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Interspecies Variation in Liver Weight, Hepatic Blood Flow, and Antipyrine Intrinsic Clearance: Extrapolation of Data to Benzodiazepines and Phenytoin

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The literature was reviewed to obtain data from 11 mammalian species on liver weight, hepatic blood flow, and antipyrine intrinsic clearance. It was demonstrated that liver weight and hepatic blood flow in all species could be readily related to body weight by a simple equation. Additionally, hepatic blood flow in all species was directly proportional to liver weight. With the exception of man, antipyrine intrinsic clearance was also directly proportional to liver weight. Man's intrinsic clearance was approximately one-seventh of that which would be predicted from other species. Data on benzodiazepines and phenytoin showed a similar pattern.

KEY WORDS: liver weight; hepatic blood flow; evolution; interspecies variation; intraspecies variation; intrinsic clearance; antipyrine; benzodiazepines; phenytoin.

INTRODUCTION

The ability of plants, microorganisms, and virtually all animal species to chemically alter exogeneously administered substances is manifestly apparent. Indeed, at the present time, it is virtually impossible to find an issue of a pharmacological journal in which some report on drug metabolism does not appear. In particular, research pertaining to the quantitative aspects of drug metabolism in different organisms seems to be increasing at an astounding rate. In most of these investigations which employ animal species, there is the tacit assumption that these data may in some way be extrapolated to other species, most notably man, and thus ultimately improve clinical medicine. Unfortunately, methodologies pertaining to the extrapolation of quantitative drug metabolism data from animal to man

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have heretofore been elusive. Much of the difficulty in the past seems to have arisen from mistaken notions regarding the biological significance of half-life ($t_{1/2}$). Recent advances on the nature of drug response as modulated by drug metabolism rates have clearly indicated that $t_{1/2}$ is a faulty parameter.

Despite the difficulties, some excellent articles pertaining to interspecies variations in drug metabolism rates have appeared in the literature (1–5). It is the purpose of this article to consider the evolutionary aspects and function of drug-metabolizing systems and thus attempt to unravel some of the complexities of interspecies variation. In particular, the drug antipyrine will, for reasons discussed later, be used as a model substance. Also, because the liver is generally considered to be the organ contributing most to the drug metabolic process, its size and blood flow will also be considered.

THEORETICAL AND HYPOTHETICAL CONSIDERATIONS

For the purposes of this inquiry, discussion will be restricted to those substances eliminated from the body solely by hepatic oxidative metabolism. This strategy is mainly adopted so as to reduce variables which would otherwise be too unwieldy to consider at the present time. Since cytochrome P_{450} is considered to mediate many of the oxidative reactions, particular consideration will be given this agent.

Wickramasinghe and Villee (6, 7) have provided some interesting insights regarding P_{450}. These investigators have postulated that a form of P_{450} was present in life forms for billions of years and that its initial function was to "mop up" traces of unwanted O_2 entering cells during the primordial stages of life existing before the advent of an oxygen atmosphere. Subsequently, it is postulated that P_{450} was involved in the formation of reactive intermediates which may have played an important role in the mutation of these life forms assisting in the successful creation of biological variations (species). As evolution proceeded, it is not difficult to imagine that the role of P_{450} once again changed to that of providing a system whereby organisms could chemically alter and thereby detoxify exogenous toxins. Brodie (5), who has provided imaginative insights in this area, has suggested that drug metabolism enzymes were necessary as a disposal mechanism to rid the body of lipid-soluble foreign compounds such as hydrocarbons, terpines, and alkaloids ingested in food, thus permitting survival of species amid a hostile chemical environment. Although there is mounting evidence that oxidative metabolism sometimes creates substances that may be more toxic than the parent compound (8), it is difficult to imagine that organisms would be better equipped for survival without such systems. That is to say, the benefits attained by way of the oxidative process far outweighted the risks.

