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Linear and Nonlinear Kinetics of Drug Elimination. I. Kinetics on the Basis of a Single Capacity-Limited Pathway of Elimination with or Without Simultaneous Supply-Limited Elimination

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The pharmacokinetic behavior of foreign substances that are completely or partially eliminated via metabolism by saturable enzyme systems is analyzed. General integrated equations are derived which describe the time course of the plasma concentration under the assumption of a saturable enzyme system according to Michaelis–Menten kinetics in combination with normal first-order elimination processes. A procedure for the estimation of initial values of the elementary kinetic parameters on the basis of the models is outlined. These initial values have been used in a nonlinear curve-fitting program in order to obtain reliable kinetic and enzyme parameters from the plasma curves. With these methods, kinetic and apparent enzyme parameters are calculated for ethanol, salicylic acid, 4-hydroxybutyric acid, and phenytoin.

KEY WORDS: Michaelis–Menten kinetics; nonlinear pharmacokinetics; supply-limited elimination; capacity-limited elimination; estimation of parameters; ethanol; salicylic acid; 4-hydroxybutyric acid; phenytoin.

INTRODUCTION

Pharmacokinetics is the study of the behavior of drugs in man or animals. It includes the kinetic processes of diffusion in and out of various compartments, with elimination usually assumed to occur from a central compartment. In general, all kinetic processes are assumed to be first order, so that the rate of drug transfer is supposed to be directly proportional to the drug concentration in the compartments. The kinetic processes may then adequately be described by a set of linear differential equations (linear pharmacokinetics).

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Elimination of drug proceeds mainly through the liver and the kidney. In these clearance organs, elimination depends on the plasma flow through the organs and the extraction or filtration efficiency. In general, the rate of elimination is proportional to the concentration of drug in the plasma entering the clearance organs:

$$dQ_{el}/dt = \dot{V}_{Cel}C \tag{1}$$

where dQ_{el}/dt is the quantity of drug (e.g., mg) eliminated per unit of time (e.g., hr), C is the concentration of drug in the plasma, and $\dot{V}_{Cel}$ is the total body clearance (e.g., liters/hr). The clearance, $\dot{V}_{Cel}$, may depend on the flow, the concentration, the condition of the organs, protein binding, etc. The clearance, therefore, in general need not be a constant. Obviously, linear kinetics is achieved only when the clearance is constant. In that case, $\dot{V}_{Cel}$ may be replaced by a clearance constant, k_{Cel}. If the clearance is constant, the rate of elimination is directly proportional to the concentration entering the clearance organs, so that linear kinetic elimination may also be termed *supply limited*. Many examples of linear kinetics are known (1).

On the other hand, a substance such as ethanol, in the concentration present in man following "normal" doses, is eliminated at a constant rate (zero-order elimination), which indicates that the elimination is merely *capacity limited* (2,3). Since most if not all drugs are in major part eliminated by enzymatic conversion into metabolites, it could be expected that several other drugs might show capacity-limited elimination in man (or animals). In such cases, the total body clearance is not constant ($\dot{V}_{Cel} \neq k_{Cel}$) and proceeds via nonlinear kinetics. This indeed has been confirmed, the best-known example being the kinetics of salicylic acid as analyzed in detail by Levy (4). It should be noted also that the renal excretion or part of this may exhibit capacity-limited behavior, *viz.*, when tubular secretion occurs. In capacity-limited elimination, the profile of the semilogarithmic plasma concentration vs. time curve will be dose dependent (3,4). Only under circumstances when the body can be regarded as a single compartment can an analytical solution of the appropriate differential equation be obtained.

Such a solution has been given by Lundquist and Wolthers (2) for when the capacity-limited pathway is the only channel of elimination. Similar solutions have been given by Levy (4) and Wagner (5,6).

In this paper, the kinetics of drug disposition will be discussed for the case where drug elimination occurs via a single, capacity-limited metabolic pathway while simultaneous supply-limited elimination occurs. A procedure will be proposed by which initial estimates of the essential kinetic parameters can be obtained from the plasma concentration curves. The theory and consequences of capacity-limited elimination via two or more metabolic pathways will be discussed in a later publication.

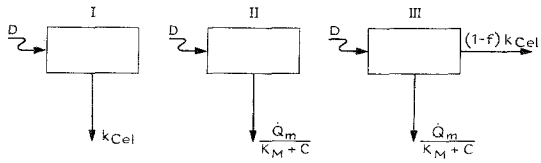

Fig. 1. Block diagrams representing linear and nonlinear kinetics of elimination from a single compartment. I, Supply-limited elimination characterized by the clearance constant. II, Capacity-limited elimination characterized by the enzyme constants K_M and $\dot{Q}_m$. In fact, the clearance is not constant but is maximally equal to $\dot{Q}_m/K_M$. III, Simultaneous supply-limited and capacity-limited elimination. A fraction is eliminated by a clearance process at all concentrations, while the rest is eliminated by a capacity-limited process. The maximum contribution to the total body clearance of this pathway is fk_{Cel}.

SUPPLY-LIMITED ELIMINATION OF DRUG FROM A SINGLE COMPARTMENT

If a drug is eliminated merely by linear elimination kinetics, i.e., a supply-limited clearance process, the total body clearance is constant, so that $\dot{V}_{Cel} = k_{Cel}$. Then the following holds true for the rate of change of drug in the body in the absence of absorption processes (see Fig. 1):

$$dQ/dt = -k_{Cel}C \qquad \text{or} \qquad dQ/dt = -kVC \tag{2}$$

where dQ/dt is the rate of disappearance of drug (e.g., mg/hr), k_{Cel} is the clearance constant (e.g., in liters/hr), C is the plasma concentration at any time t (e.g., mg/liters), k is the rate constant of elimination (e.g., hr^{-1}), and V is the volume of distribution (e.g., liters) (1).

