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Species Differences in Pharmacokinetics and Drug Teratogenesis

by Heinz Nau*

Interspecies differences in regard to the teratogenicity of drugs can be the result of differing pharmacokinetic processes that determine the crucial concentration-time relationships in the embryo. Maternal absorption, as well as distribution, of the drugs does not usually show great species differences. The first-pass effect after oral application is often more pronounced in animals than man (e.g., valproic acid, 13-*cis*-retinoic acid), although in some cases the reverse was found (e.g., hydrolysis of valpromide). Existing differences can be adjusted by appropriate choice of the administration route and measurements of drug levels. Many variables determine the placental transfer of drugs: developmental stage, type of placenta, properties of the drug. Even closely related drugs (e.g., retinoids) may differ greatly in regard to placental transfer. Maternal protein binding is an important determinant of placental transfer, since only the free concentration in maternal plasma can equilibrate with the embryo during organogenesis; this parameter differs greatly across species (e.g., valproic acid: five times higher free fractions in mouse and hamster than in monkey and man). The metabolic pattern has not yet been demonstrated to be a major cause of species differences, although recent evidence on phenytoin and thalidomide support the hypothesis that some species differences can be the result of differing activation/deactivation pathways. Laboratory animals usually have a much higher rate of drug elimination than man. Drastic drug level fluctuations are therefore present during teratogenicity testing in animals, but not to the same degree in human therapy. It must, therefore, be investigated if peak concentrations (such as for valproic acid and possibly caffeine) or the area under the concentration-time curve (AUC) (such as for cyclophosphamide and possibly retinoids) correlate with the teratogenic response. Only then is a rational and scientific basis for interspecies comparison possible. It is concluded that the prediction of the human response based on animal studies can be improved by consideration of the appropriate pharmacokinetic determinants.

Introduction

The extrapolation of teratogenicity studies of drugs or chemicals from one animal species to another, in particular from experimental animals to man, remains to be very problematic (*1–3*). Great species differences, largely unexplained up to now, are the rule rather than the exception. Established human teratogens such as thalidomide, trimethadione, lithium, and warfarin have induced a poor teratogenic response in rats. On the other hand, nonhuman primates, which closely parallel the human response with regard to the teratogenicity of thalidomide, norethindrone, testosterone, diethylstilbestrol and methylmercury (*1*), fail to respond to methotrexate, a potent human teratogen (*4*). While all human teratogens (except the coumarin anticoagulants) were shown to be teratogenic in at least one animal species, it is difficult to state at this time which animal species is to be preferred in risk assessment studies. It appears, however, that the rabbit and, particularly, the monkey offer greater predictability of the human situation than other species (*1,5*). The largest number of compounds tested was shown to be teratogenic in the mouse and hamster, on the other hand, which may indicate numerous "false positive" results (*1,2*).

Both the type of malformation produced as well as the teratogenic dose levels used may show drastic species differences. Valproic acid was shown to produce neural tube defects in man (spina bifida), mouse, and hamster (exencephaly), but not in the monkey, rabbit, and rat (Table 1). The doses resulting in human teratogenicity were shown to be in the upper therapeutic range (*6*), which was still five to ten times lower than teratogenic doses in experimental animals (Table 1).

Table 1. Teratogenic doses of valproic acid in several species.

Species	Teratogenic dose, mg/kg/day[a]		Route	References
	Neural tube defects	Skeletal defects		
Man	30	20–30	Oral	(*6–8*)
Monkey	Not observed	150	Oral	(*9,10*)
Rabbit	Not observed	150	Oral	(*11*)
Rat	Not observed	150	Oral	(*12*)
Hamster	300	Not investigated	IP	(*13*)
Mouse	200	200	IP	(*15*)
		250	SC	(*16*)
		400	Oral	(*17,18*)

[a]The lowest daily dose with teratogenic outcome is listed.

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Table 2. Teratogenic doses of retinoids in several species.[a]

Species	Lowest teratogenic dose, mg/kg/day			
	All-*trans*-retinoic acid	13-*cis*-Retinoic acid	Etretinate	Etretin
Man	?	0.5–1.5	0.5–1	?
Monkey	7.5–40	?	?	?
Rabbit	6	10	2	0.6
Hamster	7	12	3	?
Rat	6	150	2	15
Mouse	3	100	4	3

[a]Data from refs. *19–31*.

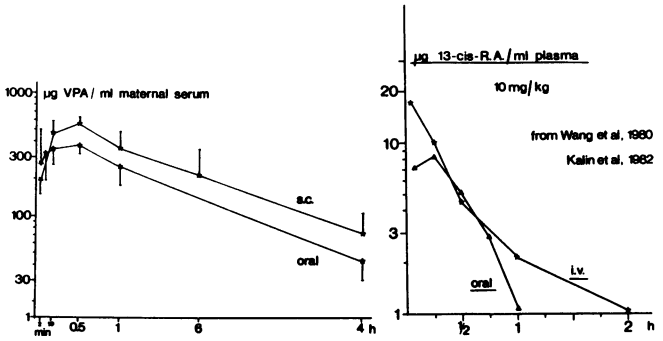

FIGURE 1. Concentration-time curves of valproic acid after oral and SC administration in pregnant mice (left) (*17,35*) and 13-*cis*-retinoic acid after oral and IV administration in nonpregnant mice (right); the data for the construction of the right panel were taken from Wang et al. (*37*) and Kalin et al. (*36*). Note the lowered bioavailability of both drugs after oral administration.

Retinoids produce a rather characteristic and uniform pattern of malformations, particularly involving the central nervous system, cardiac and facial structures in essentially all species investigated. Yet the teratogenic dose for isotretinoin (13-*cis*-retinoic acid) in mouse or rat differed from that in man by a factor of 100 (Table 2) (*20,21*). On the other hand, interspecies variation of teratogenic doses of other retinoids was much less pronounced (Table 2).

There appear to be two major reasons for the observed species differences in response to teratogens: (a) intrinsic sensitivities of the developing tissues, e.g., extreme rarity of neural tube defects in nonhuman primates, but prominence in most other species including man; (b) differences in exposure of the embryo during the sensitive stages of gestation.

This review will be mainly concerned with aspect (b), although pharmacokinetic studies are just as important in regard to aspect (a): intrinsic sensitivities of the embryos can only be evaluated at comparable exposure levels.

The main hypothesis to be evaluated can be stated as follows: Differences between species in regard to the teratogenic response to drugs or other chemicals may be the result of differing processes that determine the crucial concentration-time relationships in the embryo: maternal absorption, distribution, plasma and tissue binding, elimination via metabolism or excretion (biliary, renal...); placental transfer; embryo distribution and tissue binding, metabolism.

Thus, a number of pharmacokinetic parameters are important determinants of the drug exposure of the embryo. Since all of these factors may show considerable interspecies variations, it is not surprising that the resulting drug concentrations in the embryo—and thus the teratogenic response—may vary greatly from one species to another. It will be demonstrated, however, that species differences are particularly pronounced in regard to drug elimination rates and in regard to maternal plasma protein binding and placental transfer. The organogenesis period will be considered wherever possible, since this is the most relevant gestational period in regard to teratogenesis.

Maternal Absorption, Administration Route, First-Pass Effect

The rate as well as extent of absorption can vary depending on the species examined, the route of administration, and the drug formulation used. Since the main site for drug absorption is often the small intestine, the stomach emptying time may become important: the half-life times for emptying are in order of 10 min in mice and rats, 30 min in rabbits, 1–2 hr in dogs and man (*33*). Thus, absorption may often be more rapid in rodents as compared to man. Indeed, following oral administration of a solution of sodium valproate, maximal plasma concentrations of valproate were observed between 10 min and 30 min in the mouse (Fig. 1, left panel), but between 1 and 3 hr in man (*34*). Administration of stomach acid-resistant capsules in man resulted in a further delay of absorption of up to several hours.

