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ORAL BIOAVAILABILITY AND FIRST-PASS EFFECTS

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ABSTRACT:

Existing experimental strategies for the *in vivo* evaluation of factors affecting oral bioavailability have been reviewed. Based on concepts that have evolved, an integrated set of strategies emerges that appears capable of providing estimates of the individual contributions attributable to absorption, losses in the gut lumen, and first-pass elimination in the gut wall and the liver. The only assumptions are linear pharmacokinetics and constant clearance between treatments. These methods are also suitable for the assessment of metabolite bioavailability after drug administration and the quantitative determination of sites of biotransformation and metabolite formation *in vivo*.

Historically, the concept of bioavailability is closely, if not exclusively, associated with dosage form performance. This is because the drug entity has been defined and its absorption and disposition characteristics *per se* are fixed. Recently, the application of bioavailability principles and techniques has been extended to include animal studies in the selection of potential drug candidates for development. In particular, poor oral bioavailability is increasingly an issue in the drug discovery process. In situations where different chemical entities are under investigation, dosage form performance is just one of the possible contributing factors to poor oral bioavailability. Other possibilities include diminished access for absorption because of chemical degradation, physical inactivation, and insufficient contact time in transit through the gastrointestinal tract; poor permeability across the gastrointestinal mucosa; and elimination during the first passage through the gut wall and the liver. Reliable estimates of the relative importance of these causative factors are essential as guides to chemical modifications aimed to optimize oral bioavailability.

There is a body of literature on the subject of presystemic events and first-pass elimination and their evaluation *in vivo* (1–32). However, existing strategies and methods do not possess the flexibility and versatility that current applications demand. The main drawbacks are that key variables are incompletely resolved and parameter estimates are often confounded by simplifying assumptions attendant to their solution. The liver has been most extensively studied, and its contribution to oral bioavailability is well defined. The isolation and quantitation of the remaining components are problematic and usually predicated on assumptions such as the dose being completely absorbed unchanged, biotransformation not occurring in the gut wall; the liver being the only drug metabolizing organ; etc. While such qualifying assumptions may be appropriate in specific situations, they detract from the general applicability of a method. The purpose of this communication is to consider supplemental strategies in search of wider applicability.

Theoretical

The bioavailability of an administered substance is that fraction of the dose that reaches the general circulation unchanged. The general circulation is defined experimentally by the sampling site, usually a blood vessel in the peripheral circulation. Fig. 1 is a schematic representation of drug movement after oral ingestion. As the drug passes down the gastrointestinal tract, part of the dose may not be available for absorption because of chemical degradation, physical inactivation through binding or complexation, microbial biotransformation, etc. Of that which is absorbed at *x*, some may be metabolized in transit through the gut wall. Unchanged drug that reaches the hepatic portal vein *p* may be extracted by the liver by way of biotransformation or biliary excretion. Finally, further elimination may occur between the hepatic vein *h* and the site of measurement, say a peripheral vein *j* or a peripheral artery *a*.

Thus, the bioavailability F′ of an orally administered dose is comprised of the individual fractions that survive the various barriers encountered by the drug during its first passage from the gut lumen to the sampling site (15, 27), *viz.*,

$$F' = F_X F_G F_H F_S \quad (1)$$

where F_X is the fraction absorbed (*i.e.* net transport of unchanged drug into and around the absorptive cells of the gastrointestinal tract), F_G is the fraction that is not metabolized in a single passage through the gut wall, F_H is the fraction that is not extracted during the first passage through the liver, and F_S is the fraction that survives post-hepatic elimination *en route* to the sampling site. By definition, therefore, nonabsorption and lumenal elimination are represented by the quantity $(1 - F_X)$.

Eq. 1 formally defines oral bioavailability and its component parts. In practice, however, explicit knowledge of F_S is seldom sought. Hence, the more familiar definition of oral bioavailability, F, is that given by eq. 2.

$$F = F_X F_G F_H \quad (2)$$

The difference between eqs. 1 and 2 is in the point of reference, *i.e.* how one defines the general circulation. Whereas F′ refers to that fraction of an oral that reaches the sampling site unchanged, F is effectively a measure of drug availability to the hepatic venous circulation. Experimentally, different sites of drug administration are indicated in the determination of total body clearance (33–35).

Experimental strategies will be developed to isolate and quantify the individual components shown on the right-hand side of eq. 1. Each

