Role of Drug Metabolism in Drug Research and Development: Basic Considerations

JOHN E. BAER

Abstract □ Data from the rapid systematic study of the fate of a new drug in rats and dogs can aid the design of pharmacological and safety tests and can provide a basis for biopharmaceutic and Phase I clinical trials.

Keyphrases □ Drug metabolism—role in drug research and development, symposium □ Biopharmaceutics—drug metabolism in rats and dogs, design of pharmacological and safety tests □ Metabolism, drug—species dependence, design of pharmacological and safety tests

Drug metabolism has become a science in recent years, with its own body of knowledge and technical skills. The generalizations, facts, and techniques of this new science can be utilized in the development of new and better drugs.

The isolation of drug-related substances in urine has long attracted the attention of medically oriented chemists. However, drug metabolism is more than a recital of metabolites of various drugs isolated from the urine of various species. Williams (1), in his classic book, "Detoxication Mechanisms," gave a systematic classification of numerous chemical transformations that can occur in the body. He emphasized that these reactions usually produce less toxic, more water-soluble, and readily excretable substances.

Other generalizations about the fate of foreign compounds in the body have evolved from the studies of their absorption, excretion, and storage as well as their metabolic conversion. Fundamental studies on the role of liver microsomal enzymes on drug metabolism and on intestinal transport, renal elimination, and distribution of drugs among tissue compartments were summarized in recent reviews and treatises (2–6).

Essential to the science of drug metabolism are qualitative and quantitative techniques for the detection and determination of very small amounts of drugs (and their metabolites) in complex biological mixtures. Extraction, column chromatography, ion exchange, TLC, high pressure liquid chromatography, GC, and even electrophoresis and gel chromatography have been used. A specific sensitive quantitative analytical method is essential to a thorough investigation of the fate of a drug, yet nonspecific methods that may measure the drug and its metabolites are also important and informative (7–9).

APPLICATION TO DESIGN OF DRUGS

Drug metabolism concepts are used by the modern medicinal chemist in the design of members of the chemical series in which he works. In several series, it has been shown that the pKa and the lipid solubility of a compound influence its transport across membranes. Accordingly, he will include in his plan some congeners with varying degrees of acidity (basicity) and others with high and low partition coefficients as determined in octanol–pH 7 buffer solutions. He will make some congeners in which "metabolizable" sites are blocked. He will relate the metabolic characteristics of the drug to the target organ. Thus, water-soluble compounds may rapidly reach the kidneys (a desirable feature for diuretics), lipid-soluble compounds may have an affinity for the brain (a desirable attribute for CNS drugs), and compounds that readily conjugate with glucuronic acid may be removed by the liver and eliminated in the bile.

SELECTION AMONG CONGENERS

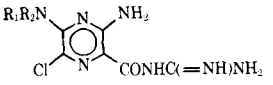
Several congeners may be equally active in the pharmacological screening tests, and a basis must be found to narrow the selection for the intensive study of a smaller number of agents or even to select a single "most probable" candidate. Drug metabolism considerations of the kind described in the previous section may be applied to ensure that representative congeners are selected. Preliminary drug metabolism studies may be made at this stage to help in the selection process. Compounds may be tested for their relative ease of metabolism in rabbit liver homogenates *in vitro*. These results may be informative but should be extrapolated with extreme caution to metabolic events that may be expected *in vivo*.

Cragoe *et al.* (10) in these laboratories made a large number of pyrazine diuretics which contained substituted amine groups. Many analogs were very active in the rat (Table I). Some basis was required to limit the number of analogs that were to be tested more intensively. TLC of urine from the rats showed that many of the simple alkyl analogs were at least partially dealkylated to the unsubstituted amine. This unsubstituted amine was, therefore, selected as one of two candidates for further intensive study. This compound went into trial in man and is now in clinical use as the potassium-sparing diuretic, amiloride.