The blood, which perfuses the site of oral absorption (stomach, intestinal tract) must first pass the liver prior to entering the systemic circulation; therefore, a significant portion of the absorbed drug can already be eliminated via hepatic metabolism during this initial liver passage (first-pass effect or presystemic clearance). Since the rate of hepatic metabolism in experimental animals often greatly exceeds that in man (see below), the first-pass effect may significantly reduce the fraction of the dose administered to experimental animals. Two examples are given in Figure 1. Oral administration of valproic acid in mice resulted in about 50% lower drug levels than subcutaneous application (Fig. 1, left panel); the oral bioavailability in man is essentially 100%. The area under the concentration-time curve (the AUC value is a good parameter of the amount of drug reaching the systemic circulation) of orally administered isotretinoin (13-*cis*-retinoic acid) was found to be only about one-fifth of the AUC value after IV application (Fig. 1, right panel). A much lower difference was found between the AUC (oral) and AUC (IV) value for tretinoin (all-*trans*-retinoic acid) (*37,38*). Thus, only 20% of the orally administered dose of isotretinoin is available to the general circulation of the mouse, which may in part explain the much lower teratogenic potency of isotretinoin as compared to tretinoin in that species (Table 2); another, probably even more impor-

tant reason was recently found to be the surprisingly low placental transfer of isotretinoin in the mouse (*21*).

In some cases, however, the human may exhibit a more pronounced first pass effect than experimental animals. Valpromide is rapidly hydrolyzed to valproic acid after oral administration in man, probably already in the stomach and intestinal mucosa as well as in the liver during absorption (*39*). Due to this very extensive first-pass effect, minimal amounts of unchanged drug reached the central circulation and valpromide can be regarded as a prodrug of valproic acid in man. On the other hand, valpromide is a major drug component in the plasma of mice (*40*) and dogs (*41*). Teratological studies with valpromide in experimental animals have therefore little relevance to the therapeutic situation.

In general, differences in the extent and rate of absorption are not that drastic, because diffusion of drugs across epithelial membranes is similar in most species. In any event, such differences can be adjusted if not only the doses, but the concentrations reached in the organism, are considered. Because of the lower plasma concentrations obtained after oral dosing of valproic acid (Fig. 1), larger doses must be administered with this route to obtain a teratogenic response comparable to that obtained with SC dosing (*17*). If drug concentrations rather than drug doses are related to the teratogenic response, a completely different picture emerges: comparable drug concentration, regardless if reached by oral or subcutaneous application, results in comparable teratogenicity. Further, it will be interesting to evaluate if tretinoin and isotretinoin elicit a comparable teratogenic response at comparable embryo exposure; experiments performed *in vitro*, when both substances are indeed of comparable toxicity, point into this direction (*21,30*).

An increasing body of evidence suggests that intraperitoneal administration (IP) of drugs can result in significantly higher embryo/fetus concentrations than other administration routes. This was shown to be the case for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*42*), alkylating agents (*43*), piperacillin (*131*), ethanol (*132*), and methadon (*133*) experiments with exterionized uterine horns of the rat suspended in drug solutions suggest a direct transuterine passage of drugs (*133*). Since an unknown portion of the dose injected IP may also pass the liver prior to entering the systemic circulation, the possible variability of the resulting first pass effect (if applicable for a particular drug) adds to the notion that IP administration is a particularly unsuitable method of drug administration from the pharmacokinetic viewpoint.

Distribution Volume of Drugs

After the drugs are absorbed and maximal concentrations are reached in the plasma (C_{max}), one, two, or more elimination phases follow (Fig. 2). If very rapid equilibrium is reached within the entire organism, then only one concentration decay phase may be observed (Fig. 1, valproic acid and, with reservations, isotretinoin). For such drugs, the distribution volume can be calculated from the dose D, the AUC value and the elimination constant k_e:

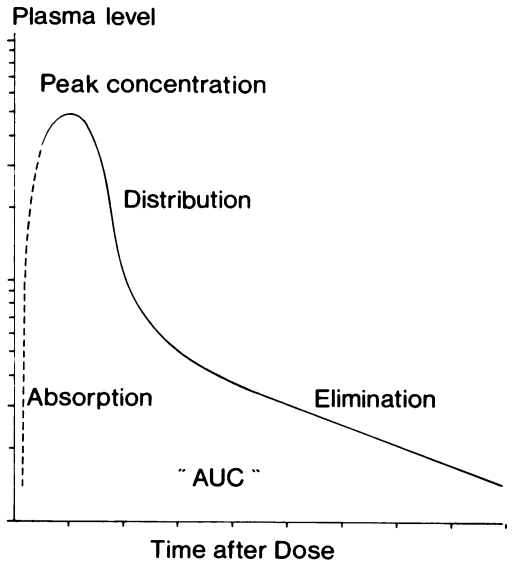

FIGURE 2. Simulated plasma concentration-time curve indicating absorption, distribution and elimination phases. Note the two phases of plasma concentration-decay: the first phase (α) corresponds to elimination of the drug plus its additional transport from plasma into tissues; the second phase (β) can be related to elimination only.

$$V_d = \frac{D}{[(AUC)k_e]}$$

In most cases, the plasma level decay curve follows two phases, the distribution phase α (where the drug is eliminated and is transferred into tissues) and the elimination phase β (pure elimination; diffusion equilibrium between plasma and tissues). The distribution volume, $V_{d,\beta}$, during the elimination phase is calculated from the dose D, the AUC value, and the elimination constant β:

$$V_{d,\beta} = \frac{D}{[(AUC)\beta]}$$

Some drugs may be stored in a "deep" or "very deep" compartment in the body which may be represented in the plasma decay curve as one or two further "bends" (the γ or δ-phases). Corresponding plasma levels are usually very low and difficult to detect during these phases after single drug administration. These stores are slowly filled up during multiple drug therapy because diffusion from plasma into the storage sites is very slow. However, once these storage sites have been loaded with drugs (it may take several weeks of therapy in the human), these sites represent a large drug reservoir, which can be slowly released following discontinuation of therapy. A good example for such pharmacokinetics is etretinate, which was found to be efficiently stored in human fat (*44*). Even several

months after discontinuation of therapy, plasma levels of etretinate and its metabolite etretin could be detected (*45*). In one case, conception three months after the last intake of etretinate may have resulted in a malformation with plasma drug levels measurable at about 5 ng/mL (*32*).

The drug distribution volumes, adjusted for body weight, generally do not appear to differ profoundly between species. Examples in Figure 3 demonstrate that the distribution volumes of trimethadione and valproic acid are rather similar across species. Data on a number of other drugs also indicate that differences between the drug distribution volumes in various species are limited usually below a factor of 2–3; larger differences are rare.

Since many drugs exhibit interspecies similarities in regard to absorption and distribution processes, the maximal concentrations (C_{max}) reached—particularly of those drugs which are rapidly absorbed—are also not expected to greatly vary across species (unless there are differences in first pass effect; cf. example of valproic acid and isotretinoin, Fig. 1) if comparable doses are administered, because

$$C_{max} = \frac{D}{V_d}$$

(for drugs with a single plasma decay curve).

This is indeed demonstrated by the two antiepileptic drugs trimethadione and valproic acid (Fig. 4). It should be noted, however, that the peak drug levels will be strongly dependent on the rate, and not only the extent of absorption. Thus, special drug formulations such as "slow release" formulations will result in broad and lowered concentration-time peaks. The rate of absorption is therefore of great significance when the teratogenicity of a drug is dependent on a particular threshold concentration reached (e.g., as peak levels). On the other hand, the rate of absorption is irrelevant when the teratogenicity of a drug is dependent on the AUC values obtained, regardless of the shape of the concentration-time curves unless there are changes in first pass elimination due to saturation. The significance of peak drug levels vs. AUC values in regard to the teratogenic response will be extensively discussed below.

