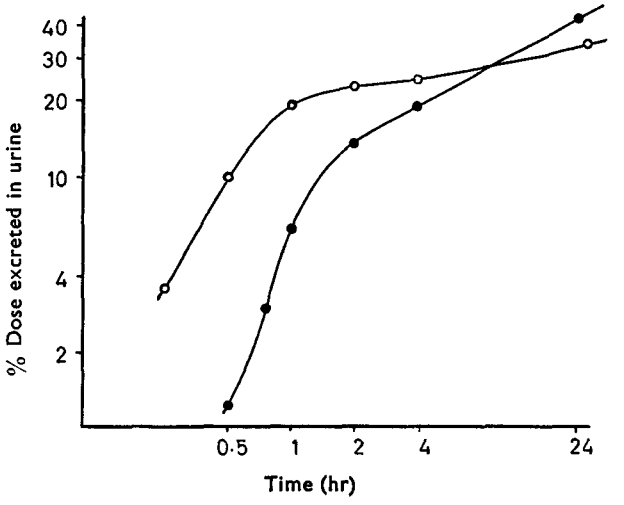

FIG. 1. Excretion in rat urine of 3-hydroxyphenyltrimethylammonium (○——○) and its glucuronide conjugate (●——●) after intramuscular injection of 100 μg of [^{14}C]3-hydroxyphenyltrimethylammonium. Each point is the mean of 3 experiments.

shows that more than half the dose was excreted as the glucuronide in 24 hr. Additional experiments using [^{14}C]neostigmine by intramuscular injection have shown that during 24 hr approximately 50% of the phenolic metabolite is excreted as the glucuronide conjugate.

The relevance of this mechanism for the metabolism and excretion of neostigmine by patients with myasthenia gravis is currently under investigation.

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The *N*-oxidation of chlorpromazine *in vitro*—the major metabolic route using rat liver microsomes

SIR,—Incubation of chlorpromazine with suitably fortified liver homogenates has been shown to result in sulphoxidation (Salzmann & Brodie, 1956; Gillette & Kamm, 1960), demethylation (Young, Ross & Maass, 1959; Ross, Flanagan & Maass, 1962), and hydroxylation (Robinson & Beaven, 1964) of the drug molecule.

Recently (Curry, 1965; Robinson, 1966), attempts were made to obtain a comprehensive picture of *in vitro* chlorpromazine metabolism after incubation

with fortified rat liver microsomes and soluble fraction; ether was used as solvent to extract chlorpromazine and its metabolites. Extraction was made from alkaline solution and the extract analysed by thin-layer chromatography. The expected products were found, namely chlorpromazine, chlorpromazine sulphoxide, demethylchlorpromazine, didemethylchlorpromazine and 7-hydroxychlorpromazine. Demethylhydroxychlorpromazine (Robinson, 1966) was also detected.

We have found, however, that *N*-oxidation is the major route in the biotransformation of chlorpromazine by rat liver microsomes, the previously described metabolic routes being relatively minor. The failure by previous workers to detect the formation of chlorpromazine *N*-oxide may be partially explained by the fact that ether is unsatisfactory as the sole extracting solvent for all chlorpromazine metabolites. For instance, when an incubation mixture comprising [^{35}S]chlorpromazine hydrochloride and rat liver microsomes fortified with nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate,

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF CHLORPROMAZINE, ITS METABOLITES, AND REFERENCE COMPOUNDS ON SILICA-GEL PLATES

Compound	Rf values and solvent systems[1] A Methanol–Diethylamine–Benzene (15:10:75)	B[2] Chloroform–Acetone–Diethylamine (88:2:10)	C Dioxan–Diethylamine–Water–Benzene (17·5:7·5:1:70)	Reaction with 50% v/v H_2SO_4 spray	Metabolite indicated
Combined ether and n-butanol extracts of alkalinized incubation mixture					
Chlorpromazine ..	0·83	0·79	0·70	Pink	
Metabolite A	0·75	0·66	0·54	Pink	Demethylchlorpromazine
„ B	0·73	0·70	0·46	Pink[3]	Chlorpromazine sulphoxide
„ C	0·58	0·31	0·24	Purple	7-Hydroxychlorpromazine
„ D	0·48	0·45	0·11	Pink[3]	Demethylchlorpromazine sulphoxide
„ E	0·18	0·08	on base line	Pink	Chlorpromazine* *N*-oxide[4]
„ F	0·10	on base line	on base line	Pink[3]	Chlorpromazine *N*-oxide sulphoxide
Chlorpromazine ..	0·84	0·79	0·70	Pink	
Demethylchlorpromazine	0·75	0·66	0·54	Pink	
Chlorpromazine sulphoxide ..	0·75	0·71	0·46	Pink[3]	
7-Hydroxychlorpromazine ..	0·58	0·33	0·26	Purple	
Demethylchlorpromazine sulphoxide ..	0·48	0·45	0·12	Pink[3]	
Chlorpromazine *N*-oxide	0·20	0·08	on base line	Pink	
Chlorpromazine *N*-oxide sulphoxide	0·11	on base line	on base line	Pink[3]	
Didemethylchlorpromazine ..	0·75	0·78	0·60 (streaks)	Pink	
7-Hydroxychlorpromazine sulphoxide ..	0·19	on base line	on base line	Purple	

[1] Thickness of silica gel 250 μ; distance of solvent development about 18 cm; measurements for Rf values made from leading edge of spot; temperature 21° ± 1°.
[2] Fishmann & Goldenberg, 1965.
[3] Colour development only on heating.
[4] Traces of 7-hydroxychlorpromazine sulphoxide are possible.
*Further confirmed by reduction with zinc and dilute hydrochloric acid to yield chlorpromazine.

magnesium chloride, nicotinamide, and glucose 6-phosphate dehydrogenase, was made alkaline with ammonia and extracted with two volumes of ether per extraction, 53, 37, 27, 25 and 22% of the initial radioactivity remained respectively after each successive extraction. Two ether extractions followed by two n-butanol extractions resulted in only 0·5% of the initial radioactivity being left in the aqueous phase.

Combined ether and n-butanol extracts were concentrated by distillation under vacuum at about 70° and examined by thin-layer chromatography; the systems and results are shown in Table 1.

The metabolic extract contained chlorpromazine, chlorpromazine sulphoxide, demethylchlorpromazine, demethylchlorpromazine sulphoxide, 7-hydroxychlorpromazine, chlorpromazine *N*-oxide, and chlorpromazine *N*-oxide sulphoxide. Didemethylchlorpromazine was found to be absent using two-dimensional thin-layer chromatography with systems B and C (see Table 1).

N-Oxidation (34%), demethylation (12%), hydroxylation (2%) plus the formation of 10% of chlorpromazine sulphoxide occurred when 2·5 μM of [^{35}S] chlorpromazine hydrochloride were incubated with male rat liver microsomes (equivalent to 5·8 mg of protein) fortified with optimal amounts of NADP, glucose 6-phosphate, magnesium chloride, nicotinamide and glucose 6-phosphate dehydrogenase, in a total volume of 4 ml. Measurements were made by scintillation counting of the spots removed from the plates.

Preliminary co-factor requirement experiments indicate that the N-oxidation, demethylation and hydroxylation reactions are NADP-dependant, whereas chlorpromazine sulphoxide formation is only partially NADP-dependant. Using the procedures outlined above, over half the chlorpromazine sulphoxide was found to be produced non-enzymatically, indicating that care should be taken in the interpretation of results where the assay of chlorpromazine sulphoxide is used as a measure of drug metabolism.

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