Table III—Plasma Levels of Amphetamine and Phentermine

t, min.	Amphetamine[a], ng./ml.	Amphetamine[a] (Nephrectomized), ng./ml.	Phentermine[a], ng./ml.
15	86.5 (1.8)[b]	83.8	65.1 (2.1)
25	69.4 (2.1)	59.5	55.7 (6.9)
45	— —	49.0	— —
60	23.0 (2.5)	44.6	21.1 (1.9)
90	— —	29.4	— —
120	10.8 ± 3.1[c]	12.0	8.5 ± 0.4[c]
180	6.8 ± 1.8[c]	7.8	4.4 ± 0.4[c]
240	3.8 (0.9)	—	2.4 (0.4)
300	2.5	3.62	1.6 (0.2)
420	— —	1.35	— —
540	— —	0.95	— —

[a] All animals were given 0.5-mg./kg. i.v. doses of drug and lightly anesthetized with ether; blood was collected as described in the *Experimental* section. [b] Numbers in parentheses are the range of two values, and the numbers given ± *SD* are the mean of four values. [c] Standard deviation.

with phentermine. Experiments examining this possibility are in progress.

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Novel Method for Bioavailability Assessment

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Abstract □ An alternative strategy is proposed for the assessment of bioavailability. It is based on estimates of renal clearance, plasma clearance, and urinary excretion of unchanged drug. The method is totally compatible with pharmacokinetic methods but lends itself to a more flexible sampling schedule and is model independent.

Keyphrases □ Bioavailability—determination with flexible, model-independent method based on estimates of renal clearance, plasma concentration, and urinary excretion of unchanged drug □ Plasma concentration and urinary excretion data—used in flexible, model-independent method for assessing bioavailability □ Urinary excretion and plasma concentration data—used in flexible, model-independent method for assessing bioavailability

There are many ways to estimate bioavailability; each one entails a set of assumptions, some of which can be experimentally verified. Oser *et al.* (1) assumed that the same fraction of the absorbed compound from different preparations is excreted unchanged in the urine. Hence, the ratio of the urinary recovery following a test preparation to that following the same dose administered as a solution is taken to be a measure of relative absorption, which they defined as "physiologic availability."

Pharmacokinetic methods of assessing bioavailability from blood plasma concentrations or urinary excretion data were summarized by Wagner (2). In essence, methods based on plasma concentration data are predicated on comparisons of the products of plasma clearance, $\dot{V}_{cl,p}$, and the total area under the plasma concentration curve, $(AUC)_\infty$. If one assumes $\dot{V}_{cl,p}$ to be constant for a given subject from one test dose to another, then the ratio of $(AUC)_\infty$ is a measure of relative absorption. But if there are intrasubject variations in elimination, the assumption of constant $\dot{V}_{cl,p}$ will not hold and an adjustment is indicated. The nature of such an adjustment depends on one's ability to estimate the terminal plasma $t_{1/2}$ in a region free from the influence of continued absorption and on one's conception of the pharmacokinetic model (3, 4). In any event, a proper

Table I—Simulated Plasma Concentration and Urinary Excretion Data following Single 500-mg. Intravenous and Oral Doses of a Drug to the Same Subject on Three Separate Occasions

Treatment	Plasma Concentration, mcg./ml., in Minutes											
	5	10	20	30	40	60	120	180	240	300	360	720
Intravenous	17.99	15.12	11.21	8.78	7.14	5.05	(2.09)	(0.89)	(0.38)	0.16	0.07	—
Oral No. 1	—	—	1.43	—	2.06	2.32	(2.22)	(1.69)	(1.17)	0.77	0.49	0.02
Oral No. 2	—	—	4.50	—	5.42	5.15	(3.15)	(1.65)	(0.82)	0.40	0.20	0

	Urinary Recovery, mg., in Hours								
	−1–0	0–1	1–2	2–3	3–4	4–6	6–12	12–24	0–24
Intravenous	0	171.97	56.30	(23.77)	(10.14)	6.18	1.37	0	(269.73)
Oral No. 1	0	21.45	31.25	(26.27)	(19.02)	21.08	12.64	0.59	(132.30)
Oral No. 2	0	50.81	48.08	(26.91)	(13.74)	10.12	3.10	0.20	(152.96)

estimate of the terminal plasma $t_{\frac{1}{2}}$ is usually required to calculate $(AUC)_\infty$ by extrapolation beyond the last data point; in some cases, this extrapolation process may also be model dependent (5).