SELECTION OF SPECIES FOR PHARMACOLOGICAL TESTS

When two screening tests are performed in two different species, the structure–activity correlations in a series of compounds may be inconsistent. Drug metabolism considerations may be applied to clarify such findings. Likewise, when an active compound is inactive in a "second-line" test in a new species, one should consider that the compound may be handled in different ways, metabolically, in the two species. Sometimes an analytical procedure can be readily

Table I—Diuretic Activity of *N*-Alkyl Analogs of Amiloride in the Rat[a]



R_1	R_2	Diuretic Activity
CH_3	H	+3
C_2H_5	H	+3
C_4H_9	H	+3
CH_3	CH_3	+3
CH_3	C_2H_5	+4
C_2H_5	C_4H_9	+3
H	H	+4

[a] *Reference 10.*

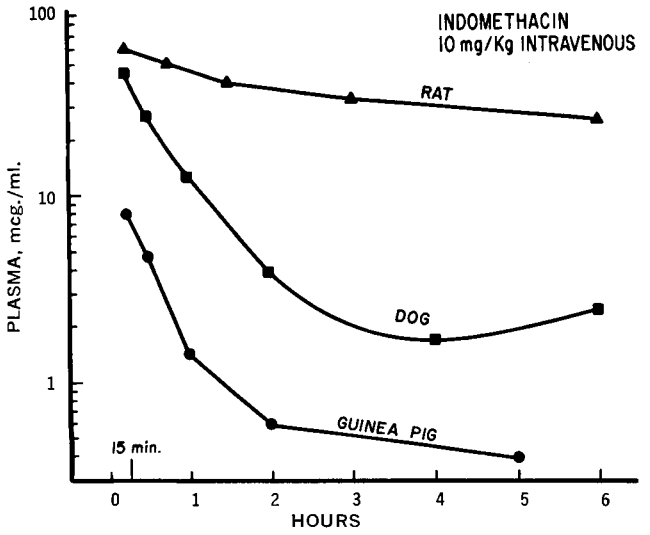


Figure 1—*The rapid decrease in plasma indomethacin (specific chemical assay) in the guinea pig is mirrored by accumulation of drug in tissue (^{14}C). At 120 min., tissue–plasma ^{14}C-ratios were: rat liver, 0.5; rat muscle, 0.06; guinea pig liver, 9.7; and guinea pig muscle, 0.5 (11).*

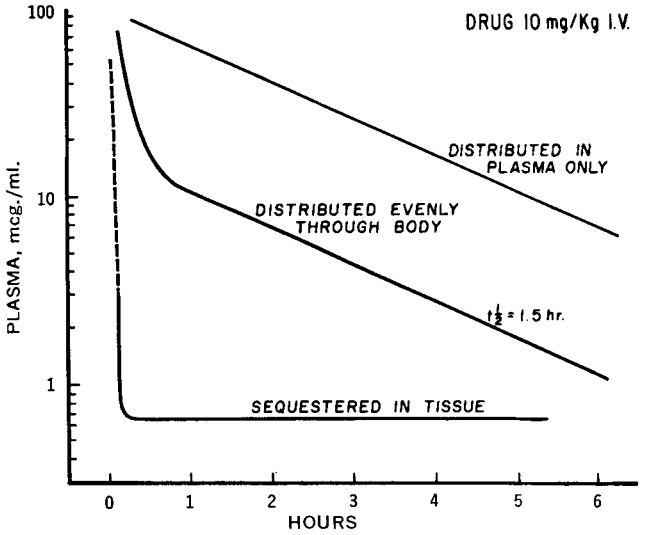


Figure 2—*In the initial phase, the concentration of a drug in plasma is determined by its distribution within the body. A drug that is stored in tissue may be released slowly and have a long plasma half-life.*

devised that will show qualitatively whether the same metabolites are excreted in both species (as by TLC of a urine extract).

