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Effect of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin on drug metabolism in the rat

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2,3,7,8-TETRACHLORODIBENZO-1,4-dioxin (dioxin),* a potential contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid, has been shown to be very toxic to various animal species[1–3] and the probable cause of industrial poisoning following accidents in 2,4,5-trichlorophenol production.[1,2,4] It also possesses foeticidal and teratogenic properties in rats and mice.[5,6] Recently French workers[7] have demonstrated that dioxin is a powerful stimulator of the enzymes that detoxify zoxazolamine in the rat; a dose of dioxin as low as 50 μg/kg being capable of shortening the zoxazolamine paralysis time by 90 per cent after 1 day. The work described here confirms this result and shows that dioxin also prolongs the action of hexobarbitone in the rat.

Male rats of the albino Porton strain weighing 180–200 g (7–8 weeks old) and with access to diet 41B and water *ad lib.* were given a single oral dose of dioxin (200 μg/kg, 100 μg/ml in Arachis oil). After either 1 or 3 days the duration of the paralysis induced by zoxazolamine hydrochloride (100 mg/kg i.p.) was measured and found to be reduced by over 54 per cent from that of oil-dosed controls (Table 1). The quantitative difference between these results and those of Buu-Hoi *et al.*[7] is most probably due to variations in the age and strain of rats used; although they quote an age of 3 months, the weight of their rats (100 g) suggests a younger animal in which the basal level of the liver microsomal oxidases would be much lower.[8]

TABLE 1. ZOXAZOLAMINE PARALYSIS TIME (MIN) OF MALE RATS TREATED WITH DIOXIN

Days after dioxin	Treated	Controls	P
1	22·0 ± 3·9 (6)	48·1 ± 8·5 (6)	0·015
3	16·9 ± 1·1 (7)	65·6 ± 6·5 (8)	0·00031

Values quoted are the mean ±S.E., the number of observations in parentheses. Significance is based on Wilcoxon's ranking test. Details of dosages are given in the text.

The zoxazolamine paralysis time is an *in vivo* measure of the activity of liver enzymes which oxidize certain aromatic substrates.[8] Since the level of these enzymes can be raised specifically by compounds such as 3-methylcholanthrene without affecting other liver oxygenases, the effect of dioxin on the hydroxylase which metabolizes hexobarbitone was determined. Male and female rats weighing 180–200 g (♂ 7–8 and ♀ 8–11 weeks old) were given a single oral dose of either dioxin (200 μg/kg, 100 μg/ml in dimethyl sulphoxide) or an equivalent volume of dimethyl sulphoxide. The sleeping time of the animals following an intraperitoneal injection of hexobarbitone sodium (♂ 150 mg/kg; ♀ 75 mg/kg) was measured either 1 or 3 days after dosing. With both sexes at 1 day there was a significant prolongation of the sleeping time, which at 3 days was more than double that of the controls (Table 2).

* Abbreviations used: 5-*cyclo*hex-1′-enyl-1,5-dimethylbarbituric acid, hexobarbitone; 2,3,7,8-tetrachlorodibenzo-1,4-dioxin, dioxin; 2-amino-5-chlorobenzoxazole, zoxazolamine.

TABLE 2. HEXOBARBITONE SLEEPING TIME (MIN) OF RATS TREATED WITH DIOXIN

Days after dioxin	Sex	Treated	Controls	P
1	♂	40·0 ± 3·1 (6)	27·4 ± 1·8 (6)	0·015
3	♂	75·5 ± 5·9 (6)	33·6 ± 3·3 (6)	0·0022
1	♀	69·4 ± 4·3 (6)	43·2 ± 1·6 (6)	0·0022
3	♀	104·2 ± 10·5 (8)	49·2 ± 3·3 (9)	$5·1 \times 10^{-5}$

Values quoted are the mean ±S.E., the number of observations in parentheses. Significance based on Wilcoxon's ranking test. Details of dosages are given in the text.