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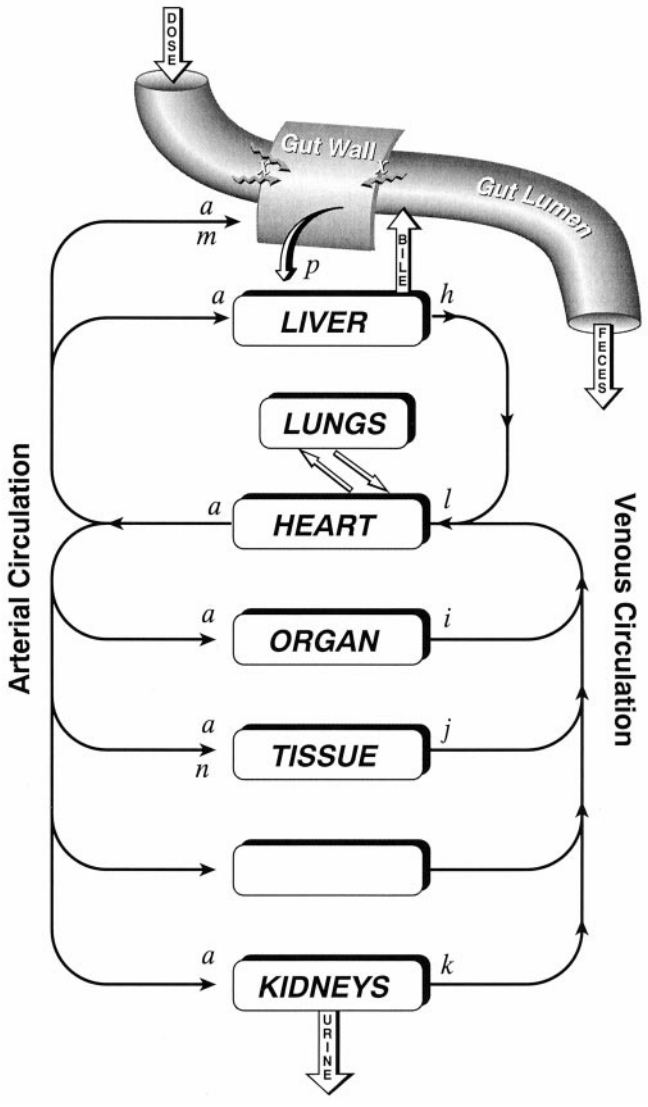

FIG. 1. *Schematic representation of drug movement in the body.*

Lower case letter mark sites of possible interest: Absorption sites *x*; the hepatic portal vein *p*; the hepatic vein *h*; peripheral veins *i, j, k, l*; peripheral arteries *a*; the mesenteric artery m; and arterial supply *n* to venous effluent at *j*.

case involves the grouping of experiments in which the sites of administration and measurement are systematically varied to yield estimates of the desired parameters (1, 13, 14, 19). Experimental designs will evolve from the familiar to the more esoteric in search of greater precision and efficiency. Assumptions are that total body clearance is independent of concentration and constant between treatments. Moreover, individual components of body clearance, especially those of primary interest, *e.g.* gut wall and liver, need to be invariant between treatments.

Measurement in the Peripheral Circulation Only. Let's begin with the evaluation of bioavailability as defined by eq. 2. The amount of drug that reaches general circulation after an oral dose, D^{po}, is

$$F\,D^{po} = CL\,AUC_j^{D^{po}} \tag{3}$$

where AUC is the area under the plasma, serum, or blood drug concentration curve from time zero to time infinity; the superscript refers to the route of drug administration, the subscript *j* denotes a venous sampling site; and CL is the total body clearance that is usually determined in a separate experiment, such that

$$CL = \frac{D^{iv,i}}{AUC_j^{D^{iv,i}}} \tag{4}$$

where $D^{iv,i}$ refers to an intravenous dose of drug administered to a peripheral vein *i*. Combining eqs. 2–4, one obtains

$$F_X\,F_G\,F_H = \frac{D^{iv,i}}{D^{po}}\,\frac{AUC_j^{D^{po}}}{AUC_j^{D^{iv,i}}} \tag{5}$$

Similarly, the bioavailability of an intravenous dose of drug administered to the portal vein, $D^{iv,p}$, can be represented by eq. 6.

$$F_H = \frac{D^{iv,i}}{D^{iv,p}}\,\frac{AUC_j^{D^{iv,p}}}{AUC_j^{D^{iv,i}}} \tag{6}$$

which, when divided into eq. 5, yields

$$F_X\,F_G = \frac{D^{iv,p}}{D^{po}}\,\frac{AUC_j^{D^{po}}}{AUC_j^{D^{iv,p}}} \tag{7}$$

To resolve $F_X\,F_G$, it would be necessary to effect an independent estimate of F_X or F_G. It is generally recognized that absorption of xenobiotics occurs mainly in the intestines, especially the upper part of the small intestine. It follows, therefore, that the intestinal wall contributes most prominently to gut-wall metabolism. Accordingly, an intra-arterial dose of drug to the superior (cranial) mesenteric artery, $D^{ia,m}$, should yield an estimate of $F_G\,F_H$. That is to say,

$$F_G\,F_H = \frac{D^{iv,i}}{D^{ia,m}}\,\frac{AUC_j^{D^{ia,m}}}{AUC_j^{D^{iv,i}}} \tag{8}$$

Dividing eq. 8 into eq. 5,

$$F_X = \frac{D^{ia,m}}{D^{po}}\,\frac{AUC_j^{D^{po}}}{AUC_j^{D^{ia,m}}} \tag{9}$$

Finally, from eqs. 7 and 9,

$$F_G = \frac{D^{iv,p}}{D^{ia,m}}\,\frac{AUC_j^{D^{ia,m}}}{AUC_j^{D^{iv,p}}} \tag{10}$$

The experimental determination of F_S should nominally include an intravenous dose to the hepatic vein, $D^{iv,h}$, as the test treatment. However, $D^{iv,i}$ is not a suitable reference in the determination of total body clearance. This is because the first-pass effects encountered by $D^{iv,i}$ are virtually identical to those operating on $D^{iv,h}$ such that

$$\frac{AUC_j^{D^{iv,h}}}{D^{iv,h}} \cong \frac{AUC_j^{D^{iv,i}}}{D^{iv,i}} \tag{11}$$

Peripheral venous sites of administration other than *i* are similarly unsuitable.

