Drug Metabolism Reviews

ISSN: 0360-2532 (Print) 1097-9883 (Online) Journal homepage: http://www.tandfonline.com/loi/idmr20

Interspecies Comparison of In Vivo Caffeine Pharmacokinetics in Man, Monkey, Rabbit, Rat and Mouse

M. Bonati, R. Latini, G. Tognoni, J. F. Young & S. Garattini

To cite this article: M. Bonati, R. Latini, G. Tognoni, J. F. Young & S. Garattini (1984) Interspecies Comparison of In Vivo Caffeine Pharmacokinetics in Man, Monkey, Rabbit, Rat and Mouse, Drug Metabolism Reviews, 15:7, 1355-1383, DOI: 10.3109/03602538409029964

To link to this article: http://dx.doi.org/10.3109/03602538409029964

DRUG METABOLISM REVIEWS, 15(7), 1355-1383 (1984-85)

Interspecies Comparison of In Vivo Caffeine Pharmacokinetics in Man, Monkey, Rabbit, Rat, and Mouse*

M. BONATI,† R. LATINI, and G. TOGNONI
Laboratory of Clinical Pharmacology
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62 - 20157 Milan, Italy

J. F. YOUNG
Division of Teratogenesis Research
National Center for Toxicological Research
Jefferson, Arkansas

S. GARATTINI
Istituto di Ricerche Farmacologiche "Mario Negri"
Milan, Italy

*This work was presented in part at the Second World Conference on Clinical Pharmacology and Therapeutics, July 31-August 5, 1983, Washington, D.C. It was refereed for publication by Harold G. Boxenbaum, Ph.D., Associate Professor, School of Pharmacy, University of Connecticut, Storrs, Connecticut 06268.

†To whom correspondence should be addressed.

 0360-2532/84/1507-1355$3.50/0

I. INTRODUCTION

With more than a century of history in the field of chemistry and pharmacology [1], caffeine could be expected to have a fairly well-defined and comprehensive profile. However, far from producing such results, the familiarity of caffeine in daily life [2-7] has apparently protected it from systematic study. Piecemeal information has accumulated in the wake of interest in the potential and real clinical effects and even more interest in the claimed toxicological risks [8-28]. The fact that no systematic picture has emerged represents a fascinating case of the casual rules that often seem to accompany development of scientific knowledge even in minor areas, and this has offered a challenging opportunity for a research protocol including comparative investigation of caffeine in five animal species: man, monkey, rabbit, rat, and mouse. The significance of this appears to extend beyond the specific case of caffeine to reach other, extensively metabolized substances.

Interspecies comparison is a major topic in toxicology. Often large differences in physiological parameters [29, 30], drug metabolism rates [31-36], and pharmacokinetic parameters [37-41]

only add to the interpretative difficulties when obtained in different experimental settings, and the data must be compiled through an exhaustive search of the literature [42, 43]. Extrapolation from animal data to man at this point risks running into major and possibly unacceptable degrees of approximation as regards estimation of either toxicity or safety.

The basic methodological aim in this study was to assess the yield of a different approach: man as both the departure and arrival point. Interspecies comparison was explicitly planned to investigate the animal species and dosage regimen that best fit the human model. Pharmacokinetic analyses were chosen and investigated not only to provide descriptive data on drug disposition but also as tools for describing (and including assessment of the importance of interspecies variation) dynamic processes and variables associated with (1) the anatomical and physiological characteristics of each species and (2) the physicochemical characteristics specific for each compound [40].

The general organization of this paper and the presentation and discussion of experimental data therefore reflect the underlying thesis that caffeine could usefully be seen as a model compound of broader methodological significance.

II. METHODOLOGY

The experimental design of the study allowed more general questions to be addressed than just the comparative description of the kinetic profile of caffeine in the five mammalian species considered. The basic assumptions are briefly discussed in the following points.

1. Caffeine is a model tool for interspecies comparison. The points supporting this statement can be compiled from the existing data:
   a. The blood/plasma concentration ratio over a large range (1-100 μg/mL) is essentially 1.0 [44, 45].
   b. In vivo and in vitro studies show that caffeine is poorly bound (10-30%) to plasma albumin over a wide range of concentrations (1-100 μg/mL), ages, and species examined [44, 46, 47]. These findings are confirmed by saliva/plasma and CSF/plasma concentration ratios [48-52].
   c. All available data indicate a first-order kinetic model at least up to 5 mg/kg for the five species considered [53-57].
   d. Age and weight ranges of subjects within a species were kept narrow and only males were studied, so that interspecies

physiological and pharmacokinetic parameters were unaffected by these variables.

e. The fraction of the administered dose eliminated unchanged is known to be <5% for all the species investigated [53, 56, 58-60], so the systemic clearance (Cl_S) is equivalent to the metabolic clearance (Cl_M) [61].

f. Gastrointestinal absorption is rapid and complete (although with different interspecies rate constants) [44, 48, 55, 62-64]. From our experimental observations, only traces of caffeine and its metabolites were found in the feces after oral doses up to 5 mg/kg (particularly for rat, mouse, and rabbit).

g. Intraspecies disposition was similar after oral and i.v. administration of the same low doses to man [63-65], rat, and rabbit [56, 66-68]. Hence the fractional systemic availability ($F = AUC_{oral}/AUC_{i.v.}$) was considered equal to unity.

h. Considering that there is no extrahepatic metabolism of the drug, we propose the relationship $Cl_S = Cl_M = Cl_H$, where Cl_H is the hepatic clearance.

i. In rats and humans, no significant first-pass effect occurs after oral caffeine [54].

2. Caffeine is efficiently eliminated by animals and humans through liver biotransformation to several dimethylxanthine metabolites (theophylline, paraxanthine, theobromine), which can be further demethylated to the respective monomethylxanthines. Xanthines can also be substrates for oxidation to the respective urate and/or for hydration to diaminouracil compounds [8, 9, 59, 69-72]. Their metabolism depends on microsomal mixed-function oxidase, particularly the various forms of cytochrome P-450 [54, 73-77]. Whether this type of activity has an exclusively defensive function [31] for the organism or a harmful one, too [78], is not discussed in this paper, even though a few metabolites have biological effects and have been suggested as risk factors for toxic effects [58, 79, 80].

3. The liver is the organ most involved in caffeine disposition [76, 81-84]. Until the early 1970s, when the role of pharmacokinetics became more important in pharmacological (and toxicological) research, the half-life was the simplest and most widely used parameter to describe and compare different drug-eliminating systems and the metabolic profiles of investigated compounds. This is true for caffeine, too [59]. But, as pointed out by Rowland [40] and Wilkinson and Shand [61], the half-life depends on two potentially independent parameters: the volume of distribution and the total clearance. Of these, the volume of distribution "has no direct physiologic meaning and does not refer to a real volume"

[85, 86]; that is, it is an apparent volume. Rowland [40] also stated that "the clearance is a meaningful parameter, it has units of flow. It is also model independent and has additive properties." Thus, clearance (for our purposes, hepatic clearance) best describes the efficiency of an organ (the liver in this case) in removing a compound (caffeine) from perfusing blood.

4. The appropriate use of hepatic clearance for interspecies comparison requires that physiological organ parameters be calculated for each species studied. We used a linear relationship in mammals between physiological parameters and body weight calculated by Boxenbaum [42] and previously reported by Adolph in 1949 [29]. Liver weight (L) was calculated from the relationship

$$L = 0.0370 \times B^{0.849} \quad (1)$$

where B is body weight (kilograms). The hepatic flow (liters/minute) was calculated from [9]

$$Q = 0.0554 \times B^{0.894} \quad (2)$$

Since Q and L are related to the same constant (B), this can be simplified to

$$Q \approx 1.5 \text{ liters/minute/kilogram liver weight} \quad (3)$$

5. Having defined the physiological parameters (Table 2 and Fig. 1; see section IV), and bearing in mind the relationship $Cl_S = Cl_M = Cl_H$, we have to consider that Cl_H is a function of liver blood flow (Q) and that organ's ability to extract the drug (E): $Cl_H = QE$. In this regard, Wilkinson and Shand [61] suggested the term "total intrinsic unbound clearance" (Clu_{int}) and the following equation:

$$Clu_{int} = Q \times Cl_H / f_u \times (Q - Cl_H) \quad (4)$$

where f_u is the fraction of drug unbound in the blood. From the previous equation, E can also be defined by

$$E = (f_u \times Clu_{int}) / (Q + f_u \times Clu_{int}) \quad (5)$$

This was the type of approach followed by Rowland et al. [87], investigated by Gillette [88], and discussed by Pang and Rowland [89-91] to characterize the "well-stirred" model that explained the function of physiological factors in the hepatic clearance of drugs.