If a preliminary quantitative analytical procedure can be quickly devised, it may show marked differences in the plasma concentration when the same dose of drug is given to different species. This may permit the selection of the "most suitable species," *i.e.*, the species in which pharmacological effects and drug levels show reasonably good correlation. It is not always known, especially at an early stage, whether the concentration of drug at the hypothetical "receptor site" is more accurately reflected by the plasma levels or by the tissue level. When a marked disparity between plasma level in two species exists, however, it can provide an explanation for interspecies differences in pharmacological activity. The plasma level of indomethacin is much greater in rats than in guinea pigs at the same dose (Fig. 1). From these dissimilar plasma level patterns, one would surmise that the guinea pig rapidly sequesters the drug in his tissues; in fact, tissue–plasma ratios in the guinea pig are much greater than those in the rat (11). The experiment has not been done, but it would be surprising if the same dose of indomethacin were to have an equal anti-inflammatory response in these two species of rodent: if tissue levels determined activity, the guinea pig would be more responsive and if plasma levels reflected concentration of drug at active sites, the rat would be more responsive.

EARLY ABSORPTION TESTS

Once a compound has been selected for serious consideration, a rapid systematic study of its fate in standard laboratory species can yield data that will help toxicologists and pharmacists. Where possible, we have prepared a labeled compound at an early stage and have measured total radioactivity in rats and dogs following intravenous and oral administration of the compound. From such data, one can determine whether the drug is well absorbed in both species, the rate of elimination from the body, and the probability of storage in body compartments. The toxicologists may use such information to establish dose levels and dosage schedules for chronic studies. The pharmacists can deduce preliminary pharmacokinetic data on absorption and elimination constants. Such considerations will influence the design of subsequent bioavailability studies: the timing of sample collections, the size of dose to be given, and perhaps the species to be preferred for animal bioavailability work.

The idealized cases shown in Fig. 2 illustrate inferences that can be drawn from plasma level data. The highest plasma levels are produced by drugs that remain in the plasma until they are eliminated. If the drug is only slowly metabolized or excreted, the concentration falls slowly. If plasma levels are moderate, the drug is probably distributed into other body compartments. If the plasma levels are low, drug (or metabolites) is concentrated in tissue and may be slowly released to the circulation and slowly excreted over a long period of time. Mepiperphenidol is an example of such a compound; the kidneys can handle the drug very efficiently, yet it requires up to 48 hr. for an intravenous dose of 2 mg./kg. to be excreted in the urine (12).

Comparison of the plasma levels after oral and intravenous administration of a drug will often indicate whether the drug is well absorbed. Conclusions based solely on excretion of radioactivity (or of drug) in urine may be misleading. For example, in the dog less than 10% of an oral dose of indomethacin is excreted in the urine, falsely indicating poor absorption (Table II). However, plasma levels are higher after an oral dose than after an intravenous one (Fig. 3), showing that the drug is actually well absorbed in the dog. Reference to the fecal elimination shows that 83% of an *intravenous* dose appears in the feces. This disparity is a consequence of the unusual ability of the dog to eliminate indomethacin (principally as its glucuronide) in the bile. Biliary secretion and enterohepatic circulation may be important metabolic pathways, and this should be kept in mind until their exact significance is specifically determined for the species under consideration.

STUDIES RELATING TO SAFETY

Differences in drug metabolism in different species may be important in interpreting toxicology data. To this end, some

Table II—Elimination of ^{14}C by Various Species following Single Doses of 10 mg./kg. of Indomethacin by Various Routes[a]

Species	Route	Percent ^{14}C-Dose Excreted Urine 72 hr.	Feces 72 hr.	Bile[b] 6 hr.
Dog	Intravenous	7.2	82.9	55.9
	Oral	3.2	—	
Rat	Intravenous	51.7	45.3	(25)[c]
	Oral	44.9	47.7	
Guinea pig	Intravenous	65.4	34.6	63.4
	Oral	57.7	37.3	
Monkey	Oral	63.8	18.8	47.9
Man[d]	Intravenous	64.2	34.9	—
	Oral	59.0	31.8	

[a] *Reference 11* and unpublished observations. [b] Separate experiments were performed to determine biliary excretion. The interspecies differences in composition of the ^{14}C-substances are under continuing study. [c] 1 mg./kg. s.c.; 2-hr. sample. [d] ~0.5 mg./kg.; 48-hr. urine, 96-hr. stool.