One way to avoid this dispositional overlap is to administer the drug intra-arterially at site *n* in fig. 1 such that

$$CL' = \frac{D^{ia,n}}{AUC_j^{D^{ia,n}}} \tag{12}$$

where CL′ is total body clearance as defined by the site of administration *n* and the site of measurement *j* (27, 33); *n* is the arterial supply to the organ or tissue for which *j* is the venous effluent. For now, let's assume that no drug elimination occurs between *n* and *j*. Under these circumstances, the product of CL′ and $AUC_j^{D^{iv,h}}$ is the amount of drug

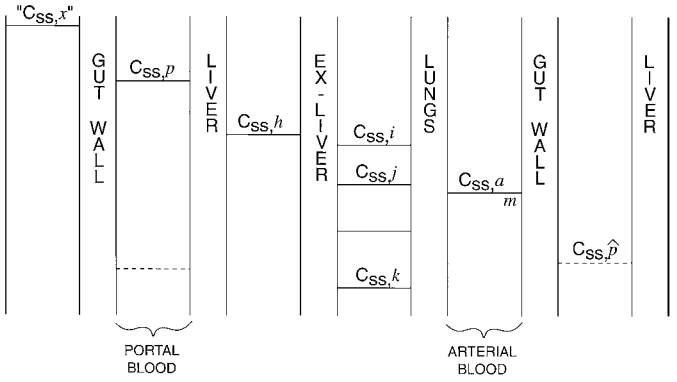

FIG. 2. *Schematic diagram of steady-state concentrations, C_{ss}, at sites of interest during a continuous perfusion of drug solution to the gastrointestinal tract at a constant rate.*

Lower case letter designation have the same meaning as in fig. 1. "$C_{ss,x}$" is the effective steady-state concentration at the absorption site *x*. See text for the definition of $C_{ss,}$.

that reaches the sampling site following $D^{iv,h}$. Hence,

$$F_S = \frac{CL'\ AUC_j^{D^{iv,h}}}{D^{iv,h}} = \frac{D^{ia,n}}{D^{iv,h}} \frac{AUC_j^{D^{iv,h}}}{AUC_j^{D^{ia,n}}} \tag{13}$$

Combining eqs. 11 and 13,

$$F_S \cong \frac{D^{ia,n}}{D^{iv,i}} \frac{AUC_j^{D^{iv,i}}}{AUC_j^{D^{ia,n}}} \tag{14}$$

Eq. 14 is experimentally preferable to eq. 13 in that a peripheral vein is more accessible than the hepatic vein. Moreover, the evaluation of F_S would entail only one additional treatment, $D^{ia,n}$, rather than two, $D^{ia,n}$ and $D^{iv,h}$.

Total body clearance, CL or CL′, may be calculated from serum, plasma, or blood concentration data as long as the same medium is used consistently in bioavailability assessment.

For clarity, ensuing discussions will dispense with F_S, the assessment of which can always be amended with an additional experiment. Furthermore, since all peripheral veins are interchangeable as sites of administration, the site qualifiers for D^{iv} are no longer necessary and will be dropped. The $D^{iv,p}$ designation is retained for drug administration to the hepatic portal vein, however. Whereas peripheral veins appear to be equivalent as sites of administration, they are not interchangeable as sampling sites. Conversely, peripheral sampling sites on the arterial side are equivalent, but administration to each artery engenders a unique first-pass effect. Therefore, data used to extract pharmacokinetic parameters should come from samples taken from a common venous sampling site. Data derived from samples taken from peripheral arteries are not similarly constrained. For this reason, subsequent developments will designate an artery *a* as the peripheral sampling site.

Measurements in the Portal and Peripheral Circulation. Concomitant measurements in the portal and peripheral blood provide a new dimension in experimental design. Suppose the gastrointestinal tract were subjected to a continuous perfusion at a constant rate of a drug solution of fixed composition. At steady state, blood concentrations C_{ss} at individual sampling sites become time invariant. Fig. 2 depicts steady-state concentrations at sampling sites of possible interest for a drug that is capable of being absorbed and the eliminating organs for which include the gut wall and the liver. The rate of drug delivery, R, from the gut lumen to the portal circulation can be estimated (23) by

$$R = Q_p(C_{ss,p} - C_{ss,\hat{p}}) \tag{15}$$

where Q_p is the blood flow rate in the hepatic portal vein, $C_{ss,p}$ is the observed concentration in portal blood at steady state, and $C_{ss,}$ is that part of $C_{ss,p}$ represented by drug returning from the general circulation. The difference between $C_{ss,p}$ and $C_{ss,}$, therefore, represents new contributions from the gut lumen. The relationship between $C_{ss,p}$ and $C_{ss,}$ can be visualized by rolling fig. 2 back on itself to form a cylinder wherein vertical bars representing "gut wall" and "liver" on the far right coincide with their counterparts on the left. In this alignment, $C_{ss,p}$ and $C_{ss,}$ appear in the same column representing the portal vein.

