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SHORT COMMUNICATIONS

The effect of carbon tetrachloride on heme components and ethylmorphine metabolism in rat liver microsomes

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THE ADMINISTRATION of carbon tetrachloride can lead to liver necrosis,[1] accumulation of neutral lipids[2] and decreased activity of microsomal enzymes which catalyze the oxidation and reduction of drugs.[3–5] It has been shown that the impairment of liver enzymes is accompanied by a decrease of liver microsomal cytochrome P-450.[6–8] Smuckler *et al.*[6] have presented evidence that the decrease seen was not due to an inability of the pigment to bind carbon monoxide when carbon tetrachloride was added to microsomes, or to a loss of this cytochrome during the isolation of the microsomes.

Nevertheless, it is not known whether the reduction of microsomal P-450 observed after carbon tetrachloride administration is due to an alteration of the native P-450, as suggested by Smuckler *et al.*,[6] or to a destruction of this cytochrome, as suggested by Sasame *et al.*[8] To distinguish between these possibilities, we have determined the amount of heme and cytochrome b_5 in addition to P-450 after administration of carbon tetrachloride. The *N*-demethylation of ethylmorphine was also studied in order to relate changes in the heme components to the functional state of the microsomal enzyme system.

Male Osborne–Mendel rats weighing from 100 to 120 g were used. They were kept on a Purina diet and fasted overnight, during which they had free access to water. Undiluted CCl_4 was given orally by means of a stomach tube in a dose of 2·5 ml/kg of body weight (control rats received an equal volume of water). At different time intervals, the rats were decapitated and the livers were removed and chilled in ice-cold Tris-HCl–KCl (1·15% KCl in 0·02 M Tris buffer, pH 7·4).

The livers were homogenized in the ice-cold Tris-HCl–KCl solution with a Teflon glass homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min in a Servall refrigerated centrifuge. The supernatant was then centrifuged for 1 hr at 78,000 *g* in a Spinco model L preparative ultracentrifuge. The microsomal pellet was washed once by resuspension in the Tris-HCl–KCl solution and centrifuged again at 78,000 *g* for 1 hr. This was done to remove traces of hemoglobin which would interfere with the microsomal heme analysis. Finally, the microsomal pellet was resuspended in Tris-HCl–KCl solution to a volume approximately twice the original liver wet weight.

Protein concentrations were determined by the method of Lowry *et al.*[9] The amounts of P-450, P-420, cytochrome b_5 and total heme were determined by the spectrophotometric methods of Omura and Sato.[10] A Shimadzu MPS 50 L spectrophotometer was used for these determinations. Ethylmorphine (EM) metabolism was determined in 3·0-ml incubation mixtures, each consisting of 1·0 mM EM, 5 mM $MgCl_2$, 8 mM sodium isocitrate, 0·33 mM NADP, 1·00 E.U. isocitric dehydrogenase, about 6 mg of microsomal protein and 50 mM Tris buffer, pH 7·4. Preliminary experiments have shown these conditions to be optimal. The mixtures were incubated in a Dubnoff metabolic shaker for 10 min at 37° in an atmosphere of air. The Nash method[11] was used to estimate the amount of formaldehyde formed from the *N*-demethylation of EM.

Student's *t*-test was used to make comparison between the controls and the treated animals.

Results reported in Table 1 confirm the findings of previous workers that the inhibitory effect of CCl_4 on the microsomal demethylation of ethylmorphine parallels a decrease in the amount of P-450. The results also show that the decrease in P-450 is closely related to the decrease in the content of heme, which is not associated with P-420 and cytochrome b_5. This indicates that there is a direct loss of P-450 as a heme component rather than a functional alteration as suggested earlier.[6] Smuckler *et al.*[6] observed no change in cytochrome b_5 during the first 2 hr; accordingly, Table 1 shows that during the first 12 hr after CCl_4 administration no significant change in cytochrome b_5 occurs. After 24 hr, however, a pronounced decrease was observed, presumably as a result of the necrotic changes of the cells, which begin after 12 hr.[12]