Under the supposition that the volume of distribution remains constant, there is a direct relationship between change in quantity (dQ) and change in the concentration (dC). The equation then may be written as follows:

$$dC/dt = -(k_{Cel}/V)C \qquad \text{or} \qquad dC/dt = -kC = (-1/\tau_{el})C \tag{3}$$

Although we prefer using the time constant (τ), as in equation 3, the following equations are also given in terms of the rate constant ($k = 1/\tau$), which is extensively used, especially in the American pharmacokinetic literature. The rate constant and the time constant (turnover time) are directly related to the clearance constant k_{Cel}, provided that the clearance constant and the volume of distribution are constant:

$$k = k_{Cel}/V \qquad \text{and} \qquad \tau_{el} = V/k_{Cel} \tag{4}$$

From both parameters, the biological half-life may be derived [$t_{1/2} = \tau_{el}(\ln 2) = (\ln 2)/k$].

The solution of equation 3 is well known:

$$\ln C = \ln A - kt = \ln A - t/\tau_{el} \tag{5}$$

or

$$C = Ae^{-kt} = Ae^{-t/\tau_{el}} = A2^{-t/t_{1/2}} \tag{6}$$

where A is the apparent initial concentration extrapolated to zero time. This implies that A depends on the boundary conditions. In the case of an intravenous administration, $A = D/V$; in the case of enteral administration and rapid absorption as compared to elimination, $A = DF/V$. Here D is the dose and F is the fraction of the dose absorbed. For reasons of simplicity, F will be considered to be equal to unity.

The total body clearance is the sum of the clearance constants representing the various metabolic pathways, renal excretion, and eventually other elimination mechanisms (saliva, sweat, bile, lungs, feces):

$$k_{Cel} = k_{Cr} + k_{Cm1} + k_{Cm2} \cdots \tag{7}$$

Here k_{Cr} is the renal and k_{Cm} the metabolic clearance constant. The latter represents various metabolic pathways, while the former represents glomerular filtration, tubular secretion, and tubular reabsorption.

CAPACITY-LIMITED ELIMINATION FROM A SINGLE COMPARTMENT VIA A SINGLE METABOLIC PATHWAY

The rate of elimination then merely depends on the rate of biotransformation via a single pathway, and in the absence of absorption it may be described by use of a Michaelis–Menten equation (2,4–6) (see Fig. 1,II). The clearance is not constant but is concentration dependent, so

$$\dot{V}_{Cel} = \dot{Q}_m/(K_M + C) = k_{Cel}[K_M/(K_M + C)] \tag{8}$$

Here $\dot{Q}_m$ is the metabolic capacity of the liver enzymes involved (in mg/hr) and is equal to V_{max} when only one enzyme is involved, K_M is the (apparent) Michaelis–Menten constant (mg/liter), and k_{Cel} is the clearance constant at low plasma concentration ($C \ll K_M$).

It is obvious that for low plasma concentrations, such that $C \ll K_M$, the disappearance rate will again be directly proportional to the plasma concentration. Then the clearance is again concentration independent ($\dot{V}_{Cel} = k_{Cel}$). So the total body clearance under the condition that elimination proceeds merely via a single metabolic pathway depends only on the metabolic capacity and the dissociation constant, provided that C is small compared

to K_M. Then we obtain

$$k_{Cel} = \dot{Q}_m/K_M \quad \text{while} \quad k = \dot{Q}_m/VK_M \quad \text{and} \quad \tau_{el} = VK_M/\dot{Q}_m \tag{9}$$

On the other hand, as long as $C \gg K_M$, it is clear that the clearance is inversely proportional to the plasma concentration:

$$\dot{V}_{Cel} = k_{Cel}K_M/C \quad \text{and} \quad dQ/dt = -\dot{Q}_m \tag{10}$$

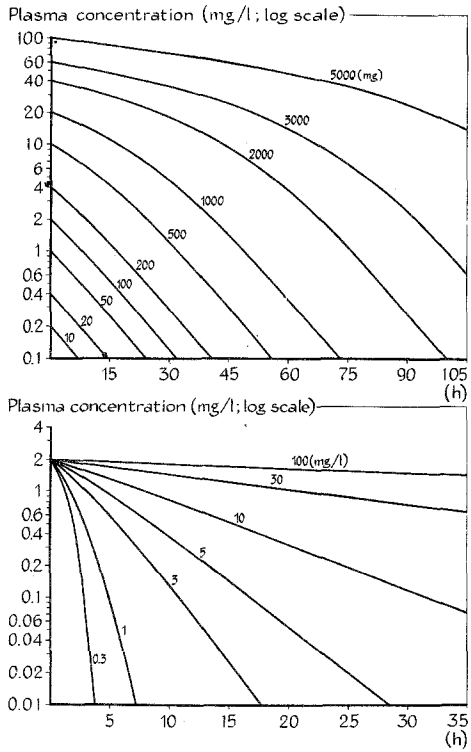

Fig. 2. Plasma concentration curves based on equation 13 showing nonlinear kinetics of elimination. The volume of distribution and the metabolic capacity are kept constant: $V = 50$ liters and $\dot{Q}_m = 50$ mg/hr. Top: The dose is varied as indicated from 10 to 5000 mg. K_M is constant (10 mg/liters). The plasma curves become flat at a higher dose, but they are straight lines with a slope determined by k (or τ_{el}) when $C \leq 0.1K_M$. ($k = 0.1\ \text{hr}^{-1}$, $\tau_{el} = 10$ hr). Bottom: K_M is varied from 0.3 to 100 mg/liter while the dose is constant ($D = 100$ mg). The whole curve becomes flatter when K_M becomes higher. For $K_M \geq 10$ mg/liter, practically no capacity limitation can be seen.

