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Hepatotoxicity of CS_2 in rats: relation to postexposure liver weight and pre-exposure cytochrome P-450 level

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MICROSOME mixed function oxidase activity and cytochrome P-450 levels are depressed in animals given CS_2.[1,2] Such animals are less sensitive to carbon tetrachloride liver injury.[3,4] In animals pretreated with phenobarbitone, CS_2 causes a greater fractional decrease in both drug metabolizing enzymes and cytochrome P-450 levels.[1] In contrast to this biochemical effect, CS_2 does not induce liver lesions after a single toxic oral dose[5] but does so after phenobarbitone pre-treatment[5,6] and this effect is aggravated by short-term starvation.[6] In the present work we examined the relation between histological damage and the level of cytochrome P-450 at the time of CS_2 exposure. Different liver cytochrome P-450 levels were achieved by feeding or starving rats given an identical phenobarbitone dose but varying the time before exposure to CS_2.

Albino rats of Porton–Wistar strain (200–220 g) were given two doses of phenobarbitone sodium (BDH) i.p., the first 80 mg/kg and the second 50 mg/kg 6 hr later. Control animals were given saline. Animals were killed decapitation 24 or 48 hr after the first injection and liver cytochrome P-450 estimated using the procedures described by Bond and De Matteis,[1] or were subjected to a 4 hr inhalation exposure to 2·0 mg/l. CS_2 in an inhalation chamber described earlier.[7] Animals were divided into six groups and treated as shown in Table 1. All animals were males except in Group F. Food was withdrawn at the start of exposure but for some animals food was withdrawn at either at the time of the first phenobarbitone injection or 24 hr later. Exposed animals were killed 18 hr after the end of exposure, livers were removed, weighed and fixed in formol alcohol or formol saline. Paraffin sections were prepared and stained with Harris' haematoxylin and eosin. Sections were scored as follows: indistinguishable from normal, 0; few hydropic cells in the region of central veins, 1+; approximately half of the lobules were changed to hydropic cells, 2+.

Table 1 shows that phenobarbitone pretreatment significantly increased the cytochrome P-450 level in every group, while starvation of the phenobarbitone pretreated animals caused a further significant increase. In control rats 1 day fasting resulted only in a slight change in cytochrome P-450 levels but after 2 days starvation P-450 increased significantly (Group E saline-treated controls) and was even higher than in phenobarbitone-treated fed rats (Group A) ($t = 3{\cdot}19$; $P < 0{\cdot}05$). However, these animals which were starved for 2 days without phenobarbitone treatment (Group E) did not develop histological change on exposure to CS_2.

In phenobarbitone-treated rats the lowest cytochrome levels were found in the female 24 hr starved group (Group F) or in the male fed group (Group A) and the highest in the male 48 hr starved group (Group E). The female group had no liver damage, only 4 of the 8 fed male had slight liver damage, while the last group developed the most extensive hydropic degeneration. However, within these two extremes, no correlation was found between the pre-exposure cytochrome P-450 levels and post-exposure liver damage. Thus only one animal in Group C which had approximately the same cytochrome level as in Group B developed a slight liver lesion. In group D with significantly higher cytochrome P-450 concentrations than in Group B ($t = 4{\cdot}31$ $P < 0{\cdot}0125$) the extent of liver damage was less pronounced.

Liver weight after exposure increased in starved animals in the same order as liver damage, that is F < C < D < B < E. The correlation between liver weights and liver damage can be seen in Table 2.

TABLE 1. RELATIONSHIP BETWEEN PRE-EXPOSURE CYTOCHROME P-450 LEVELS IN LIVER AND THE DEGREE OF HYDROPIC DEGENERATION AFTER A SINGLE 4 hr EXPOSURE TO 2·0 mg/l CS_2

Group	Pre-exposure treatment (48–24 hr*) Phenobarb.	Pre-exposure treatment (48–24 hr*) Starvation	Pre-exposure treatment (24–0 hr*) Phenobarb.	Pre-exposure treatment (24–0 hr*) Starvation	Pre-exposure cytochrome P-450 (0 hr) nmole/g liver wet wt ± S.E.M.	Liver weight 18 hr after the end of exposure g/100 g b.w.	Degree of hydropic degeneration 0	1+	2+
A♂	−	−	+	−	31·3 ± 2·4 N = 4 (18·0 ± 0·9 N = 4)	3·77 ± 0·06 N = 8	4	4	
B♂	−	−	+	+	44·3 ± 2·0 N = 8 (23·8 ± 1·1 N = 7)	4·69 ± 0·11 N = 8		2	6
C♂	+	+	−	−	46·7 ± 2·3 N = 4 (19·1 ± 1·5 N = 4)	3·79 ± 0·06 N = 12	11	1	
D♂	+	−	−	+	58·8 ± 2·7 N = 8 (21·3 ± 1·0 N = 4)	4·26 ± 0·11 N = 12		6	6
E♂	+	+	−	+	76·0 ± 4·7 N = 4 (40·2 ± 1·4 N = 4)	5·27 ± 0·20 N = 8			8
F♀	−	−	+	+	25·1 ± 1·4 N = 4 (18·8 ± 1·9 N = 4)	3·52 ± 0·05 N = 8	8		

Phenobarbitone sodium was given i.p. in a dose of 80 mg/kg and 6 hr later 50 mg/kg. First injection was given either 48 or 24 hr before exposure to CS_2 or estimation of cytochrome P-450. Numbers in parentheses give cytochrome P-450 concentration in saline-treated animals. Scoring of hydropic degeneration is described in the method section.

* Time before killing for estimation of P-450 or start of CS_2 exposure.

TABLE 2. RELATION OF RELATIVE LIVER WEIGHT TO HYDROPIC DEGENERATION IN MALE PHENOBARBITONE-PRETREATED RATS 18 hr AFTER THE END OF EXPOSURE TO CS_2

Extent of hydropic degeneration	Liver wet weight per 100 g b.w. (g) (18 hr after exposure)	Significance of difference from middle group
0	3·79 ± 0·054 $N = 16$	$t = 2·01$ $P < 0·05$
1+	4·01 ± 0·095 N = 12	
2+	4·90 ± 0·109 N = 20	$t = 6·14$ $P < 0·005$

Bond and De Matteis[1] have shown that in phenobarbitone pretreated rats, CS_2 increased liver weight by increasing its water content and Butler, Hempsall and Magos (in preparation) have found a correlation between hydropic degeneration and liver water content. Table 2 shows that liver weight correlates well with the presence and degree of hydropic degeneration observed in rats after exposure to CS_2. Comparing preexposure cytochrome levels with either liver weight or hydropic degeneration it seems that the level of cytochrome P-450 is not, or at least not alone, a measure of sensitivity to the hepatotoxic carbon disulphide effect. First of all cytochrome P-450 levels can be increased by starvation to a level which in some of the phenobarbitone-treated rats was followed by hydropic degeneration, nevertheless these animals were not affected by CS_2. Secondly by modifying the time interval between phenobarbitone treatment and the starvation schedule, cytochrome P-450 levels can be increased and hydropic degeneration decreased.

At present it is impossible to define the role of phenobarbitone and starvation in the sensitization of liver against CS_2. It has been reported that the excretion of free and acid labile CS_2 and bivalent sulphur metabolite in CS_2 exposed rats was influenced only by starvation but not by phenobarbitone,[8] and it was suggested that the effect of phenobarbitone on carbon disulphide hepatotoxicity might not be mediated through the formation of a more toxic metabolite. Based on the relationship between cytochrome P-450 and drug metabolizing enzymes the results reported here have given a further support to this hypothesis.

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