TABLE 1. INFLUENCE OF ADMINISTRATION OF CCl_4 ON ETHYLMORPHINE DEMETHYLASE ACTIVITY AND HEME COMPONENTS OF RAT LIVER MICROSOMES*

Time after treatment (hr)		HCHO (mμmoles formed in 10 min/mg protein)	Cytochrome P-450 (mμmoles/mg protein)	Cytochrome P-420 (mμmoles/mg protein)	Cytochrome b_5 (mμmoles/mg protein)	Total heme (mμmoles/mg protein)	Total heme minus (cyto. P-420 + b_5) (mμmoles/mg protein)
3	Control	93·1 ± 8·9	0·909 ± 0·036	<0·025	0·286 ± 0·007	1·440 ± 0·032	1·154 ± 0·023
	CCl_4	47·6 ± 3·9	0·536 ± 0·025	<0·025	0·285 ± 0·018	1·060 ± 0·021	0·775 ± 0·020
	% of Control	51·2	59·0	—	99·7	—	67·1
12	Control	135·0 ± 6·0	0·909 ± 0·052	<0·025	0·452 ± 0·051	1·423 ± 0·084	0·971 ± 0·069
	CCl_4	39·1 ± 2·6	0·313 ± 0·015	<0·025	0·401 ± 0·023	0·810 ± 0·045	0·409 ± 0·035
	% of Control	28·9	35·0	—	88·8	—	42·0
24	Control	125·0 ± 11·6	1·190 ± 0·059	<0·025	0·427 ± 0·017	1·466 ± 0·030	1·039 ± 0·025
	CCl_4	22·4 ± 3·3	0·162 ± 0·028	0·228 ± 0·022	0·205 ± 0·016	0·570 ± 0·022	0·137 ± 0·012
	% of Control	17·9	13·8	—	48·0	—	13·2

* The results are expressed as the mean ± S.E.M. of five animals. The values from the CCl_4-treated animals were significantly different ($P < 0·01$) from the corresponding control values, except in the case of cytochrome b_5 in the 3-hr and 12-hr groups.

P-420 was measurable in the CCl_4-treated animals only after 24 hr, when a sharp peak at 420 mμ, was observed along with the peak at 450 mμ. At 3 and 12 hr, only a slight shoulder occurred at 420 mμ both in the controls and in the CCl_4-treated animals; this shoulder would account for less than 0·025 mμmole P-420.

No characteristic spectral changes were seen after addition of either CO or KCN to a microsomal suspension from control animals, indicating that the samples were free from hemoglobin or methemoglobin.

The sum of P-450, P-420 and cytochrome b_5 was less than the amount of total heme in the 3-hr or 12-hr experiments, but greater than the amount of total heme in the 24-hr experiment. Since these discrepancies cannot be explained by the presence of hemoglobin or methemoglobin, it is possible that the extinction coefficient of P-450 varies markedly and that the value (91 $mM^{-1}cm^{-1}$) derived from rabbit liver microsomes[10] is not always applicable. Despite these discrepancies, however, there is clearly a relationship between the decrease in the amount of P-450 and a loss of a heme component.

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Cholesterol biosynthesis in the liver of experimentally induced porphyric mice*

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ACUTE porphyria is an inherited metabolic disease characterized by urinary excretion of excessive amounts of the porphyrin precursors, delta-aminolevulinic acid (ALA) and porphobilinogen as the result of an elevated activity of ALA synthetase in the liver.[1,2] Moreover, recent investigations have revealed the participation of changes of lipid metabolism in addition to those of porphyrin metabolism in acute human porphyria and experimental porphyria in animals. In 1961 Labbe, Hanawa and Lottsfeldt[3] reported more than two-fold increase in the synthesis of fatty acids by the liver of rats injected with the porphyrinogenic drug, allylisopropylacetamide (AIA). Recently Taddeini,

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