Therefore, if we may ostensibly view oxidative metabolism as a protective (adaptive) reaction, the query arises as to what pharmacokinetic parameter best gauges an organism's ability to protect itself from oral ingestion of toxic substances. In arriving at a decision, let us assume that exogenous substances produce their effects in a manner related to unbound concentration in the blood. In this regard, Wilkinson and Shand (9) and Rowland *et al.* (10) have provided equations leading to a logical approach. Assuming a linear system, for orally ingested substances completely metabolized by the liver, the following equations are appropriate measures of drug exposure:

$$\mathrm{AUC}_u = (F_L \cdot \mathrm{dose})/\mathrm{CL}_{\mathrm{int}} \tag{1}$$

$$\bar{C}_{u,\mathrm{ss}} = (F_L \cdot \mathrm{dose})/(\mathrm{CL}_{\mathrm{int}} \cdot \tau) \tag{2}$$

where AUC_u is the area under the unbound blood concentration–time curve, F_L is the fraction of the dose reaching the liver intact, $\bar{C}_{u,\mathrm{ss}}$ is the average unbound blood concentration at steady state, $\mathrm{CL}_{\mathrm{int}}$ is the intrinsic clearance of the drug, and τ is the dosing interval during chronic dosing. It is immediately apparent that the one biological parameter affecting drug exposure of the organism to unbound pharmacologically active substance is $\mathrm{CL}_{\mathrm{int}}$. Quite noticeably, chemical exposure is unrelated to the extent of plasma protein binding and hepatic blood flow. Liver size, however, does effect $\mathrm{CL}_{\mathrm{int}}$.

Intrinsic clearance may readily be calculated from the following relationship (9, 10):

$$\mathrm{CL}_{\mathrm{int}} = (Q \cdot \mathrm{CL}_h)/f_b(Q - \mathrm{CL}_h) \tag{3}$$

where f_b is the fraction of drug in blood unbound to blood components (e.g., plasma proteins), Q is hepatic blood flow, and CL_h is hepatic clearance.

In the determination of $\mathrm{CL}_{\mathrm{int}}$, values for Q, CL_h, and f_b are required. With this in mind, consideration was given as to which substance would offer sufficient data to calculate $\mathrm{CL}_{\mathrm{int}}$ in a variety of species. The obvious candidate was antipyrine. This drug was selected for a variety of reasons: (1) all known metabolic pathways are oxidative, with only small amounts of drug excreted intact in the urine; (2) all available data indicate first-order kinetics in usual doses; (3) the difference between Q and CL_h is sufficiently large so as to permit accurate determination of the denominator of equation 3; (4) f_b is essentially unity in all species; (5) assay methods used in blood, serum, or plasma determinations were essentially specific and accurate; (6) in those species where $t_{1/2}$ was the only parameter reported, CL_h could be calculated by assuming a volume of distribution equal to total body water; and (7) there existed in the literature an abundance of data in different species.

METHODOLOGY

The methodology used in the acquisition of data consisted of exhaustively searching the literature; the literature search ended in August 1977. Initially the literature was reviewed for data on any species concerning renal excretion of intact antipyrine, protein-binding information, metabolic products, and blood-to-plasma ratios (λ). Next, wherever possible, intravenous data were sought for the calculation of CL_h (dose/AUC). In some few instances only $t_{1/2}$ values were reported; in others, administration was by the intraperitoneal route. In these cases, CL_h was calculated from $t_{1/2}$ assuming the volume of distribution to be equal to total body water. When it was ascertained in which species CL_h calculations could be made, data were sought on liver weights and hepatic blood flows in these species. In the entire literature search, all reliable data that could be found were used. Average values of parameters were calculated from the means from each study. Because of the great number of references used, appropriate citations will be omitted; detailed tabular data (referenced) used in all calculations are

Table I. Average Values of Liver Weights and Hepatic Blood Flows in Various Mammalian Species