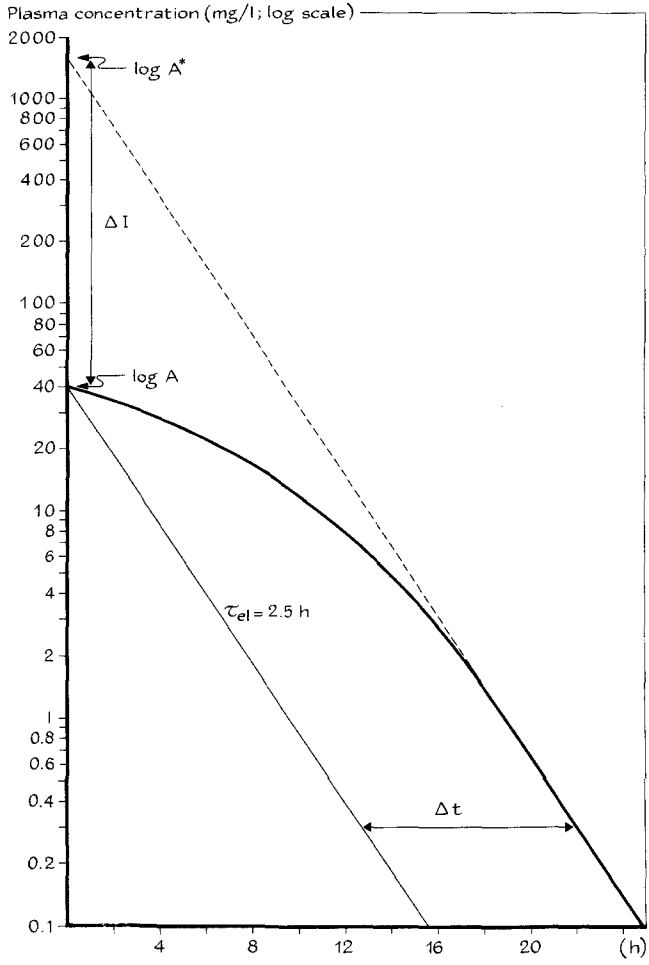

Fig. 3. Outline of the procedure for the initial estimation of the values of K_M and $\dot{Q}_m$. The parameters for the given curve are $D = 2000$ mg, $V = 50$ liters, $K_M = 10$ mg/liters, $Q_m = 200$ mg/hr, and $k = 0.4\ \text{hr}^{-1}$

which means that elimination under these circumstances proceeds as a zero-order process. The equation then becomes

$$C = A - t\dot{Q}_m/V \tag{11}$$

This obviously holds true for the disappearance of ethanol from plasma in man following the intake of two glasses of whiskey (2,3,7).

Under the given conditions of equation 8, the differential equation governing the change of drug concentration in the body becomes

$$dC/dt = -k[K_M/(K_M + C)]C = -(1/\tau_{el})[K_M/(K_M + C)]C \qquad (12)$$

Here the rate constant k and the time constant τ_{el} are related to the clearance constant at low plasma concentration according to equation 4.

Equation 12 can be integrated (2,4–5,6), but the solution is implicit with respect to the plasma concentration:

$$\ln C = \ln A + (A - C)/K_M - kt = \ln A + (A - C)/K_M - t/\tau_{el} \qquad (13)$$

In Fig. 2 theoretical plasma concentration curves are given, based on equation 13. The profile of the curve is dose dependent. However, for low plasma concentrations a straight line is always obtained for which the slope is defined merely by k or τ_{el}. Linear pharmacokinetic behavior, of course, is obtained only when the plasma concentration becomes negligible with respect to K_M. As a rule, this will require the plasma concentration to be smaller than about $0.1K_M$ (see also Ref. 5). At such low concentrations, equation 13 reduces to the following:

$$\ln C = \ln A^* - kt = \ln A^* - t/\tau_{el} \qquad (14)$$

Here $\ln A^*$ refers to the apparent initial concentration obtained by extrapolating the straight line to the ordinate (see Fig. 3). The value for $\ln A^*$ may be estimated from equation 13 when C becomes sufficiently small so that it may be neglected with respect to A, so that

$$\ln A^* = \ln A + A/K_M \qquad (15)$$

The apparent dissociation constant, K_M, can be calculated from the difference between the intercept, $\ln A^*$, of the extrapolated straight line for which the slope is determined by τ_{el} and the real intercept of initial plasma concentration $\ln A$ (see Fig. 3). After transformation to decimal logarithm, the following relation may be obtained:

$$K_M = A\,0.4343/[\log(A^*/A)] = 0.434A/\Delta I \qquad (16)$$

where $\Delta I = \log A^* - \log A$ is the difference between the extrapolated and real intercepts when the data are plotted on a decimal, semilogarithmic scale. Experimentally, k and τ_{el} (and therefore also $t_{1/2}$) may be calculated from the straight part of the semilogarithmic plasma curve at sufficiently low plasma concentrations. However, it is essential that sensitive assay procedures be available which allow us to obtain unambiguous data in the region where dose-independent kinetics apply ($C \ll K_M$). Subsequently, the metabolic

capacity, $\dot{Q}_m$, may be calculated from K_M and k_{Cel} or k (or τ_{el}) and V:

$$\dot{Q}_m = k_{Cel}K_M \qquad \text{or} \qquad \dot{Q}_m = VK_Mk = VK_M/\tau_{el} \tag{17}$$

K_M and $\dot{Q}_m$ may also be calculated from the difference in time, representing the displacement of the straight line of the semilogarithmic plasma curve in relation to a linear parallel to that line, starting from A.

It can easily be derived that (see Appendix I)

$$K_M = A/k\Delta t = A\tau_{el}/\Delta t \qquad \text{and} \qquad \dot{Q}_m = AV/\Delta t \tag{18}$$

Obviously, the constants K_M and $\dot{Q}_m$ calculated in this way bear a relation to the real values only if the requirements set before have been fulfilled. For ethanol and to some extent also for phenytoin (diphenylhydantoin), the equation holds reasonably true so that the kinetic and enzyme parameters of these drugs have been calculated with the procedure outlined above (see Table I and Discussion). However, it is in general unlikely that a single capacity-limited pathway is the only route of drug elimination. For most drugs, supply-limited elimination will occur simultaneously with capacity-limited elimination via one or more pathways. So dose-dependent elimination then occurs simultaneously with dose linear elimination kinetics.

