plasma cholinesterase systems. At these concentrations, the enzyme-substrate reaction follows classical Michaelis-Menten kinetics, suggesting a single enzyme–substrate interaction.[5] Although the apparent K_m value obtained from such plots appears reliable and reproducible, it does not necessarily represent a true K_m value but rather one intermediate between those for the two enzyme components present.

A complete kinetic analysis of pseudo (butyryl) cholinesterases must await separation and purification of each of the active enzyme species.

[5] The above suggestion does not apply to any rigorous kinetic treatment but is offered only as a guide for determining the relative potencies of cholinesterase inhibitors. Although unlikely, it is possible that specific inhibitors may have abnormally high affinities for minor enzyme components in impure cholinesterase preparations. Such a possibility cannot be ruled out completely on the basis of the available data.

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Keyphrases

Cholinesterase systems—human plasma
Michaelis constants—acetylcholine Cl hydrolysis
Acetylcholine Cl—autohydrolysis
Hydrolysis—cholinesterase fractions

Correlation of Ratios of Drug Metabolism by Microsomal Subfractions with Partition Coefficients

By ERIC J. LIEN and CORWIN HANSCH

A strong correlation between ratios of drug metabolism by microsomal subfractions with 1-octanol/water partition coefficient was found. Explanation for the spread of ratios is presented.

A VARIETY OF drug-metabolizing enzymes appear to be distributed between rough and smooth particles derived from the endoplasmic reticulum of liver cells (1–6). Using density gradient centrifugation, Fouts' group has separated the smooth-surfaced particles from the denser, rough-surfaced particles and then studied the metabolism of various drugs by the two types of particles. The rates of metabolism for each type of particle were obtained in terms of micromoles of drug metabolized per hr. per mg. of microsomal nitrogen. The ratios of enzymic activity in the two types of particles differed by almost 10-fold (depending on the drug). Fouts interpreted the different ratios as indicating different concentrations (activities) of enzymes in the two types of tissues. The authors wish to show that an alternative explanation can be advanced from a consideration of the physical properties of the substrates.

Equation 1 was derived *via* the method of least squares from the data of Fouts (2) and Gram *et al.* (6) in Table I. These data were obtained using Rothschild's method of preparation of microsome fractions. In Eq. 1, $R_{(s/r)}$ stands for the ratio of enzyme activity in the two types of particles (smooth/rough) and P is the partition coefficient of the drug in 1-octanol/water (7, 8), n is the number of data points used in deriving the equation, r is the correlation coefficient, s is the standard deviation from regression, and the figures in parentheses are the 90% confidence intervals. Addition of a term in $(\log P)^2$ to Eq. 1 does not result in an improved correlation (9). Three of the $\log P$ values in Table I were calculated (7, 10, 11). The value for benzpyrene was based on $\log P = 3.37$ for naphthalene, codeine was based on 0.76 for morphine, and amphetamine was based on 1.41 for 2-phenylethylamine.

$$\log R_{(r/s)} = \underset{(\pm 0.035)}{-0.101} \log P + \underset{(\pm 0.108)}{0.859}$$

n	r	s	
10	0.885	0.116	(Eq. 1)

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TABLE I—CORRELATION OF RATIOS OF ENZYMIC ACTIVITY (SMOOTH *Versus* ROUGH-SURFACED MICROSOMAL FRACTIONS OF RABBIT LIVER) WITH PARTITION COEFFICIENTS

	Substrate	Type of Reaction	Log P	Log $R_{(s/r)}$ Obs.[b]	Log $R_{(s/r)}$ Calcd.[d]
1	Benzpyrene	Aromatic hydroxylation	6.92	0.18[c]	0.16
2	Chlorpromazine	Ring-sulfur oxidation	5.35[a]	0.30	0.32
3	Zoxazolamine	Aromatic hydroxylation	2.46[a]	0.72[c]	0.61
4	*p*-Nitrobenzoic acid	Reduction of NO_2	1.83[a]	0.48	0.67
5	*l*-Amphetamine	Deamination	1.71	0.70	0.69
6	Hexobarbital	Aliphatic hydroxylation	1.47[a]	0.62	0.71
7	Codeine	*O*-Dealkylation	1.41	0.70	0.72
8	Acetanilide	Aromatic hydroxylation	1.16[a]	0.90	0.74
9	Aniline	Aromatic hydroxylation	0.90[a]	0.67[c]	0.77
10	Aminopyrene	*N*-Demethylation	0.76[a]	0.90	0.78

[a] Experimentally determined values. Other values calculated. [b] From *Reference 2*. [c] From *Reference 6*. [d] Calcd. using Eq. 1.

Using Dallner's method of preparing microsomal subfractions from rabbit liver, Gram *et al.* (6) studied the metabolism of eight drugs. Using log P values from Table I Eq. 2 has been derived, omitting data on azosulfamide[1] for which log P is not available.

$$\log R_{(s/r)} = \underset{(\pm 0.034)}{-0.091} \log P + \underset{(\pm 0.103)}{0.553}$$

$$n = 7 \quad r = 0.923 \quad s = 0.089 \quad \text{(Eq. 2)}$$

DISCUSSION

Equation 1 indicates that the more lipophilic the drug is (the higher log P is), the smaller the ratio of metabolism or, the more hydrophilic, the larger the ratio. The linear relation between log P and log $R_{(s/r)}$ does *not* imply the higher the ratio, the faster the metabolic change. In each case we are comparing the same drug in two different types of particles. In Table I there are 10 drugs of quite different chemical structure being metabolized by an unknown number of enzymes. Although the ratios in Table I can be explained by postulating different concentrations (or activities) of different enzymes in the two types of particles, this approach does not lend itself to rationalizing Eq. 1. An alternative postulate is that approximately the same activities of enzymes are in each type of particle, but the availability of the drug to the enzyme is rate limiting and depends on the milieu in which the enzyme is set. If one postulates that each type of particle contains enzymes in a very lipophilic setting, this would explain why hydrophobic drugs such as benzpyrene and chlorpromazine are metabolized at ratios near 1. The smooth particles would then appear to have a set of enzymes accessible to hydrophilic drugs which would explain why drugs with low log P values yield higher ratios. In this respect it is interesting to compare the four examples of aromatic hydroxylation in Table I. It seems likely that the same type of enzyme (12) would be involved in each case. If this is so, then the differences in R must be due to differences in availability of drug to enzyme. Most interesting is the fact that log $R_{(s/r)}$ is correlated with log P regardless of the type of reaction. This also points to access of drug to enzyme rather than a difference in enzyme activity in the two particles.

The correlation with Eq. 1 still leaves 22% of the variance in the data "unexplained" ($r^2 = 0.78$). This of course must be attributed to the second rate-limiting process, that of action of the enzyme on drug. For these processes no stereo or electronic terms have been included in Eq. 1. Although the slope of Eq. 2 is very close to that of Eq. 1, the intercept is different. Thus for *isolipophilic* drugs one would expect higher ratios using Rothschild's method of preparation. The dependence of log $R_{(s/r)}$ on log P appears to be the same.

Fouts' work is quite important for an ultimate understanding of the role of the liver in drug metabolism. Looked at from the point of view of Eq. 1, it appears to indicate two different kinds of setting for microsomal enzymes. It is further support for Brodie's (13) observation of the great dependence of drug metabolism on lipophilic character of the drugs (14).

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Keyphrases

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