Species	Body weight (kg)	Liver weight (% of body weight)
Mouse	0.0304	5.06
Rat	0.223	4.04
Guinea pig	0.344	4.57
Rabbit	2.88	4.78
Dog	16.5	2.91
Pig	91.8	1.97
Sheep	49.6	1.65
Goat	27.7	1.90
Cattle	760.0	1.06
Monkey	4.12	3.25
Human	62.8	2.42
		Hepatic blood flow (liters/min)
Mouse	0.0304	0.00262
Rat	0.249	0.0172
Guinea pig	0.344	0.0214
Rabbit	2.75	0.122
Dog	16.5	0.676
Pig	76.8	3.36
Sheep	50.2	2.43
Goat	24.1	0.480
Cattle	124.	6.06
Monkey	4.84	0.250
Human	70.0	1.78

available on request. Table I gives average values of liver weight and hepatic blood flow obtained from the literature review.

RESULTS AND DISCUSSION

Interspecies Variation in Liver Weight and Hepatic Blood Flow

Prior to the discussion on antipyrine, let us consider interspecies variation in liver weight and hepatic blood flow. Adolph (11) was probably the first investigator to thoroughly inquire into the nature of relationships in mammals between physiological parameters and body weight. In general, he found that such physiological values as tidal volume, creatinine clearance, and basal oxygen consumption could be related to the animal's body weight. Additionally, organ weights could also be related in the same fashion. Linear relationships were generally obtained whenever log-log plots were made (by convention, body weight was considered the independent variable). The so-called heterogonic relationship thus indicated for organ weights is (11)

$$I = aB^k \tag{4}$$

where I is organ weight, B is body weight, and a and k are calculable parameters. For the data collated in the present study (Fig. 1), the relationship obtained was

$$L = 0.0370B^{0.849} \tag{5}$$

where L and B are liver and body weights expressed in kilograms. When hepatic blood flow was correlated with body weight (Fig. 1), the relationship obtained was

$$Q = 0.0554B^{0.894} \tag{6}$$

where Q is hepatic blood flow in liters/minute. Since both L and Q are related to B through the general heterogonic relationship expressed in equation 4, they may be related to each other accordingly (Fig. 1):

$$Q = 0.0554(L/0.0370)^{0.894/0.849} \tag{7}$$

If we approximate the exponential term as unity, the relationship simplifies to

$$Q \approx 1.50 \text{ liters/min/kg liver weight} \tag{8}$$

Thus it appears that in all mammalian species investigated hepatic blood flow is approximately equal to 1.5 liters/min/kg liver weight.