By analogy to eq. 15, the total amount of drug that reaches the portal circulation from the gut following a single oral dose is

$$F_X\ F_G\ D^{po} = Q_p(AUC_p^{D^{po}} - AUC_{\hat{p}}^{D^{po}}) \tag{16}$$

$AUC^{D^{po}}$ is not an experimentally observable entity, but its value can be deduced from the corresponding area measured in samples taken from a peripheral blood vessel, say, $AUC_a^{D^{po}}$.

From an intravenous dose, one obtains $AUC_a^{D^{iv}}$ and $AUC_p^{D^{iv}}$. Since there is no lumenal source of drug after an intravenous dose,

$$AUC_{\hat{p}}^{D^{iv}} = AUC_p^{D^{iv}} \tag{17}$$

Furthermore, in a linear system with constant clearance between treatments, the ratio of AUC to AUC_a is invariant regardless of the route of administration and numerically equal to that following an iv dose; *i.e.*

$$\frac{AUC_{\hat{p}}^{D^{po}}}{AUC_a^{D^{po}}} = \frac{AUC_{\hat{p}}^{D^{ia,m}}}{AUC_a^{D^{ia,m}}} = \ldots = \frac{AUC_{\hat{p}}^{D^{iv}}}{AUC_a^{D^{iv}}} \tag{18}$$

Combining eqs. 16–18,

$$F_X\ F_G = \frac{Q_p}{D^{po}}\left[AUC_p^{D^{po}} - \frac{AUC_a^{D^{po}}}{AUC_a^{D^{iv}}}\ AUC_p^{D^{iv}}\right] \tag{19}$$

Given that

$$F_X\ F_G\ F_H = \frac{D^{iv}}{D^{po}}\ \frac{AUC_a^{D^{po}}}{AUC_a^{D^{iv}}} \tag{20}$$

dividing eq. 19 into eq. 20 yields

$$F_H = \frac{D^{iv}}{Q_p}\left\{\frac{AUC_a^{D^{po}}}{AUC_p^{D^{po}}\ AUC_a^{D^{iv}} - AUC_a^{D^{po}}\ AUC_p^{D^{iv}}}\right\} \tag{21}$$

Similarly, the amount of drug that reaches the portal circulation after a dose to the mesenteric artery is

$$F_G\ D^{ia,m} = Q_p(AUC_p^{D^{ia,m}} - AUC_{\hat{p}}^{D^{ia,m}}) \tag{22}$$

which, when combined with eqs. 17 and 18, yields

$$F_G = \frac{Q_p}{D^{ia,m}}\left(\frac{AUC_p^{D^{ia,m}}\ AUC_a^{D^{iv}} - AUC_a^{D^{ia,m}}\ AUC_p^{D^{iv}}}{AUC_a^{D^{iv}}}\right) \tag{23}$$

Finally, dividing eq. 23 into eq. 19, one obtains

$$F_X = \frac{D^{ia,m}}{D^{po}}\left\{\frac{AUC_p^{D^{po}}\ AUC_a^{D^{iv}} - AUC_a^{D^{po}}\ AUC_p^{D^{iv}}}{AUC_p^{D^{ia,m}}\ AUC_a^{D^{iv}} - AUC_a^{D^{ia,m}}\ AUC_p^{D^{iv}}}\right\} \tag{24}$$

Simultaneous measurement in the portal vein and a peripheral artery eliminates the need for an iv,p treatment. There are, however,

twice as many measurements in the remaining treatments. In addition, the application of eqs. 21 and 23 requires explicit knowledge of the blood flow rate in the portal vein; implicit in eq. 24 is the assumption of constant Q_p between treatments. Depending how precisely one needs to separate the parameters F_X, F_G, and F_H, one can either measure Q_p directly (35–38) or rely on literature values (39). With Q_p defined as blood flow rate, drug concentrations in whole blood should be used in eqs. 21 and 23.

Simultaneous Measurement of Drug and Metabolite. Let's define F_{m_i} as the bioavailability of a specific metabolite m_i after oral administration of the parent drug, *viz.*

$$F_{m_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_{m_i,a}^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}}} \tag{25}$$

where M_i is the dose administered as m_i and AUC_{m_i} is the area under the m_i concentration curve from time zero to time infinity. Other superscripts and subscripts have the same meaning as before. Molar equivalents of drug and metabolite should be used throughout to account for the difference in their molecular weight.