SIMULTANEOUS SUPPLY- AND CAPACITY-LIMITED ELIMINATION FROM A SINGLE COMPARTMENT

If one metabolic pathway becomes capacity limited by increase in the dose, then at the low concentration range ($C \ll K_M$) a fraction, f, of the total body clearance occurs via that pathway. The clearance or clearance function then becomes

$$\dot{V}_{Cel} = (1 - f)k_{Cel} + \dot{Q}_m/(K_M + C) \tag{19}$$

Here $(1 - f)k_{Cel}$ represents that part of the total body clearance that remains concentration independent, while the other part fk_{Cel} equals $\dot{Q}_m/K_M$ only at low plasma concentration ($C \ll K_M$) and decreases at higher concentrations (saturation effect). The change in the plasma concentration now can be described by the following differential equation:

$$\begin{aligned} dC/dt &= -k[(1 - f) + fK_M/(K_M + C)]C \\ &= -(1/\tau_{el})[(1 - f) + fK_M/(K_M + C)]C \end{aligned} \tag{20}$$

where τ_{el} and k are the time constant and the rate constant, respectively, at sufficiently low plasma concentrations ($C \ll K_M$) where linear elimination kinetics apply. They still relate to the clearance constant according to equation 4. Integration with the boundary condition ($t = 0$, $C = A$) leads

to an implicit solution for C:

$$\ln C = \ln A + \frac{f}{1-f} \ln \left[\frac{1 + (1-f)\, A/K_M}{1 + (1-f)\, C/K_M} \right] - kt$$

or

$$\ln C = \ln A + \frac{f}{1-f} \ln \left[\frac{1 + (1-f)\, A/K_M}{1 + (1-f)\, C/K_M} \right] - t/\tau_{el} \tag{21}$$

A similar differential equation and solution have been given by Wagner (6).

Theoretical plasma concentration curves for a drug with $K_M = 3$ mg/liter are shown in Fig. 4 (left). At low plasma concentrations ($C \ll K_M$), parallel straight lines are always obtained whose slope is solely determined by k (or τ_{el}). However, at very high plasma concentrations, parallel straight lines are ultimately obtained which have a shallower slope than at the very low concentrations. The concentration-dependent component ($Q_m/K_M + C$) of the apparent clearance in equation 19 progressively becomes less important with respect to the concentration-independent component $(1 - f)k_{Cel}$ as the

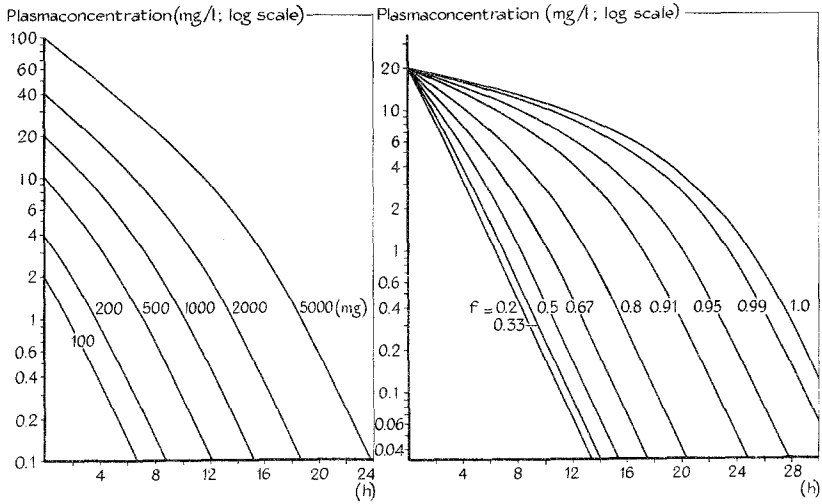

Fig. 4. Plasma concentration curves in combined linear and nonlinear kinetics of elimination. The volume of distribution and the metabolic capacity of the saturable system are kept constant: $V = 50$ liters and $\dot{Q}_m = 50$ mg/hr. Left: Dose variation from 100 to 5000 mg for a theoretical drug where two-thirds is eliminated by a potentially capacity-limited pathway ($f = 0.67$). $K_M = 3$ mg/liter and $k = 0.5\,\mathrm{hr}^{-1}$ ($\tau_{el} = 2$ hr). At concentrations below $0.1K_M$, the curves are parallel. Right: Variations of the fraction of the total body clearance that proceeds via a capacity-limited pathway, from $f = 0.2$ to $f = 1$. The clearance constant k_{Cel} is kept at 25 liters/hr, which means that $k = 0.5\,\mathrm{hr}^{-1}$ ($\tau = 2$ hr). So K_M is varied along with f according to $K_M = \dot{Q}_m/fk_{Cel} = 2/f$ (mg/liters). It is clear that capacity limitations will not be detected when $f \leq 0.2$.

plasma concentration increases. Obviously, this will occur earlier if f is smaller.

However, it is worthwhile to consider first the situation where the plasma concentration is large with respect to K_M. $\dot{Q}_m/C$ is not yet negligible relative to $(1 - f)k_{Cel}$. Under these conditions, equation 19 reduces to

$$\dot{V}_{Cel} = (1 - f)k_{Cel} + \dot{Q}_m/C \tag{22}$$

and equation 20 becomes

$$dC/dt = -k[(1 - f) + fK_M/C]C = -(1 - f)kC - fkK_M \tag{23}$$

or

$$dC/dt = -(1/\tau_{el})[(1 - f) + fK_M/C]C = -[(1 - f)C]/\tau_{el} - fK_M/\tau_{el}$$

Integration with the boundary condition ($t = 0$, $C = A$) leads to

$$C = Ae^{-k^*t} - \frac{f}{1 - f}K_M(1 - e^{-k^*t}) \tag{24}$$

or

$$C = Ae^{-t/\tau^*_{el}} - \frac{f}{1 - f}K_M(1 - e^{-t/\tau^*_{el}})$$

where $k^* = (1 - f)k$ and $\tau^*_{el} = \tau_{el}/(1 - f)$ are the rate constant and time constant, respectively, corresponding to the dose-independent part of the clearance $(1 - f)k_{Cel}$. This equation describes the plasma concentration curve for concentrations about 10 times K_M. The influence of the second term on the right-hand side of equation 24 becomes less significant relative to the value of its coefficient, $K_M f/(1 - f)$. At sufficiently high concentration values, equation 24 will appear to approximate a straight line on a semi-logarithmic plot, the slope of which will be close to k^* $(1/\tau^*_{el})$. This may be seen by examination of equation 22, where ultimately $\dot{Q}_m/C$ may be neglected with respect to $(1 - f)k_{Cel}$, so that a concentration-independent clearance results. Therefore, at very high plasma concentrations, the overall elimination will appear to be supply limited. In these extreme case, the clearance process can be simply described as