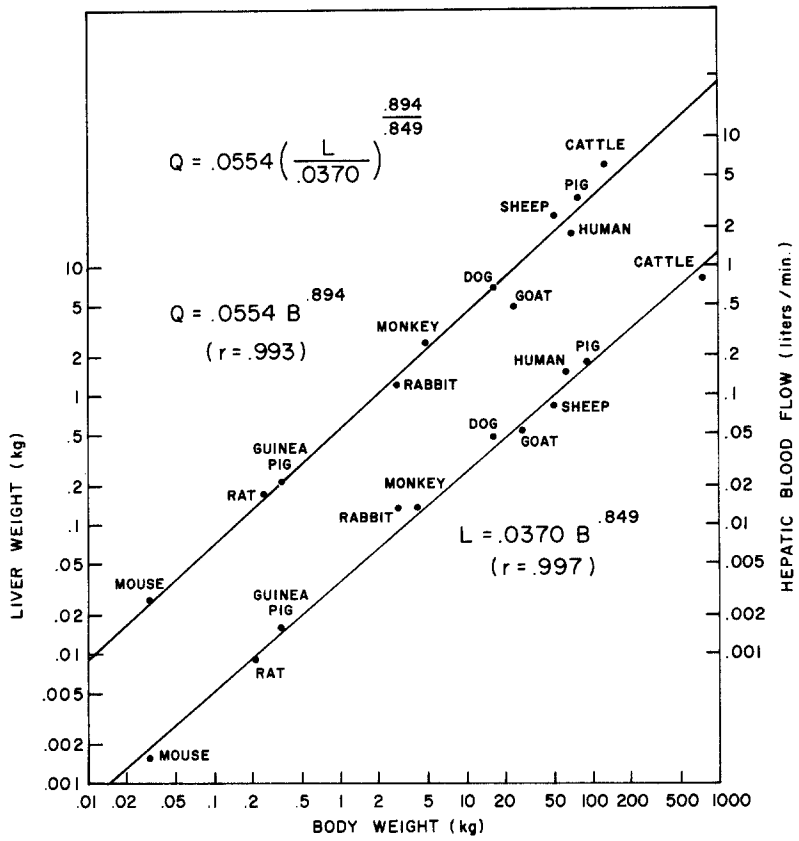

Fig. 1. Liver weight and hepatic blood flow in mammals as a function of body weight. Equations fitted using the method of least squares on unweighted logarithmically transformed data. See text for discussion.

Interspecies Variation in Antipyrine Intrinsic Clearance

As mentioned previously, all recognized metabolic pathways in the disposition of antipyrine are oxidative reactions (12–17).

Antipyrine CL_{int} was calculated from the available data using equation 3. Q was taken as the average from all studies on the individual species, i.e., the values indicated in Table I. CL_h values in species were determined using appropriate pharmacokinetic equations, primarily dose/AUC following i.v. injection. Free fraction in blood was taken as unity, and it was assumed that all doses were completely metabolized by the liver. Some minor difficulties arise from these assumptions. For example, in some species drug may be bound to plasma proteins as much as 16%. Also, some species may excrete as much as 5% of the dose intact in the urine. Nonetheless, considering the

nature of the more serious difficulties, the errors arising from these assumptions were considered acceptable. In particular, serious errors may have been introduced because of intraspecies variations in L, Q, and CL_h. These parameters may vary greatly as influenced by species strain, age, sex, weight, diet, nutritional state, and exposure to environmental factors such as enzyme inducers. It was hoped that by averaging all available data within species that the influence of these factors would tend to be randomly scattered.

Figure 2 illustrates the relationship between antipyrine CL_{int} and body weight. Data from humans were omitted from the analysis. The dashed line represents the regression based on nonlogarithmically treated data. It appears curved only because it is illustrated on log-log coordinates. This regression, however, does have a major theoretical drawback, in that a finite value of CL_{int} is predicted when body weight is zero. That is, the y intercept is not equal to zero. In the case of a 25-g mouse, this artifact would

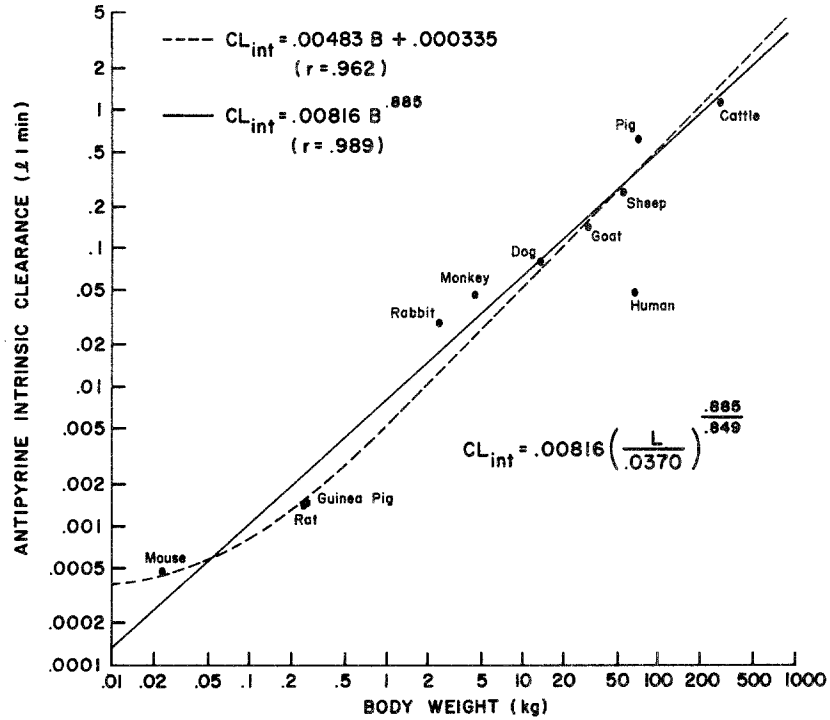

Fig. 2. Antipyrine CL_{int} in mammals as a function of body weight. Dashed line is the least-squares fit of nonlogarithmically transformed data weighted by the factor $1/y^2$. Solid line is from equation fitted using the method of least squares on unweighted logarithmically transformed data. See text for discussion.