6. Only a tentative comparison can be made of the metabolic profile of caffeine in blood, as only a few of its less polar metabolites can be measured in the bloodstream after parent drug administration. Comparative urinary patterns of caffeine metabolites tend to be more species specific.

7. A specific case was made for testing the hypothesis that saturation of the caffeine-metabolizing enzyme system could be the cause of the nonlinear kinetics documented in the rat [53, 54], suggested for the rabbit [56, 66-68], but not found over a comparable dose range in man [55].

8. Man and his natural exposure to caffeine are formally assumed as the reference species and category.

III. EXPERIMENTAL

A. Human Volunteers

Four healthy male volunteers, 26 to 36 years of age and 69 kg mean body weight, participated in the study. They abstained from all methylxanthine-containing foodstuffs for 10 days before and up to the end of the study [92]. Each subject was given, in a random sequence, four oral doses of caffeine (0.22, 1.0, 1.54, and 5 mg/kg) in different preparations. Two volunteers also received a higher fifth dose (10 mg/kg). Details of the study are given elsewhere [55].

B. Animals

Five male monkeys (Macaca cynomolgus), 5 kg mean body weight (housed at RBM, Ivrea, Italy) were used; three received three doses of caffeine (2.5, 10, and 50 mg/kg), and the other two were given only two of these doses.

Thirteen male New Zealand white rabbits (Charles River, Italy), 3 kg mean body weight, were used to obtain pharmacokinetic findings after five different doses of caffeine (1, 5, 10, 50, and 100 mg/kg). Each animal was given only one of the doses. Before the beginning of the study, one central ear artery was cannulated [93].

Male CD-COBS rats (Charles River, Italy), 280 g mean body weight, were used. The day before the study their right common carotid artery was cannulated [94]. Caffeine doses were 1, 2.5, 5, 10, 25, 50, and 100 mg/kg.

Male CD-COBS mice (Charles River, Italy), 30 g mean body weight, were given caffeine at three oral doses (1, 10, and 100 mg/kg).

All animals were housed in air-conditioned rooms with constant humidity and maintained on a 12-h light-dark cycle. They received stock diet and water ad libitum. Before the experiment each animal was fasted overnight.

C. Drug Administration, Sampling, and Assay

Human volunteers were given caffeine orally as an aqueous solution, as mocha coffee, or as a soft drink. Animals received caffeine (supplied by National Soft Drink Association, Washington, D.C., USA) in 0.1% or 1.0% water solution by stomach tube. A few rats and rabbits received caffeine in physiological saline solution through their cannula, into the common carotid artery (rat) or central ear artery (rabbit), after which the tube was flushed with an equal volume of saline solution. The doses and numbers of animals are shown in Table 1.

TABLE 1

Doses of Caffeine Administered to Mammalian Species

Caffeine dose (mg/kg)	Man	Monkey	Rabbit	Rat	Mouse
0.22	+	-	-	-	-
1.00	+	-	+	+	+
1.54	+	-	-	-	-
2.50	-	+	-	+	-
5.00	+	-	+	+	-
10.00	+	+	+	+	+
25.00	-	-	-	+	-
50.00	-	+	+	+	-
100.00	-	-	+	+	+
Total of performed kinetics	18	10	13	38	3[a]

[a]Five animals were killed at each time after caffeine administration. n = 160.

Blood samples were drawn at different time intervals after administration but followed a similar schedule in all experiments. Blood was sampled from the antecubital vein (man and monkey), via the cannulated arteries (rat and rabbit), or by killing the animals (mouse) by decapitation at intervals after drug administration (5 in each group). Urine samples were collected for 48 h after dosing. Caffeine and its metabolites were assayed in blood, plasma, and urine by high-pressure liquid chromatography, as previously described [55, 76, 95].

D. Data Analysis

Blood or plasma concentrations versus time were analyzed using a one-compartment open model system after linear dosing. Experimental points were fitted by a nonlinear regression iterative program (Carl Peck, Uniformed Services University, Bethesda, Md., USA) and run according to Sacchi-Landriani et al. [96] on a HP-85 desk computer (Hewlett-Packard, USA). Areas under blood or plasma concentration versus time curves (AUC) were calculated by the trapezoidal rule and extrapolated to infinity. The apparent volume of distribution (aVd) and the systemic clearance (Cl_S) were calculated as

$$aVd = F \times D/(AUC_{0\rightarrow\infty} \times k_{el}) \quad (6)$$

and

$$Cl_S = aVd \times k_{el} = F \times D/AUC_{0\rightarrow\infty} \quad (7)$$

assuming the bioavailable fraction (F) of the administered caffeine dose (D) equal to 1.0 when the drug was administered orally (see section II, point 1.g). When blood or plasma concentration versus time curves did not show linear elimination kinetics, only AUC values were calculated, extrapolating them to infinity using the slope of the last portion of the curve that was linear and thus dose independent [86]. All slopes were obtained by linear regression analysis [97], and differences between results were tested by ANOVA, randomized block design [98].

Apparent in vivo K_m and V_{max} values were determined by two different approaches:

1. Once the rate of change of the blood (or plasma) caffeine concentrations was determined in the postabsorptive and/or postdistributive phase from one sampling time to the next, $\Delta C/\Delta t$, as a function of concentration (C) at the midpoint of the sampling

interval [86], the data were plotted according to the Lineweaver-Burk expression:

$$\frac{1}{\Delta C/\Delta t} = \frac{K_m}{V_{max} C} + \frac{1}{V_{max}} \tag{8}$$

2. For all drugs eliminated by first-order kinetics, the total area under the blood (or plasma) level versus time curves (AUC) should be proportional to the dose, so values for the ratios AUC/D are similar within each investigated species. Since this was not true in our case for the rat and rabbit, and to some extent for the mouse, we tried an interspecies comparison using the AUC-dose relationship by applying the following equation [99, 100]:

$$AUC = [D/(aVd \times V_{max})] \times [(D/2aVd) + K_m] \tag{9}$$

Data were fitted by a nonlinear regression iterative program. To use this equation (correct for intravenous administration), we assumed that no statistical differences were noted after i.v. or oral administration (the basis of this assumption for the case of caffeine has been discussed earlier). A mean value of 0.75 (liters/kilogram) for aVd was used for all five species (see section IV.B.1).

IV. INTERSPECIES RELATIONSHIPS

A. Interspecies Variation in Physiological Parameters

Linear relationships were obtained (Table 2, Fig. 1) by log-log plots of surface area, liver weight, and hepatic blood flow versus body weight, confirming the reliability of the heterogonic relationship for the five mammalian species under study. Biological similarities can thus be found which correlate physiological parameters with body weight [101].