$$\dot{V}_{Cel} = (1 - f)k_{Cel} \tag{25}$$

so

$$dC/dt = -k^*C = -(1 - f)kC = -C/\tau^*_{el} = -(1 - f)C/\tau_{el} \tag{26}$$

which on integration leads to

$$\ln C = \ln A - k^*t = (\ln A - t/\tau^*_{el}) \tag{27}$$

and

$$C = Ae^{-k^*t} = (Ae^{-t/\tau_{el}^*})$$

So in general the contribution of the capacity-limited pathways can be calculated from the ratio of the slopes of the two straight-line segments in a semilogarithmic plasma concentration–time plot. It should be emphasized, however, that the correct slope for k^* or τ_{el}^* is found only at very high plasma concentrations. Consequently, f will be underestimated from most experimental data (see Discussion). When $f = 0.5$, so that 50% of the total body clearance (at $C \ll K_M$) occurs via a potentially capacity-limited pathway, the limiting slope at high plasma concentrations is one-half the slope found at the low concentration range where linear kinetics prevail.

The correctness of the estimate will be strongly dependent on the number of plasma data points available and the total dose administered. However, if the potentially capacity-limited pathway contributes less than 20% to the overall clearance, the difference in slope can hardly be detected (see Fig. 4). This implies that capacity-limited elimination pathways may easily be overlooked. Nevertheless, a capacity-limited pathway which contributes little to the overall body clearance may be of importance if administered with another drug which is metabolized via the same enzymatic processes.

At *low* plasma concentrations where $C \ll K_M$, equation 21 converges on

$$\ln C = \ln A + \frac{f}{1-f} \ln [1 + (1-f)\, A/K_M] - kt \tag{28}$$

or

$$\ln C = \ln A + \frac{f}{1-f} \ln [1 + (1-f)\, A/K_M] - t/\tau_{el}$$

The intercept of this straight line, $\ln A^*$, is larger than the real intercept, $\ln A$, and can be represented by the following equation, analogous to equation 15:

$$\ln A^* = \ln A + \frac{f}{1-f} \ln [1 + (1-f)\, A/K_M] \tag{29}$$

The value of f can be calculated from the ratio of the slopes of the straight-line segments at very high ($C \gg K_M$) and at very low ($C \ll K_M$) plasma concentrations. Therefore, K_M may be calculated from the difference between the real and the extrapolated intercepts in a semilogarithmic plot according to the following relations, obtained from equation 29, by rearrangement and transformation to decimal logarithms:

$$\Delta I = \frac{f}{1-f} \log [1 + (1-f)\, A/K_M] \tag{30}$$

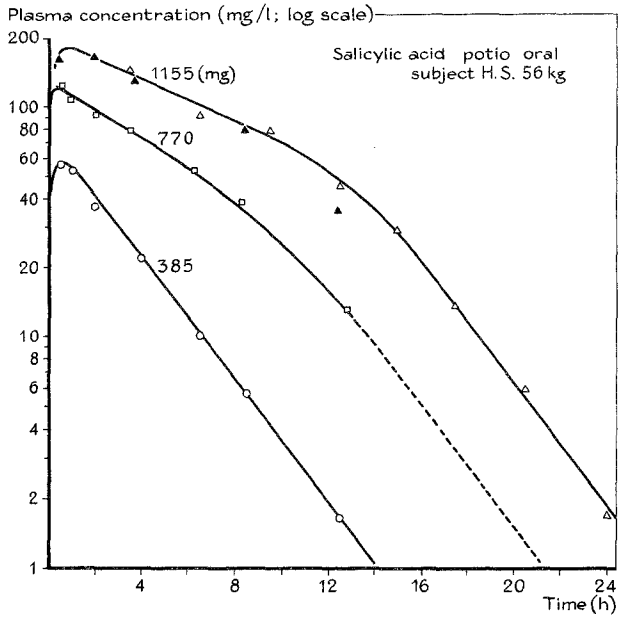

Fig. 5. Plasma curves of different doses of salicylic acid in the same human subject. The experiment with the largest dose was done twice (the open triangles 3 months after the filled ones). At low plasma concentrations, parallel straight lines are obtained from which k or τ_{el} can be estimated. At high concentrations, straight lines are also obtained. See Table I and text for further explanation.

or

$$K_M = (1 - f)A/\text{antilog}\left[\frac{(1 - f)\Delta I}{f} - 1\right] \tag{31}$$

The apparent Michaelis–Menten constant, K_M, can also be calculated from the time shift, Δt, between the straight line (equation 28) and a parallel straight line starting at A. It can be derived that (see Appendix I)

$$\Delta t = \frac{f}{k(1 - f)0.4343}\log\left[1 + (1 - f)\,A/K_M\right] \tag{32}$$

$$= \frac{f\tau_{el}}{(1 - f)0.4343}\log\left[1 + (1 - f)\,A/K_M\right]$$

or

$$K_M = (1 - f)A/\text{antilog}\left[\frac{0.434k\Delta t(1 - f)}{f} - 1\right] = (1 - f)A/\text{antilog}\left[\frac{0.434\Delta t(1 - f)}{f\tau_{el}} - 1\right] \quad (33)$$

Once K_M has been determined, the metabolic capacity term, $\dot{Q}_m$, of the enzyme system concerned becomes quantitatively accessible on the basis of

$$\dot{Q}_m = fk_{Cel}K_M = fkVK_M = f(V/\tau_{el})K_M \quad (34)$$

Some examples of combined capacity- and supply-limited elimination are shown in Figs. 5 and 6. Although salicylic acid was administered orally as a solution, absorption is very fast with respect to elimination so that equation 21 becomes a good approximation. However, 4-hydroxybutyric acid was given by intravenous injection (11). In this case, the distribution phase appears to be fast with respect to the terminal log linear slope so that equation 21 may be applied in this case, also. Kinetic and enzyme parameters

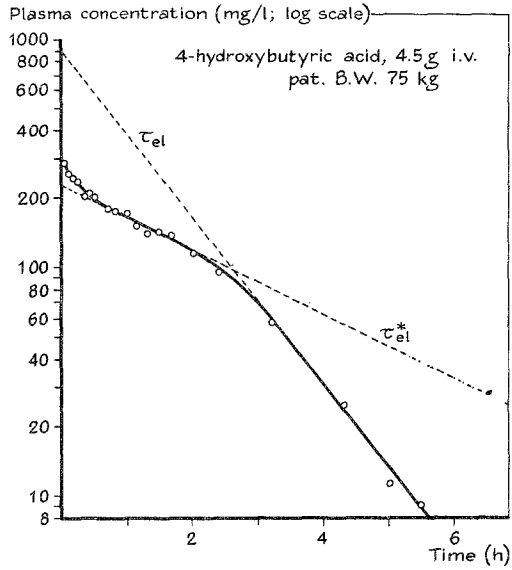

Fig. 6. Plasma curve of 4-hydroxybutyric acid in a patient. Capacity-limited elimination and supply-limited elimination occur here, also. Data from van der Pol *et al.* (11). The extrapolated lines for estimation of k (or τ_{el}) and k^* (or τ^*_{el}) are drawn. See Table I and text for further explanation.

