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Effect of Chloroform Ingestion on Some Carcinogen-Metabolising Enzyme Systems of Rats

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Despite the original work of ESCHENBRENNER (1945), which demonstrated that large doses of chloroform induces hepatic tumours in rodents, the solvent has been widely used as a bacteriocidal agent in toothpaste and numerous other products. More recent studies led to the proposed ban of the use of the solvent in the U.S.A. (WINSLOW and GERSTNER 1978) whereas experiments conducted in the U.K. yielded contradictory results (PALMER et al. 1977 ; ROE et al. 1977). It has been estimated that a 65 Kg woman using toothpaste containing 3.5% w/v chloroform receives an oral dose of 0.15 mg/Kg/day (GLASS et al. 1975). Thus, most of the carcinogenecity investigations conducted previously have been concerned with the determination of the number of tumours which develop following administration of chloroform at various multiples of concentrations equivalent to 0.15 mg/Kg/day. It is possible, however, that chloroform is not carcinogenic per se but alters the metabolic response to endogenous or exogenous chemicals which are pro or proximate carcinogens. The hepatic cytochrome P_{450}-dependent mixed function oxidase system is largely responsible for the metabolism of many drugs and carcinogens (JERINA and DALY 1974). Therefore, in this experiment, the effect of chloroform ingestion at the 0.15 and 100-fold level on the activity of the enzymes responsible for the oxidation and conjugation of polycyclic aromatic hydrocarbons was investigated using benzo(a)pyrene as an example of these carcinogenic compounds.

MATERIALS AND METHODS

Chemicals: [G^{3}H] Benzo(a)pyrene (specific activity 40 Ci/mmol) and [8-^{14}C] styrene oxide (specific activity 16.4 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, Bucks., U.K. 1,1,1 Trichlorpropene 2,3-oxide (TCPO) was obtained from Alldrich Chemicals, Gillingham, Dorset, U.K. Unlabelled benzo(a)pyrene and all other chemicals were of the purest grade available and supplied by the Sigma Chemical Company, Poole, Dorset, U.K.

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"Analar" chloroform was redistilled at 60-62°C in 2.5 L portions discarding the first and last 250 ml.

Animals and Treatment: Three groups of 3 male Sprague-Dawley rats of body weight 330± 20 g, supplied by Olac, Bicester, Oxon., U.K. and maintained on PRM diet (Dixons, Ware, Herts., U.K.), were used at each dose level. It was found that a cage of 6 of these animals consumed an average of 240 mls of water daily so that chloroform was included in the drinking water in sufficient quantity to give a final dose of either 0.15 or 15 mg/Kg/40 ml/rat/day. Any remaining "chloroform-water" was discarded and replenished with a fresh mixture daily. The water-feed bottles were of all glass design and covered to protect the contents from the light. Control animals received no solvent in their drinking water. After 14 days chloroform ingestion, the animals were sacrificed by cervical dislocation and their livers excised, homogenised separately and microsomal pellets prepared by centrifugation.

Incubations: Microsomal protein, cytochrome P_{450}, aryl hydrocarbon hydroxylase (AHH) and Uridine diphosphoglucuronic acid (UDPGA) transferase, were all determined by the familiar techniques previously described (CAPEL et al 1978). DNA binding of benzo(a)pyrene was also estimated essentially as described except that the specific inhibitor of epoxide hydratase activity TCPO (ALEXANDROV 1977) was included in some of the incubates. Glutathione transferase was determined by the technique of JAMES et al. (1976).

Radiochemical Analyses: Duplicate assays of all samples were determined by counting for 2 cycles in a Packard 2650 liquid scintillation spectrometer. Counting efficiency was determined by external standardisation.

RESULTS

The results in Table 1 indicate that ingestion of chloroform at the 0.15 or 15 mg/Kg/day did not affect gross liver weight or microsomal protein content. Table 2 indicates that cytochrome P_{450} was significantly decreased at the higher intake level but there was no significant difference in AHH activity between the control and chloroform-treated animals.

In the presence of TCPO, which inhibited epoxide hydratase activity, there was a significant increase in the DNA binding of benzo(a)pyrene, the greatest increase being observed in the chloroform-treated animals. It was concluded, therefore, that chloroform increases epoxide hydratase activity since in the absence of this enzyme there was an increase in the binding of the carcinogen to DNA. Chloroform ingestion at the higher and lower level significantly decreased the activity of microsomal UDPGA transferase but there was no significant difference in the level of glutathione transferase.