Placental Transfer of Drugs

The rate of transfer of drugs to the embryo and fetus should clearly be differentiated from the extent of drug transfer, since different factors determine these two pharmacokinetic variables (Table 3). It has often been generalized that drugs with high lipophilicity, low degree of ionization, and molecular weights below 1000 are rapidly transferred across the placenta (*51*). Such generalizations may not always be valid, which is demonstrated just for two cases. Valproic acid is present at physiological pH in the ionized form to 99.9%; in spite of this high degree of ionization, the transfer rate was found to be rapid, and equilibration between maternal plasma and gestational tissue was established within 30 min in the mouse (*17*). The highly lipophilic substance

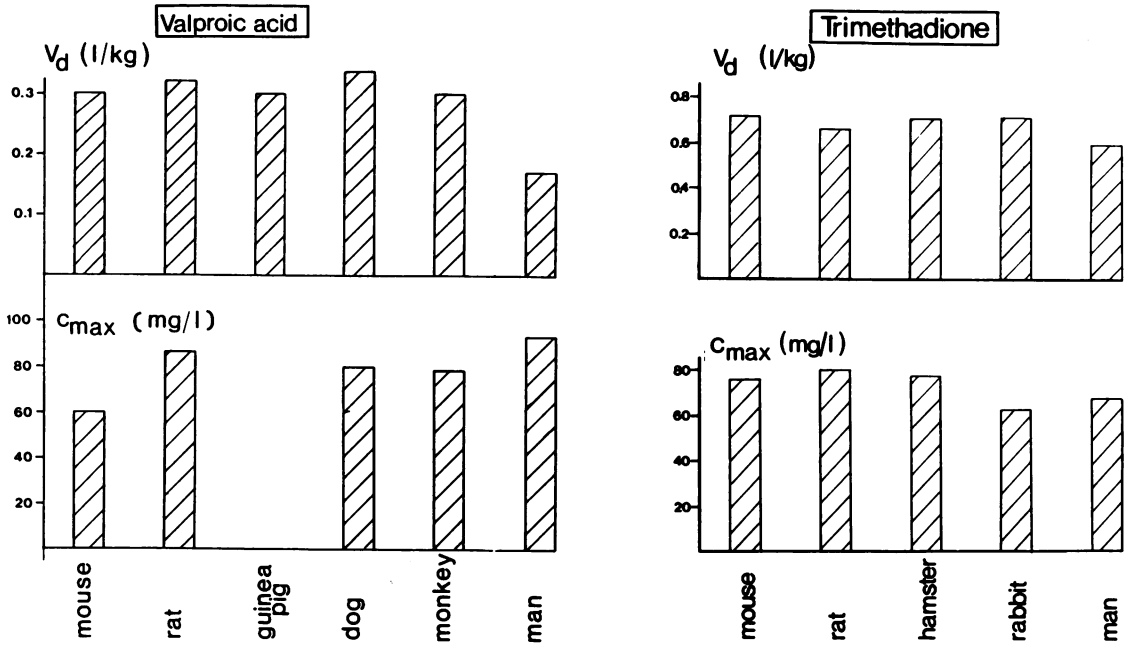

FIGURE 3. Volume of distribution V_d and maximal concentrations C_{max} of (left) valproic acid (*46,47*) and (right) trimethadione (*48*) in various species. An oral dose of 50 mg/kg was used for valproic acid (except in man, 25 mg/kg), and an IV dose of 50 mg/kg for trimethadione (except in man, oral). Note the relatively small interspecies variation of V_d and C_{max}, except for the relatively low V_d of valproic acid in man because of extensive plasma protein binding in this species (Fig. 7). A full listing of pharmacokinetic parameters of these two teratogens is given elsewhere (*46–48*).

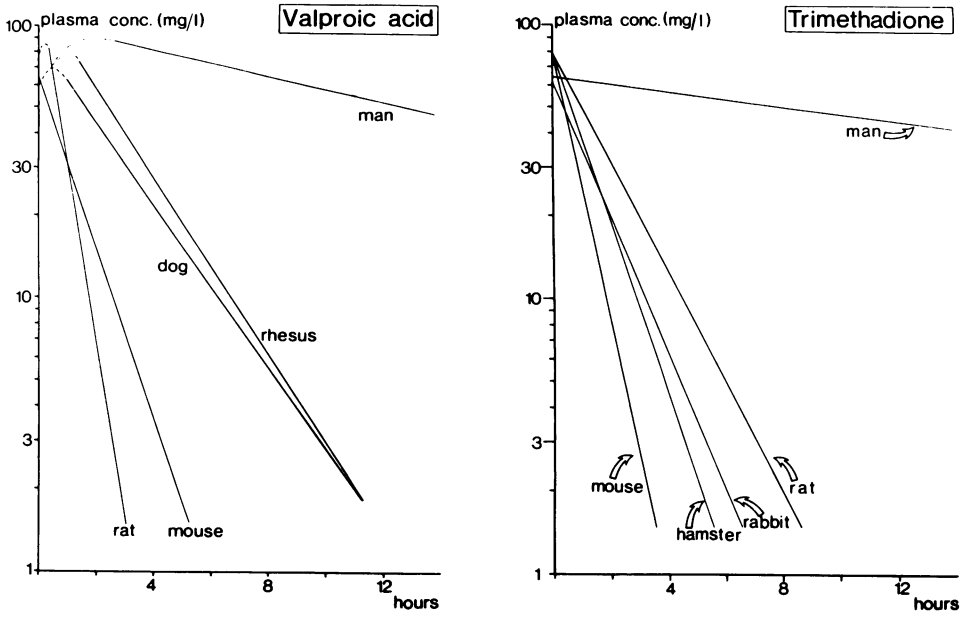

FIGURE 4. Plasma concentration–time curves of (left) valproic acid following an oral dose in various species (50 mg/kg) (*10,14,46,47,49, 50*) and (right) trimethadione following a dose of 50 mg/kg to various species (IV: mouse, hamster, rabbit, rat; oral: man). The right figure was plotted from data reported by Tanaka et al. (*48*). Note the relatively comparable drug peak concentrations in spite of the drastically different half-lives in the various species.

Table 3. Parameters determining the rate and extent of drug transfer to the embryo.[a]

Drug transfer	Parameters
Rate	Lipid solubility of drugs[b]
	Molecular weight of drugs[b]
	Placental blood flow
	Placental structure and function
	Active transport of drugs
Extent	PK_a of the drug
	Maternal/embryonic pH gradient
	Protein binding of drugs
	Active transport of drugs

[a]Adapted from Mirkin and Singh (*51*).
[b]And other physicochemical characteristics determining the diffusion constants of drugs.

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (not ionized) reached very low concentrations in the embryo and fetus of the mouse, presumably because of the low fat content of the conceptus (*42*). Thus, lipophilicity and degree of ionization do not correlate in each case with the rate or extent of placental transfer, respectively.

Active transport mechanisms have not clearly been demonstrated to be of major importance for the rate or the extent of placental drug transfer except in a few cases (*52*). Dencker (*53*) has recently shown that tretinoin (all-*trans*-retinoic acid) may have specific binding sites in the embryo, particularly in the neural tube area. It has also been speculated that this compound is actively transported across the mouse placenta via specific binding proteins (*21*). Further experimental evidence for the specific binding must be obtained in future studies.

The pH of the embryo or fetal blood can be important in regard to the extent of transfer of acidic and basic drugs. The pH of fetal blood was shown to be lower than maternal blood (*51*); consequently, the concentrations of free drug (unbound to proteins) of acidic drugs such as valproic acid (*54*) will be lower in fetal than in maternal blood. The reverse is true for basic drugs such as local anesthetic agents (*55*), where free drug concentrations in fetal blood can exceed maternal values, particularly in fetal acidosis.

Since the pH of the early mammalian embryo is higher than maternal plasma, acidic drugs can accumulate in these embryos during organogenesis which may be of significance in regard to the teratogenicity of this class of compounds (*40,56*). Examples of teratogens accumulating in the early mammalian embryo include valproic acid and dimethadione in the early mouse and rat embryo (*40*), salicylic acid (*57*), and methoxyacetic acid in the rat embryo (*40,76*), thalidomide (a glutamic acid metabolite) in the rabbit blastocyst (*58*), and a halothane metabolite (probably trichloroacetic acid) in the mouse embryo (*59*).