Table I. Estimated and Computer-Fitted Parameters for Some Drugs That Show Capacity-Limited Elimination[a]

	Dose (mg)							
	Ethanol		Salicylic acid		Phenytoin	4-Hydroxybutyric acid		
	48,000[b]	64,000[c]	770[d]	1155[d]	250[e]	2900[f]	4000[f]	4500[f]
A^*_{est} (mg/liter)	6.6×10^5	7.2×10^5	600	2500	5.90	1170	9900	900
A_{est} (mg/liter)	1330	1220	125	200	4.55	220	250	230
A_{fit} (mg/liter)[g]	1310	1320	129	191	4.66	201	244	238
"V"$_{fit}$ (liters)[h]	36.7	48.5	5.98	6.0	53.7	14.4	16.4	18.9
	(1.7)	(1.4)	(0.16)	(0.8)	(1.4)	(0.9)	(0.8)	(1.2)
$\tau^*_{el,est}$ (hr)	—	—	7.2	9.7	—	1.3	2.7	2.1
k^*_{est} (hr^{-1})	—	—	0.14	0.10	—	0.77	0.37	0.48
$\tau_{el,est}$ (hr)	0.43	0.68	3.3	3.3	21	0.62	0.83	0.61
k_{est} (hr^{-1})	2.33	1.47	0.30	0.30	0.048	1.61	1.20	1.64
$\tau_{el,fit}$ (hr)	0.42	0.60	2.8	2.5	20.0	0.44	0.46	0.56
	(0.06)	(0.08)	(0.3)	(0.6)	(0.8)	(0.18)	(0.20)	(0.49)
k_{cal} (hr^{-1})	2.38	1.67	0.36	0.4	0.05	2.27	2.17	1.97
f_{est}	—	—	0.54	0.66	1.00	0.48	0·70	0.71
f_{fit}[h]	—	—	0.79	0.91	1.00	0.85	0.88	0.85
			(0.09)	(0.16)		(0.11)	(0.03)	(0.07)
$K_{M,est}$ (mg/liter)	210	190	21	25	16	25	14	36
$K_{M,fit}$ (mg/liter)[h]	200	140	26	27	10	31	10	15
	(45)	(30)	(17)	(30)	(4)	(43)	(9)	(28)
V_{est} (liters)	27.1	43.4	6.0	6.0	.54	14	16	19
$k_{Cel,cal}$ (liters/hr)	64.5	72.3	2.1	2.4	2.7	31.8	34.8	33.9
$\dot{Q}_{m,cal}$ (mg/hr)	12900	10100	44	59	27	840	310	430

[a]The symbols A^*, A, V, τ, k, f, K_M, k_{Cel}, and $\dot{Q}_m$ are explained in the text and in the glossary of terms. The subscripts "est," "fit," and "cal" mean "estimated," "fitted," and "calculated," respectively. "Estimated" refers to the graphical parameter determination, "fitted" values are those obtained directly from the computer-fitting program, and "calculated" values are derived from estimated and fitted parameters according to the equations described in the text. V_{est} is the best possible estimation of the actual volume of distribution and is used for calculation of $k_{Cel,cal}$ and $\dot{Q}_{m,cal}$, while "V"$_{fit}$ is just an operational magnitude, leaving out consideration of distribution or absorption processes, and is used for descriptions according to equations 13 or 21.

[b]Data from Wagner and Patel (7). For estimation of the parameters as well as for the curve fitting, the zero time is taken after absorption can be expected to be completed. Therefore, the intercept A and the volume "V"$_{fit}$ do not correspond to the actual volume of distribution V_{est}, which is estimated from the whole plasma concentration curve.

[c]Data from Haggard *et al.* (3). Same remarks as for footnote *b*.

[d]Two oral doses were given to the same subject. Absorption is very rapid (see Fig. 5). Therefore, a close agreement exists between V_{est} and "V"$_{fit}$ and the curves can be handled as if intravenous administration were used.

[e]Data from Glazko *et al.* (9). Based on mean plasma concentration values in six persons who received 250 mg of phenytoin by intravenous infusion. Concentration points in the distribution phase have been left out for estimation of the parameters and curve fitting.

[f]Data from van der Pol *et al.* (11) in three patients following intravenous infusion. The distribution phase has been left out for estimation of the parameters and curve fitting (see Fig. 6).

[g]The magnitude of A_{fit} is calculated from "V"$_{fit}$ and the dose D ($A = D/$"V"), since by the computer program used the experimental points are fitted to an equation in which A is written explicitly as $D/$"V" so that "V" is fitted directly.

[h]The numbers in parentheses represent the errors in the fitted parameter values. See text for further explanation. Errors are given only for parameters that were directly fitted and not for those that were calculated from fitted values, since such errors would hardly be meaningful.

calculated according to the procedure outlined before are summarized in Table I. Since experimentally f is underestimated, the initial values of K_M and $\dot{Q}_m$ so obtained will not be optimal. Therefore, the initial values obtained for K_M and $\dot{Q}_m$ have been used in a nonlinear curve-fitting computer program[2] in order to obtain more reliable values of the *in vivo* enzyme parameters. The fit to the data is seen in Figs. 5 and 6. The data summarized in Table I indicate that the initial estimates calculated from the procedure outlined above give a reasonable estimation of the values obtained by curve fitting.

