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.

The authors are indebted to the Medical Research Council for financial support.

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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.

Hg²⁺, Zn²⁺, Fe³⁺ and Cu²⁺ 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).

C. R. L. acknowledges the award of a grant from Chelsea College.

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A Study of the Possible Metabolism of Trichlorofluoromethane

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Trichlorofluoromethane (CCl₃F), although structurally similar to CCl₄, is regarded as inert and non-toxic (Jenkins *et al.*, 1970), and as such has been used in combination with other chlorofluoroalkanes