Similarly, F_{X,m_i}, F_{G,m_i}, and F_{H,m_i} are, respectively, the fraction that is absorbed as m_i from the lumen following an oral dose of drug, the fraction that reaches the portal vein as m_i following a single passage of drug through the gut wall, and the fraction that reaches the hepatic vein as m_i following a single passage of drug through the liver. Finally, $F_{m_i}^{M_i}$, $F_{X,m_i}^{M_i}$, $F_{G,m_i}^{M_i}$, and $F_{H,m_i}^{M_i}$ are to m_i following metabolite m_i administration as F, F_X, F_G, and F_H are, respectively, to drug following drug administration.

The quantity F_{m_i} is comprised of m_i that is derived from parent drug that reached the general circulation initially as drug and m_i that reaches the sampling site for the first time as m_i *per se*. That fraction of the total body clearance of drug that is available as m_i is f_{m_i}. The bioavailability of drug after an oral dose D^{po} is F. Hence, the systemic source of F_{m_i} is f_{m_i} F. The nonsystemic component of F_{m_i} is the sum of the bioavailability of m_i that is formed in the gut lumen, $F_{X,m_i} F_{G,m_i}^{M_i} F_{H,m_i}^{M_i}$, and m_i that is formed and survived during first passage of the parent through the gut wall and liver. The latter is composed of m_i molecules that are formed in the liver and survived, $F_X F_G F_{H,m_i}$, and m_i molecules in the portal circulation that survived a single passage through the liver, $F_X F_{G,m_i} F_{H,m_i}^{M_i}$. Thus,

$$F_{m_i} = F_{X,m_i} F_{G,m_i}^{M_i} F_{H,m_i}^{M_i} + F_X(F_G F_{H,m_i} + F_{G,m_i} F_{H,m_i}^{M_i}) + f_{m_i} F \tag{26}$$

The fraction f_{m_i} of drug clearance that is bioavailable as m_i is given by eq. 27.

$$f_{m_i} = \frac{M_i^{iv}}{D^{iv}} \frac{AUC_{m_i,a}^{D^{iv}}}{AUC_{m_i,a}^{M_i^{iv}}} \tag{27}$$

Therefore,

$$f_{m_i} F = \frac{M_i^{iv}}{D^{po}} \frac{AUC_{m_i,a}^{D^{iv}} AUC_a^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}} AUC_a^{D^{iv}}} \tag{28}$$

By analogy to eqs. 25 and 26, the bioavailability of m_i after a dose of drug to the mesenteric artery is given by eq. 29.

$$f_{m_i} F_G F_H + F_G F_{H,m_i} + F_{G,m_i} F_{H,m_i}^{M_i} = \frac{M_i^{iv}}{D^{ia,m}} \frac{AUC_{m_i,a}^{D^{ia,m}}}{AUC_{m_i,a}^{M_i^{iv}}} \tag{29}$$

The fraction of D^{po} that is absorbed as drug is F_X, such that

$$F_X = \frac{D^{ia,m}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_a^{D^{ia,m}}} \tag{30}$$

The product of eqs. 29 and 30 is, therefore,

$$f_{m_i} F + F_X F_G F_{H,m_i} + F_X F_{G,m_i} F_{H,m_i}^{M_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}} AUC_{m_i,a}^{D^{ia,m}}}{AUC_a^{D^{ia,m}} AUC_{m_i,a}^{M_i^{iv}}} \tag{31}$$

The difference between eq. 31 and eq. 28 is the contribution of first-pass metabolism to the bioavailability of m_i from an oral dose of drug, *viz.*

$$F_X F_G F_{H,m_i} + F_X F_{G,m_i} F_{H,m_i}^{M_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}}} \left\{ \frac{AUC_{m_i,a}^{D^{ia,m}}}{AUC_a^{D^{ia,m}}} - \frac{AUC_{m_i,a}^{D^{iv}}}{AUC_a^{D^{iv}}} \right\} \tag{32}$$

The two terms on the left-hand side of eq. 32 represent the respective contributions of m_i formed in the liver and the gut wall. Separate estimates for each can be effected by administering drug to the portal vein, following which the bioavailability of m_i is given by eq. 33.

$$f_{m_i} F_H + F_{H,m_i} = \frac{M_i^{iv}}{D^{iv,p}} \frac{AUC_{m_i,a}^{D^{iv,p}}}{AUC_{m_i,a}^{M_i^{iv}}} \tag{33}$$

Since

$$F_X F_G = \frac{D^{iv,p}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_a^{D^{iv,p}}} \tag{34}$$

the product of eqs. 33 and 34 is

$$f_{m_i} F + F_X F_G F_{H,m_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}} AUC_{m_i,a}^{D^{iv,p}}}{AUC_a^{D^{iv,p}} AUC_{m_i,a}^{M_i^{iv}}} \tag{35}$$

