calcium, magnesium, sodium and potassium in acute carbon-tetrachloride poisoning.

Material and methods

Adult albino rats of both sexes (Sprague Dawley strain) weighing 150–200 g and maintained on stock diet were used. Food and water were allowed ad libitum.

The animals were divided into three groups: Control group, Group treated with CCl_4 and Group treated with AMP $^1/_2$ hour before CCl_4. Each group consisted of 10 animals.

The animals were injected intraperitoneally with CCl_4 (1:1 V/V) in mineral oil) 0.5 ml mixture per 100 g body wt. The control animals were similarly given corresponding amounts of mineral oil.

Where protection by AMP was studied, AMP was given intraperitoneally (30 mg/animal) $^1/_2$ hour before the injection of carbon tetrachloride. Twenty-four hours after administration of carbon tetrachloride, animals were killed by decapitation and blood was collected for serum minerals determination.

The method of *Sinaha* and *Gabrielli* (25) was used for determination of serum zinc. 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* (29).

Results and discussion

The fact that iron is an essential constituent of hemoglobin and of cytochrome and other components of respiratory enzyme systems makes it an element of great fundamental importance. Its chief functions lie in the transport mechanisms (cytochrome system) (8).

In the present work, the mean serum iron in CCl_4 treated rats when compared with control rats there is a statistically significant increase. The injection of AMP 30 minutes before CCl_4 led to a significant decrease of serum iron. This may be due to some protective action of AMP on the liver (27).

The increase in serum iron may result from the disintegration of hepatic cells or may be due to haemolysis of the red blood corpuscles.

Tab. 1.

		Control	CCl_4	AMP + CCl_4
Zinc	M	133.4	203.3	158.2
(μg/100 ml)	S.D.	± 13.4	± 25.1	± 36.5
P : C vs. CCl_4		–	< .05	> .05
CCl_4 vs. AMP + CCl_4				< .05
Copper	M	118.9	198.2	144.0
(μg/100 ml)	S.D.	± 15.5	± 31.2	± 37.8
P : C vs. CCl_4		–	< .05	> .05
CCl_4 vs. AMP + CCl_4				< .05
Iron	M	135.3	208.5	156.0
(μg/100 ml)	S.D.	± 16.9	28.1	31.0
P : C vs. CCl_4		–	< .05	> .05
CCl_4 vs. AMP + CCl_4				< .05
Calcium	M	8.57	9.95	10.74
(mg/100 ml)	S.D.	± 1.11	± 0.73	± 0.95
P : C vs. CCl_4		–	< .05	< .05
CCl_4 vs. AMP + CCl_4				> .05
Magnesium	M	4.02	3.71	3.86
(mg/100 ml)	S.D.	± 0.39	0.30	0.60
P : C vs. CCl_4		–	> .05	> .05
CCl_4 vs. AMP + CCl_4				> .05
Potassium	M	22.82	26.56	26.35
(mg/100 ml)	S.D.	± 2.26	± 2.90	± 1.66
P : C vs. CCl_4		–	< .05	< .05
CCl_4 vs. AMP + CCl_4				> .05
Sodium	M	228.7	270.7	253.0
(mg/100 ml)	S.D.	± 9.5	± 17.9	± 27.8
P : C vs. CCl_4		–	< .05	< .05
CCl_4 vs. AMP + CCl_4				> .05

Acute liver damage resulted in an increase in plasma iron (21), while plasma iron was not elevated in rats with various degrees of chronic liver damage (5).

The acute liver damage due to CCl_4 in the present work may be accompanied with haemolysis of the red blood corpuscles.

Copper is an essential constituent of animals and plants and its absence may lead to severe derangement of growth physiology and metabolism. Variations in plasma copper level have been encountered in a variety of diseases (7).

In CCl_4 treated rats the serum copper was higher than that of the control. The high level of serum copper in CCl_4 treated rats is related to hepatic damage. Injection of AMP before CCl_4 led to normalization of serum copper. This may also be due to the protective effect of AMP.