A study of 18 metabolites and analogs of valproic acid showed that the teratogenicity is a very specific property of this class of weak acids (*60*). Only the 4-en metabolite (2-propyl-4-pentenoic acid) exhibits teratogenicity comparable to the parent drug. A shift of the double bond to the 2-position (2-en-valproic acid; 2-propyl-2-pentenoic acid) as well as further branching of the 2-position (the α-carbon is now quartenary) essentially abolishes teratogenicity (*60*) (Fig. 5). Since latter compounds exhibit anticonvulsant activity (*61*), the possibility of developing a structural analog to valproic acid with good anticonvulsant activity, but low teratogen-

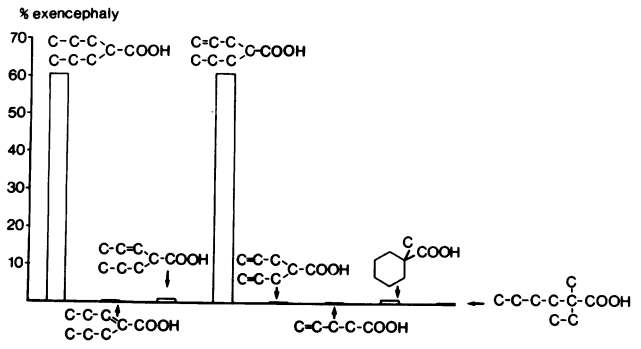

FIGURE 5. Incidence of exencephaly (% of live fetuses) of various metabolites of valproic acid and related carboxylic acids. Only the 4-en-metabolite is of teratogenicity comparable to the parent drug (*60*).

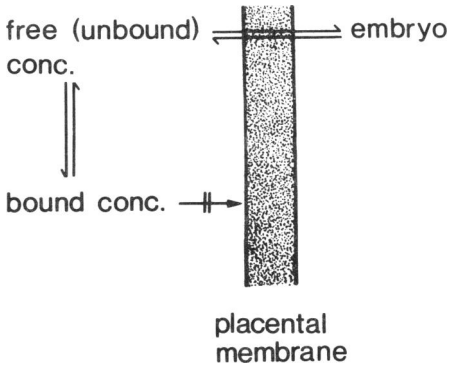

FIGURE 6. Schematic representation of the influence of maternal plasma protein binding on the embryonic drug exposure. Only the free plasma concentration is expected to equilibrate with embryonic tissue.

icity (*62*) exists. Furthermore, these substances should provide valuable tools for a study of the exact nature of the structural requirements for teratogenic activity.

Exposure of the embryo will also be dependent on the route of drug administration. Administration IP often results in higher concentrations in the embryo and more extensive teratogenic effects as compared to other application regimens (see above).

Although morphological studies (*134*) have defined differences between several mammalian placentas and their comparative development, these results did not provide information in regard to the possible differences in the function of these placentas. Thus, essentially we do not know how and if differences between the placental structures influence the transfer of drugs to the embryo in the various species. Comparative studies on the placental transfer of drugs in different species at comparable gestational stages are available in few cases only.

Digoxin readily crossed the rodent and human placenta, however, the ovine placenta was surprisingly impermeable to this drug (*51*). Gentamycin was efficiently transferred by the human, but not the goat placenta (*63*). These comparative studies were performed during the fetal gestational stages.

It becomes increasingly clear, however, that even substances which are closely related from a structural viewpoint can differ greatly in regard to placental transfer and teratogenicity. This was recently demonstrated in the case of 13-*cis*- and all-*trans*-retinoic acids, which show drastic differences in regard to placental transfer in the mouse (*21*) that may explain the greatly differing teratogenic potencies of these two retinoids. Also the teratogenicity of benzodiazepins in the rat was shown to be related to the relative extent of placental transfer: nitrazepam showed much higher teratogenicity as well as placental transfer as compared to diazepam (*64*). It was also reported that the relative teratogenic potency of synthetic and naturally ocurring steroids in the rat was related to the relative exposure of the embryo to these two compounds (*65*).

There are also some examples which demonstrate that the intrinsic sensitivities of the embryo can greatly vary among different species. The monkey was shown to be less sensitive to methotrexate (*135*) and hydroxyurea (*136*) than the rat: relatively small maternal doses of these drugs produced a teratogenic effect in the rat in spite of low embryonic concentrations, while even high embryonic levels in the monkey embryo elicited only a limited teratogenic effect.

Maternal Plasma Protein Binding and Placental Transfer

It is an attractive, but still unproven hypothesis that the extent of protein binding will be an important determinant of placental drug transfer and teratogenesis, since only the free drug (unbound to macromolecules) is expected to equilibrate with the embryo (Fig. 6).

Binding of drugs to macromolecules in blood or plasma (commonly termed plasma protein binding) or tissues is a major factor in drug distribution. If drug tissue binding exceeds plasma proteins binding (often observed with basic drugs), then large distribution volumes (adjusted for body weight) result ($V_d > 1$ L/kg). If the reverse is true (as with many acidic drugs), small distribution volumes are obtained ($V_d < 0.4$ L/kg). If a drug (such as ethosuximide, trimethadione, or dimethadione) is not bound to either plasma or tissue macromolecules, or if plasma and tissue binding are similar (phenytoin, diazepam), then a drug distributes rather uniformly throughout the organism ($V_d \sim 0.5$–1 L/kg).

It has been noted that tissue binding may be rather similar across species, while plasma protein binding can vary greatly and unpredictably. The plasma protein binding of drugs is often lower in experimental animals than in man which is demonstrated in the case of valproic acid in Figure 7. This may result in somewhat larger distribution volumes of drugs in animals as compared to man (Fig. 3, left). Exceptions to this rule, however, do exist (*33*): the protein binding of indomethacin in the rat is 99%, in man 97%. This extensive protein binding may partly explain the relatively long

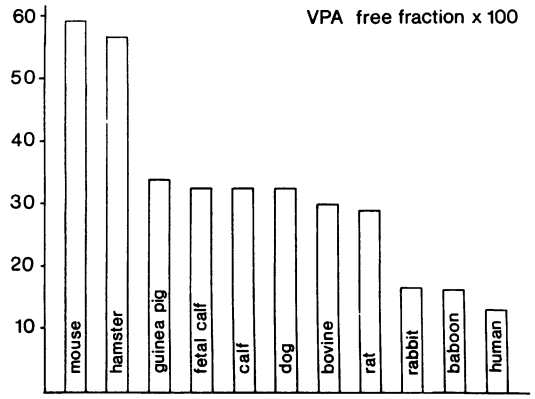

FIGURE 7. Protein binding (expressed as free fractions × 100) of valproic acid in serum (reconstituted from lyophilized material commercially available) of various species. Added total concentration was 100 μg/mL. Note the much higher free fractions in mouse and hamster serum as compared to primate serum. From Nau (*47*).

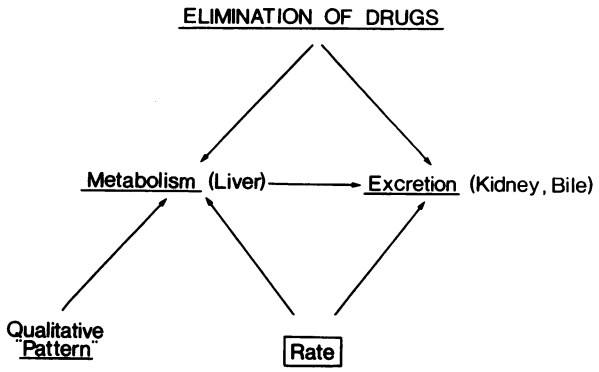

FIGURE 8. Schematic representation of drug elimination; the qualitative patterns and quantitative rates are differentiated.

half-life of this drug in the rat (8–22 hr) which even exceeds that in man (4 hr).