B. Interspecies Variation in Pharmacokinetic Parameters after Low Doses

Table 3 shows the pharmacokinetic profile of caffeine at relatively low doses (1.0 and 2.5 mg/kg), so as to fall into the linear range of its disposition for all five species investigated. The elimination rate constant (k_{el}) is inversely proportional to body weight while the half life ($t_{1/2}$), systemic clearance (Cl_S), and apparent volume of distribution (aVd) are directly proportional to the body

TABLE 2

Physiological Parameters (Mean ± SE) in Mammalian Species

Physiological parameter	Man	Monkey	Rabbit	Rat	Mouse[a]
Body weight (kg) [B]	68.75 ± 1.70	5.09 ± 0.38	2.42 ± 0.15	0.28 ± 0.2	0.03
Surface area (m^2) [A][b]	1.778 ± 0.030	0.349 ± 0.018	0.162 ± 0.002[c]	0.038 ± 0.002	0.009
Liver weight (kg) [L][d]	1.343 ± 0.028	0.147 ± 0.009	0.082 ± 0.001	0.012 ± 0.001	0.002
Hepatic blood flow [Q][e] (L/min)	2.014 ± 0.042	0.221 ± 0.013	0.129 ± 0.002	0.018 ± 0.001	0.003

[a]Average values.
[b]$A = K \times B^{2/3}/10^4$; from Ref. 136.
[c]$A = (0.0014 \times B + 8.6104) \times B^{0.667}$, where B is in grams and A is in square centimeters; from Ref. 137.
[d]According to Eq. (1); from Ref. 42.
[e]According to Eq. (2); from Ref. 42.

weight, which is in accordance with the findings of Weiss et al. [39] on the relation between pharmacokinetic parameters and body weight. Elimination rate constant and half-life values (the two kinetic parameters most frequently reported) are in good agreement with those given by others over a 30-year period for all five species [9, 39, 69, 70, 72].

1. Apparent Volume of Distribution

Once corrected for body weight, values were calculated for the five species, and a mean volume of distribution of 0.75 L/kg was obtained, which leads to the conclusion that caffeine does distribute into the total body water. This is in agreement with findings that caffeine rapidly moves into and out of cells (blood/plasma ratio equal to unity) [56] and tissues [102]. Animal and human studies showed that no physiological "barriers" limit the passage of caffeine throughout tissues so that easy and rapid equilibrium

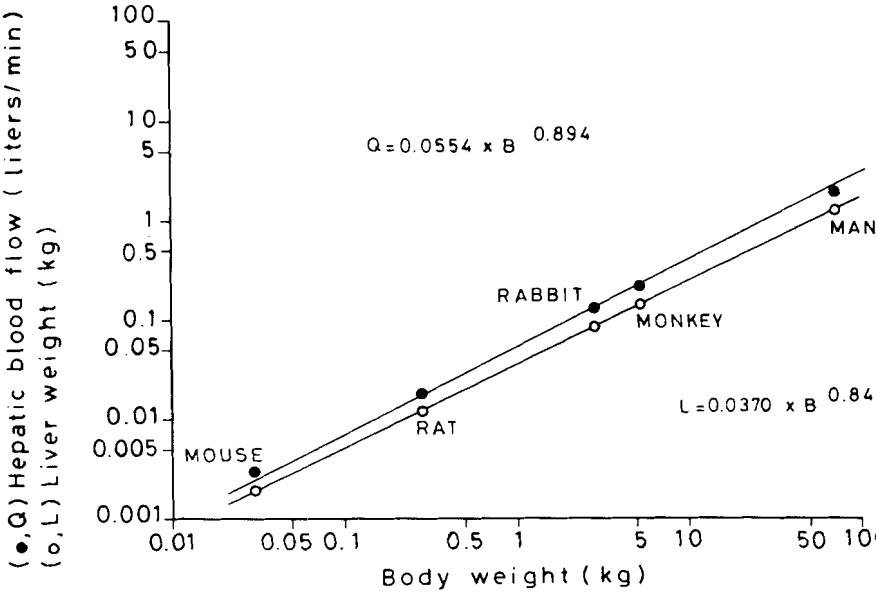


FIG. 1. Liver weight and hepatic blood flow in mammalian species as a function of body weight. According to Eqs. (1) and (2); from Ref. 42.

is reached between mother and fetuses, and blood and all tissues, including brain and testes [59]. Generally, caffeine enters and leaves tissues by simple diffusion, although recently a partial carrier-mediated blood-brain barrier has been reported [103]. Brain/blood ratios, calculated for the peak levels (after oral administration) or the AUC, did not show any significant difference between rat and mouse or at different dose levels (1, 10, 100 mg/kg); this suggests the hypothesis that interspecies differences in caffeine disposition (see later discussion) depend more on clearance than on distribution, at least for the mouse and rat [66].

2. Intrinsic Clearance

Extraction ratio and intrinsic clearance (Table 4) showed similar patterns in comparison with elimination rate constant and systemic clearance (Table 3) for man and monkey versus rabbit, rat, and mouse. The extraction ratio for the rat was similar to that calculated in vitro using perfused liver (the only available data regarding the species considered) [81] and confirmed the reliability

TABLE 3

Pharmacokinetic Parameters (Mean ± SE) for Caffeine in Mammalian Species, in a Range of Doses for Which First-Order Kinetics Is Applicable

Pharmacokinetic parameter	Man	Monkey	Rabbit	Rat	Mouse[a]
Dose (mg/kg)	1.0	2.5	1.0	2.5	1.0
K_{el} (h^{-1})	0.16 ± 0.01	0.22 ± 0.05	0.45 ± 0.11	0.82 ± 0.08	0.96
$t_{1/2}$ (h)[b]	4.19	3.16	1.62	0.84	0.72
Cl_S (mL/min)[c]	137.42 ± 14.49	15.25 ± 2.93	15.33 ± 4.19	3.55 ± 0.35	0.36
Cl_S (mL/min/kg)[c]	1.96 ± 0.21	3.06 ± 0.73	5.88 ± 1.49	12.54 ± 1.06	12.19
aVd (L)[d]	50.02 ± 2.50	4.23 ± 0.28	2.02 ± 0.13	0.26 ± 0.03	0.02
aVd (L/kg)[d]	0.73 ± 0.02	0.83 ± 0.01	0.78 ± 0.05	0.92 ± 0.05	0.76

[a]Average values.
[b]Harmonic mean half-life.
[c]According to Eq. (7).
[d]According to Eq. (6).

of this approach. Comparison of the intrinsic clearance of man and the other species (Table 5, Fig. 2) confirmed that from a kinetic point of view, linear dosages may give similar caffeine disposition profiles in the monkey and even in the rabbit, rat, and mouse, which metabolize caffeine 2-3 times faster than man or monkeys. The findings are in good agreement with other data reported for antipyrine, benzodiazepines, and phenytoin [42]. To Brodie's statement [31] that man metabolizes drugs more slowly than animals, we would add that the hepatic metabolic enzyme activity is

TABLE 4

Hemodynamic Parameters of Caffeine (Mean ± SE) in Mammalian Species

Hemodynamic parameter	Man	Monkey	Rabbit	Rat	Mouse[a]
Intrinsic clearance (mL/min) [Clu_{int}][b]	153 ± 15.62	18.36 ± 3.86	19.93 ± 6.24	4.94 ± 0.56	0.45
Liver blood flow (mL/min) [Q][c]	2014 ± 42	221 ± 13	129 ± 2	18 ± 1	3
Extraction ratio [E][d]	0.07 ± 0.01	0.07 ± 0.02	0.11 ± 0.03	0.13 ± 0.02	0.12

[a]Average values.
[b]According to Eq. (4); from Ref. 61.
[c]According to Eq. (2); from Ref. 42.
[d]According to Eq. (5); from Ref. 61.

TABLE 5

Caffeine Intrinsic Clearance Comparison in Mammalian Species

Kinetic parameter	Man	Monkey	Rabbit	Rat	Mouse[a]
Clu_{int} (mL/min)[b]	153.31 ± 15.62	18.35 ± 3.86	19.93 ± 6.24	4.94 ± 0.56	0.45
Clu_{int} (mL/min/L)[c]	118.99 ± 12.01	126.74 ± 31.69	239.20 ± 70.72	401.07 ± 52.35	225.00
Clu_{int}(animal)/ Clu_{int}(man)		1.06	2.01	3.37	1.89

[a]Average values.
[b]According to Eq. (4); from Ref. 61.
[c]According to Eqs. (1) and (4); from Refs. 42 and 61.