Subtracting eq. 28 from eq. 35 and then eq. 36 from eq. 32, one gets

$$F_X F_G F_{H,m_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}}} \left\{ \frac{AUC_{m_i,a}^{D^{iv,p}}}{AUC_a^{D^{iv,p}}} - \frac{AUC_{m_i,a}^{D^{iv}}}{AUC_a^{D^{iv}}} \right\} \tag{36}$$

and

$$F_X F_{G,m_i} F_{H,m_i}^{M_i} = \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}}} \left\{ \frac{AUC_{m_i,a}^{D^{ia,m}}}{AUC_a^{D^{ia,m}}} - \frac{AUC_{m_i,a}^{D^{iv,p}}}{AUC_a^{D^{iv,p}}} \right\} \tag{37}$$

Finally, the bioavailability of lumenally formed m_i is the difference between eq. 26 and eq. 31, *viz.*

$$\begin{aligned} F_{X,m_i} F_{G,m_i}^{M_i} F_{H,m_i}^{M_i} &= F_{m_i} - f_{m_i} F - F_X F_G F_{H,m_i} - F_X F_{G,m_i} F_{H,m_i}^{M_i} \\ &= \frac{M_i^{iv}}{D^{po}} \frac{AUC_a^{D^{po}}}{AUC_{m_i,a}^{M_i^{iv}}} \left\{ \frac{AUC_{m_i,a}^{D^{po}}}{AUC_a^{D^{po}}} - \frac{AUC_{m_i,a}^{D^{ia,m}}}{AUC_a^{D^{ia,m}}} \right\} \end{aligned} \tag{38}$$

The key expressions are eqs. 25, 28, 32, and 38. They indicate that the overall bioavailability F_{m_i} can be separated into its lumenal, first-pass, and systemic components through the simultaneous measurement of drug and m_i following D^{po}, $D^{ia,m}$, D^{iv}, and M_i^{iv}. The gut-wall and hepatic contributions to first-pass drug elimination and m_i bioavailability can be separated from each other by the addition of $D^{iv,p}$. Further resolution is possible through the additional treatments of M_i^{po}, $M_i^{ia,m}$, and $M_i^{iv,p}$ to obtain estimates of m_i formation in the lumen and bioavailability across the gut wall and liver. Assumptions that

pertain to m_i disposition are the same as those for drug, *i.e.* clearances are independent of concentration and constant between treatments. Reversibility in the biotransformation of drug and m_i to each other is not excluded conceptually but may require some change in form to accommodate the definition of clearance (40).

Discussion

Experimental strategies have been outlined to isolate and quantify the individual elements that contribute to the bioavailability of drug and metabolite after an oral dose of drug. They represent an integration of and extensions to existing methods (1, 7, 15, 17, 19, 23, 25, 29, 32). Depending on the kind of information being sought, experiments may involve drug and metabolite administration orally, intravenously to the hepatic portal vein and a peripheral vein, and intra-arterially to the mesenteric artery followed by the measurement of drug and metabolite in blood samples taken from the hepatic portal vein, a peripheral vein, and a peripheral artery. Assumptions are linear pharmacokinetics and constant clearance between treatments.

Questions encountered in the selection of compounds with optimal oral bioavailability for further development as a potential drug are different from those in support of clinical evaluation of a selected compound in man or other target animals. Instead of whether the bioavailability of a compound has been adversely affected by formulation or whether the intended effect of the formulation to enhance or modulate has been achieved, one is more likely to be interested in the factors affecting oral bioavailability and their respective contributions to the observed value. In drug discovery, therefore, it would be highly desirable to be able to separate and quantify lumenal events from systemic ones and permeability issues from first-pass effects for individual compounds under investigation. Coincidentally, there is also greater flexibility in experiment design in the preclinical evaluation of drug candidates.

Heretofore, estimates of fraction absorbed have been based on the assumption of no gut-wall metabolism or on nonspecific measures such as drug-related substances recovered in the urine or AUC of total radioactivity. Such estimates are not useful in the resolution of F_XF_G into its components. On the other hand, drug administration to the arterial supply of the gut provides an opportunity to assess metabolism by the gut-walls free from the confounding effects of gastrointestinal absorption. First choice among such arteries is the superior (cranial) mesenteric because it has the widest coverage of absorptive surfaces along the gut. To the extent that a reasonable estimate of F_G can be effected, the magnitude of the corresponding F_X provides useful direction for new compound synthesis. For example, a high value of F_X would indicate good membrane permeability while a low value suggests poor net transport or significant lumenal loss, $1\text{-}F_X$. The relative magnitudes of F_X and F_GF_H would distinguish transport problems from first-pass elimination.

Because experimental parameters are often dependent on the method for their determination, they can be defined more precisely after the fact. The assessment of oral bioavailability and its components is typical. The definition of F is dependent on the choice of reference for the determination of body clearance and the sampling site. Insofar as F_H can be determined under well-defined experimental conditions, F_XF_G is precisely defined by the ratio F/F_H. Conversely, by sampling hepatic portal blood, one can estimate F_XF_G directly and define F_H as F/F_XF_G. By definition, F_XF_G is clearly the net effect of drug absorption and first-pass gut-wall metabolism. However, the experimental separation and quantitation of F_X and F_G are seldom, if ever, attempted. Most of the reported strategies are based on the assumption that the drug is either completely absorbed (8, 12, 15–18, 25, 27, 28) or not metabolized in the gut wall (7, 8, 12, 15, 31). In effect, F_XF_G has been experimentally defined either as drug absorption or gut-wall metabolism. Similarly, the experimental evaluation of F_X and F_G by dose administration to a mesenteric artery are subject to a different set of design constraints and dependencies. In subsequent discussion, it may be useful to treat bioavailability F and its components as net transport and survival to their respective experimentally defined reference points. By considering the formal definitions of each parameter and their possible dependencies on method, one may be better able to interpret the results. For example, would a molecule be registered as part of F_X, F_G, or F_H if it enters an enterocyte as drug, is conjugated there, and is deconjugated in the liver? What about a molecule that is absorbed in the stomach and is metabolized in the liver?

