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Effect of phenobarbitone and propionyl-promazine on serum enzymes in carbon-tetrachloride hepatotoxicity

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With 6 figures and 1 table

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Carbon-tetrachloride (CCl_4) has a wide-spread use in the various industries. It has been used also in the treatment of hookworm infestations in man and liver flukes in animals.

In recent years much work has appeared about the induction of liver damage by carbon-tetrachloride and protection against it by various agents like antihistaminics, phenothiazines quinine and procaine (28).

Potentiation of CCl_4 toxicity by phenobarbital has been reported by *Garner* and *McLean* (13), *Stenger* et al. (31), *Lal* et al. (23) and recently *Cornish* et al. (6).

However, *Gadgil* et al. (12) showed that phenobarbitone had a definite protective effect on the hepatotoxicity produced by carbon-tetrachloride in rats.

Jenkins et al. (17) have reported that pretreatment with phenobarbital protects against the hepatotoxicity of 1,1-dichlorethylene. *Welch* and *Coon* (34) have reported substantial decrease in the toxicity of a number of organo-phosphorus insecticides as a result of pretreatment of mice with phenobarbital.

An attempt to clear the mechanism of liver damage the study of the influence of various hepatic microsomal enzyme inducers on the hepatotoxic effect of CCl_4 is of value.

The present work was undertaken to investigate the influence of phenobarbital when it is given in repeated doses together with small doses of CCl_4 on serum enzymes. The same experiment was repeated to investigate the influence of propionyl-promazine (phenothiazine derivative) on CCl_4.

It is also recognized that the barbiturates and phenothiazine tranquilizers are in general use by a considerable part of the working population. Thus the potentiation of CCl_4 toxicity by these materials represents a potential hazard that should be recognized.

Serum enzymes and histopathologic studies were utilized as a measure of hepatotoxic response to the drugs employed.

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Material and methods

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

Ten animals were included in each treatment group. The six experimental groups were arranged as in table 1. Carbon-tetrachloride (0.1 ml/kg s.c.) diluted in the ratio of 1:1 with paraffin oil was administered daily for ten days, phenobarbitone (60 mg/kg i.p.) and propionyl-promazine (2 mg/kg i.m.) were administered daily for a period of 10 days. Two other groups were administered either phenobarbitone plus CCl_4 or propionyl-promazine plus CCl_4. The animals were then sacrificed by light ether anesthesia and blood samples were taken from the orbital plexus. Serum was obtained by centrifugation and immediately frozen for subsequent enzyme analysis.

Serum glutamic oxaloacetic transaminases (SGOT) and serum glutamic pyruvic transaminases (SGPT) were assayed by the method of *Reitman* and *Frankel* (29). Serum alkaline phosphatase was assayed by the method of *King* and *Armstrong* (20).

Liver samples were taken for histopathologic studies.

Results

The effect of daily administration of phenobarbitone or propionyl-promazine together with s.c. injection of CCl_4 is shown in table 1.

In group 2 when CCl_4 was administered alone, SGOT, SGPT and alkaline phosphatase were significantly increased in comparison to those in the control group.

The activity ratio between serum GOT and GPT in the normal control group was 2.44. The activity of SGPT increases nearly 6.1 fold after CCl_4 administration and thus the activity ratio between GOT and GPT is sharply reduced to 0.56. The activity of serum GPT when CCl_4 and phenobarbitone were administered together showed value of about 50 % of the value when CCl_4 was administered alone, while it remained high when CCl_4 administration was combined with propionyl promazine.

Serum alkaline phosphatase increased significantly in all the groups especially in group 4 when phenobarbitone was administered with CCl_4.

Regarding the pathological examination of the liver, it was found that the liver in the group in which CCl_4 was administered alone, the liver showed the typical picture of central fatty necrosis, the lobular pattern was preserved. The hepatic cells showed severe degree of degeneration, mainly central, with big vacuoles of fat which occupied most of the bulk of the cytoplasm. The cell nuclei were pyknotic and pushed to the periphery of the cells. The central veins and interlobular vessels were congested.

When phenobarbitone was administered alone (group 3), the lobular pattern was preserved. The hepatic cells showed variable degrees of degeneration as cloudy swelling in the central zone and vacuolation of variable sizes due to fatty change more apparent in the peripheral zone. Central veins and sinusoids were dilated and congested.