contribute 74% of the value to the CL_{int}. On the other hand, the heterogonic relationship predicts no CL_{int} when body weight is zero. Considering the problems involved and the assumptions made, the correlation coefficient of 0.989 and the randomness of scatter suggests a rather good fit. Combining the heterogonic relationships of L and CL_{int}, Fig. 2 indicates that antipyrine CL_{int} may be approximated as 0.22 liter/min/kg liver weight (assumes an exponent of unity). Of course, it is apparent that humans deviate by a factor of approximately one-seventh from the theoretical relationship. This is consistent with the statement of Brodie (5) that "drugs are metabolized in man more slowly than in laboratory animals." If antipyrine CL_{int} truly reflects interspecies variation for exogenous substances in general, then it would seem that man indeed is unique in that he lacks the quantitative capacities of other mammalian species.

Intraspecies Variation in Drug Metabolic Rates

From even a cursory examination of recent clinical pharmacokinetic literature, it is readily apparent that healthy individuals of the human species differ greatly in drug metabolism rates. From an evolutionary standpoint, this is a desirable situation which would be expected. As discussed by Mayr (18), any population which lacks diversity is more narrowly adapted, more specialized, and therefore more vulnerable to extinction under adverse conditions. In this regard, drug metabolism polymorphism is an example of a genetic mechanism which produces variation and is therefore a component of adaptiveness.

Interestingly, during the evolutionary development of divergent life forms, it was the transition from asexual to sexual reproduction which dramatically contributed to a genetic means of creating and transmitting individual differences within a population, primarily through the vast numbers of possible gene recombinations. It is this immensity of genotypic variation that produces and ensures intraspecies variation in drug metabolism rates.

Of course, civilization has brought with it a variety of manmade factors causing even greater variability. Variations caused by nutritional state, diet, body position, presence of disease, influence of other drugs, environmental conditions, etc., provide a myriad of research possibilities.

Extrapolation of Benzodiazpepine Intrinsic Clearance Data from Dog to Man

The antipyrine CL_{int} data (Fig. 2) indicated that, based on liver weight, man's CL_{int} for this compound is approximately one-seventh that of other mammalian species. Based on a broad reading of the pharmacokinetic

literature, it seems reasonable that an approximate sevenfold difference between man and other mammalian species might very well exist for CL_{int} of other drugs. Even if there were considerable error spanning several orders of magnitude, some reasonable factor for extrapolation of animal data to man would be potentially useful.

It was therefore decided to compare interspecies variation for some other drugs. Initially, benzodiazepine pharmacokinetics (intrinsic clearances) in man and beagle dog were compared. The benzodiazepines were selected since necessary data for the calculation of CL_{int} in dog and man were available on at least five members of the series (4, 19–28). Two compounds, diazepam and flunitrazepam, were discarded from further analysis when data from beagle dogs (19) indicated that blood clearance exceeded Q by a factor of at least twofold. Interestingly, Klotz *et al.* (4), in a splendid study on interspecies variation of diazepam pharmacokinetics, provided strong evidence for extrahepatic metabolism in some species, including dog. The remaining drugs used in the analysis were bromazepam, clonazepam, and chlordiazepoxide. Bromazepam and chlordiazepoxide are virtually completely metabolized in both dog and man, ostensibly by oxidative pathways (24–27). Clonazepam is also virtually completely metabolized in both dog and man, but by both oxidative demethylation and nitro reduction (22,28). For the purpose of these calculations, Q in dog and man was taken as 41.0 and 25.4 ml/min/kg body weight, respectively; liver weight in dog and man was taken as 2.91% and 2.42% of body weight (Fig. 1, Table I).