Methods based on urinary excretion data for unchanged drug or metabolites embody the same assumptions as those of Oser *et al.* (1). In pharmacokinetic terms, the assumptions are that component rates of total elimination are individually constant or that the ratio of the rate of urinary excretion to the overall rate of elimination of that which is absorbed is constant. Intrasubject variations in the rate of urinary excretion are manifested as changes in the slopes of the amounts remaining to be excreted *versus* time plots. Compensations are subject to the same considerations as with plasma $t_{\frac{1}{2}}$ and are, therefore, also model dependent.

Depending on the biological disposition of the drug, bioavailability studies are designed to utilize one or more of these methods. Such studies should include means for testing some of the assumptions. Where it is safe and experimentally feasible, it is generally desirable to include at least one intravenous dose as a reference of maximum bioavailability. The purpose of this report is to describe a different experimental strategy which utilizes plasma concentration and urinary excretion data together so as to permit greater flexibility in the design of bioavailability protocols.

THEORETICAL

Plasma and renal clearances are physiological phenomena which should not be model dependent. Estimates of plasma and renal clearances become model dependent when their values are inferred from pharmacokinetic parameters. Observed plasma concentrations are manifestations of the net effect of absorption, distribution, metabolism, and excretion; hence, sets of plasma concentration data points by themselves are not model dependent. But mathematical reconstructions of plasma concentration data may be model dependent or uncertain when projections are made concerning AUC beyond the last data point. The proposed strategy for the assessment of bioavailability is based on determinations of renal clearance and assumptions about the constancy of plasma clearance or parts thereof following test doses of a drug.

Suppose the bioavailability of an oral dosage form is to be assessed by comparing it with an intravenous injection of the same drug. From the total urinary recovery of unchanged drug following the intravenous dose:

$$Ff = \frac{U_\infty}{D} \qquad \text{(Eq. 1)}$$

For an intravenous dose, $F = 1.0$; hence:

$$f^{iv} = \frac{U_\infty{}^{iv}}{D^{iv}} \qquad \text{(Eq. 2)}$$

where F is the fraction of the dose D that reaches the general circulation unchanged, f is the fraction of F that is excreted in the urine unchanged, U_∞ is the total amount of unchanged drug recovered in the urine, and the superscripts refer to the treatment. Renal clearance, $\dot{V}_{cl,r}$, of unchanged drug can be estimated from plasma samples taken over intervals coinciding with one or more fractional urine collection periods, *i.e.*:

$$\dot{V}^{iv}_{cl,r} = \frac{U_{t_2} - U_{t_1}}{\int_{t_1}^{t_2} Cp\, dt} \qquad \text{(Eq. 3)}$$

where U_{t_1} and U_{t_2} are the cumulative amounts of unchanged drug excreted in the urine at times t_1 and t_2, respectively; and $\int_{t_1}^{t_2} Cp\, dt$ is the area under the plasma curve from t_1 to t_2, which can be estimated by the trapezoidal rule or other suitable means. Plasma clearance is estimated by:

$$\dot{V}^{iv}_{cl,p} = \frac{\dot{V}^{iv}_{cl,r}}{f^{iv}} = \frac{D^{iv}\dot{V}^{iv}_{cl,r}}{U_\infty{}^{iv}} \qquad \text{(Eq. 4)}$$

Following oral administration, the observed fraction of the unchanged drug excreted in the urine is the product of the fraction absorbed and the fraction of the compound that is absorbed and excreted unchanged, *i.e.*:

$$F^{po}f^{po} = \frac{U_\infty{}^{po}}{D^{po}} \qquad \text{(Eq. 5)}$$

If plasma clearance is assumed to be constant between test doses, $\dot{V}^{iv}_{cl,p} = \dot{V}^{po}_{cl,p}$, then:

$$f^{po} = \frac{\dot{V}^{po}_{cl,r}}{\dot{V}^{po}_{cl,p}} = \frac{\dot{V}^{po}_{cl,r}}{\dot{V}^{iv}_{cl,p}} \qquad \text{(Eq. 6)}$$

and the fraction absorbed:

$$F^{po} = \frac{F^{po}f^{po}}{f^{po}} = \frac{U_\infty{}^{po}\dot{V}^{iv}_{cl,p}}{D^{po}\dot{V}^{po}_{cl,r}} \qquad \text{(Eq. 7)}$$