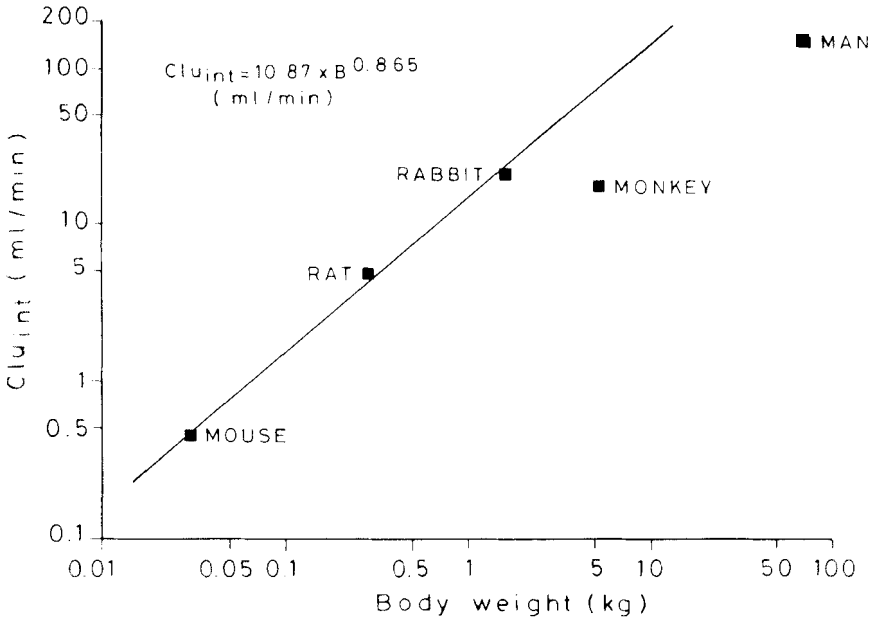


FIG. 2. Caffeine Clu_{int} in mammalian species as a function of body weight. Line does not utilize man and monkey points.

not per se linearly related to body weight in different mammalian species. To explore further the potential toxicological implications of our approach, "physiological time" as proposed by Boxenbaum [43] was applied to caffeine as the variable which makes physiological events species independent. The basic assumption of the approach is known: Longevity, or aging rate, is characteristic of each species [104-109] and is the sum of wide-spanning vital processes and exogenous metabolic functions (e.g., drugs). The application of the maximum potential lifespan (MPL, as the maximum documented longevity for a species) [110] is shown in Fig. 3. Assuming constant drug exposure over years (the unit of MPL), a proportional relationship was found between the total volume from which drug would be cleared, (Clu_{int}) X MPL, and body weight. While no practical use can as yet be suggested for the resulting good fit, it is worth stressing the potential utility of a similar comprehensive framework when trying to compare,

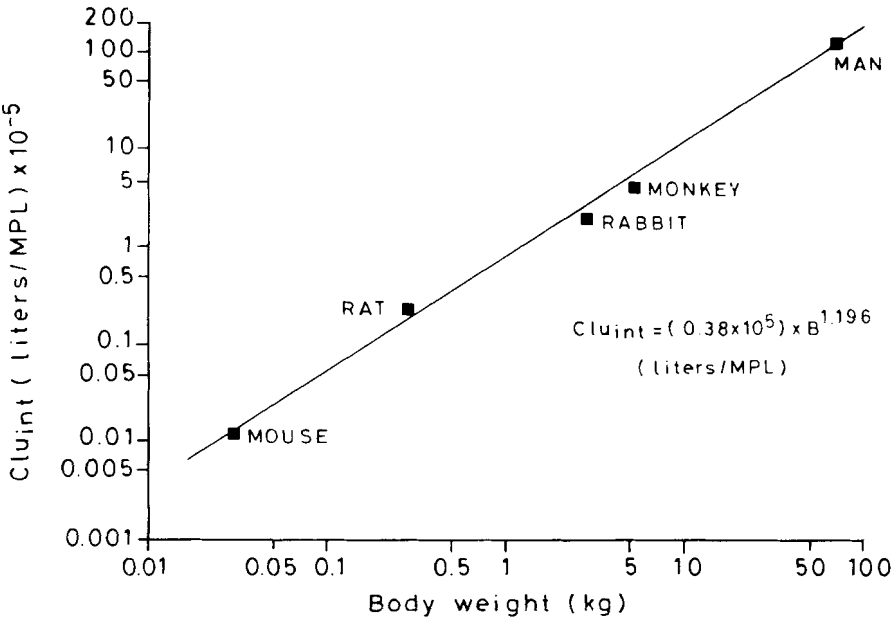


FIG. 3. Caffeine Clu_{int} per Maximum Potential Lifespan (MPL) in mammalian species as a function of body weight. MPL values were calculated for monkeys, rabbits, rats, and mice employing Sacher's equation [110]: MPL = 10.389 X (brain weight)$^{0.636}$ X (body weight)$^{-0.225}$. For man, because the brain weight of our volunteers was obviously not available, previous reported data were utilized [43, 109].

predict, or extrapolate data related to nonsaturable kinetic conditions from one species to another.

C. Circulating Metabolites

AUC values can be used as the basis for comparison between species by also taking into account circulating metabolites (Table 6). Paraxanthine is the primary caffeine metabolite found at high levels in the bloodstream of man and rabbits; theophylline, in the monkey; and theobromine, in the mouse. Since in rats, caffeine disposition is dose dependent around 5-10 mg/kg doses [53, 54],

TABLE 6

Average Bloodstream $AUC_{0 \rightarrow 24\ h}$ (mg/L/min) for Caffeine and Its Metabolites after Normalized Doses (to 1 mg/kg)

Compound	Man (5 mg/kg)	Monkey (10 mg/kg)	Rabbit (5 mg/kg)	Rat (10 mg/kg)	Mouse (10 mg/kg)
Caffeine	941	464	100	188	102
Theophylline	79	649	24	19	4
Paraxanthine	394	42	174	—	20
Theobromine	83	75	12	27	48

these data must be viewed with reserve. No dose-AUC proportion exists, and at lower doses bloodstream kinetics of caffeine metabolites cannot be accurately described. However, in the rat, theophylline and theobromine production is of the same order, while paraxanthine formation is less [66]. A similar metabolic profile was reported in relation to brain concentrations in the rat and mouse [56]. Thus from Tables 3-6 it may be deduced that man and monkey have the same pharmacokinetic profile but different metabolic profiles in blood.

D. Urinary Metabolism

Table 7 shows comparative patterns of caffeine metabolites after low doses of the compound. These data, because more than one set was available, represent the averages of published values for man [44, 55, 58, 69, 111-113], rat [56, 58, 60, 66-68, 114], and mouse [56, 66-68, 115]. Even casual analysis shows that the pattern differs qualitatively and quantitatively for the various species. In adult humans most of the administered caffeine undergoes 3-N-demethylation to paraxanthine (the primary metabolite found in the blood). The major metabolite excreted in urine is 1-methyluric acid. An acetylated diaminouracil derivative (5-acetylamino-6-amino-3-methyluracil) was recovered exclusively in human urine [113, 116-118]; it was recently suggested that the

polymorphic enzyme N-acetyltransferase could be responsible for its production [119]. Caffeine is biotransformed in the monkey (Macaca cynomolgus) by 7-demethylation. Theophylline (as in the blood) and 1,3-dimethyluric acid account for almost the whole metabolic excretion pattern of the compound [58]. Comparison of caffeine disposition in three different monkey strains revealed different profiles in each [120]. The Macaca profile resembled the metabolic pattern in beagle dogs, the only species in which 3-methylxanthine was identified in serum after single doses of caffeine [73].

