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