To the extent that the superior mesenteric vein is only one of the tributaries feeding the hepatic portal circulation, the proposed treatment of dosing to the superior mesenteric artery does not completely capture metabolic activities of the entire gut wall. Among the unrepresented regions are the stomach and the upper portion of the rectum. However, absorption must take place through these tissues for the metabolic activities therein to manifest. In consideration of factors such as tissue permeability, effective surface area, dwell time, and fecal impaction, contribution of the rectum to drug absorption after an oral dose is usually thought to be negligible while that of the stomach is about 10%; the remainder is attributed to the intestines, particularly the upper part of the small intestine (41–43). Since only a fraction of that which is absorbed through the stomach wall contributes to the overall bioavailability, the error associated with its neglect seems acceptably small. In situations where another segment of the gut is deemed to contribute more significantly than the small intestine, the site of administration should then be the artery supplying that segment. Dosing simultaneously to two or more loci may be more encompassing but is conceptually less desirable than dosing only to the region mostly responsible for metabolism in the gut wall. This is because such an undertaking would engender the need to apportion the relative contribution of each segment *a priori*. The consequences of incomplete coverage, albeit by design, is that estimates of the fraction absorbed F_X are somewhat biased on the high side to the extent that F_G is underestimated. This is because their product F_XF_G is unaffected by the nature of the experimental approximation. Drug molecules that are absorbed from the lower rectum directly into the inferior vena cava would also positively bias estimates of F_X. This source of error is probably insignificant after an oral dose but may be significant after rectal administration or in situations when drug is absorbed directly into the lymphatic system.

In addition, there may be situations in which not all of the metabolic activity extant in the intestinal epithelium is accessible to drug entering from the serosal side. This would result in an overestimate of F_G and a corresponding underestimation of F_X. Inasmuch as the product of F_X and F_G is unaffected, the decrement in F_X would appear as a corresponding increase in the fraction of the dose metabolized in the gut lumen. For example, a qualitative difference in biotransformation after oral and parenteral routes of administration may indicate that the enzyme system responsible for the orally-specific metabolite is not accessible to substrate delivered from the serosal side of the gut or that said metabolite is formed in the lumen and absorbed as such. Few attempts have been made to distinguish between the alternatives. In isolated intestinal segments, absence of conjugates in the effluent after the administration of phenol (44) and isoprenaline (20) to the arterial supply may indicate lack of penetration by these substrates. On the other hand, the presence of only nonconjugated metabolites of testosterone (44) in the effluent suggests transport into enterocytes but not

to the relevant phase II enzymes therein. Indirectly, substrate permeability into enterocytes from the systemic circulation may be inferred from studies on the inductive and inhibitory effects of xenobiotics on intestinal enzymes following inhalation or parenteral administration. Substances presumably polycyclic aromatic hydrocarbons from cigarette smoke (45) and intraperitonially administered dexamethasone (46) and some combination of phenobarbital, polyhalogenated biphenyls, and organochlorine pesticides (47) seem to have ready access to enterocytes while erythromycin (48) apparently does not. In view of the paucity and inconclusive nature of the evidence, the possibility of limited access should be considered in the design and interpretation of experiments. If one suspects incomplete access, comparative turnover rates in gut-wall homogenates *vs.* lumenal contents may be revealing. Also, high rates of metabolism in gut-wall homogenates may be incompatible with high estimates of F_G. Finally, as a practical matter, an acceptably high estimate of F_X or an unacceptably low estimate of $F_X F_G$ may be sufficiently decisive despite misapportionment.

Available evidence seems to suggest that systemic access to drug-metabolizing enzymes in enterocytes is compound specific (25). Complete access leads the best estimate of F_G and the highest resolution among F_X, F_G, and F_H. At the other end of the spectrum, total inaccessibility would result in no resolution between F_G and its neighbors F_X and F_H. Nonetheless, dose administration to the mesenteric artery, $D^{ia,m}$, is the preferred treatment in situations in which the primary objective is to assess the relative contribution of nonabsorption *vs.* first-pass elimination. In the worst case, one would obtain a valid estimate of F_H as if the dose had been administered to the hepatic portal vein. Except in the worst case, the result should be closer to the target than that following the alternative treatment $D^{iv,p}$.

