A Comparison of the Interactions of Trichlorofluoromethane and Carbon Tetrachloride with Hepatic Cytochrome P-450

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CCl_3F , unlike CCl_4 , is relatively non-toxic (Jenkins *et al.*, 1970). It has been suggested that the toxicity of CCl_4 may be due in part to a ligand interaction with cytochrome P-450 (Reiner & Uehleke, 1971), affecting metabolism. CCl_3F also binds to hepatic cytochrome P-450, giving a type I interaction, similar to that produced by CCl_4 (K_s values: CCl_3F , 2.3 mM; CCl_4 , 1.1 mM). Extraction of the microsomal phospholipid by the iso-octane procedure of Leibman & Estabrook (1971) abolished this spectrum. This suggests that CCl_3F interacts with the phospholipid environment of cytochrome P-450.

CCl_3F shows a novel spectrum with microsomal preparations that have been reduced with $\text{Na}_2\text{S}_2\text{O}_4$. This difference spectrum shows peaks at 420 and 452 nm and appears similar to that obtained with CO and reduced cytochrome P-450. Reiner & Uehleke (1971) reported a similar spectrum for CCl_4 and rabbit hepatic cytochrome P-450. With rat hepatic cytochrome P-450 the magnitude of the 452 nm peak obtained with CCl_3F is approximately five times that observed with CCl_4 . The formation of this spectral change is time-dependent, reaching a maximum for CCl_3F at approx. 20 min, and is not abolished by iso-octane treatment of the microsomal preparation. One possible explanation of this observation is that CCl_3F is also capable of liganding to the Fe^{2+} of the reduced form of cytochrome P-450. In view of this, CCl_3F might be expected to inhibit competitively cytochrome P-450-dependent drug oxidations, as does CO (Estabrook *et al.*, 1970).

Experiments *in vivo* have shown a significant increase (40%) of hexobarbital sleeping time in rats at high dosages of CCl_3F (1 g/kg body wt.). CCl_4 shows a similar effect (Dingell & Heimberg, 1968). Experiments with CCl_3F *in vitro* have shown no significant inhibition of type I substrate metabolism (biphenyl hydroxylation, ethylmorphine *N*-demethylation and nitroanisole *O*-demethylation) but an apparent activation of the hydroxylation of aniline, a type II substrate. CCl_4 inhibits the metabolism of all these substrates. A solvent effect or a configurational change in cytochrome P-450 (Van Dyke & Rikans, 1971) may account for the effect of CCl_3F on aniline hydroxylation.

CCl_3F therefore binds to the phospholipid of microsomal preparations, and also to another site that appears similar to the CO-binding site.

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The Effect of Carbenoxolone on the Subcellular Distribution of [^3H]Aldosterone in Rat Kidney

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Carbenoxolone [3β -(3-carboxypropionyloxy)-11-oxo-olean-12-en-30-oic acid] is used in the treatment of gastric ulcer (Baron & Sullivan, 1970), and the side effects of Na^+ retention and hypokalaemia may occur in about 20% of patients treated with the drug (Doll *et al.*, 1968). Three possible mechanisms for this aldosterone-like effect of carbenoxolone have been discussed (Hausmann & Tarnoky, 1968): (1) possession of intrinsic aldosterone-like activity; (2) increased secretion of aldosterone; (3) displacement of aldosterone from protein binding sites. Previous work has indicated that the first two mechanisms are unlikely (Porter, 1970; Baron *et al.*, 1969). We have therefore investigated the distribution of [^3H]aldosterone in the kidney of adrenalectomized rats 30 min after simultaneous injections of [^3H]aldosterone (0.04–10 nmol subcutaneously) and carbenoxolone (50 mg intraperitoneally/kg body wt.).