As in man, paraxanthine is the major metabolite measured in the bloodstream and urine of rabbits, though the complete urinary and kinetic profiles are quite different [56, 66-68]. The urinary metabolic pattern in the rat is diffuse, involving all the primary routes of caffeine degradation. A few minor metabolites, such as trimethylallantoin, were detected in this species alone, in which the main significant metabolite is 6-amino-5-[N-formylmethylamino]-1,3-dimethyluracil [58, 114, 121, 122]. As in the rat, initial degradation of caffeine in the mouse involves 1-3-7-demethylation and 8-oxidation pathways to the same extent (confirming blood data). Unlike other species investigated, urine of mice (and questioned in beagles [73, 123]) contained no detectable levels of 6-amino-5-[N-formylmethylamino]1,3-dimethyluracil.

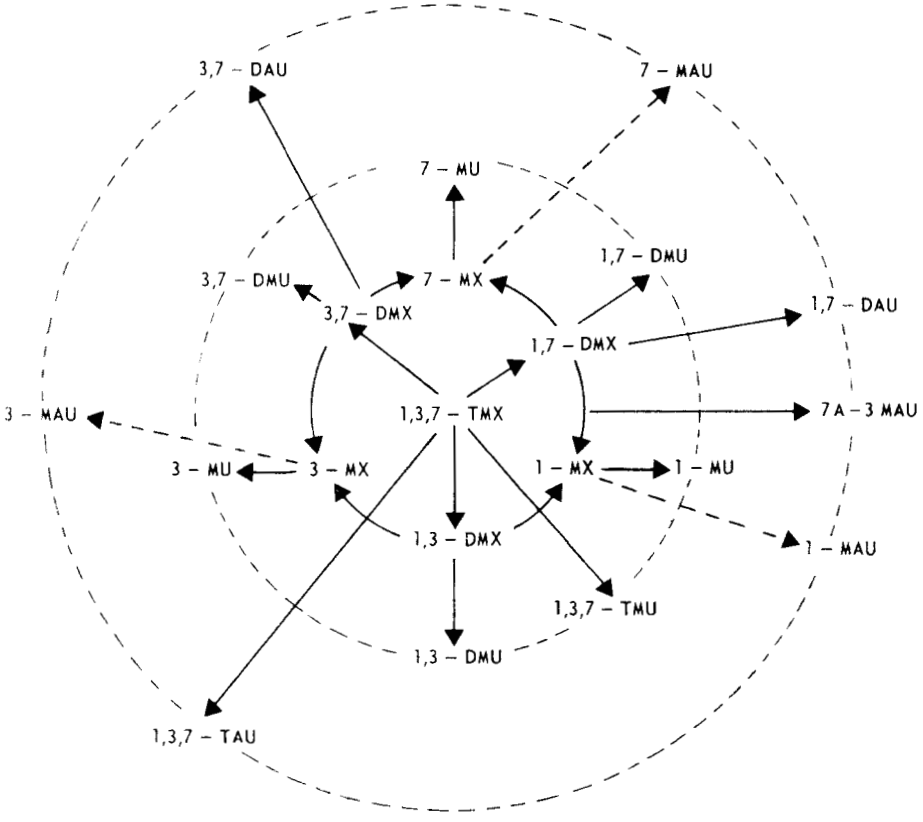
E. Nonlinear Kinetics

Plasma and brain concentrations of caffeine given at different doses to rats indicated a limited capacity to cross the blood-brain barrier and metabolize the drug [53, 56, 66-68]. Ratios between doses were much lower than between AUC in plasma and in brain, because of a disproportionate increase in the dose-concentration relationship (46-fold instead of 10-fold from 1- to 10-mg/kg doses). After oral administration, peak level and rate constant of absorption (k_{abs}) also rose faster than the dose [53, 56]. Thus the rat's metabolic capacity is saturable at relatively low doses. The consequences of saturation kinetics in rats are that exposure to caffeine increases disproportionately after either single or repeated dosing and metabolic profiles can change with the dose. A similar trend was suspected for rabbits [56, 66-68].

Since data were available after dose-range kinetic studies in the same subject (man) and in the same species (rat, rabbit, monkey, mouse), we undertook an interspecies comparison of caffeine pharmacokinetics. Using the AUC-dose relationship for a one-compartment model with Michaelis-Menten metabolism [99, 100], the nonlinear kinetic elimination of caffeine was

TABLE 7

Comparative Urinary Excretion of Caffeine Metabolites in Five Mammalian Species (% of Administered Dose)



Compound[a]	Man	Monkey	Rabbit	Rat	Mouse
1,3,7-TMX	1.1	1.6	1.5	2.9	5.1
3,7-DMX	2.5	1.3	2.7	7.1	–
1,3-DMX	2.3	27.6	–	4.4	5.0
1.7-DMX	5.4	1.4	13.7	8.6	10.4
1-MX	13.1	–	10.8	3.0	4.2

(continued)

Table 7 (continued)

Compound[a]	Man	Monkey	Rabbit	Rat	Mouse
3-MX	2.5	7.6	–	1.5	2.3
7-MX	6.6	–	–	–	4.1
1,3,7-TMU	1.2	1.0	1.9	5.8	15.0
1,3-DMU	2.7	44.0	–	2.1	15.0
3,7-DMU	0.8	–	6.5	–	–
1,7-DMU	8.2	–	3.0	1.8	6.4
1-MU	24.1	2.0	–	3.6	9.7
3-MU	0.1	–	–	–	7.8
7-MU	–	–	–	–	2.1
1,3,7-TAU	1.1	0.4	2.1	19.5	–
3,7-DAU	1.9	–	–	3.0	–
1,7-DAU	2.4	–	–	–	–
7A-3MAU	17.8	–	–	–	–
% recovery	94.1	76.9	42.2	63.3	87.1

[a] 1,3,7-TMX: 1,3,7-trimethylxanthine(caffeine); 3,7-DMX: 3,7-dimethylxanthine (theobromine); 1,3-DMX: 1,3-dimethylxanthine (theophylline); 1,7-DMX: 1,7-dimethylxanthine (paraxanthine); 1-, 3-, 7-MX: 1-, 3-, 7-methylxanthine; 1,3,7-TMU: 1,3,7-trimethyluric acid; 1,3-, 3,7-, 1,7-DMU: 1,3-, 3,7-, 1,7-dimethyluric acid; 1-, 3-, 7-MU: 1-, 3-, 7-methyluric acid; 1,3,7-TAU: 6-amino-5-[N-formylmethylamino]1,3-dimethyluracil; 3,7-DAU: 6-amino-5-[formylmethylamino]1-methyluracil; 1,7-DAU: 6-amino-5-[N-formylmethylamino]3-methyluracil; 7A-3MAU: 6-amino-5-[N-acetylamino]3-methyluracil; 1-MAU: 6-amino-5-[N-formylamino]1-methyluracil; 3-MAU: 6-amino-5-[N-formylamino]3-methyluracil; 7-MAU: 6-amino-5-[N-formylmethylamino]uracil.