Several lines of recent evidence support the hypothesis that plasma protein binding may be a major and important factor determining the extent of placental transfer and possibly also the teratogenicity of highly protein-bound drugs. The transfer of drugs such as diazepam, valproic acid, salicylic acid and several local anesthetic agents to the human fetus was shown to be determined by the gradient between maternal and fetal plasma protein binding (Table 4). The total concentrations of valproic acid, diazepam, and salicylic acid were higher in fetal blood than in maternal blood (Table 4), because of more extensive fetal plasma protein binding; maternal plasma protein binding of these drugs was diminished because of increased free fatty acid levels acting as displacers. The situation was reversed for local anesthetic agents such as lidocaine and bupivacaine (low fetal concentrations because of low fetal binding) (Table 4). The free (unbound and possibly active) concentrations of all these compounds are expectedly similar on both sides of the placenta. Chlorthalidone reaches very low levels in fetal blood, probably because of low concentrations of carbonic anhydrase in fetal erythrocytes. The relatively low levels of dicloxacillin, erythromycin, and cimetidine have not been explained (Table 4). Because of more extensive protein binding of valproic acid in the rat as compared to the mouse, the exposure of the rat embryo with this drug was found to be lower than of the mouse embryo (*77*). These results may help to explain the increased sensitivity of the mouse in regard to valproic acid teratogenesis. Interestingly, neural tube defects were produced in the two species (mouse, hamster) with the lowest maternal protein binding (highest free concentration available for placental transfer). Also the results of *in vitro* experiments with valproic acid and its more highly bound active metabolites suggest that the free concentrations (in this case in the culture medium) and not the total concentrations are relevant determinants of the concentrations in the embryo as well as the teratogenicity observed (*78*). The comparative teratogenicity of salicylic acid in rat and rhesus monkey could also be related to the extent of maternal protein binding and placental transfer (*137*). This drug was more teratogenic in the rat as compared to the monkey, probably because of lower maternal plasma protein binding and more extensive placental transfer in the rat as compared to the monkey (*137*). Furthermore, administration of bacterial endotoxin was shown to reduce the maternal plasma protein binding of salicylic acid which resulted in increased placental transfer and teratogenicity of this drug (*79*).

While all these results provide circumstantial evidence for the importance of maternal protein binding in teratogenesis, it appears of importance to study factors which can modify maternal protein binding, e.g., coadministration of other drugs, hormonal and nutritional factors, stress, etc. Some of these factors have been shown to decrease maternal plasma protein binding which may result in increased drug exposure of the embryo (*66–70, 80*).

Table 4. Transfer of drugs during fetal development in the human.

Fetal/maternal plasma concentration ratio	Drug	Reference
> 1	Diazepam	(*66,67*)
	Valproic acid	(*54,68*)
	Salicylic acid	(*69,70*)
	Nalidixic acid	(*71*)
0.5–1	Most drugs	
< 1	Dicloxacillin	(*72*)
	Erythromycin	(*73*)
	Chlorthalidone	(*74*)
	Lidocaine, bupivacaine	(*55*)
	Tubocurarine, suxamethonium	(*75*)
	Cimetidine	(*76*)

Drug Elimination

Drugs are eliminated predominantly by metabolism in the liver and excretion via bile and urine (Fig. 8). It

Table 5. Pattern of drug metabolism in rat and monkey as model for man.[a]

Pattern as model for man	Percent of drugs studied: Rat	Other nonprimates (dog, rabbit, guinea pig)	Rhesus monkey
Good	29	32	73
Fair	12	27	19
Poor	20	9	4
Invalid	42	32	4

[a]Data collected for 32 substances by Caldwell et al. (*82*) and references therein.

must therefore be differentiated between the qualitative pattern of metabolites produced predominantly in the liver and the quantitative rates of drug elimination and excretion, predominantly via hepatic metabolism and renal and biliary excretion.

Pattern of Drug Metabolism

Drug metabolism is widely considered to be an important reason for the species differences observed in regard to the toxic response to drugs. The pattern of metabolites produced is often complex and unpredictable, although some generalizations have been made for some metabolic pathways: the rat and the dog are particularly efficient biliary excretors; glucuronic acid conjugation is usually deficient in the cat; the dog is a poor acetylator; and the rat and guinea pig are poor hydroxylators of aliphatic amines and aromatic amides, respectively (*81*,*82*). The old world monkey resembles man most closely in drug metabolic terms. A compilation of the metabolic pattern of 32 compounds showed that the rhesus monkey was a good model for man in 73% of cases and an invalid model in only 4% of cases (Table 5).

On the other hand, the pattern of valproic acid metabolites was rather similar in a variety of species, although also here some quantitative differences occur between the mouse, rat, dog, monkey, and man (*50*). It is therefore highly unlikely that the species differences observed in valproic acid teratogenesis are the result of different metabolic patterns in the species investigated.

Nevertheless, it is tempting to speculate that, if major interspecies differences occur in regard to drug metabolism, such differences may be of significance for the teratogenic response. Indeed, indirect evidence has been obtained for the responsibility of reactive metabolites for the teratogenicity of phenytoin (*83*) and thalidomide (*84*). However, direct proof that differences in metabolic pattern or metabolic activation pathways ("toxification") are responsible for species differences in the teratogenic drug response is not available up to now. In other words, it could not be demonstrated that a particular drug metabolic pathway or the deficiency of a pathway is the reason for the teratogenicity of a drug in one species, and for the lack of response in another. Indirect, although very interesting evidence for the importance of reactive metabolites of thalidomide was presented by Gordon et al. (*84*) using an *in vitro* lymphocyte system. These authors found that microsomes from rabbit, monkey, and man (sensitive species), but not from the rat (insensitive species) can increase the toxicity of thalidomide in this lymphocyte system. Furthermore, the addition of epoxide hydrolase decreased the toxicity of thalidomide. These results indicate that the putative toxic metabolite of thalidomide—produced by the rabbit, monkey and man but not the rat—may be an epoxide (*84*). If these promising results can be extrapolated to the embryo in order to explain the species differences of thalidomide teratogenicity, they must await further studies.

In a recent report, Finnell (*85*) has speculated that the intraspecies variation of phenytoin teratogenicity in the mouse may be the result of differing epoxide hydrolase activities of the various strains. This enzyme may be responsible for the detoxification of the putative epoxide metabolite of phenytoin which could conceivably be the ultimate teratogenic metabolite. It should be mentioned that other studies have implicated the parent drug and not a metabolite or the teratogenic agent (*86*); the phenytoin metabolism to the *p*-hydroxy and dihydrodiol metabolite, both likely to be produced via an epoxide intermediate, did not differ between a susceptible (A/J) and resistant (C57 BL/6J) strain of mice (*86*). Furthermore, the marked intraspecies differences of the mouse with respect to phenytoin teratogenicity could not be explained by Atlas et al. (*87*), by genetic differences in phenytoin elimination rates *in vivo* or by liver microsomal metabolism *in vitro*.

Further evidence for the importance of the epoxide detoxification pathway in phenytoin teratogenesis was obtained by Spielberg's group (*88*) in clinical studies. These authors incubated lymphocytes from patients in the presence of phenytoin which was activated *in vitro* by a microsomal enzyme preparation. Lymphocytes from children, which had been exposed to maternal phenytoin *in utero* and who had birth defects exhibited increased susceptibility to activated phenytoin *in vitro*.

There is a major species difference in regard to the presence of the hepatic cytochrome P-450 enzyme system in the fetus: primate species have considerable amounts of these enzymes, while nonprimate fetuses (and embryos) have none or have them only to a minor degree (*89*,*90*,*108*). Since this enzyme system might be responsible for the toxification or detoxification of a number of drugs, these findings are of considerable potential importance. However, such pathways have not been related to species differences in the teratogenic response. Most importantly, most major malformations are produced during early gestation (less than 8 weeks after conception in man) and studies with embryonic tissues during that period in primate species including man are essentially lacking. Should the embryonic cytochrome P-450 system prove to be responsible for the activation of certain drugs to proximate or ultimate teratogens, then non-human primate species would be the

preferred species for testing such substances, if this enzyme system should indeed be present in the embryo at the relevant early gestational periods (*3,5*).

Studies by Kastner et al. (*94*) showed that the isolated and reconstituted cytochrome P-450 can be successfully incorporated into the *in vitro* limb bud system.

Studies by Juchau and co-workers (*91*) showed that some xenobiotic-metabolizing enzymes can indeed be induced in the early rodent embryo *in vivo*. Subsequent *in vitro* culture experiments in the presence of *N*-hydroxy-2-acetaminofluorene indicated that this compound was more toxic to the preinduced embryos than to control embryos. Also the production of metabolites of this substance by preinduced embryos *in vitro* was demonstrated.

In a recent study, Flint and Brown (*109*) showed the presence of both constitutive and inducible levels of cytochrome P-450 isozymes in cultured rat embryo limb cells by staining with specific antibodies. The implication of these findings in regard to the toxicity of phenytoin, cyclophosphamide and triazoles was discussed by these authors who convincingly showed that rat embryo cells are capable of xenobiotic metabolism during organogenesis.

