acid reductase. However, adaptive increased specific activities of two hepatic tetrahydrofolate-dependent enzymes, formyltetrahydrofolic acid synthetase and formiminotetrahydrofolic acid transferase, could not be demonstrated.

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In vivo effects of carbon tetrachloride and chloroform on liver and kidney glucose-6-phosphatase in mice

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ALTHOUGH biochemical changes in the liver following the administration of carbon tetrachloride and chloroform are wellknown,[1–8] little is known about biochemical changes occurring in the kidney. We have compared the effects of $CHCl_3$ and CCl_4 on glucose-6-phosphatase activity in mouse liver and kidney.

Experimental

Animals. CFLP mice were obtained from Carworth Europe, Alconbury, Hunts; TF1 mice from A. Tuck, Rayleigh, Essex and CBA mice from the Laboratory Animal Centre, Carshalton, Surrey. They were used when they were between 20 and 30 g in weight.

Treatment. The pelleted diet FFG (Dixon & Son, Ware, Herts) and water were available *ad lib.* CCl_4 and $CHCl_3$ were given orally as 50% (v/v) solutions in liquid paraffin. Animals were killed by cervical dislocation 21 hr after dosing.

Enzyme preparation. Livers or kidneys were homogenized in 9 vol. of ice-cold 0·25 M sucrose–0·1 M cacodylate–0·001 M EDTA buffer (pH 7·0) in a Teflon-glass homogeniser. The homogenate was filtered through surgical gauze and the filtrate was used directly for enzyme assay.

Enzyme assay. The enzyme incubation mixture contained 0·4 ml of 0·1 M sodium cacodylate buffer (pH 6·1), 0·5 ml of 0·05 M disodium glucose-6-phosphate (Wessex Biochemicals, Bournemouth) and 0·1 ml homogenate. After 15 min incubation at 30°, the reaction was terminated by the addition of 2 ml trichloroacetic acid. Inorganic phosphate was determined in a 1-ml aliquot of protein-free supernatant by the method of Fiske and Subbarow.[9]

Protein concentration. This was measured by the method of Lowry *et al.*[10]

Liver lipid. This was determined by application of the methods of Folch *et al.*[11] and Bligh and Dyer[12] as described by Diplock *et al.*[13]

Results

Comparative effects of CCl_4 and $CHCl_3$ on liver glucose-6-phosphatase in male and female mice. The results are shown in Table 1. CCl_4 produced a significant fall in liver glucose-6-phosphatase in both sexes but $CHCl_3$ had no effect. These results agree with previous findings.[1,2,8]

Comparative effects of CCl_4 and $CHCl_3$ on kidney glucose-6-phosphatase in male and female mice. The results are shown in Table 2. Both CCl_4 (2 ml/kg) and $CHCl_3$ (1 ml/kg) decreased kidney glucose-6-phosphatase activity in male mice, but had little effect in female mice.

TABLE 1. EFFECTS OF CCl_4 AND $CHCl_3$ ON LIVER GLUCOSE-6-PHOSPHATASE*

Sex	Halohydrocarbon (2 ml/kg)	Glucose-6-phosphatase (μmole PO_4/g liver/hr) Control	Treated	Change (%)
M	CCl_4	981 ± 28	711 ± 72†	−28
F	CCl_4	987 ± 55	651 ± 69†	−35
M	$CHCl_3$	1205 ± 56	1165 ± 89	−3
F	$CHCl_3$	1028 ± 22	1227 ± 92	+19

* Results are the mean ± S.E. of three individual liver analyses.
† Significantly lower than controls; $P < 0.05$.

Effect of $CHCl_3$ on liver lipid accumulation. Although $CHCl_3$ did not affect liver glucose-6-phosphatase it did, like CCl_4, increase liver lipids. We found an increase in the neutral lipid content from (mean ± S.D.) 28·1 ± 8·7 mg/g to 48·5 ± 10·6 in male CFLP mice, from 31·8 ± 1·6 to 53·7 ± 7·0 in female CFLP mice, from 14·2 ± 1·6 to 44·0 ± 16·5 in male TF1 mice and from 22·2 ± 1·7 to 50·4 ± 8·3 in female TF1 mice.

Discussion

The administration of CCl_4 to animals causes, within a few hours, a loss of glucose-6-phosphatase[1,2] and, within 24 hr fatty liver and hepatic necrosis. The toxicity of CCl_4 has been shown to be dependent on its metabolism via a toxic intermediate to CO_2.[14] After CCl_4 is given to rats, the presence in microsomal lipids of a material having the optical characteristics of a conjugated diene has been demonstrated.[15] Pretreatment of rats with phenobarbitone, although increasing the toxicity of CCl_4,[14] prevents the CCl_4-induced loss of glucose-6-phosphatase.[16]

$CHCl_3$ also produces fatty liver and necrosis but, unlike CCl_4, does not affect either hepatic glucose-6-phosphatase or produce detectable amounts of diene in liver.[8] In our experiments the *in vivo* effects of CCl_4 and $CHCl_3$ on hepatic glucose-6-phosphatase have been confirmed. In addition, however,

TABLE 2. THE EFFECTS OF ORAL CCl_4 (2·0 ml/kg) AND $CHCl_3$ (1·0 ml/kg) ON KIDNEY GLUCOSE-6-PHOSPHATASE*

Strain	Sex	Halohydrocarbon	Kidney glucose-6-phosphatase (μmole PO_4/100 mg protein/hr) Control	Treated
CFLP	M	CCl_4	613 ± 59 (3)	483 ± 25 (3)
CFLP	F	CCl_4	768 ± 48 (3)	757 ± 81 (3)
CFLP	M	$CHCl_3$	621 ± 22 (9)	392 ± 18 (15)†
CFLP	F	$CHCl_3$	699 ± 27 (4)	588 ± 43 (7)
TF1	M	$CHCl_3$	589 ± 37 (4)	339 ± 15 (8)†
TF1	F	$CHCl_3$	482 ± 40 (4)	494 ± 21 (10)
CBA	M	$CHCl_3$	501 ± 17 (2)	228 ± 7 (5)†
CBA	F	$CHCl_3$	463 (1)	424 ± 20 (2)

* Mice were killed 21 hr after dosage and kidneys excised. Results are given as means ± S.E. with the no. of mice in parentheses.

† Significantly lower than control value: $P < 0{\cdot}001$.

it has been shown that both halohydrocarbons produce loss of kidney glucose-6-phosphatase in mice and this effect is sex-dependent, being greatest in males. These results demonstrate that hepatic fat accumulation and loss of glucose-6-phosphatase cannot be directly due to a common primary lesion and, when coupled with previous studies[17] on the protective action of antioxidants against CCl_4 toxicity, reinforce the view[6] that "changes seen with glucose-6-phosphatase and lipoperoxidation after CCl_4 are only secondary effects and have little to do with the initiating event in the development of the lesion".

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