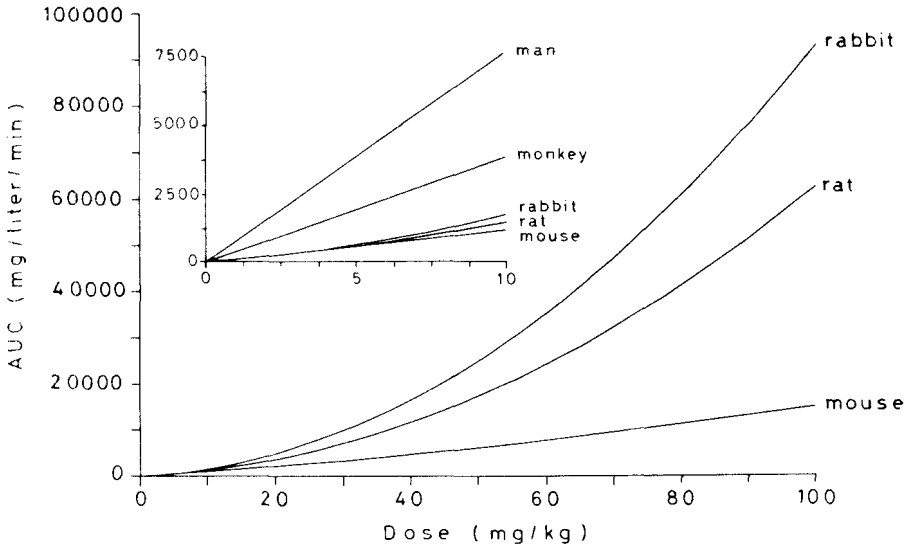


FIG. 4. AUC-dose relationship after caffeine administration to five mammalian species. Insert shows AUC-dose relationship up to 10-mg/kg dose.

investigated in all five species (Fig. 4). For rabbits and rats, the AUC increased proportionally more than the dose. For the rat, rabbit, and mouse, the in vivo apparent K_m and V_{max} values were estimated. Similar values were obtained from Lineweaver-Burk plots (Table 8). In the rat and rabbit, caffeine was eliminated by a saturable process with a similar affinity constant (K_m) of about 10 μg/mL, while for mice a mean value of 84 μg/mL was calculated. Close values were calculated for the theoretical maximum rate of the process (V_{max}) for these three species, although variability was broader (particularly for the mouse because data on only three doses were available). Calculated values for rats were in good agreement with those reported by Aldridge et al. [54], the only other published information. These findings have important implications in extrapolation of caffeine toxicological data across species. Ratios between animals and man must be calculated for rats and rabbits on the basis of the caffeine AUC and not on the dose, and it must be taken into account that first-order kinetics prevails in man (at least up to 10 mg/kg, the

TABLE 8

Estimates of K_m and V_{max} in Three Mammalian Species after 1-100 mg/kg Caffeine

	AUC-dose relationship[a]		Lineweaver-Burk plot[b]	
Species	K_m (μg/mL)	V_{max} (μg/mL/min)	K_m (μg/mL)	V_{max} (μg/mL/min)
Rat	8.74	0.159	9.48	0.043
Rabbit	13.03	0.068	10.22	0.056
Mouse	85.24	0.133	82.13	0.570

[a]According to Eq. (9); from Refs. 99 and 100.
[b]According to Eq. (8); from Ref. 86.

highest administrable dose [55]), monkey, and mouse (up to around 100 mg/kg).

V. CONCLUSIONS

These data from systematic studies by the same group form a more reliable basis for interspecies comparison of in vivo caffeine pharmacokinetics, against which to weigh and compare the variety of piecemeal information in the literature. Although numerous physiopathological factors (age, sex, smoking, pregnancy, diseases, etc.) can affect caffeine disposition in both humans [64, 124-133] and animals [76, 134-136], significant information can be obtained from the comparison made here employing healthy young adult males. The combined analyses of original data and of findings from the literature provide the following picture:

1. Caffeine was eliminated by first-order kinetics by all five species considered after doses up to 5 mg/kg.
2. Comparison of pharmacokinetic parameters showed closer similarity between man and monkeys than between monkeys and rabbits, rats, and mice.

3. A linear relationship was suggested between pharmacokinetic time (a variable in terms of chronological time) and body weight across all five mammalian species investigated taking into account species-longevity differences.

4. Species differences were seen in the caffeine metabolic profile in blood: Man was more similar to the rabbit (with paraxanthine formation as primary metabolite), while the other three species seemed to have species-specific patterns.

5. The total urinary excretion pattern of caffeine metabolites was more species specific, with each species presenting a unique metabolic profile.

6. Nonlinear kinetic ranges showed similar profiles for saturated caffeine elimination in the rat and rabbit, very different from the profiles for the other three.

In conclusion, broad interspecies variability was found in caffeine kinetic and metabolic relationships. These findings might prove helpful in experimental planning and extrapolation, although the pharmacokinetic approach alone, without pharmacodynamic studies (as a measure of therapeutic and/or toxic effects), is only one of the two sides of any interspecies comparison.

Acknowledgments

Supported in part by grants from the International Life Sciences Institute (ILSI), Washington, D.C., and CNR (National Research Council), Rome, Italy: Grant on Clinical Pharmacology and Rare Diseases. We thank Judy Baggott, Vanna Pistotti, Rose Huber, and Cindy Hartwick for help in preparing and editing the manuscript.

REFERENCES

[1] C. G. Lehman, ed., *Lehrbuch der Physiologischen Chemie*, 2nd ed., Leipzig, 1850.

[2] M. L. Bunker and M. McWilliams, *J. Am. Diet. Assoc.*, *74*, 28 (1979).

[3] J. F. Greden, *The Sciences*, *19*, 6 (1979).

[4] D. M. Graham, *Nutr. Rev.*, *36*, 97 (1978).

[5] T. W. Rall, in *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gilman, eds.), 6th ed., Macmillan, New York, 1980, pp. 592-607.

[6] J. H. Andresky, *Consumers Dig.*, September-October 1981, p. 31.

[7] M. Dong, D. Hoffman, D. C. Locke, and E. Ferrand, *Atmos. Environ.*, *11*, 651 (1977).
[8] P. B. Dews, *Annu. Rev. Nutr.*, *2*, 323 (1982).
[9] S. M. Tarka, Jr., *CRC Crit. Rev. Toxicol.*, *9*, 275 (1982).
[10] P. W. Curatolo and D. Robertson, *Ann. Int. Med.*, *98*, 641 (1983).
[11] Caffeine and pregnancy, *FDA Drug Bull.*, *10*, 19 (1980).
[12] M. F. Jacobson, A. S. Goldman, and R. H. Syme, *Lancet*, *1*, 1415 (1981).
[13] L. Rosenberg, A. A. Mitchell, S. Shapiro, and D. Slone, *J. Am. Med. Assoc.*, *247*, 1429 (1982).
[14] S. Linn, S. C. Schoenbaum, R. R. Monson, B. Rosner, P. G. Stubblefield, and K. J. Ryan, *N. Engl. J. Med.*, *306*, 141 (1982).
[15] J. C. Martin, *Neurobehav. Toxicol. Teratol.*, *4*, 421 (1982).
[16] P. S. Thayer and P. E. Palm, *CRC Crit. Rev. Toxicol.*, *3*, 345 (1975).
[17] G. A. Nolen, *J. Toxicol. Environ. Health*, *10*, 769 (1982).
[18] T. Sugimura, *Cancer*, *49*, 1970 (1982).
[19] S. Rowner, *Washington Post*, January 18, 1980.
[20] D. Trichopoulos, M. Papapostolou, and A. Polychronopoulou, *Int. J. Cancer*, *28*, 691 (1981).
[21] L. D. Marrett, S. D. Walter, and J. W. Meigs, *Am. J. Epidemiol.*, *117*, 113 (1983).
[22] E. L. Wynder, N. E. L. Hall, and M. Polansky, *Cancer Res.*, *43*, 3900 (1983).
[23] R. L. Phillips and D. A. Snowdon, *Cancer Res.*, *43*, 2403s (1983).
[24] M. F. Stanton, R. A. Ahrens, and L. W. Douglass, *Experientia*, *34*, 1182 (1978).
[25] D. Robertson, J. C. Frolich, R. K. Carr, J. T. Watson, J. W. Hollifield, D. G. Shand, and J. A. Oates, *N. Engl. J. Med.*, *298*, 181 (1978).
[26] D. J. Dobmeyer, R. A. Stine, C. V. Leier, R. Greenberg, and S. F. Schaal, *N. Engl. J. Med.*, *308*, 814 (1983).
[27] T. B. Graboys and B. Lown, *N. Engl. J. Med.*, *308*, 835 (1983).
[28] D. A. Sawyer, H. L. Julia, and A. C. Turin, *J. Behav. Med.*, *5*, 415 (1982).
[29] E. F. Adolph, *Science*, *109*, 579 (1949).
[30] E. J. Freireich, E. A. Gehan, D. P. Rall, L. H. Schmidt, and H. E. Skipper, *Cancer Chemother. Rep.*, *50*, 219 (1966).
[31] B. B. Brodie, *Pharmacologist*, *6*, 12 (1964).
[32] B. B. Brodie and W. D. Reid, *Fed. Proc.*, *26*, 1062 (1967).