The assumption of constant plasma clearance may be reassessed by comparing observed values for $\dot{V}^{po}_{cl,r}$ and $\dot{V}^{iv}_{cl,r}$. If they are equal, it may be reasonable to accept the hypothesis of constant plasma clearance in the absence of contrary evidence. This is tantamount to saying that the ratio of fractional urinary recovery, Ff, is a measure of bioavailability when the ratio of $\dot{V}_{cl,r}$ to $\dot{V}_{cl,p}$ is constant between treatments. However, if they are not equal, several obvious alternatives can be considered. First, the observed differences may be ignored on the premise that the nonrenal components of plasma clearance would be compensating. Second, one may assume that only the renal components change but that the nonrenal components remain constant, in which event:

$$\dot{V}^{po'}_{cl,p} = \dot{V}^{po}_{cl,r} + \dot{V}^{iv}_{cl,p} - \dot{V}^{iv}_{cl,r} \qquad \text{(Eq. 8)}$$

and:

$$f^{po} = \frac{\dot{V}^{po}_{cl,r}}{\dot{V}^{po'}_{cl,p}} \qquad \text{(Eq. 9)}$$

Third, the nonrenal component of $\dot{V}_{cl,p}$ may be thought to vary in

Table II—Sample Calculation of Bioavailability Using the Intravenous Dose as Reference

Treatment	Clearance, ml./min. $\dot{V}_{cl,r}^{iv}$[a]	Clearance, ml./min. $\dot{V}_{cl,p}^{iv}$[b]	Clearance, ml./min. $\dot{V}_{cl,r}^{po}$[a]	Clearance, ml./min. $\dot{V}_{cl,p}^{po'}$[c]	Fraction of Dose Excreted in Urine, fF^d	Fraction of Dose Absorbed, F^e
Intravenous	266.2 (282.1)[f]	493.9 523.4			0.539	
Oral No. 1			223.1 (222.9)	450.8 (464.2)	0.265	0.536 (0.552)
Oral No. 2			186.6 (192.4)	414.3 (433.7)	0.306	0.680 (0.690)

[a] By Eq. 3. [b] By Eq. 4. [c] By Eq. 8. [d] By Eq. 1. [e] By Eq. 7. [f] See text.

Table III—Sample Calculation of Relative Bioavailability Using Oral Dose No. 2 as Reference

Treatment	Clearance, ml./min. $\dot{V}_{cl,r}^{s}$[a]	Clearance, ml./min. $[\dot{V}_{cl,p}^{s}]_{ex}$[b]	Clearance, ml./min. $\dot{V}_{cl,r}^{x}$[a]	Clearance, ml./min. $[\dot{V}_{cl,p}^{x}]_{ex}'$[c]	Fraction of Dose Excreted in Urine, fF^d	Relative Absorption, F^xF^{se}
Oral No. 1			223.1	646.3	0.265	0.768
Oral No. 2	186.6	609.8			0.306	

[a] By Eq. 3. [b] By Eq. 13. [c] By Eq. 17. [d] By Eq. 1. [e] By Eq. 16.

direct proportion to changes in the renal clearance, so that:

$$\dot{V}_{cl,p}^{po''} = \frac{\dot{V}_{cl,r}^{po}}{\dot{V}_{cl,r}^{iv}} \cdot \dot{V}_{cl,p}^{iv} \qquad \text{(Eq. 10)}$$

and:

$$^{po} = \frac{\dot{V}_{cl,r}^{po}}{\dot{V}_{cl,p}^{po''}} \qquad \text{(Eq. 11)}$$

Finally, one may take the view that none of the first three alternatives is reasonable; hence, no estimate of bioavailability is warranted. The choice of alternatives may be dictated by one's understanding of the physicochemical and pharmacological properties of the drug and prior knowledge of its disposition in animals and in man. For example, changes in cardiac output would influence $\dot{V}_{cl,p}$ and $\dot{V}_{cl,r}$ and the other excretory components of $\dot{V}_{cl,p}$ proportionately, whereas changes in urinary pH may affect only $\dot{V}_{cl,r}$ of some drugs.