The results of the analysis are indicated in Table II. The dog/man ratios of CL_{int} per unit liver weight for bromazepam, clonazepam, and chlordiazepoxide are 4.20, 9.33, and 11.4, respectively. The average ratio is 8.31 to 1.

Extrapolation of Phenytoin Intrinsic Clearance Data from Animals to Man

From an analysis of the antipyrine data, it was established that the human species metabolizes this drug at approximately one-seventh the rate of other mammalian species (i.e., using intrinsic clearance as an index of drug metabolic rate). Data on benzodiazepine showed a similar quantitative pattern.

For further support of the quantitative differences between man and other mammalian species, CL_{int} of phenytoin was calculated (29–35) in several species (from equation 3), and the data are illustrated in Fig. 3. This drug is eliminated ostensibly by oxidative metabolism. An effort was made to obtain and utilize data in which phenytoin was eliminated by linear (as opposed to saturable) kinetics, i.e., data from low-dose studies were sought.

Table II. Comparison of Benzodiazepine Pharmacokinetic Data in Dog and Human

Compound	Average body Weight (kg)	CL_h (ml/min)	CL_{int} (ml/min)	CL_{int} (ml/min/Kg LW)[a]
		Dog data		
Bromazepam	12.5	47.3	61.3	168.0
Clonazepam	10.0	112.0	688.0	2360.0
Chlordiazepoxide	11.5	92.0	1010.0	3010.0
		Human data		
Bromazepam	84.2	65.1	81.6	40.0
Clonazepam	67.9	98.6	415.0	253.0
Chlordiazepoxide	80.9	45.7	516.0	263.0
		Ratio CL_{int} (dog)/CL_{int} (human)		
		Bromazepam	4.20	
		Clonazepam	9.33	
		Chlordiazepoxide	11.4	
		Average	8.31	

[a]LW, Liver weight.

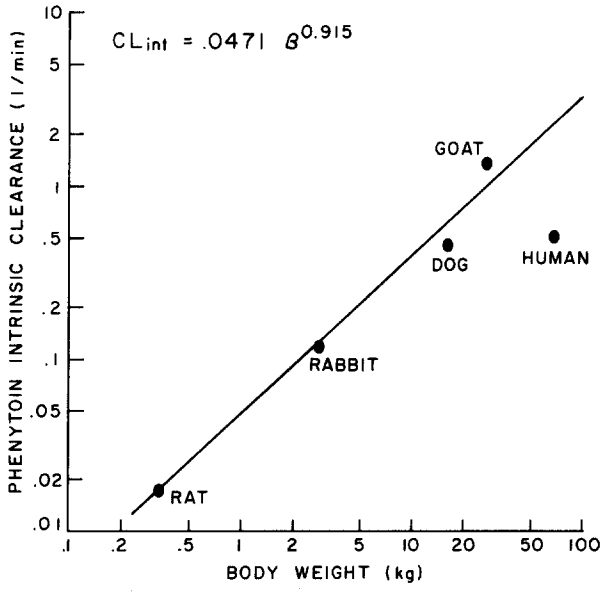

Fig. 3. Phenytoin CL_{int} in mammals as a function of body weight. Regression line does not utilize human data point. See text for discussion.

Values of Q were taken from collated data presented previously (Table I). Once again, it is apparent that the human species metabolizes slower than expected. Extrapolation of the regression line predicts an CL_{int} which is 4.4 times greater than observed.

Although the data presented in the present work are limited, it is hoped that the beginnings of a pattern may have been established. However, let this caveat be offered. The vast numbers of drugs not investigated by these procedures make it difficult at this time to gauge the significance of these limited findings. Also, it cannot be overemphasized that any interspecies comparisons of drug metabolism rates should use intrinsic clearance as the parameter of choice.

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