Table III—Slow Elimination by Man of a Compound, Halofenate, that is Extensively and Firmly Bound to Plasma Protein

	Percent of Oral Dose Excreted					
	Subject 1		Subject 2		Subject 3	
Hours	Urine	Feces	Urine	Feces	Urine	Feces
4	2.9	—	2.5	—	2.0	—
8	3.7	—	3.9	—	2.9	—
12	3.2	—	4.2	—	2.0	—
24	7.2	8	7.2	26	6.1	—
48	8.9	—	7.3	7	8.9	6
72	13.0	12	7.3	—	12.7	6
96	5.5	3	2.3	2	6.1	2
120	4.7	1	1.7	1	3.7	1
144	3.5	—	1.7	—	3.4	1
Total	56.2	24	37.0	36	48.7	16

qualitative and quantitative data should be obtained on the fate of the drug in the species selected for chronic toxicity studies. This may be accomplished in a preliminary way by comparing the behavior of the labeled material in urines of the different species as they are subjected to sequential extraction, TLC, two-dimensional radioautography, or other separation techniques.

Equally important, preliminary information in man on the fate of the drug should be obtained at as early a stage as possible in the development of the compound. If, as is often the case, there is no obvious difference in the fate of the drug in rat, dog, and man, one can be more confident in extrapolating the pharmacological and toxicological data from animals to man as Phase I studies proceed.

If the species differences exist, timely modifications in the design of chronic toxicity studies can often be made, and clinical protocols may be modified appropriately.

Drug metabolism studies can contribute to a prediction of possible drug interactions. The extent of binding of the drug to plasma protein can be readily measured; if it is highly bound, it may displace other drugs *in vivo*, increasing their effective concentration, as with certain anticoagulants. The ability of the compound to depress the metabolism of other drugs can be measured, as by observing whether the drug increases the plasma half-life of antipyrine. The drug may also, upon repeated administration, stimulate or "induce" drug-metabolizing enzymes, leading to a shortened effective duration of drugs that are so metabolized.

Highly bound drugs may be eliminated slowly over many days (Table III). Halofenate is actually secreted by the kidney, but the effective "free" concentration of drug is so low, in milligrams per milliliter, that very little drug is eliminated per unit time and the half-life in plasma is >24 hr. in man (13, 14).

ANALYTICAL METHODOLOGY

Development of quantitative analytical methods for measuring drugs in biological fluids is an essential function of the drug metabolism scientist. As medicinal chemists have devised compounds of greater potency, the ingenuity of drug metabolism workers to develop sensitive methods has been severely tested. Colorimetry and UV spectrometry have been supplemented by fluorometry, flame ionization, and electron-capture gas chromatography and more recently by reverse isotope dilution and radioimmunoassay techniques. Levels of less than 10 ng./ml. of plasma can be measured in some cases using these techniques. The distinctive utility of specific methods (for drug alone) or nonspecific methods (drugs plus metabolites) should be recognized. Specific methods are needed for pharmacokinetic studies; nonspecific methods lend themselves to balance studies. Having devised such methods, the drug metabolism worker will doubtless find his skill in demand in the support of bioavailability studies as final formulations of the drug are made available.

ISOLATION OF METABOLITES

Concurrently, the drug metabolism worker will make a preliminary effort to isolate and characterize the major metabolites that may be present in urine, plasma, or feces. At this stage, the medicinal synthetic chemist may be asked to synthesize an authentic sample of the metabolite. Shen and Rugianesi (15) were thus impelled to devise a procedure for the synthesis of acyl glucuronides. As an incentive, there is the hope, not always fulfilled, that the metabolite itself will be biologically active. An additional bonus may be the detection of a novel metabolic pathway previously not recognized to exist (16).