Table 1. Effect of daily administration of phenobarbital or propionyl-promazine together with s.c. injection of CCl_4 on serum enzymes.

Experimental procedure	Doses given daily for 10 days	SGOT I.U./L	SGPT I.U./L	GOT/GPT	AlK Phosph K.A.U.
1 Control group		64.2 ± 3.8	26.3 ± 1.20	2.44	17.5 ± 4.71
2 CCl_4 group	.1 ml/kg s.c.	89.8 ± 7.6	160.0 ± 6.58	0.56	38.8 ± 2.22
3 Phenobarbitone group	60 mk/kg i.p.	98.4 ± 5.5	42.4 ± 2.89	2.32	33.3 ± 2.80
4 CCl_4 + Phenobarbitone	60 mg/kg i.p.	114.2 ± 4.5	71.6 ± 11.97	1.59	105.4 ± 14.24
5 Propionyl-premazine	2 mg/kg i.m.	77.6 ± 7.1	37.0 ± 4.56	2.06	27.0 ± 5.48
6 CCl_4 + Propionyl-promazine	2 mg/kg i.m.	142.2 ± 8.5	138.0 ± 6.04	1.03	38.4 ± 1.36

In group 4, when CCl_4 was administered together with phenobarbitone, the lobular pattern of the liver was maintained with scattered few areas of degeneration showing slight degrees of cloudy swelling and small vacuoles of fatty degeneration mainly in the periphery of the lobules. Blood vessels appeared normal.

In group 5, when propionyl-promazine was administered alone, the liver appeared within normal. The cells showed minimal cloudy swelling, some nuclei were pale, sinusoids were slightly dilated.

In the last group 6 (CCl_4 and propionyl-promazine) the lobular architecture of the liver was intact. The cells showed massive fatty degeneration especially at the centre of the lobules, with pyknotic shifted nuclei, but to a lesser degree than in group 2 with CCl_4 alone.

Discussion

Considerable evidence is now available suggesting that the production of liver damage is linked with an increased activity of the liver microsomal enzymes (24). Increases in microsomal enzymes can be brought about by pretreatment of the experimental animals with one of a number of drugs. Phenobarbital is one of the substances commonly used for this purpose (4). Inhibition of hepatic microsomal enzymes is also produced by certain compounds as phenothiazenes (36).

The importance of the activity of the microsomal drug-metabolizing enzymes in the CCl_4-induced liver injury has been previously emphasized. In some experiments where the activity was reduced, the animals proved resistant to the poison (24, 7); by contrast the increase of the enzyme activity leads to increased sensitivity to CCl_4 (24). Potentiation of CCl_4 toxicity by phenobarbital has been reported by various authors (6, 13, 23, 31).

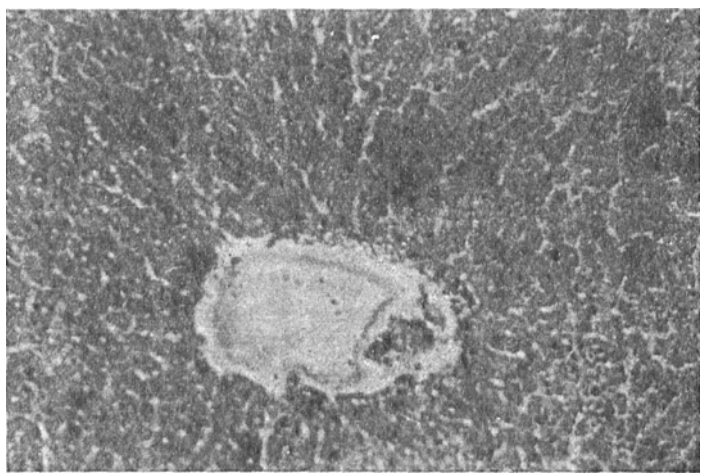

Fig. 1. A section in the liver stained with H & E of normal liver pattern and vasculature.