Numerous studies have indicated that cyclophosphamide must be activated by cytochrome P-450 dependent enzymes to teratogenic metabolites; the reader is referred to a recent comprehensive review (*98*). Labeling with stable isotopes afforded a convenient way to identify the metabolic pathways leading to the proximate or ultimate teratogenic metabolites (*92*).

Another related enzyme system, the cytochrome P_1-450, P-448 or aryl hydrocarbon hydroxylase (AHH), responsible for the activation of polycyclic aromatic hydrocarbons, is much more ubiquitously distributed in various tissues and also was shown to be present in the early embryo (*93*). Studies in mice have shown that the affinities of the Ah-receptor in the embryos can be correlated with the incidence of teratogenicity. Mice having a high affinity Ah receptor (C57 BL/6N) are much more susceptible to AHH induction and teratogenicity after IP administration of benz[a]pyrene than mice having a poor affinity Ah receptor (AKR/J or DBA/2N)(*95*). Interestingly, the teratogenicity of benz[a]pyrene was found to be lower in responsive than in nonresponsive strains of mice following oral application of the toxin. Apparently, extensive first-pass metabolism after oral intubation reduced the amount of the toxin reaching the embryos in the responsive strain, but not the nonresponsive strain of mice (*96*).

Placental AHH may play a protective role for embryonic and fetal development. In a study involving smoking women it was found that placentas from normal infants contained high levels of AHH activity, whereas placentas from abnormal infants had significantly lower AHH activities (*97*). Thus, the AHH-related enzymes may detoxify polycyclic aromatic hydrocarbons derived from cigarette smoke.

Pretreatment with agents inducing or inhibiting drug metabolism have provided some information on the importance of metabolic activation in teratogenesis. Phenobarbital and barbital were shown to increase the teratogenicity of aminopyrine, furylfuramide, and tetrahydrocannabinol possibly by enhancing the metabolic pathway leading to the proximate or ultimate teratogenic agent (Table 6). On the other hand, phenobarbital pretreatment was shown to decrease the teratogenicity of valproic acid, and pretreatment with β-naphthoflavone reduced the teratogenicity of caffeine. Results of pretreatment experiments are often difficult to evaluate because inducing or inhibiting agents cannot only modify the production of a particular metabolite, but also its further biotransformation. In this regard, polychlorinated biphenyl pretreatment was shown to increase the teratogenicity of cyclophosphamide in the mouse, but to decrease the teratogenicity of this drug in the rat (Table 6). Apparently, the balance between toxification and detoxification pathways of cyclophosphamide are differently affected by phenobarbital in these two species. Precise measurements of the parent drug and/or their putative active metabolites in the embryo will be necessary to decide if the parent drug or a particular metabolic pathway can be related to teratogenesis.

The conclusion that valproic acid itself is the teratogenic agent (Table 6) is consistent with the teratogenic activity of this drug *in vitro* (*15,78*) where little metabolic activity can be expected. Furthermore, drug levels were high, but the metabolite concentrations were very low in the embryo (*17*). Also, the drug levels following various administration regimens (oral, subcutaneous, IP, minipump infusion) could be related to the teratologic response (*17*). All these data taken collectively point to the drug as the ultimate or proximate teratogen (*17*).

Rates of Drug Elimination

It becomes increasingly clear that most drugs are eliminated much more rapidly in experimental animals than in man. This is true both for hydrophilic compounds which are predominantly excreted via kidney or bile, and for lipophilic drugs which are extensively metabolized (Table 7).

Small laboratory animals have relatively larger body surface area/body weight ratios than man (Table 8). Because of this relatively large body surface area, increased metabolic functions to maintain body temperature is required in laboratory animals. It has been shown that many biological characteristics such as respiratory rate, long volume, kidney and liver size, heart rate and blood flow rates can be more readily related to the body surface area than to the body weight. An inverse relationship was found between the longevity of a species and the activity of the elimination processes.

Such concepts have proved useful for a comparison of the toxicity of a wide range of anticancer agents which are not metabolized and can be effectively excreted because of the presence of polar groups. It is quite reasonable that the toxicity of anticancer agents (*115*) can be correlated with the doses calculated on a mg/m^2 basis

Table 6. Effect of pretreatment of experimental animals on the teratogenicity and pharmacokinetics of some drugs.

Drug	Species	Pretreatment	Effect on teratogenicity	Possible explanation	Reference
Caffeine	Mouse	β-Naphthoflavone	Decreased	Decreased drug level	(*99*)
Ethanol	Mouse	4-Methylpyrazole	Increased	Increased drug level	(*100,101*)
Valproic acid	Mouse	Phenobarbital[a]	Decreased	Decreased drug levels	(*17*)
Tetrahydrocannabinol	Mouse	Phenobarbital	Increased	Increased formation of	(*102*)
Aminopyrine	Mouse	Phenobarbital, barbital	Increased	reactive (teratogenic)	(*103*)
Furfurylamide	Mouse	Phenobarbital	Increased	metabolite(s)	(*104*)
Cyclophosphamide	Mouse	PCBs	Decreased	Species-dependent effect on metabolic balance	(*105*)
	Rat	PCBs	Increased	between activation/deactivation pathways	(*106*)
Phenytoin	Mouse	Phenobarbital	Decreased	Complex influence on	(*87,107, 108*)
		3-Methylcholanthrene	Unchanged	activation pathways and	
		SKF-525A[b]	Increased	detoxification of putative	
		TCPO[c]	Increased	epoxide metabolite	
		DEM[d]	Increased		

[a] In one dosing regimen (1 × 600 mg/kg SC on day 8, mouse) phenobarbital pretreatment (0.1% in drinking water from day 7–8) reduced the incidence of exencephaly from 61% to background levels (0–1.5%) (*17*). Epidemiological studies (retrospective) also indicate that phenobarbital comedication may reduce the incidence of spina bifida in the offspring of valproate-exposed human epileptics (Robert, Lindout, and Rosa, personal communication).
[b] Inhibitor of cytochrome P-450 catalyzed reactions.
[c] Inhibitor of epoxide hydrolase.
[d] Glutathione-depleting substance.

Table 7. Half-lives of some drugs in various species.[a]

	Predominantly eliminated via		Half-lives, hr							
Drug	Liver	Kidney	Mouse	Rat	Hamster	Guinea pig	Rabbit	Dog	Monkey	Man
Phenytoin	×			3–5				2–6	10–15	10–60
Phenobarbital	×	×	3				30–50	40–70		50–150
Primidone	×	×	1–3	1.7			2–3	2–10	10	3–17
Trimethadione	×		0.7	1.5–2.6	1		1–2	8		20–24
Valproic acid	×		0.8	0.3		1		1–4	0.7–3	12
Diazepam	×		1	1		2.4	3	8		20–50
Hexobarbital	×		0.3	1–2[b]			1	3		6
Antipyrine	×		0.2–0.6	1–2[b]		2	0.9	1–2	1–2	12
Phenazon	×		0.2	2	2	1		2	2	10
13-*cis*-Retinoic acid	×		0.3	1				3–6		10–30
All-*trans*-retinoic acid[c]	×		0.5–1	0.6–2				4–5		
Cyclophosphamide	×		0.2	0.7	0.2			0.5	0.7	7
Caffeine	×		0.7	0.8			1.6		3.2	4.2
Digoxin	×	×		9		16		27		44
Phenylbutazone	×			6	1–5		0.5–3	1–6	1–8	72
Oxyphenylbutazone				6			3	0.5–6	8	72
Flurobufene					0.4			0.6		3
Cefotetan	(+)[d]	+	0.2	0.3			0.5	1	1.3	3.4
Cefmetazole	(+)	+	0.1	0.1			1.5	1	0.5	1
Cefoperazone	(+)	+	0.2	0.2			1	0.7	2.4	2.5
Moxalactam	(+)	+	0.3	0.4			1	1	1	1.3
Cefpiramide	(+)	+	0.2	0.4			1.1	1.2	2.5	5
Cefazolin	(+)	+	0.2	0.4			0.4	1	0.8	1.5

[a] Adapted from data in the literature (*46, 47, 110–113*).
[b] Large sex difference.
[c] Saturation kinetics.
[d] (+), biliary excretion of β-lactam antibiotics significant in rat, but usually very low in other species.

because these compounds interfere with growth processes which can be related to the body surface area and thus the activity of the metabolic processes. With other classes of drugs such a close correlation cannot be expected since they interfere with specific physiological functions or produce some specific structural or biochemical lesion in the target cells (*110,111*). In such cases it can be expected that comparable drug concentrations in the various species should result in comparable pharmacological and toxicological effects. This has

Table 8. Body weights, surface areas and conversion factors of dosing from mg/kg into mg/m².