There exists the possibility that this decrease in the activity of certain hydroxylases might be secondary to some other effect of dioxin on the animals. One consequence of dosing rats with dioxin is an immediate and prolonged reduction in their food intake.* Starvation is known to depress the metabolism of hexobarbitone in male mice[9] and rats.[10] Accordingly groups of 6 rats (♂ 180–200 g) were given an oral dose of either dioxin in oil (200 μg/kg) or an equivalent volume of oil. Immediately after dosing, food was removed from the cages and the hexobarbitone sleeping time measured 24 hr later. The mean value for the dosed group, 53·7 ± 3·6 min, was significantly higher than that of their controls, 39·4 ± 3·0 min (P = 0·026); therefore the difference in food consumption of dosed animals and controls is not the sole factor affecting the liver metabolism.

In a further experiment, the hexobarbitone sleeping time of a group of rats (♀ 19–21 weeks old) which had recovered from the observable effects of an oral dose of dioxin (75 or 120 μg/kg), given 89 days previously, was compared with that of their control group. There was no significant difference between the groups, indicating that the inhibition of the hexobarbitone hydroxylase is reversible.

The similarity in the degree of inhibition of hexobarbitone metabolism in rats of both sexes suggests that, either this effect is mediated by dioxin without any requirement for its metabolism, or that such metabolism, like that of zoxazolamine and aniline,[10] is not sex dependent.

Many compounds are known[8] which can enhance the action of liver oxygenases by increasing either generally or selectively the levels of the enzymes involved. Conversely it is possible to inhibit barbiturate metabolism by the action of compounds such as piperonyl butoxide,[11] carbon tetrachloride[12] or carbon disulphide.[13] Chronic treatment of male rats with thyroxin[14] has the same divergent effect on zoxazolamine and hexobarbitone metabolism as dioxin; however, *in vitro* hexobarbitone oxidation by liver preparations from thyroxin-treated female rats is unaffected.[15] Whether these present observations have any bearing on the toxic action of dioxin has yet to be investigated.

The demonstration that dioxin has simultaneous stimulatory and inhibitory effects on different pathways of oxidative drug metabolism in the liver suggests that it may help in the study of the enzyme system involved.

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The influence of anticonvulsant drugs on formyl tetrahydrofolic acid stimulation of rat brain respiration *in vitro*

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THE TREATMENT of anti-epileptic drug-induced megaloblastic anaemia with folic acid may aggravate epilepsy[3]. A possible explanation of this is that folic acid, or one of its derivatives, may directly affect neuronal excitability. In favour of such a possibility, 5-fluorouracil (which blocks the utilization of active folate) lowers the convulsive threshold in rats.[1,4] Also formyl tetrahydrofolic acid (f-THF) in the presence of noradrenaline (NA) stimulates the respiration of brain synaptosomes and restores the respiration of synaptosomes which have been inhibited with phenobarbitone or phenytoin.[6] The following is an *in vitro* experiment in which the actions of phenobarbitone, phenytoin, primidone and sulthiame on brain respiration are observed and the influence of these drugs on f-THF stimulation of cerebral oxygen uptake examined.

Young adult, female, white Wistar rats weighing 150–190 g were used. A mitochondrial–synaptosomal suspension in 0·25 M sucrose was prepared and its oxygen consumption measured in an oxygen electrode as previously described.[6] The composition of the incubating medium was: glucose 10 mM; NaCl, 124 mM; KCl, 5 mM; KH_2PO_4 1·2 mM; $MgSO_4$, 1·3 mM; $CaCl_2$, 0·75 mM; NaH_2PO_4-Na_2H PO_4 buffer (pH 7·4) 5 mM. The final volume was 2·0 ml. To this solution various additions were made in volumes up to 0·2 ml. These gave final concentrations of up to the following values:

NA	7×10^{-4}M
f-THF	7×10^{-4}M
Primidone	$2{\cdot}5 \times 10^{-4}$M
Sulthiame	$2{\cdot}3 \times 10^{-4}$M
Phenytoin	$8{\cdot}0 \times 10^{-4}$M
Phenobarbitone	$8{\cdot}0 \times 10^{-5}$M

Concentration–response curves of enhancement of oxygen consumption by NA and f-THF are shown in Fig. 1. After the initial (ascending steeply) part of the curves, that for NA continued to rise but at a diminished rate, whereas that for f-THF reached a maximum response at about 4×10^{-4}M and above this concentration produced less effect.