The proposed strategy applies even when an intravenous dose is not included. In such cases, one treatment is usually designated as the standard (s) to which all other treatments (x) are compared. Accordingly:

$$\frac{U_\infty^s}{D^s} = F^s f^s \qquad \text{(Eq. 12)}$$

and:

$$[\dot{V}_{cl,p}^{s}]_{ex} = \frac{\dot{V}_{cl,r}^{s}}{F^s f^s} \qquad \text{(Eq. 13)}$$

The quantity $[\dot{V}_{cl,p}^{s}]_{ex}$ denotes an experimentally derivable value which is only proportional to the true plasma clearance, $\dot{V}_{cl,p}^{s}$, in that:

$$\dot{V}_{cl,p}^{s} = F^s[\dot{V}_{cl,p}^{s}]_{ex} \qquad \text{(Eq. 14)}$$

For $\dot{V}_{cl,r}^{s} = \dot{V}_{cl,r}^{x}$:

$$f^x = \frac{\dot{V}_{cl,r}^{x}}{\dot{V}_{cl,p}^{x}} = \frac{\dot{V}_{cl,r}^{s}}{\dot{V}_{cl,p}^{x}} = \frac{\dot{V}_{cl,r}^{x}}{F^s[\dot{V}_{cl,p}^{s}]_{ex}} \qquad \text{(Eq. 15)}$$

Relative absorption is given by:

$$\frac{F^x}{F^s} = \frac{F^x f^x}{F^s f^x} = \frac{U_\infty^x \cdot [\dot{V}_{cl,p}^{s}]_{ex}}{D^x \dot{V}_{cl,r}^{x}} \qquad \text{(Eq. 16)}$$

Corrective measures, when indicated, should be applied to $[\dot{V}_{cl,p}^{s}]_{ex}$. Thus, Eqs. 8 and 10 are revised to read as follows:

$$[\dot{V}_{cl,p}^{x}]_{ex}' = \dot{V}_{cl,r}^{x} + [\dot{V}_{cl,p}^{s}]_{ex} - \dot{V}_{cl,r}^{s} \qquad \text{(Eq. 17)}$$

and:

$$[\dot{V}_{cl,p}^{x}]_{ex}'' = \frac{\dot{V}_{cl,r}^{x}}{\dot{V}_{cl,r}^{s}} \cdot [\dot{V}_{cl,p}^{s}]_{ex} \qquad \text{(Eq. 18)}$$

DISCUSSION

Experimentally, the proposed method calls for the complete collection of urine in planned increments. Plasma sampling times should at least coincide with selected incremental urinary collection periods. Intermediate plasma samples may also be indicated to permit accurate determinations of the incremental areas under the plasma curve, which is essential to the calculations of renal clearance. Assays of plasma and urine specimens should be specific for unchanged drug. The number of plasma points may be minimized by choosing relatively short urine collection periods at times when plasma concentrations do not change rapidly. It is also prudent to include more than one estimate of $\dot{V}_{cl,r}$ per treatment. In this regard, it might be advantageous to make estimates of $\dot{V}_{cl,r}$ from contiguous urine collection periods. This is so in respect both to economy in the required number of plasma data points and to the modulation of experimental difficulties in voiding completely and on time. (See Appendix for sample calculations.)

The description of the proposed method is confined to the case where bioavailability is defined as the amount, or relative amount, of drug reaching the general circulation unchanged. In some cases, such as with prodrugs, it may be more appropriate to define bioavailability as that fraction, or relative fraction, of the dose reaching the general circulation as a specific metabolite. The proposed strategy accommodates these situations with the following provisions. Estimates of $\dot{V}_{cl,r}$ and $\dot{V}_{cl,p}$ are those of the metabolite. Equation 4 applies only if the intravenous dose is administered as the metabolite; otherwise, only estimates of relative absorption would be possible, and Eq. 13 should be used regardless of the route of administration. Because the method relies on estimates of $\dot{V}_{cl,p}$ or $[\dot{V}_{cl,p}]_{ex}$, it may be considered only when bioavailability can be appropriately defined in terms of drug or metabolite(s) reaching the general circulation. Thus, if metabolism is route dependent, then, by definition, drug metabolized on the first pass through the liver is nonavailable. For this reason, this method is generally inappropriate if estimates of bioavailability of a drug are attempted through measurements of metabolite(s).