TABLE 1 - The effect of chloroform on the livers of rats

Treatment (Dose)	Average Body weight (g)	Liver Weight (g)	Microsomal Protein (mg/g)
0 (control)	331 ± 33	13.5±1.3	27.8±0.6
0.15 mg/Kg	358 ± 13	14.3±0.5	29.7±2.2
15 mg/Kg	342 ± 29	16.0±1.9	26.5±1.2

Results expressed are the means ± S.D. of duplicate assays for 3 groups of 3 animals at each dose level.

DISCUSSION

The metabolism of chloroform is well documented, the solvent being excreted largely unchanged via the lungs in man (FRY et al. 1972). Some microsomal oxidation leading to the formation of electrophilic metabolites such as phosgene has been demonstrated in vitro (MANSUY et al. 1977). It is possible that such metabolites could be responsible for any carcinogenic effects of chloroform by a direct alkylating action on cellular macromolecules. Orally-administered chloroform for prolonged lengths of time is hepatotoxic (WINSLOW and GERSTNER 1978). The toxicity of the related chlorinated alkane carbon tetrachloride is enhanced by its metabolism and prior administration of this solvent reduces its toxicity by impairing its own metabolism (NAYAK et al. 1975). If chloroform is similar in this respect, then daily administration would presumably decrease its metabolism and thus reduce its potential carcinogenic action mediated via microsomal oxidation. The decreased cytochrome P_{450} observed at the higher treatment level in this experiment would not only decrease the oxidation of chloroform but might also reduce the P_{448}-mediated activation of other procarcinogens. There are a number of species of cytochrome P_{450} however, and it is possible that chloroform does not effect the level of the cytochrome concerned with carcinogen activation.

The P_{450}-dependent mixed function oxidase-mediated oxidation of many compounds, including polycyclic aromatic hydrocarbons, to form reactive electrophilic intermediates is regarded as the carcinogen-activation process (JERINA and DALY 1974). Further metabolism of the resultant epoxide with either glutathione transferase or epoxide hydratase form less reactive compounds and may be regarded as detoxication reactions (JERINA and DALY 1974). In this experiment chloroform had no effect on AHH activity but stimulated epoxide hydratase. Thus, chloroform should result in the conversion of activated carcinogens to less reactive diols.

TABLE 2 - The effect of chloroform on some hepatic carcinogen-metabolising enzymes in rats

Treatment (Dose)	Cyto-chrome P450 a	AHH activity b	DNA binding of [^{3}H] benzo(a)pyrene -TCPO c	DNA binding of [^{3}H] benzo(a)pyrene +TCPO c	UDPGA transferase b	Glutathione transferase b
0 (control)	1.3±0.08	0.12±0.02	5.0±1.2	6.6±0.8	1.41±0.30	99.5±16.5
0.15mg/Kg	1.2±0.09	0.14±0.03	4.4±0.5	7.4±0.6 *	1.02±0.08 *	113.7±16.8
15mg/Kg	1.0±0.09 *	0.13±0.02	5.8±1.3	9.1±0.8 *	1.06±0.25 *	93.3±11.6

Results expressed are the means ± S.D. of duplicate assays for 3 groups of 3 animals at each dose level.

a expressed as nmol/mg protein.

b expressed as nmol/mg protein/min.

c expressed as dpm x 10^4 [^{3}H] benzo(a)pyrene bound per mg DNA.

* significantly different from the controls ($P < 0.05$).

There was no effect on glutathione transferase but the decrease in UDPGA transferase could lead to a decrease in the excretion of hydroxylated carcinogens. UDPGA transferase is probably a multiple enzyme with differing substrate specificities however (DUTTON 1971), and the 4-nitrophenol used in this experiment might not be indicative of the enzyme species which glucuronidates polycyclic aromatic hydrocarbons. Thus, estimation of the likely in vivo effect on carcinogen disposition from observations of changes in enzyme activity measured in vitro within a species is complicated.

Prediction of the consequences of these changes for man is even more uncertain but this experiment does serve to indicate that ingestion of chloroform even at this low level can affect the activity of carcinogen-metabolising enzymes. Previous experiments with alcohol demonstrated that induction of these microsomal enzymes alters with duration of solvent intake (CAPEL et al. 1978a). Clearly further experiments are necessary to determine if this is also the case with chloroform before any firm decisions regarding the fate of this solvent are taken.

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