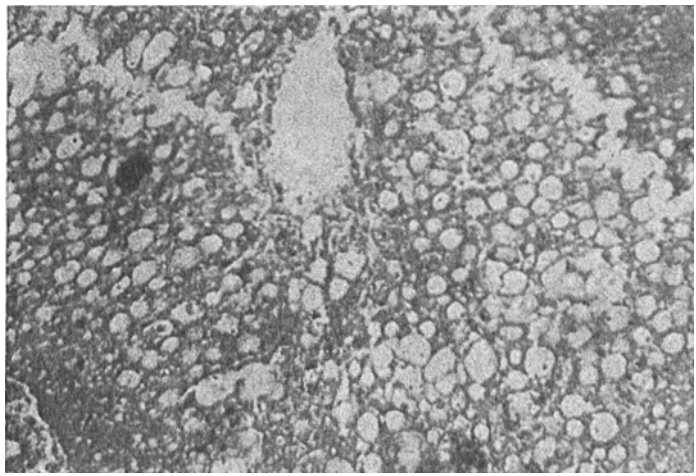

Fig. 2. A section in the liver stained with H & E of CCl_4 group showing massive fatty degeneration, mainly central vessels are congested and dilated.

However, the protective effect of phenobarbital on CCl_4-induced fatty liver was demonstrated histologically in the present work. Thus when CCl_4 was administered alone, the liver showed the typical picture of central fatty necrosis. However, when CCl_4 was administered together with phenobarbitone, the lobular pattern of the liver was maintained, showing slight degree of fatty degeneration. This is in accord with the previous results of *Gadgil* et al. (12).

Both phenobarbital, which is known to be an inducer of microsomal enzymes (3), and SKF 525A, which is an inhibitor (30), afford a clear protection of fatty liver in ethanol-poisoned animals (22).

Also pretreatment of mice with phenobarbital or chlorcyclizine decreases the toxicity of a number of organophosphorus insecticides (34). Chlorcyclizine is a potent inhibitor of drug-metabolizing enzymes (19), while phenobarbital is a potent inducer (4).

Taking into account that the two drugs, phenobarbital and SKF 525 A or phenobarbital and chlorcyclizine, have an opposite action on drug-metabolizing enzyme activity, the assumption that these enzymes have a role in the genesis of fatty liver seems untenable.

Study of serum enzymes is useful in detecting the point of action of toxic substance. Estimation of SGPT and SGOT are found to be very sensitive indices of hepato-cellular injury, and it seems that SGPT is a more specific and sensitive index than SGOT.

From table 1 it is clear that SGPT activity rises sharply after subcutaneous injection of CCl_4 and when CCl_4 administration was combined with propionyl-promazine. However, SGPT decreased when CCl_4 administration was combined with phenobarbitone. Thus the activity of serum GPT when CCl_4 and phenobarbitone were administered together showed value of about $^1/_2$ of the value when CCl_4 was administered alone.

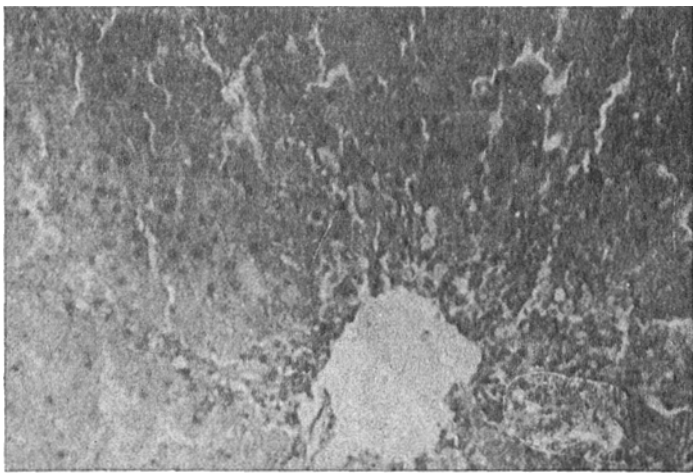

Fig. 3. A section in the liver stained with H & E of phenobarbitone group showing variable degree of degeneration as clowdy swelling, fatty vacuoles of variable size more in the peripheral zone. Vessels and sinusoids are dilated.

The serum glutamic pyruvic transaminases level shows that phenobarbitone had a protective effect on the hepatotoxicity produced by carbon tetrachloride, as shown from the pathological examination of the liver (fig. 2 and 4).

Marked fatty necrosis could be demonstrated when high values of SGPT were found, which is not the case with serum GOT.