DISCUSSION

In general, the main importance of graphical methods for estimation of pharmacokinetic parameters lies in the possibility of obtaining initial estimates which can be used for subsequent digital computer fitting procedures. Of course, the precision of these initial approximations is dependent on the number of experimental data points available and the accuracy and the sensitivity of the method used for the assay of the plasma concentration. For instance, in the well-known method of residuals used in making initial estimates in multiple exponential equations, it is essential to have enough data points to make a good estimate of the slope of the extrapolated straight lines. Also, the so-called deeper compartments can easily be overlooked when plasma concentration has not been followed long enough. At least the same degree of accuracy, sensitivity, and specificity of the assay method is required for optimal application of the procedure outlined above. For the simplest model of capacity-limited elimination (equations 12–18) where the saturable metabolic pathway is the only mechanism of elimination, the graphically estimated values of K_M and $\dot{Q}$ may result in a good approximation of the real value. These are of course dependent on accurate estimation of the slope of the straight line at low plasma concentrations and of the extrapolated intercept A^*. This will require estimation of the slope of the straight line from concentration data in the region where the plasma levels are negligible with respect to K_M. When enough data are available below $0.1K_M$, it will be possible to obtain K_M and $\dot{Q}_m$ values with an error of only a few percent. Of course, the magnitude of the deviation of the estimated parameters from the real ones will become larger if the assay cannot accurately define the low plasma concentration. Nevertheless, the method will always indicate the order of magnitude of the parameters and provide an initial estimate for a subsequent computer fitting.

As far as the second model including simultaneous supply- and capacity-limited elimination (equations 20–34) is concerned, another complicating factor may arise. The estimated value of K_M from equation 31 is dependent not only on the accurate determination of ΔI but also on f. The fraction, f, is estimated from the ratio of the slopes k^* and k of the two straight lines

[2]FARMIT, a nonlinear curve-fitting program, in use at the Computer Centre of the University of Nijmegen. Details available on request.

which arise at very high and at very low plasma concentrations. Yet k^* can be determined accurately only at such high plasma concentrations that contribution of the saturated mechanism to the overall elimination is negligible with respect to the contribution of the mechanism(s) that remains linear over the whole experimental concentration range. It is doubtful whether this situation will ever be reached. For instance, the maximum dose that can safely be administered may be a serious restriction in this respect. Therefore, the estimated values for the parameters assuming combined capacity- and supply-limited mechanisms will as a rule show greater deviation from the real values than in the case of the simpler model. Once again it should be stressed that computer fitting starting with good initial graphical estimates of the parameters will greatly improve the accuracy of the estimates.

Several examples of kinetic parameters calculated from the experimental data from our laboratory and the literature are discussed below.

In the case of ethanol data obtained by Wagner and Patel (7), it is assumed that absorption is both complete and rapid with respect to elimination. These suppositions seem to be reasonable. Ethanol is known to be oxidized in man to the extent of 90–98 % so that f may be assumed to approach unity. However, two distinct mechanisms are available: the liver alcohol dehydrogenase and the liver microsomal oxidizing systems. The relative contributions of these systems to the overall metabolism of ethanol are dependent on the plasma concentration that exists (8). Some problems arise with regard to the calculation of the kinetic parameters in this case. As has been shown in data from this laboratory (C. A. M. van Ginneken and J. M. van Rossum, unpublished), an apparent K_M is expected to come out which is a function of the two K_M values for both systems and their relative importance or weight. For the moment, it is sufficient to note that the K_M value given in Table I is of the expected order of magnitude. The two metabolic systems have different K_M values as follows from *in vitro* studies. For alcohol dehydrogenase, K_M is about 90 mg/liter; for the microsomal oxidizing system, K_M is about 400 mg/liter (8). Unfortunately, no information is available with which to estimate the value of the metabolic capacity terms $\dot{Q}_m$ for the two enzymes. In our analysis of ethanol, we utilized the simplest model (equation 13). It should be noted that the calculated metabolic capacity $\dot{Q}_m$ seems to be a very reasonable overall estimate. As far as K_M is concerned, however, one must bear in mind that the calculated values actually may be the result of some combination of two elementary constants. Further analysis can be done only when more and more accurate data become available.

Phenytoin (diphenylhydantoin) seems to be about 80% hydroxylated (9). Assuming this to be due to a saturable metabolic system, one would expect the fraction f to be about 0.8. However, a good computer fit to the data

requires $f = 1$, as shown in Table I, so we are forced to conclude that the data are not sufficient for discriminating among the several possibilities. Furthermore, evidence is accumulating that phenytoin metabolism is product-inhibited (10). However, the present data agree well with the procedure outlined above, whereas they do not allow a more thorough analysis.

In the case of salicylic acid, absorption from the oral route is fast with respect to elimination, but at least two capacity-limited pathways are involved (4). This implies that equation 21 may not be applied unless additional requirements have been fulfilled. When two different capacity-limited pathways occur simultaneously with a concentration-independent fraction, the mathematical relationship between plasma concentration and time becomes more complicated, and the calculation of the kinetic parameters is no longer straightforward. However, in the case of salicylic acid elimination in man, the K_M values of the two metabolic systems that are easily saturated are of the same order of magnitude, as may be seen from the renal excretion data of Levy *et al.* (4). It may be calculated that if two saturable pathways are involved with the same K_M value the differential equation may be written as a single pathway while the overall capacity is the sum of the two capacities. Our data do not allow a more detailed analysis. The overall values obtained for salicylic acid from plasma data agree reasonably with the data obtained from renal excretion of metabolites by Levy *et al.* (4).

In the case of 4-hydroxybutyric acid (11) injected in various patients during anesthesiology with a fast distribution phase, it is not known whether one or more capacity-limited pathways are involved. The available plasma data, however, do not allow a further analysis. Analysis of combined supply- and capacity-limited elimination via more pathways will be done in a forthcoming paper.

The errors in the kinetic and enzyme parameters as given in Table I reflect the goodness of fit of the data to the model used. When many data points are used, this error can be regarded as the standard error. In the cases that are described here, the exact meaning of the value of the error is somewhat obscure, since the error is strongly dependent on the number of data points available. This may contribute to the fact that some parameters show a rather large error, although on inspection the calculated curves appear to describe the experimental points rather well. As a matter of fact, the magnitude of the error in a parameter reflects the degree of sensitivity of the fitted curve to changes in that parameter. The relative error in K_M and τ_{el} might still be related to the strong positive correlation which appears to exist between these two parameters. Therefore, it may be possible to obtain a nearly equally good fit to the data by certain simultaneous changes in K_M and τ_{el}.

