the method of Omura & Sato (1964), was 0.7 nmol/mg of protein in microsomal fractions derived from rats given each of the dietary treatments. The lowered activity could not be explained by a defect in the electron-transport path by which the cytochrome is reduced, since the total reduction of the cytochrome by NADPH was found to be the same in microsomal fractions derived from rats given each of the dietary treatments.

When the rate of oxidation of NADPH was examined in the presence of aminopyrine, the nucleotide was oxidized more slowly by the microsomal preparation from vitamin E-deficient rats.

These results suggest that the mixed-function oxidase system exhibits a specific requirement for vitamin E. Carpenter (1967, 1968) has also reported abnormal kinetics in microsomal drug metabolism in vitamin E-deficient animals. However, details of her work are not available for comparison with ours.

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Inhibition of the Alternative Pathways of Chlorpromazine Metabolism *in vitro*

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Chlorpromazine and its four main metabolites, namely monodesmethylchlorpromazine, chlorpromazine sulphoxide, 7-hydroxychlorpromazine and chlorpromazine *N*-oxide, were determined, by a radio-t.l.c. technique, after microsomal oxidation by a guinea-pig hepatic preparation in the presence of various compounds as possible inhibitors of drug metabolism *in vitro*.

Inhibition of chlorpromazine demethylation was found with *N*-ethylmaleimide and *p*-chloromercuribenzoate at 1 mM, whereas these compounds, which react with thiol groups, had no effect on *N*-oxidation at this concentration. Sulphoxidation and ring hydroxylation were moderately affected. Compound SKF 525A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) at 1 mM inhibited all routes of metabolism except *N*-oxidation.

1,10-Phenanthroline strongly inhibited all routes of metabolism except *N*-oxidation. 2,2′-Bipyridyl, another chelating agent, also inhibited demethylation and ring hydroxylation of chlorpromazine, but had little or no effect on sulphoxidation or *N*-oxidation.

Metyrapone [2-methyl-1,2-bis-(3-pyridyl)propan-1-one] and 4-aminobiphenyl inhibited the formation of chlorpromazine *N*-oxide to a greater extent than they did the other routes of chlorpromazine metabolism, whereas imidazole, another compound that binds with microsomal cytochromes, had no effect on *N*-oxidation of chlorpromazine but strongly inhibited the other routes.

Diphenylamine and the water-soluble free-radical scavenger *NN*-diphenyl-*N*′-2,4-dinitro-6-sulphophenylhydrazyl inhibited all routes of chlorpromazine metabolism, but *N*-oxidation was the least affected.

Whereas propyl gallate produced a large decrease in the demethylation of chlorpromazine but had no effect on the formation of chlorpromazine *N*-oxide, another antioxidant, ascorbic acid, had no effect on any route of chlorpromazine metabolism.

Hg^{2+}, Zn^{2+}, Fe^{3+} and Cu^{2+} at 1 mM caused inhibition of demethylation of chlorpromazine but not of *N*-oxidation.

Some inhibition by cysteamine of *N*-oxidation of chlorpromazine was found [cf. oxidation of nicotine (Gorrod *et al.*, 1971) and normethadone (Beckett *et al.*, 1971)], but this route was not inhibited by dithiothreitol, unlike the *N*-oxidation of normethadone or nicotine. Little or no inhibition of *C*-oxidation by cysteamine or dithiothreitol was reported with nicotine or normethadone, in agreement with our finding with chlorpromazine.

The results indicate that demethylation, ring hydroxylation and sulphoxidation are probably mediated by different enzyme systems from that which catalyses *N*-oxidation of chlorpromazine, a result in accordance with oxidation mechanisms for other xenobiotics (Beckett, 1971).

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A Study of the Possible Metabolism of Trichlorofluoromethane

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Trichlorofluoromethane (CCl_3F), although structurally similar to CCl_4, is regarded as inert and non-toxic (Jenkins *et al.*, 1970), and as such has been used in combination with other chlorofluoroalkanes

as an aerosol propellant. The toxicity of CCl_4 is believed to be a function of its metabolism (McLean & McLean, 1966), possibly as a result of the formation of a free radical (Butler, 1961; Slater, 1966). Similarly, the low toxicity of CCl_3F has been attributed to its inertness due to the stabilizing effect of the fluorine atom on the C–Cl bond (Slater, 1965; Clayton, 1966).