In control rats a plot of [^3H]aldosterone in the kidney crude nuclear fraction against plasma [^3H]aldosterone concentration yielded a characteristic biphasic curve, as described by Fanestil & Edelman (1966). A similar plot was obtained in carbenoxolone-treated rats, but the [^3H]aldosterone concentrations in the crude nuclear fraction and in the 100000g supernatant were depressed by about 30%. The pattern of kidney nuclear uptake is considered to be a combination of high-affinity saturatable binding with a lower-affinity non-saturatable component. In control rats saturation of nuclear binding occurred at a plasma concentration of 0.83 nM, whereas carbenoxolone treatment decreased this value to 0.52 nM.

After the subcutaneous injection of [^3H]aldosterone (0.04–0.28 nmol) rat kidney nuclei from

control and carbenoxolone-treated adrenalectomized rats were fractionated by the method of Herman *et al.* (1968) into nuclear residue, a 50%-(w/v)-(NH₄)₂SO₄ precipitate and a 50%-(w/v)-(NH₄)₂SO₄ supernatant. After administration of [³H]aldosterone (0.15 nmol) the concentrations (fmol/mg of protein) of [³H]aldosterone in these three fractions were 1.5, 16.0 and 9.6 respectively in control rats and 1.4, 12.0 (*P* < 0.05) and 7.2 (*P* < 0.05) respectively in carbenoxolone-treated rats. Herman *et al.* (1968) have demonstrated that the (NH₄)₂SO₄-precipitate fraction contains stereospecific mineralocorticoid-binding macromolecules. Our experiments with rat kidney as a model system indicate that carbenoxolone can inhibit the binding of aldosterone in the kidney. Therefore alterations in the distribution of aldosterone may be the basis of the mineralocorticoid side effects exhibited by some patients treated with the drug.

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Hepatic and Extrahepatic Reduction of Nicotine 1'-N-Oxide in Rats

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Reduction of nicotine 1'-N-oxide [7:3 mixture of *trans* and *cis* diastereoisomers from *S*-(−)-nicotine] to nicotine has already been demonstrated in humans after oral administration (Beckett *et al.*, 1970) and by guinea-pig liver preparations (Booth & Boyland, 1971). We now report the hepatic and extrahepatic reduction of nicotine 1'-N-oxide by rats, and present evidence for the localization and characterization of

the enzymes involved. Enzyme activity was measured by determining the nicotine produced. The analytical procedures used were as described by Beckett *et al.* (1971).

Nicotine 1'-N-oxide was reduced anaerobically by all tissues studied, the liver being the most active. The small intestine was the most active, on a wet-weight basis, of the extrahepatic tissues, having 40-65% of the activity found in the liver, whereas kidney, heart, lung and spleen had only 5-10% of the hepatic activity. Blood or brain had no significant reducing ability towards nicotine 1'-N-oxide under the conditions used. Reduction was also observed on anaerobic incubation of nicotine 1'-N-oxide with suspensions of rat intestinal contents.

Almost all the activity from either liver or intestine was present in the 1000g supernatant, and the bulk of this was not sedimented at 10000g. The activity was present in both the microsomal and the soluble fractions, the former having 2-3 times the activity of the latter. The microsomal and soluble-fraction reductases were non-specific with regard to their requirements for NADH or NADPH, although the latter produced a greater degree of reduction.

Both reducing systems of the soluble and microsomal fractions were partially inhibited by either SKF 525-A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) or EDTA (1 mM) and nearly completely inhibited by KCN (1 mM) or CO with either NADH or NADPH as cofactor. Practically all reducing activity was abolished by boiling the tissue or by incubation in the presence of air. NADH or NADPH in the absence of tissue failed to reduce nicotine 1'-N-oxide.

It appears that the reducing systems involve an NADPH or NADH cytochrome reductase, and possibly cytochrome P-450. The behaviour of the systems towards inhibitors, heat and O₂ suggests a similarity with nitro reductase.

R. M. D. is presently on sabbatical leave from The American University, Beirut, Lebanon.

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