This proves that serum GPT is a more specific and sensitive index than SGOT of hepatocellular injury. Regarding SGOT it was increased

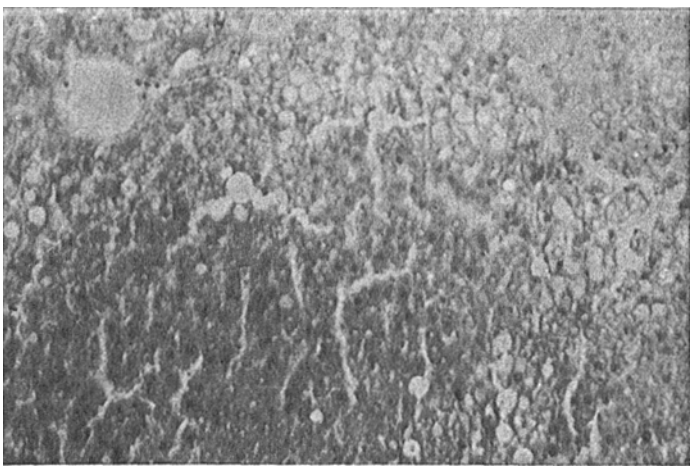

Fig. 4. A section in the liver stained with H & E of phenobarbitone plus CCl_4 group showing scattered few areas of degeneration and small vacuoles of fat mainly at the periphery.

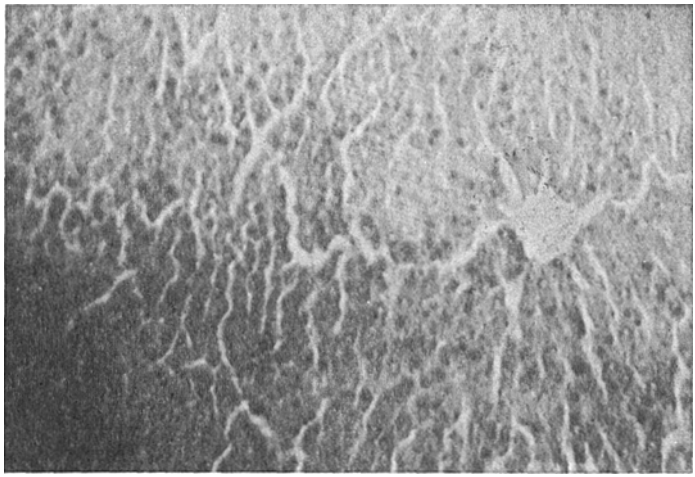

Fig. 5. A section in the liver stained with H & E of propionyl-promazine group showing more or less normal architecture, with minimal cloudy swelling, some pale nuclei and slight dilatation of sinusoids.

significantly after daily administration of phenobarbital or propionyl-promazine together with s.c. injection of CCl_4 in comparison to those when CCl_4 was administered alone.

This may be due to myocardial damage or ischaemia, since in the case of phenobarbitone it afforded protection to the liver against the hepatotoxic effect of CCl_4.

Large doses of barbiturates can depress the cardiac and vasomotor centers in the brain stem with resultant diminution in cardiac output and hypotension (26). And when barbiturate intoxication is severe, myocardial metabolism may be adversely affected resulting in congestive heart failure (32, 15).

Conrad (5) considered serum GOT a specific test for myocardial and hepatocellular damage or necrosis, and not a nonspecific indicator of inflammation or tissue reaction.

The increase in SGOT in case of phenobarbitone or propionyl-promazine when it was administered together with CCl_4 was significantly more than CCl_4, phenobarbitone or propionyl-promazine when administered alone. This shows that the interaction between CCl_4 and phenobarbitone or propionyl-promazine potentiated their toxicity on the heart.

The activity ratio between serum GOT and GPT in the normal control group was 2.44. The activity of GPT increases nearly 6.1 fold after CCl_4 administration and thus the activity ratio between SGOT and SGPT is sharply reduced to 0.56. This is in accord with the previous work of *Goldbergi* et al. (16) who found that SGPT activity increases 8.3 fold after subcutaneous injection of 1 mg/kg body wt. of CCl_4 and the activity ratio between GOT and GPT is sharply reduced.