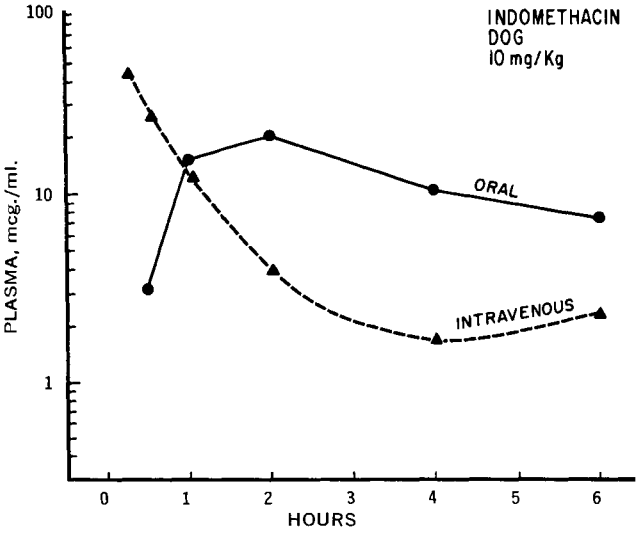


Figure 3—*Comparison of plasma concentrations of drug after oral or intravenous administration of 10 mg./kg. of indomethacin indicates rapid and complete oral absorption of indomethacin by the dog (11).*

It does not seem necessary or useful to isolate and characterize absolutely all of the urinary metabolites of a drug. Of more theoretical importance is the goal of determining the concentration of a drug or metabolite at the pharmacological receptor site and the correlation of concentration with activity.

EXTENDED STUDIES

Species differences with respect to metabolism may exist and may be of interest, but the relevance of these data to safety or efficacy in man may not be clear even after considerable effort. As an example, the species variation with respect to intestinal irritancy of indomethacin has not yet been elucidated by drug metabolism studies, even though the drug has been in clinical use since 1965. Studies that continue in this area must be classified as long term and fundamental in nature.

Early safety studies with indomethacin gave evidence of intestinal (as distinct from gastric) lesions in the rat at low doses. The rhesus monkey, guinea pig, and rabbit did not show such lesions even at moderately high repeated doses. In the dog and the rat, intestinal lesion occurrence was clearly dose related. The human is less sensitive in this respect than the rat or dog. In all species studied, drug-related material appeared in the bile (Table II). Even though the bile was the sole route of elimination of ^{14}C in the dog but not in the rat, this was not reflected in the magnitude of the minimal dose that produced the lesion in the two species.

Hucker *et al.* (11) showed that the principal drug-related component in the bile of the dog is indomethacin glucuronide. In an elegant series of experiments, he showed that 50% of the glucuronide was reabsorbed by the small intestine, undergoing hydrolysis to indomethacin. The reabsorbed indomethacin, in turn, was conjugated by the liver and resecreted in the bile. The intestinal lesions seemed to be related to the direct application of drug- (or metabolite-) containing bile to the mucosal surface of the small intestine. Dogs that received moderate or large doses of indomethacin intravenously or by rectal suppository developed small intestinal lesions even though no drug was ingested. In addition, Brodie *et al.* (17) showed that bile duct ligation in rats completely prevented the intestinal lesions, even when the drug was given orally at a dose (12 mg./kg.) that would be expected to give 100% incidence of lesions in normal rats. Thus, the evidence favors the existence in dog and rat bile of a component that can cause the lesions. The known metabolites were much less toxic than indomethacin for the rat, chronic doses as high as 20 mg./kg./day being without effect in the GI tract.

Duggan *et al.* (18) and Hogans *et al.* (19) continued to investigate the composition and kinetics of the indomethacin metabolites in various species. Indomethacin is demethylated to 1-(*p*-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid and is also deacylated to 5-methoxy-2-methylindole-3-acetic acid; the latter pathway is predominant in the rat but not in man. The concentration of unconjugated unchanged indomethacin is relatively high in the bile of rats, although the bile contains substantial free glucuronic acid as well as the glucuronides of the parent drug and of its metabolites. In the guinea pig, half of the drug-related radioactivity in the bile has not been characterized chemically; preliminary data suggest that this reflects a "detoxication mechanism" with respect to intestinal ulceration in the rodent. The concentration of free indomethacin in dog bile is very low; data on the concentration of drug or metabolites in human bile are fragmentary to date but suggest that little free indomethacin is present. The occurrence of lesions quite possibly depends on unchanged indomethacin, in bile, applied to the duodenal mucosa in appropriate concentration. Although much information has been obtained about indomethacin, its enterohepatic circulation, and its metabolism to date, additional studies are still required to verify the hypothesis.