Species	Body weight, kg	Surface area, m²	Conversion factor[a,b]	Dose equivalent/ kg[c]
Mouse	0.02	0.0066	3.0	12.0
Rat	0.15	0.025	5.9	6.0
Dog	8	0.40	20	1.7
Monkey	3	0.24	12	3.0
Humans				
Child	20	0.80	25	1.5
Adult	60	1.60	37	1.0

[a] Literature data (*114,115*).
[b] To convert a mg/kg dose in a given species into an equivalent mg/m² dose, multiply the dose by the conversion factor.
[c] Dose equivalent for the adult human is set as 1.0.

indeed been shown to be the case for barbiturates: in spite of a 50-fold difference in rates of metabolism and duration of action of these drugs, various animal species and man recover from the effects of these drugs at similar plasma levels (*111*). In teratology, such data are essentially lacking at this time.

In Table 8 the factors for the conversion of mg/kg doses to mg/m² doses are listed for several species. If the relationships above hold true, equieffective doses (on a mg/kg basis) in mice, rats and rhesus monkeys will be 12×, 6×, and 3× higher than in man, respectively. As demonstrated below, such factors should be used with extreme reservations.

The elimination of drugs which are predominantly metabolized is not only dependent on liver size and hepatic blood flow—parameters which can be related to body surface area—but also on specific enzymes or isoenzymes present. Therefore, predictions are very difficult, but also here, man exhibits usually lower or much lower hepatic drug metabolizing enzyme activities than the laboratory animals. A detailed comparison between various enzyme activities in mouse, rat, hamster, and man showed that man exhibited lower microsomal cytochrome P-450 dependent monooxygenase activities in liver (and extremely low activities in lung) as compared to the rodent species (*116*).

Walker (*81*) has demonstrated a reasonably good correlation between the relative monooxygenase activity and the relative half-life of compounds predominantly eliminated by monooxygenase attack. Such drugs should be 5 to 30 times more persistent in man than in the male rat.

Epoxide hydrolase activities, on the other hand, were low in mouse and comparable in rat, hamster, and man. Glutathione-S-transferase activities were low in rat and man and substantially higher in mouse and hamster (*116*). These few examples show that generalizations are very difficult in this area and drug metabolism activity will depend on the particular metabolic reaction, the nature of the substrate, the possible influence of inhibitors or inducers (Table 6), and *in vivo*, the availability of the drug at the site of metabolism (e.g., liver concentrations, plasma protein binding, volume of distribution).

It should, however, be noted that the generalization that animals eliminate drugs more or much more rapidly than man does not always hold true, and a number of exceptions exist (*33*): the elimination of indomethacin was found to be slower in rat than in man, perhaps because of more extensive protein binding in the rat; zomepirac was also eliminated more slowly in rat than in man, while the elimination of proxicromil and furosemide were about equal in both species.

Clearance and Half-life as Parameters

Plasma (or blood) clearance (Cl) is the most appropriate parameter to characterize drug elimination. This parameter, which denotes the total capacity of the organism to eliminate drugs, via metabolism and/or excretion, can best be calculated from the dose administered (D), the fraction of drug absorbed (F) and the area under the concentration-time curve (AUC):

$$\mathrm{Cl} = \frac{DF}{\mathrm{AUC}}$$

On the other hand, the elimination half-life ($t_{1/2}$) is dependent not only on drug elimination, but also on the drug distribution volume (V_d).

$$t_{1/2} = 0.69 \frac{V_d}{\mathrm{Cl}} \text{ (one plasma elimination phase)}$$

Thus, a long (short) half-life is not necessarily related only to low (high) drug clearance, but may additionally be caused by a high/low volume of distribution. Also, the kinetics of many drugs cannot be described by a single-compartment model, but by a two- (or three-) compartment model, where several phases of the plasma concentration-time curve decay can be observed (Fig. 2), described by $t_{1/2,\alpha}$ (distribution phase) and $t_{1/2,\beta}$ (elimination phase).

$$t_{1/2,\beta} = 0.69 \frac{V_{d,\beta}}{\mathrm{Cl}} \text{ (two plasma elimination phases)}$$

The initial phase (α) of rapid drug concentration decline in plasma results from distribution of the drug from the central compartment into the peripheral compartment (e.g., storage sites in tissues). Exceptions here are high-clearance drugs which are also eliminated during the α phase. After distribution is complete, the plasma level curve is described by the elimination phase (β) with irreversible drug clearance. The precise value for the β-elimination half-life is sometimes difficult to obtain because of the low concentrations present during this elimination phase.

Although plasma (or blood) clearance values would have some advantages over half-lives for the characterization of the total capacity of the organism for drug elimination, it was decided to compile half-lives in Table 7 since this pharmacokinetic parameter gives a good estimate for the duration of drug action and the extent

of drug accumulation with a given multiple dosing regimen.

Saturation Kinetics (Nonlinear Pharmacokinetics)

If linear kinetics are followed, then the plasma concentrations and AUC values will be proportional to the dose. It follows that nonlinear kinetics are obtained if a linear relationship between dose and plasma concentrations (or AUC values) does not exist. Several reasons may be responsible for this nonlinearity with increasing doses: (a) the first-pass effect becomes saturated; (b) the plasma protein binding becomes saturated resulting in increased volume of distribution and tissue drug levels; (c) the hepatic and renal elimination capacities become saturated. All three factors may result in an overproportional rise of drug levels (or AUC values) with increasing doses. Small increments in dose may cause an unexpectedly large increase of the teratogenic response. Very steep dose-response curves with a pronounced threshold are the result which are indeed often seen in experimental teratology.

Examples of drugs with pronounced saturation kinetics include all-*trans*-retinoic acid, salicylic acid, valproic acid, caffeine, coumarins, phenylbutazone, and phenytoin (*117*). Interestingly, all-*trans*-retinoic acid exhibits saturation kinetics already at very low doses (*118*) (< 1 mg/kg, rat), while 13-*cis*-retinoic acid does not (mouse).

Such considerations demonstrate most clearly the value of concentration (or AUC value)-effect relationships in addition to dose-effect plots for the evaluation of teratogenicity tests (*16*). The considerable interindividual variation observed even in closely controlled laboratory animal species also call for the attempt to correlate individual maternal drug exposure with the teratologic response (*119*).

Peak Levels vs. AUC Values as Teratological Correlates

Since, as discussed in detail above, many drugs exhibit shorter half-lives in animals than in man, the pharmacokinetics obtained during teratogenicity testing—drug application once or twice a day during organogenesis—are grossly different from those seen in the human therapeutic situation. In experimental animals, a series of narrow and high drug concentration peaks are obtained while in man drug fluctuations are usually much less pronounced and blood levels are kept within a given therapeutic range. This is demonstrated in Figure 9 where the pharmacokinetics of valproic acid in man (therapeutic doses) are compared with those in the mouse (teratogenic doses) throughout the sensitive period for interference with neural tube closure in both species.