Serum alkaline phosphatase increased significantly in all the groups especially in the group when phenobarbitone was administered with CCl_4.

Fig. 6. A section of the liver stained with H & E of propionyl-promazine + CCl_4 group showing massive fatty degeneration, specially at the central zones. The fatty change is, however, less than that produced by CCl_4 alone.

Alkaline-phosphatase increases 24–48 hrs after administration of high doses of CCl_4 when hepatobiliary injury is produced (33). Increased alkaline phosphatase was also reported by *Koch-Weser* (21) in blood serum and liver tissue of CCl_4-poisoned rats.

An elevated value of serum alkaline phosphatase has been considered to originate from bone or from liver, and in some cases (of metastatic cancer) from both. Now factors which regulate levels in the circulation of intestinal alkaline phosphatase become relevant in the interpretation of abnormal values of the serum alkaline phosphatase (10).

No direct correlation was found by *Rees* et al. (27) between degree of liver injury and serum enzyme levels in CCl_4 poisoning. There is an initial lesion which results in leakage of cytoplasmic enzymes. Mitochondrial damage is a late manifestation in CCl_4 poisoning. Only recent injury may be measured by the enzyme levels in serum since the rise in most cases being transitory.

Wirtschafte (35) found that serum glutamic oxaloacetic transaminase activity rises sharply at 36 hrs after subcutaneous injection of CCl_4, reaches a peak at 48 hrs and then declines rapidly. Low values were observed in 1 of 7 animals and are therefore not specific indicators of absence of hepatic necrosis.

Chinsky et al. (2) stated that the amount of transaminase liberated into the serum probably reflects the amount of tissue involved, multiplied by its transaminase concentration.

The results of the present work show that phenobarbitone had a protective effect on the hepatotoxicity produced by carbon tetrachloride, while propionyl-promazine had not.

Administration of the tranquillizer propionyl-promazine was accompanied by a marked decrease in number of RBCs, packed cell volume and

haemoglobin level (11). This fact was attributed to splenic storage of RBCs. This splenic storage withdraws an important quantity of oxygen transporting RBCs from the circulation. Hence the application of tranquillizers in anaemic and shocked animals is contraindicated (8).

Liver damage due to carbon tetrachloride has been attributed by *Calvert* and *Brody* (1) to sympathetic overflow leading to hepatic ischemia. This may explain the potentiation of propionyl-promazine to the toxic effect of CCl_4 on the heart and liver due to its hypoxic effect, since rats exposed to low oxygen tension show far more severe lesions than the control animals in air (14).

Many theories have been postulated regarding the mechanism of the hepatotoxic action of carbon tetrachloride. It is suggested that CCl_4-induced liver injuries have a mechanism which involves the peroxidative decomposition of structural lipids, particularly at the level of the endoplasmic reticulum (9, 18).

Phenobarbital (25) is known to be bound to liver microsomes, thus inhibiting the activity of the enzymes which provoke the peroxidation of microsomal structural lipids.

From this point of view the protective mechanism of phenobarbitone in the present experimental conditions seems to be due to an antagonistic action of phenobarbitone in carbon-tetrachloride-induced decomposition at the level of the endoplasmic reticulum.

Summary

The influence of phenobarbitone given in ten repeated doses simultaneously with small doses of CCl_4 on serum enzymes was investigated in albino rats.

The same experiment was repeated to investigate the influence of propionyl-promazine (phenothiazine derivative).

The results proved that SGPT is a more specific and sensitive index than SGOT of hepato-cellular injury.

The activity ratio between serum GOT and GPT in the normal control group was 2.44. The activity of SGPT increased nearly 6.1 fold after CCl_4 administration and thus the activity ratio between GOT and GPT is sharply reduced to 0.56.

The activity of serum GPT when CCl_4 and phenobarbitone were administered together showed value of about $^1/_2$ of the value when CCl_4 was administered alone, while it remained high when CCl_4 administration was combined with propionyl-promazine.

Serum GOT and alkaline phosphatase increased significantly in all the groups.

Regarding the pathological examination of the liver it was found that marked fatty necrosis could be demonstrated when high values of SGPT was found, which is not the case with serum GOT.

It is concluded that in the present experimental conditions phenobarbitone protected the liver from the hepatotoxic effect of CCl_4, while propionyl-promazine did not.

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