ANCILLARY DATA

In support of a new drug application, a section on drug metabolism is submitted. The basic premise is that such information will aid the evaluation of the safety and efficacy of the drug in human medicine. Experiments should be designed and presentations should be prepared with this goal in mind. The National Research Council Committee on Problems of Drug Safety, Drug Research Board, National Academy of Sciences (20), prepared a detailed guideline including specific questions that may be appropriate to the particular drug. Among these questions are the effect of the drug on metabolism of other drugs that may be given concurrently, distribution of the drug (or metabolites) in various tissues, effect of prolonged administration on plasma levels or metabolic patterns, and the transport of drug (or metabolites) into milk or fetal tissue. It may be proper to anticipate requests from poison control centers for methods of drug detection in cases of overdosage and data on whether dialysis or alteration of urine pH will facilitate drug elimination.

REFERENCES

(1) R. T. Williams, "Detoxication Mechanisms," Wiley, New York, N. Y., 1959.

(2) B. B. Brodie and J. R. Gillette, "Handbook of Experimental Pharmacology," vol. 28, Springer, Berlin, Germany, 1971.

(3) J. R. Gillette, "Progress in Drug Research," vol. 6, Birkhauser Verlag, Basel, Switzerland, 1963, pp. 13–73.

(4) B. N. LaDu, H. G. Mandel, and E. L. Way, "Fundamentals of Drug Metabolism and Drug Disposition," Williams & Wilkins, Baltimore, Md., 1971.

(5) M. M. Reidenberg, "Renal Function and Drug Action," W. B. Saunders, Philadelphia, Pa., 1971.

(6) E. S. Vesell, *Ann. N. Y. Acad. Sci.*, **179**, 1(1971).

(7) F. J. Wolf, "Separation Methods in Organic Chemistry and Biochemistry," Academic, New York, N. Y., 1969.

(8) J. A. F. deSilva, "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," Lea & Febiger, Philadelphia, Pa., 1970, pp. 203–264.

(9) J. L. Hirtz, "Analytical Metabolic Chemistry of Drugs," Marcel Dekker, New York, N. Y., 1971.

(10) E. J. Cragoe, Jr., O. W. Woltersdorf, Jr., J. B. Bicking, S. F. Kwong, and J. H. Jones, *J. Med. Chem.*, **10**, 66(1967).

(11) H. B. Hucker, A. G. Zacchei, S. V. Cox, D. A. Brodie, and N. H. R. Cantwell, *J. Pharmacol. Exp. Ther.*, **153**, 237(1966).

(12) K. H. Beyer, E. K. Tillson, H. F. Russo, and S. F. Paulson, *Amer. J. Physiol.*, **175**, 39(1953).

(13) G. M. Fanelli, Jr., D. L. Bohn, S. S. Reilly, and J. E. Baer, *J. Pharmacol. Exp. Ther.*, **180**, 377(1972).

(14) H. B. Hucker, L. T. Grady, B. M. Michniewicz, S. C. Stauffer, S. E. White, G. E. Maha, and F. G. McMahon, *ibid.*, **179**, 359 (1971).

(15) T. Y. Shen and R. L. Rugianesi, *Carbohyd. Res.*, **19**, 179 (1971).

(16) H. B. Hucker, B. M. Michniewicz, and R. E. Rhodes, *Biochem. Pharmacol.*, **20**, 2123(1971).

(17) D. A. Brodie, P. G. Cook, B. J. Bauer, and G. E. Dagle, *Toxicol. Appl. Pharmacol.*, **17**, 615(1970).

(18) D. E. Duggan, A. F. Hogans, K. C. Kwan, and F. G. McMahon, *J. Pharmacol. Exp. Ther.*, **181**, 563(1972).

(19) A. F. Hogans, K. F. Hooke, and D. E. Duggan, *Pharmacologist*, **13**, 237(1971).

(20) J. J. Burns, *Clin. Pharmacol. Ther.*, **10**, 607(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received from *Merck Sharp & Dohme Research Laboratories, West Point, PA 19486*