Acknowledgement—This work was supported by the Medical Research Council and the National Cancer Institute of Canada.

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On the relationship between lipid solubility and microsomal metabolism of drugs

(*Received* 20 *July* 1964; *accepted* 30 *July* 1964)

GAUDETTE and Brodie,[1] in a study of 28 drugs and natural products containing methyl or other alkyl groups, found that only those compounds which were highly lipid soluble were oxidatively dealkylated by rabbit liver microsomes *in vitro*. They suggested that either the enzymes involved were protected by a lipid barrier which only fat-soluble substances could penetrate or that only nonpolar compounds could intereact with their active sites. McMahon and Easton[2,3] also observed a correlation between lipid solubility and rate of liver microsomal demethylation of other drugs. They concluded that the ipid solubility of a substrate was rate-limiting for this process, and accepted the suggestion of Gaudette and Brodie concerning the localization of the enzymes involved behind a lipid barrier.

The results of these three studies have been emphasized by reviewers of the subject of drug metabolism, who have attempted to generalize concerning the nature and function of the microsomal drug-metabolizi- g enzymes. Thus Gillette[4,5] and Brodie and Maickel[6] concluded that all foreign compounds that are oxidized by liver microsomal enzymes are nonpolar and that the oxidation products are less lipid soluble than the parent compounds. They considered the function of this process as increasing urinary excretion of foreign compounds by decreasing tubular reabsorption, a process that depends on solubility in the lipoidal membranes of the kidney tubules.

Exceptions can be found to many generalizations, including the one under consideration here, and the examination of these exceptions may lead to clarification and extension of the concepts involved. We wish to present here some data that are not compatible with the generalization that there is a correlation between rate of metabolism and degree of lipid solubility of the substrates of microsomal oxidative enzymes. These exceptions were encountered during studies of the metabolism of S-methyl compounds and of certain antimetabolites[7, 8].

The demethylation of a variety of substrates was followed by determining the amount of formaldehyde formed during incubation with microsomes plus soluble fraction from mouse or rat liver homogenates. Incubation conditions and analytical procedures have been described previously[7, 9] and are summarized in Table 1. Lipid solubility was estimated by determining at 25° the partition of each substrate between chloroform and 0·1 M phosphate buffer, pH 7·4 and is expressed as the per cent present in the chloroform phase after equilibration.

The representative data presented in Table 1 clearly indicate that there is no obvious correlation between the rate of demethylation of the substrates studied and their solubility in chloroform. Outstanding are the high rates of demethylation of the water-soluble compounds, puromycin aminonucleoside and 1-methylguanosine.

TABLE 1. COMPARISON OF LIPID SOLUBILITY AND RATE OF DEMETHYLATION BY MICROSOMES

Substrate	Formaldehyde formed (mμmoles)			Percent in $CHCl_3$
	Rat		Mouse	
	Exp. 1	Exp. 2	Exp. 3	
Methitural	50			99
Methylthiobenzothiazole	1,020			99
Caffeine			93·4	95
2-Methylthio-4-hydroxytrimethylene-pyrimidine			460	88
Puromycin		588		88
Dimethylaminopurine		267	2,580	42
Methylthiopurine	360		1,525	32
Theophylline			90	12
Theobromine			30	11
2-Amino-6-methylthiopurine			714	10
Methylaminopurine		0		8·2
Puromycin aminonucleoside	974			4·8
Tyrosine analog of puromycin		374		4·0
1-Methylguanosine		508		1

Microsomes plus soluble fraction obtained from 1 g liver were incubated 1 hr at 37° with substrate, 500 μg NADP, 500 μg glucose-6-phosphate, 5 mg nicotinamide, 5 mg semicarbazide hydrochloride, 25 μmoles $MgCl_2$, 3·0 ml 0·5 M phosphate buffer (pH 7·4), in a total volume of 6·0 ml. Substrate concentration was 4 mM. Formaldehyde analyses in experiments 1 and 3 have been published previously.[7, 8] The results presented are averages of separate analyses of two to four flasks. Variation between duplicate formaldehyde analyses averaged 3·4%. Similar experiments have been performed 6 to 12 times.

The discrepancy between these results and those of previous workers does not appear to be due to variations in experimental procedures. Demethylation requiring NADPH and oxygen was the reaction observed in the other work cited, although Gaudette and Brodie also measured the oxidative removal of other alkyl groups. Whereas Gaudette and Brodie used rabbit livers, McMahon found no essential differences in results, using the livers of rats, mice, or guinea pigs. Chloroform was used to measure lipid solubility by Gaudette and Brodie, whereas McMahon used heptane.

The compounds employed in this study cover a wide range of lipid solubilities, intermediate between the very high or very low lipid solubilities of substrates studied by others. The use of compounds closely related in structure but differing widely in solubility may be more informative than the use of compounds which differ widely both in structure and solubility. Although several substituted purines are included, there is no reason to believe that this fact alone should make a difference in the results. These compounds have been shown to be demethylated by reactions having the same cofactor requirements and intracellular localization as those metabolizing other drugs.

The key role of lipid solubility in determining rate of microsomal drug metabolism is not in fact entirely supported by close examination of the data quoted in favor of this concept. Thus, Gaudette and Brodie found a fivefold difference in the rates of dealkylation among compounds which they considered to be highly lipid soluble, and only substances that were quite water soluble were not dealkylated. McMahon found that the rate of metabolism varied only two- to three-fold for compounds whose heptane solubilities varied over a 300-fold range, and none of the compounds he studied was completely inert.

Thus, although it is undoubtedly true that many drugs which are metabolized by microsomal enzymes are lipid soluble, it is evident from these studies that this is not a necessary property of substrates for the demethylation reaction. Substrate structure or other, as yet unknown, factors may be more important considerations in microsomal metabolism than is usually assumed. This conclusion is also supported by the observation that different species metabolize substrates at different relative rates. It also appears to be necessary to qualify the concept that these microsomal enzymes are separated from the aqueous phase by a lipid layer through which a potential substrate must first

pass. It may be possible to reconcile all these data by assuming that the less lipid-soluble substrate forms a complex with lipoid elements of the microsomes, thereby becoming lipid soluble. Although this type of complex has been shown to be formed between amino acids and cell membranes,[9] no such evidence is at present available for the compounds under consideration here.

Acknowledgements—This research was supported by Research Grant CY 2978 from the National Cancer Institute, Bethesda, Md., and by The Burroughs Wellcome Fund. The authors wish to express their thanks to Dr. H. George Mandel, in whose laboratory this work was done, and to Mr Algimont Kerza-Kwiatecki for expert technical assistance.

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