[33] L. B. Mellett, *Prog. Drug Res., 13*, 136 (1969).
[34] A. H. Conney, C. Coutinho, B. Koechlin, R. Swarm, J. A. Cheripko, C. Impellizeri, and H. Baruth, *Clin. Pharmacol. Ther., 16*, 176 (1974).
[35] J. R. Gillette, in *Drug Metabolism: From Microbe to Man* (D. V. Parke and R. L. Smith, eds.), Taylor and Francis, London, 1977, pp. 147-168.
[36] D. V. Parke, in *Animals in Toxicological Research* (I. Bartosek, A. Guaitani, and E. Pacei, eds.), Raven Press, New York, 1982, pp. 127-145.
[37] R. L. Dedrick, K. B. Bischoff, and D. S. Zaharko, *Cancer Chemother. Rep., 54*, 95 (1970).
[38] J. R. Gillette, *Clin. Toxicol., 9*, 709 (1976).
[39] M. Weiss, W. Sziegoleit, and W. Förster, *Int. J. Clin. Pharmacol., 15*, 572 (1977).
[40] M. Rowland, in *Drug Metabolism: From Microbe to Man* (D. V. Parke and R. L. Smith, eds.), Taylor and Francis, London, 1977, pp. 123-145.
[41] J. W. Daniel, in *Animals in Toxicological Research* (I. Bartosek, A. Guaitani, and E. Pacei, eds.), Raven Press, New York, 1982, pp. 115-126.
[42] H. Boxenbaum, *J. Pharmacokinet. Biopharm., 8*, 165 (1980).
[43] H. Boxenbaum, *J. Pharmacokinet. Biopharm., 10*, 201 (1982).
[44] J. Axelrod and J. Reichenthal, *J. Pharmacol. Exp. Ther., 107*, 519 (1953).
[45] J. Blanchard, *J. Pharm. Sci., 71*, 1415 (1982).
[46] M. L. Eichman, D. E. Guttman, Q. Van Winkle, and E. P. Guth, *J. Pharm. Sci., 51*, 66 (1962).
[47] P. V. Desmond, R. Patwardhan, R. Parker, S. Schenker, and K. V. Speeg, Jr., *Life Sci., 26*, 1261 (1980).
[48] W. D. Parsons and A. H. Neims, *Clin. Pharmacol. Ther., 24*, 40 (1978).
[49] C. E. Cook, C. R. Tallent, E. W. Amerson, M. W. Myers, J. A. Kepler, G. F. Taylor, and H. D. Christensen, *J. Pharmacol. Exp. Ther., 199*, 679 (1976).
[50] S. M. Somani, N. N. Khanna, and S. B. Bada, *J. Pediatr., 96*, 1091 (1980).
[51] R. Newton, L. J. Broughton, M. J. Lind, P. J. Morrison, H. J. Rogers, and I. D. Bradbrook, *Eur. J. Clin. Pharmacol., 21*, 45 (1981).
[52] S. M. Somani and N. N. Khanna, in *Drug Metabolism in the Immature Human* (L. F. Soyka and G. P. Redmond, eds.), Raven Press, New York, 1981, pp. 199-215.
[53] R. Latini, M. Bonati, D. Castelli, and S. Garattini, *Toxicol. Lett., 2*, 267 (1978).

[54] A. Aldridge, W. D. Parsons, and A. H. Neims, *Life Sci.*, *21*, 967 (1977).
[55] M. Bonati, R. Latini, F. Galletti, J. F. Young, G. Tognoni, and S. Garattini, *Clin. Pharmacol. Ther.*, *32*, 98 (1982).
[56] S. Garattini, M. Bonati, and R. Latini, in *Proceedings 1st International Caffeine Committee Workshop*, November 8-10, 1978, Keauhou-Kona, Hawaii, 1979.
[57] A. H. Neims, *Nutr. Rev.*, *38*, 197 (1980).
[58] R. Latini, M. Bonati, E. Marzi, and S. Garattini, *Toxicol. Lett.*, *7*, 267 (1981).
[59] A. W. Burg, *Drug Metab. Rev.*, *4*, 199 (1975).
[60] M. J. Arnaud, *Biochem. Med.*, *16*, 67 (1976).
[61] G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, *18*, 377 (1975).
[62] T. E. Chvasta and A. R. Cooke, *Gastroenterology*, *61*, 838 (1971).
[63] J. Blanchard and S. J. A. Sawers, *Eur. J. Clin. Pharmacol.*, *24*, 93 (1983).
[64] J. Blanchard and S. J. A. Sawers, *J. Pharmacokinet. Biopharm.*, *11*, 109 (1983).
[65] G. Sant'Ambrogio, P. Mognoni, and L. Ventrella, *Arch. Int. Pharmacodyn.*, *150*, 259 (1964).
[66] S. Garattini, M. Bonati, and R. Latini, in *Proceedings 2nd International Caffeine Committee Workshop*, October 22-24, 1979, Munich, West Germany, 1980.
[67] S. Garattini, M. Bonati, R. Latini, and F. Galletti, in *Proceedings of the 3rd International Caffeine Committee Workshop*, October 27-28, 1980, Hunt Valley, MD, 1980.
[68] S. Garattini, M. Bonati, and R. Latini, in *Proceedings 4th International Caffeine Committee Workshop*, October 17-21, 1982, Athens, Greece, 1982.
[69] M. J. Arnaud and C. Welsch, in *ASIC 9e Colloque*, London, 1980, pp. 385-396.
[70] A. H. Neims and R. W. von Borstel, in *Nutrition and the Brain* (R. J. Wurtman and J. J. Wurtman, eds.), vol. 6, Raven Press, New York, 1983, pp. 1-30.
[71] M. P. Lachance, *J. Food Safety*, *4*, 71 (1982).
[72] B. L. Oser and R. A. Ford, *Drug Chem. Toxicol.*, *4*, 311 (1981).
[73] A. Aldridge and A. H. Neims, *Drug Metab. Dispos.*, *7*, 378 (1979).
[74] C. Mitoma, L. Lombrozo, S. E. LeValley, and F. Dehn, *Arch. Biochem. Biophys.*, *134*, 434 (1969).
[75] K. Szczawinska, E. Ginelli, I. Bartosek, C. Gambazza, and C. Pantarotto, *Chem.-Biol. Interact.*, *34*, 345 (1981).

[76] A. Guaitani, R. Abbruzzi, M. Bianchi, M. Bonati, P. Catalani, R. Latini, C. Pantarotto, and K. Szczawinska, *Biochem. Pharmacol.*, *21*, 53 (1984).
[77] M. Bonati, A. Celardo, F. Galletti, R. Latini, F. Tursi, and G. Belvedere, *Toxicol.. Lett.*, in press.
[78] J. R. Mitchell and D. Jollows, *Gastroenterology*, *68*, 392 (1975).
[79] S. Garattini, E. Erba, L. Morasca, G. Peri, A. Mantovani, S. Filippeschi, F. Spreafico, and M. Arnaud, *Toxicol. Lett.*, *10*, 313 (1982).
[80] G. Peri, N. Polentarutti, S. Filippeschi, C. Malfiore, G. Amato, S. Pepe, M. Bonati, S. Garattini, and A. Mantovani, *Toxicol. Lett.*, *18*, 133 (1983).
[81] R. Latini, M. Bonati, E. Marzi, M. T. Tacconi, B. Sadurska, and A. Bizzi, *J. Pharm. Pharmacol.*, *32*, 596 (1980).
[82] M. J. Arnaud and C. Welsch, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis* (M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, eds.), Academic Press, New York, 1980, pp. 813-816.
[83] D. Warszawski, B. Ben-Zvi, and R. Gorodischer, *Biochem. Pharmacol.*, *30*, 3145 (1981).
[84] G. Belvedere, F. Tursi, L. Jiritano, F. Galletti, and M. Bonati, in *Cytochrome P-450: Biochemistry, Biophysics, and Environmental Implications* (E. Hietanen, M. Laitinen, and O. Hanninen, eds.), Elsevier, Amsterdam, 1982, pp. 689-694.
[85] M. Rowland and T. N. Tozer, *Clinical Pharmacokinetics: Concepts and Applications*, Lea and Febiger, Philadelphia, 1980.
[86] M. Gibaldi and D. Perrier, *Drug and Pharmaceutical Sciences*, vol. 1: *Pharmacokinetics*, Dekker, New York, 1975.
[87] M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, *1*, 123 (1973).
[88] J. R. Gillette, in *Handbook of Experimental Pharmacology* (O. Eichler, A. Farah, H. Herken, and A. D. Welch, eds.), vol. 28/3, Springer-Verlag, Berlin, 1975, pp. 33-85.
[89] S. K. Pang and M. Rowland, *J. Pharmacokinet. Biopharm.*, *5*, 625 (1977).
[90] S. K. Pang and M. Rowland, *J. Pharmacokinet. Biopharm.*, *5*, 655 (1977).
[91] S. K. Pang and M. Rowland, *J. Pharmacokinet. Biopharm.*, *5*, 681 (1977).
[92] R. N. Warren, *J. Chromatogr.*, *40*, 468 (1969).