In summary, the proposed method requires estimates of U_∞ and $\dot{V}_{cl,r}$, from which $\dot{V}_{cl,p}$ or $[\dot{V}_{cl,p}]_{ex}$, f, and F can be calculated. Some salient features are:

1. It is model independent. The only assumptions are those related to plasma clearance or components thereof. It should be recalled that the same assumptions concerning $\dot{V}_{cl,p}$ and/or $\dot{V}_{cl,r}$ are implicit in all pharmacokinetic methods; however, the proposed method does not entail further assumptions concerning individual

or hybrid model parameters. Since estimates of $\dot{V}_{cl,p}$ and $\dot{V}_{cl,r}$ are not influenced by the nature of the absorption process, this method should apply even when absorption is inordinately slow or defies mathematical description. At the same time, the techniques are not only compatible with but also may be applied advantageously in conjunction with pharmacokinetic techniques.

2. A complete definition of the time course of change in plasma concentrations is not needed; thus, the required number of plasma data points is flexible. It is, therefore, particularly well suited in situations where large volumes of plasma are required per sample, such as when the assay method lacks sensitivity or when the dosages are low. If necessary, several doses may be given repetitively so that plasma levels will be sufficiently high before estimates of $\dot{V}_{cl,r}$ are made. In this event, one would obtain an assessment of average bioavailability upon repetitive dosing, but one does not need to wait for the attainment of steady state before terminating the study.

3. Since $\dot{V}_{cl,p}$ is an estimate of the net result of physiological disposition, the method should yield valid assessments of bioavailability even when enterohepatic recirculation exists or is suspected.

4. Even though the present discussion on intrasubject variations, manifested as changes in $\dot{V}_{cl,r}$, considers only alternative assumptions, concomitant measurements of metabolic turnover rates and renal clearances of endogenous substances, particularly those with both renal and extrarenal components of elimination, are seen as interesting possibilities as independent indicators of change in $\dot{V}_{cl,r}$ and $\dot{V}_{cl,p}$. This would entail tracer doses of the indicator, which should be selected among candidates having elimination patterns resembling those of the drug being studied. Various applications of the proposed method will be discussed in future reports.

APPENDIX

Sample calculations using the proposed method are performed on a set of simulated data for a subject who received the same dose of a drug intravenously once and orally on two other occasions. Except for the renal clearance of unchanged drug, all other aspects of drug disposition remained constant for each of the three test doses. Absorption was arbitrarily fixed at 55.0 and 68.8% for the two oral doses. Typical plasma concentration and urinary excretion data for unchanged drug simulated according to the prescribed conditions are shown in Table I.

Application of the proposed method does not require all of the data in Table I. An assessment of bioavailability can be attempted from just a few selected points, such as those shown in parentheses. Sample calculations using the intravenous dose as the standard reference are shown in Table II. Renal clearances are calculated from incremental urinary recoveries at 2–3 and 3–4 hr. and trapezoid areas under the plasma curve for the corresponding times. Since they are different for each test dose, estimates of $\dot{V}_{cl,p}^{po\prime}$ are made with the aid of Eq. 8. The fractions absorbed, F^{po}, are then calculated from $\dot{V}_{cl,r}^{po}$, $\dot{V}_{cl,p}^{po\prime}$, and U_{∞}^{po} using Eq. 7. The difference between the calculated and the true fraction absorbed is due to the trapezoid approximation of areas under the plasma curve. Had the true areas been used, the answers would have been exact; these are shown in parentheses.

With the assumption that there were no data from an intravenous dose, calculations of relative absorption from the two oral doses are shown in Table III in which the second oral dose is designated as the standard. The answer, $F^2/F^1 = 0.767$, is slightly different from the ratio of $F^1/F^2 = 0.788$ obtained in Table II using the intravenous dose as the standard. This is because the appropriate expressions for estimating $\dot{V}_{cl,p}^{po\prime}$ (Eq. 8) and $[\dot{V}_{cl,p}^{2}]_{ex}{}'$ (Eq. 17) in this example are not linearly related to $\dot{V}_{cl,p}^{iv}$ and $[\dot{V}_{cl,p}^{1}]_{ex}$, respectively; hence, an exact proportionality is not expected when different standards are used. Conversely, when linear expressions, such as Eqs. 9 and 18, are appropriate, exact correspondence between ratios should result regardless of how the standards are chosen.

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