APPENDIX I

Derivation of Equations 18 and 32

With respect to Fig. 3, one can conceive of two points with the same concentration, namely one on the straight line starting from $\log A^*$ and one on the parallel line from $\log A$, which are a time distance Δt apart. One can write for these points:

$$\ln C = \ln A^* - kt = \ln A + A/K_M - kt \tag{14/15}$$

and

$$\ln C = \ln A - k(t - \Delta t) = \ln A - kt + k\Delta t \tag{5a}$$

From comparison, we see that

$$A/K_M = k\Delta t \qquad \text{so} \qquad K_M = A/k\Delta t \tag{18}$$

Essentially the same procedure can be applied for deriving equation 32. Then the two points are given by

$$\ln C = \ln A^* - kt = \ln A + \frac{f}{1-f} \ln [1 + (1 - f)\, A/K_M] - kt \tag{28}$$

and

$$\ln C = \ln A - k(t - \Delta t) = \ln A - kt + k\Delta t \tag{5a}$$

From comparison, it follows that

$$k\Delta t = \frac{f}{1-f} \ln [1 + (1 - f)\, A/K_M]$$

or

$$\Delta t = \frac{f}{k(1-f)0.4343} \log [1 + (1 - f)\, A/K_M] \tag{32}$$

APPENDIX II

Integration of equation 20 proceeds as follows:

$$dC/dt = -k[(1 - f) + fK_M/(K_M + C)]C \tag{20}$$

Rearrangement leads to

$$\frac{K_M + C}{[K_M + (1 - f)C]C}\, dC = \left[\frac{1}{C} + \frac{f}{K_M + (1 - f)C}\right] dC = -k\, dt \tag{35}$$

Now integration is straightforward and, with the boundary condition

($t = 0$, $C = A$), the solution is

$$\ln C = \ln A + \frac{f}{1-f} \ln \left[\frac{1 + (1-f)\, A/K_M}{1 + (1-f)\, C/K_M} \right] - kt \tag{21}$$

APPENDIX III

Equation 13 is just a special form of equation 21, namely, for the case where $f = 1$.

Equation 21 reduces to equation 13 as f approaches unity. Starting with equation 21, we have to calculate

$$\lim_{f \to 1} (\ln C) = \ln A - kt - \lim_{f \to 1} \left\{ \frac{f}{1-f} \ln \left[\frac{1 + (1-f)\, C/K_M}{1 + (1-f)\, A/K_M} \right] \right\} \tag{21a}$$

Simple rearrangement gives

$$\lim_{f \to 1} \left\{ \frac{f}{1-f} \ln \left[\frac{1 + (1-f)\, C/K_M}{1 + (1-f)\, A/K_M} \right] \right\} = \lim_{f \to 1} \left\{ \frac{f}{1-f} \ln \left[1 + \frac{(1-f)(C-A)}{K_M + (1-f)\, A} \right] \right\} \tag{36}$$

Since the following equation obviously holds true:

$$-1 < \frac{(1-f)(C-A)}{K_M + (1-f)\, A} \leq 0 \tag{37}$$

the logarithm in equation 36 can be expanded into a Taylor series, so that we can write

$$\begin{aligned} &\lim_{f \to 1} \left\{ \frac{f}{1-f} \ln \left[1 + \frac{(1-f)(C-A)}{K_M + (1-f)\, A} \right] \right\} \\ &\quad = \lim_{f \to 1} \left\{ \frac{f}{1-f} \left[\frac{(1-f)(C-A)}{K_M + (1-f)\, A} - \frac{(1-f)^2 (C-A)^2}{2[K_M + (1-f)\, A]^2} \right. \right. \\ &\qquad \left. \left. + \frac{(1-f)^3 (C-A)^3}{3[K_M + (1-f)\, A]^3} - \cdots \right] \right\} \\ &\quad = \lim_{f \to 1} \left\{ \frac{f(C-A)}{K_M + (1-f)\, A} - \frac{f(1-f)(C-A)^2}{2[K_M + (1-f)\, A]^2} \right. \\ &\qquad \left. + \frac{f(1-f)^2 (C-A)^3}{3[K_M + (1-f)\, A]^3} - \cdots \right\} \\ &\quad = \frac{C-A}{K_M} \end{aligned} \tag{38}$$

By substituting this result into equation 21a, we obtain $\lim_{f \to 1} (\ln C) = \ln A - kt - (C - A)/K_M$, which is identical to equation 13.

GLOSSARY

dQ_{el}/dt: the quantity of drug (e.g., mg) eliminated per unit of time (e.g., hr).
C: the concentration of drug in the plasma (mg/liter).
$\dot{V}_{Cel}$: the total body clearance (e.g., liters/hr).
k_{Cel}: the clearance constant (e.g., liters/hr), assuming linear elimination kinetics.
k_{Cr}: the renal clearance constant (e.g., liters/hr) (glomerular filtration, tubular secretion, tubular reabsorption), assuming first-order processes (see equation 7).
k_{Cm}: the metabolic clearance constant (various metabolic pathways, e.g., liters/hr), assuming first-order processes (see equation 7).
dQ/dt: the rate of disappearance of the drug (e.g., mg/hr).
k: the rate constant of elimination (e.g., hr^{-1}).
τ: the time constant, sometimes referred to as the mean turnover time ($\tau = 1/k$).
V: the volume of distribution (assuming a one-compartment-body model).
$\dot{Q}_m$: the metabolic capacity of the liver enzymes involved (mg/hr).
K_M: the (apparent) Michaelis–Menten constant (mg/liters) (dissociation constant).
A^*: the apparent initial concentration obtained by extrapolating the straight line to the ordinate.
$\Delta I = \log A^* - \log A$: the difference between the extrapolated and real intercepts when the data are plotted on a decimal, semilogarithmic scale.
f: a fraction of the total body clearance that occurs via the metabolic pathway.
F: fraction of administered dose absorbed intact.

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