[93] R. Latini, M. Bonati, F. Gaspari, G. L. Traina, L. Jiritano, A. Bortolotti, J. E. Borzelleca, S. Tarka, M. J. Arnaud, and S. Garattini, *Toxicology, 30*, 343 (1984).
[94] M. Bonati, R. Latini, B. Sadurska, E. Riva, F. Galletti, J. E. Borzelleca, S. Tarka, M. J. Arnaud, and S. Garattini, *Toxicology, 30*, 327 (1984).
[95] M. Bonati, D. Castelli, R. Latini, and S. Garattini, *J. Chromatogr., 164*, 109 (1979).
[96] G. Sacchi-Landriani, V. Guardabasso, and M. Rocchetti, *Comput. Programs Biomed., 16*, 35 (1983).
[97] G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th ed., Iowa State University Press, Ames, Iowa, 1967.
[98] M. Rocchetti and M. Recchia, *Comput. Programs Biomed., 14*, 7 (1982).
[99] J. G. Wagner, *J. Pharmacokinet. Biopharm., 1*, 103 (1973).
[100] N. P. Chau, *J. Pharmacokinet. Biopharm., 4*, 537 (1976).
[101] B. Günther, in *Biogenesis Evolution Homeostasis* (A. Locker, ed.), Springer-Verlag, Heidelberg, 1973, pp. 127-133.
[102] C. P. Bianchi, *J. Pharmacol. Exp. Ther., 138*, 41 (1962).
[103] A. L. McCall, W. R. Millington, and R. J. Wurtman, *Life Sci., 31*, 2709 (1982).
[104] A. Carrel, *Science, 74*, 618 (1931).
[105] S. Brody, *Growth, 1*, 60 (1937).
[106] K. Schmidt-Nielsen, *J. Exp. Zool., 194*, 287 (1975).
[107] W. A. Calder III, *Annu. Rev. Physiol., 43*, 301 (1981).
[108] S. L. Lindstedt and W. A. Calder III, *Q. Rev. Biol., 56*, 1 (1981).
[109] E. Armstrong, *Science, 220*, 1302 (1983).
[110] G. A. Sacher, in *CIBA Foundation Colloquia on Ageing* (G. E. W. Wolstenholme and M. O'Connor, eds.), Churchill, London, 1959, pp. 115-141.
[111] A. Aldridge, J. V. Aranda, and A. H. Neims, *Clin. Pharmacol. Ther., 25*, 447 (1979).
[112] D. Dan-Shya Tang-Liu, R. L. Williams, and S. Riegelman, *J. Pharmacol. Exp. Ther., 224*, 180 (1983).
[113] D. M. Grant, B. K. Tang, and W. Kalow, *Clin. Pharmacol. Ther., 33*, 591 (1983).
[114] M. J. Arnaud, *Experientia, 32*, 1238 (1976).
[115] A. Burg and M. Stein, *Biochem. Pharmacol., 21*, 909 (1972).
[116] M. M. Callahan, R. S. Robertson, M. J. Arnaud, A. R. Branfman, M. F. McComish, and D. W. Yesair, *Drug Metab. Dispos., 10*, 417 (1982).
[117] D. M. Grant, B. K. Tang, and W. Kalow, *Clin. Pharmacol. Ther., 33*, 355 (1983).

[118] B. K. Tang, D. M. Grant, and W. Kalow, *Drug Metab. Dispos.*, *11*, 218 (1983).
[119] A. S. Tu, R. S. Robertson, M. M. Callahan, and P. S. Thayer, *Mutat. Res.*, *95*, 475 (1982).
[120] J. Caldwell, J. O'Gorman, and R. H. Adamson, *Pharmacologist*, *23*, 212 (1981).
[121] M. Bonati, A. Bortolotti, L. Jiritano, R. Latini, and S. Garattini, *Drug Metab. Dispos.*, *11*, 509 (1983).
[122] M. J. Arnaud, I. Bracco, J.-L. Sauvageat, and M.-F. Clerc, *Toxicol. Lett.*, *16*, 271 (1983).
[123] D. Warszawski, Z. Ben-Zvi, G. Gorodiescher, M. J. Arnaud, and I. Bracco, *Drug Metab. Dispos.*, *10*, 424 (1982).
[124] J. V. Aranda and T. Turmen, *Clin. Perinatol.*, *6*, 87 (1979).
[125] B. M. Assael, M. Bonati, R. Latini, G. Marra, and R. Parini, in *Drug Metabolism in the Immature Human* (L. F. Soyka and G. P. Redmond, eds.), Raven Press, New York, 1981, pp. 249-263.
[126] C. M. McCay, F. Lovelace, G. Sperling, L. L. Barnes, C. H. Liu, C. A. H. Smith, and J. A. Saxton, Jr., *J. Gerontol.*, *7*, 161 (1952).
[127] J. M. Trang, J. Blanchard, K. A. Conrad, and G. G. Harrison, *Am. J. Clin. Nutr.*, *35*, 487 (1982).
[128] W. D. Parsons and J. G. Pelletier, *Can. Med. Assoc. J.*, *127*, 377 (1982).
[129] R. V. Patwardhan, P. V. Desmond, R. F. Johnson, and S. Schenker, *J. Lab. Clin. Med.*, *95*, 603 (1980).
[130] K. J. Acheson, B. Zahorska-Markiewicz, P. Pittet, K. Anantharaman, and E. Jéquier, *Am. J. Clin. Nutr.*, *33*, 989 (1980).
[131] B. E. Statland and T. J. Demas, *Am. J. Clin. Pathol.*, *73*, 390 (1980).
[132] A. N. Kotake, D. A. Schoeller, G. H. Lambert, A. L. Baker, D. D. Schaffer, and H. Josephs, *Clin. Pharmacol. Ther.*, *32*, 261 (1982).
[133] D. Warszawski, R. Gorodischer, S. W. Moses, and H. Bark, *Biol. Neonate*, *32*, 138 (1977).
[134] H. D. Christensen, C. V. Manion, and O. R. Kling, in *Drug Metabolism in the Immature Human* (L. F. Soyka and G. P. Redmond, eds.), Raven Press, New York, 1981, pp. 163-181.
[135] S. J. Wilson, J. Ayromlooi, and J. K. Errick, *Obstet. Gynecol.*, *61*, 486 (1983).

[136] W. S. Spector, ed., *Handbook of Biological Data*, Saunders, Philadelphia, 1956.
[137] C. Jelenko III, A. P. Anderson, and T. H. Scott Jr., *Am. J. Vet. Res.*, *32*, 1637 (1971).