Imamura (9) reported that the elevation of total serum copper was seen in cancer of the liver, stomach, biliary tract and lung and in leukemia. Serum copper was also high in hepatitis.

Zinc is necessary for normal growth and general health (20). It has been shown to be an integral constituent and-factor of a number of enzymes (17) and is receiving wide-spread attention as a possible limiting factor in normal wound healing (19). It seemed to play a catalytic role in insulin production (12).

Under normal conditions concentration of zinc is maintained at a relatively stable level in the fluids and tissues of the body despite its continual and rapid turnover. However changes in zinc metabolism, notably a significant decrease in serum values, have been reported to occur in response to the stress of a variety of diseases (28).

These changes in zinc metabolism may possibly prove to be of diagnostic, prognostic or even therapeutic usefullness in various disease states (18).

In this work the serum zinc was higher in rats treated with CCl_4 than in normal rats. This increase was normalized under the influence of AMP.

Hyperzincemia was found in hyperthyroidism and hypertension. The level of blood zinc was also raised by the administration of adrenalin, thyroxine and thyrotropic hormones (4). A portion of this increase was due to haemolysis of erythrocytes (30).

The decrease in serum zinc under the influence of AMP may be due to the action of AMP on hormone secretion. In recent years a large body of data has been accumulated showing that cyclic AMP is the critical intracellular mediator of the actions of many hormones on their target tissues (26).

Potassium is an important catalyst and co-factor in many enzyme processes in carbohydrate metabolism.

When signs of intracellular dehydration occur, the plasma potassium may be somewhat higher than normal (3). The increase in serum potassium may also be from the red cell haemolysis.

Carbon-tetrachloride administration induced a significant increase in the level of plasma potassium. The serum potassium is still high and does not return to the normal level under the influence of AMP.

The mean serum sodium in CCl_4 treated rats was considerably higher than normal. The serum sodium remained high and did not return to the normal level 24 hours after treatment with AMP plus CCl_4.

The serum calcium was significantly high in CCl_4 treated rats. In pretreatment with AMP, serum calcium was still high.

Malaisse et al. (14) stated that glucose-induced insulin release is probably associated with a concomitant release of calcium.

AMP promote insulin secretion (11). These findings are in agreement with those of *Jün-Bor* (10) who found that alloxan decreased blood calcium levels in both diabetic rats and mice.

It is proposed that Ca^{++} is somehow required for the structural integrity of the cell membrane, whereas Mg^{++} probably participates in the mechanism whereby insulin promotes the binding of glucose to the sugar transport carrier (16).

On treatment with carbon tetrachloride or CCl_4 plus AMP we found that the serum magnesium was normal after 24 hours.

A slight decrease in serum magnesium was observed in patients with liver disease (23).

Calvert and *Brody* (1) suggested that a predominant factor in the hepatotoxicity of CCl_4 is an anoxia produced through the mediation of the sympathetic nervous system. Release of epinephrine from the adrenal medulla under sympathetic stimulation is also suggested.

Early changes in minerals content of mitochondria have been reported (23) 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 carbon-tetrachloride poisoning appears to follow the increase in concentration of calcium in liver mitochondria (24). Changes in mitochondrial oxidation still required about 18 hours to be manifest.

From the results of the present work and from previous findings it is concluded that the disturbance in minerals metabolism is one of the earliest lesions in CCl_4 poisoning.

Summary

Carbon tetrachloride twenty-four hours after its administration resulted in a significant increase in serum iron, copper, zinc, calcium, potassium and sodium, while for magnesium no significant change was observed. A portion of this rise was due to the known hepatotoxic effect of CCl_4 on the liver.

Pretreatment with adenosine-5-monophosphate led to a normalization of the level of serum iron, copper and zinc, while in case of calcium, magnesium, potassium and sodium there was no significant change from that found in CCl_4.

The normalization of serum copper and iron under the influence of AMP may be due to some protective action of AMP on the liver.

However, the disturbance in minerals metabolism may be considered as one of the earliest lesions in CCl_4 poisoning.

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