Distribution studies show that after a single dose of CCl_3F to Wistar rats a total of 97% of the dose is expired unchanged within 6h, whereas CCl_4 is excreted more slowly, only 75% being expired unchanged in 18h (Paul & Rubinstein, 1963). Possible metabolites of CCl_3F are dichlorofluoromethane ($CHCl_2F$) and tetrachlorodifluoroethane ($C_2Cl_4F_2$), which could be formed by reductive dehalogenation, via the production of a free radical. No dichlorofluoromethane was detected (<0.2% of substrate) in extracts of incubations of CCl_3F with microsomal preparations from rat and chicken, or from rat, mouse, guinea-pig and hamster pretreated with phenobarbital (100mg/kg body wt. daily for 3 days). The effect of CCl_3F on lipid peroxidation was investigated as an index of the formation of free radicals. Using the production of thiobarbituric acid-positive materials as a measure of lipid peroxidation, we found no evidence of free-radical formation from CCl_3F in rats and mice even after pretreatment of the animals with phenobarbital to stimulate any potential metabolism, but as reported by Slater & Sawyer (1971) CCl_4 did produce large increases of thiobarbituric acid-positive material.

However, CCl_3F is not completely inert biologically and has been shown to exhibit characteristic binding spectra with hepatic microsomal preparations, giving a type I spectrum, as does CCl_4, with a similar K_s value (for CCl_4, 1.1mM; for CCl_3F, 2.3mM). This binding constant (K_s) has been closely associated with the Michaelis constant for the affinity of compounds for the drug-metabolizing enzyme cytochrome *P*-450 (Schenkman *et al.*, 1967; Degkwitz *et al.*, 1969). Thus, although there is no evidence of significant metabolism of CCl_3F, it has been shown to interact with hepatic cytochrome *P*-450, which is believed to be concerned with the metabolic dechlorination of CCl_4 (McLean & McLean, 1966).

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The Metabolism of (+)- and (−)-Amphetamine and (+)- and (−)-Dimethylamphetamine in Rabbits *in vivo*

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After the intraperitoneal administration of either (+)- or (−)-amphetamine to male New Zealand white rabbits, the following compounds were detected in ethereal extracts of pooled urine: amphetamine, benzyl methyl ketone, α-methyl-β-phenylethanol, α-methyl-β-phenylethylhydroxylamine and *syn*- and *anti*-phenylacetone oximes. The urine was adjusted to pH7.4 before exhaustive extraction.

When the aqueous phase was made alkaline (pH12–13) further ether extraction gave α-methyl-β-phenylethylhydroxylamine and the above oximes, indicating the presence in urine of the hydroxylamine and the oximes in conjugated form.

When (+)- or (−)-dimethylamphetamine was administered as above, the following compounds were identified in addition to those derived from (+)- or (−)-amphetamine: dimethylamphetamine, dimethylamphetamine *N*-oxide, methylamphetamine and *N*-methyl-α-methyl-β-phenylethylhydroxylamine.

The urinary excretion products of amphetamine were determined as already reported (Beckett & Al-Sarraj, 1972). Dimethylamphetamine *N*-oxide was characterized by t.l.c. and by reduction to dimethylamphetamine with $TiCl_2$ (cf. Beckett *et al.*, 1971*a*). The presence of the *N*-methylhydroxylamine was demonstrated by comparison with authentic material (A. H. Beckett, J. W. Gorrod & H. N. Chissick, unpublished work) on g.l.c., t.l.c. and alkaline treatment to give the above *syn*- and *anti*-phenylacetone oximes in constant ratio.

Only the *N*-oxide and the two hydroxylamines detected result from direct metabolic oxidation; the other compounds are metabonates (Beckett *et al.*, 1971*b*) arising from chemical breakdown of metabolites or further enzymic attack on the chemical breakdown products.

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