It can be observed from Figure 9 and the results of calculations listed in Table 9, that the teratogenic levels,

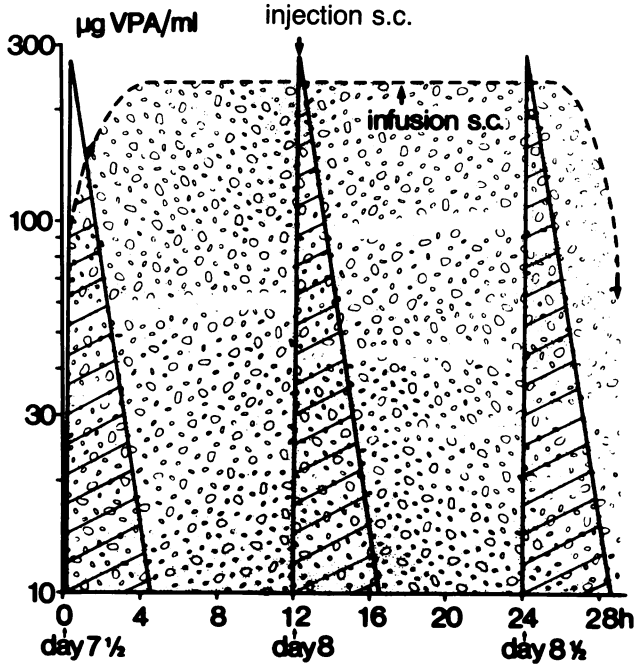

FIGURE 9. Simulated plasma concentration-time curves of valproic acid following a teratogenic drug administration regimen in the mouse (resulting in 10% exencephaly formation) as compared to a therapeutic drug administration schedule in man. Note the much more drastic drug concentration fluctuations in the mouse as compared to man. The time periods on the abscissa correspond to the length of the organogenesis period sensitive to interference with neural tube defect formations. From Nau (*47*).

particularly the free levels, are much higher in the mouse than the "therapeutic peak levels" in man. On the other hand, the AUC values during human therapy are higher than the corresponding values obtained after application of teratogenic doses in the mouse (Table 9). For a rational interspecies comparison or risk assessment it is therefore essential to elucidate if peak levels or the AUC values ("total exposure") are the relevant factors determining the teratogenicity of this drug.

We have therefore administered valproic acid not only by conventional injection regimens, but also at a constant rate via subcutaneous implanted osmotic mini-

Table 9. Comparison of pharmacokinetic parameters of valproic acid in mouse (teratogenic dose) with man (therapeutic dose).

Parameters	Mouse[a]	Man[a]
Teratogenic dose	180[b] (4 ×/24 hr)	30 (per 24 hr)
Cl (mL/hr × kg)[c]	1000	10
AUC (µg/mL × hr)[d]	720	3000
C_{max} (µg/mL)		
Total drug	230	100–150
Free drug	120	10–20

[a]Literature data (*14,16,46,47,50*).
[b]In this regimen SC doses were used which resulted in a significant formation of exencephaly (10% of live fetuses).
[c]Plasma clearance.
[d]Area under plasma concentration-time curve.
[e]Maximal concentration.

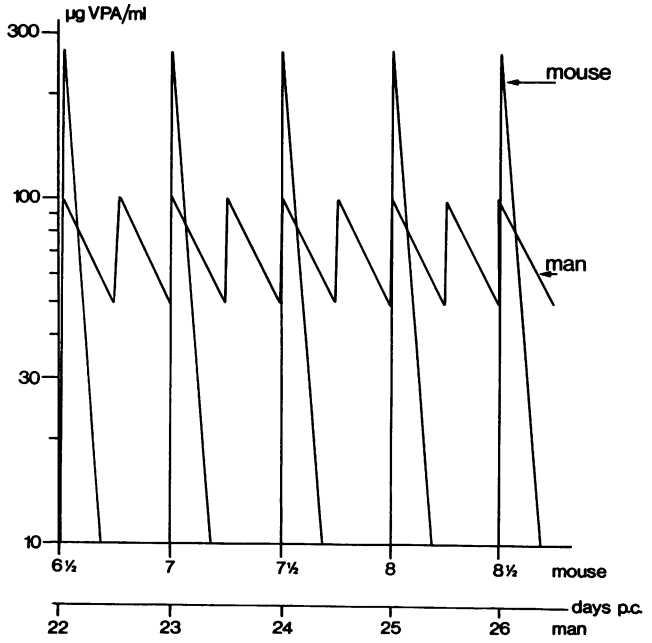

FIGURE 10. Comparison of two different administration regimens of valproic acid which both result in 10% incidence of exencephaly formation in the mouse. The drug was either injected SC (220 mg VPA-Na/kg × 12 hr; maternal peak plasma levels of 270 µg/mL) or infused by SC implanted minipumps (3800 mg VPA-Na/kg × day; maternal steady-state plasma levels of 250 µg/mL). Calculated by probit analysis from dose/concentration-response curves (*16*) and adapted from Nau (*47*).

pumps; in this way the teratogenic effects of peak drug levels (injection regimen) can be compared with those of steady-state concentrations (infusion regimen) (*14,16,120–122*). Particular emphasis was given to the formation of neural tube defects (exencephaly in the mouse) since, as Table 1 shows, these malformations may also be produced by valproic acid in man.

The dose-response curves for the formation of exencephaly was shifted greatly to the right with the infusion regimen as compared to the injection regimen. Less pronounced shifts were obtained for the dose-response curves for fetal weight retardation and embryolethality. In fact, high rates of exencephaly with the infusion regimen could only be observed within a very narrow "dose window" not covered up by embryolethality. On the other hand, the concentration-response curves were rather similar for both regimens. Here, steady-state levels after infusion were compared with peak levels after injection. These results indicate that the total drug exposure, as measured by doses or AUC values, was not the decisive factor in regard to valproic acid-induced exencephaly formation in the mouse. A particular threshold concentration must be reached—either by a series of short-lived drug concentration peaks or by steady-state concentrations—to produce the teratogenic effect in the embryo (Fig. 10).

These results should have important implications for a rational interspecies comparison. As Table 9 shows, the peak concentrations, particularly of the free drug—are much higher in the mouse after teratogenic dose as compared to man during therapy, implicating some margin of safety. On the other hand, a comparison of the AUC values would have indicated that man is actually exposed to valproic acid to a larger extent than the mouse. It seems therefore prudent to suggest that the peak drug levels which are still present in human therapy (Fig. 9) should be reduced as much as possible either by dividing the daily dose or by using slow-release formulations to minimize the teratogenic risk of antiepileptic therapy with this drug.

A comparison of the pharmacokinetics as well as teratogenicity of caffeine following injection of single massive doses with the results obtained after multiple application of divided doses suggest that the teratogenicity of this drug is also related to peak concentrations (*123,124*).

The situation with other drugs may be quite different. We have recently observed that the teratogenicity of cyclophosphamide can be related to the maternal AUC and not the peak levels (*125*). There, it makes little difference if a particular AUC value is reached with narrow and high concentration peaks (injection) or with low steady-state concentrations for a prolonged time (infusion).

Also the embryotoxicity of saliclate (*126*), cyanide (*127*), arsenate (*128*), and tetracycline (*129*) has been investigated following infusion of these substances. It appears that embryotoxicity is produced by the infusion of these compounds which could indicate the importance of AUC values in regard to the teratogenic response, although dose- and concentration-response relationships of the injection and infusion regimens have not been reported with these substances.

The administration of drugs via drinking water or food is very convenient; however, the results obtained are often difficult to interpret. Even if the presence of drugs does not change the food and water consumption, the relatively short half-lives of many drugs (Table 7) and the variability of food and water consumption prevents a controlled pharmacokinetic study. Plasma drug levels will depend greatly on the time of food/water intake in relation to the time of sampling (*130*).

Concluding Remarks

It is very unlikely that an animal species will ever be found that will consistently metabolize and excrete drugs with the same rates and metabolic pattern as man and is suitable for laboratory experimentation. We should therefore accept that there are great differences and rather concentrate to study the significance of these differences and their effects on the teratological response which could greatly improve our present extrapolation techniques. The example of valproic acid discussed above clearly shows that it is not sufficient to use doses, AUC values or peak levels for interspecies comparison. We must first determine which pharmacokinetic parameter correlates with the teratogenic response. If the AUC values (or doses) are the decisive

factors (as with cylcophosphamide), then toxicity is related inversely to clearance of the drug (where usually great differences exist between animals and man and "scaling factors" such as those listed in Table 8 may be useful). On the other hand, if peak drug levels (such as the case with valproic acid) correlate with teratogenicity, then the toxic response will be related to absorption and distribution phenomena (which are usually not too different in the various species). If we do not know which parameter to use for comparison, then completely misleading results may be obtained (Table 9).

In this way interspecies comparisons will be based on a more rational and scientific basis and the prediction of the human response based on animal studies could be improved.

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