In the assessment of F_S by eqs. 13 or 14, $D^{ia,n}$ should best be administered by infusion rather than as a bolus. This is to maximize the opportunities for representative sampling at site *j* and to ensure thereby a competent estimate of CL′. The assumption that the organ spanning sites *n* and *j* should be noneliminating was dictated by eq. 1, wherein the general circulation was defined by the sampling site *j*. If this assumed condition were not satisfied, the application of eq. 13 or 14 would result in a different estimate of post-hepatic elimination, say F_S', that is numerically larger than F_S. In effect, the reference point has again been changed such that general circulation is now synonymous with the arterial supply. Although seldom indicated, the decrement between F_S' and F_S can be restored by assessing the extraction ratio across sites *n* and *j*. Alternative experimental strategies have been described for the determination of F_S or, more generally, first-pass effects across organs (49–52). Different combinations of sites of administration and measurement may result in estimates that are independently valid but differ because of their point of reference. In any event, the implementation of experimental strategies that require total accountability at the site of administration should be undertaken with care (19, 27, 53–56).

Simultaneous sampling in the portal and peripheral circulation represents an alternative experimental strategy wherein the reference point shifts from a peripheral site to the portal vein. The prominent role of the portal measurements is evident by comparing eq. 16 to eq. 19 and eq. 22 to eq. 23. They clearly show the subordinate nature of the peripheral samples which are used mainly to effect estimates of $AUC_{\hat{p}}$. An intravenous dose is needed in the estimation of $AUC_{\hat{p}}$. By design this treatment is to emulate only drug molecules returning to the portal vein from the general circulation. Sampling in a peripheral artery ensures the registration of drug molecules from the intravenous dose prior to their entry to the portal circulation for the first time. Similarly, drug molecules that enter the portal vein *via* the enterohepatic circulation are not entering for the first time and, therefore, should register as part of $AUC_{\hat{p}}$ as well. To fully account for the effects of enterohepatic circulation the sampling scheme should be adequate to effect competent measures of AUC_a and AUC_p. Overall, there are no apparent strategic advantages or experimental expediencies associated with simultaneous measurements in the portal circulation.

There is renewed interest in the use of the portal-to-peripheral concentration gradient as a measure of intestinal absorption (41–45). Notwithstanding the confounding effects of gut-wall metabolism, the validity of this approach depends on how closely the drug concentration profile measured in a peripheral blood vessel resembles that which is occurring at $\hat{p}$. Fig. 2 shows that steady-state concentrations at peripheral sampling sites *i*, *j*, *k*, and *a* may differ from each other and from the expected value at for one drug at a fixed rate of input. The relative magnitudes at these same sites will differ from drug to drug since they depend on where drug elimination occurs and the relative contributions of each eliminating organ. After a single oral dose of drug, the difference in concentration between *p* and varies with time and is proportional to the time course of change in drug input to the portal circulation; it starts at zero at time zero, goes through a series of finite values, and returns to zero eventually. Differences in concentration between *p* and peripheral sites *i*, *j*, *k*, or *a* must undergo similar changes with time but not coincidently with each other. Also unlike the differences between those at $\hat{p}$ and , they are not necessarily zero in the absence of input from the gut and, therefore, generally not proportional to the drug input profile. The remoteness with which concentrations at a peripheral site can emulate those at $\hat{p}$ suggests that the valid use of portal-to-peripheral concentration gradients *per se* would be limited. Empirically, applicability is limited to situations in which AUC's measured in the portal vein and the peripheral site after an iv dose are equal. In other words, differences in drug concentration between the portal and the peripheral blood are not indicative of ongoing absorption except in highly specialized situations, *e.g.* the drug is metabolically inert. They are especially inappropriate as indices of comparative absorption across compounds.

There are many situations in which one would be interested in the bioavailability of a metabolite in addition to or instead of the drug. For example, in the evaluation of prodrugs, bioavailability of the drug is germane. In this context, F is a measure of the bioavailability of the prodrug after prodrug administration while F_{m_i} is the bioavailability of drug after prodrug administration. On another occasion one may wish to know how much of the administered drug reaches the general circulation as an active metabolite. The experimental strategy for metabolite bioavailability assessment is the same as that for drug. Drug bioavailability involves the accounting of a single sequence of events in which drug molecules move serially through the gut lumen, the gastrointestinal mucosa, the gut wall, and the liver. Drug molecules that survive each tissue are a continuing source of metabolite while metabolite formed in each tissue must survive the remaining tissues sequentially to be counted. Metabolite bioavailability, therefore, consists of the tracking of parallel sequences representing the serial survival of drug and metabolite. Since there are no constraints on the relationship between drug and metabolite, *i.e.* primary or n^{th} generation, the same strategy applies for any other metabolite. Furthermore, more than one metabolite can be studied simultaneously. For example, the bioavailability of metabolites m_i and m_j after drug administration would nominally entail one additional treatment M_j^{iv} and analyzing all samples for drug, m_i and m_j. How the results should be analyzed to yield the appropriate parameters would depend on the

relationship between m_i and m_j. If they are products of parallel and mutually exclusive metabolic pathways, the components of m_j bioavailability would be represented by expressions analogous to eqs. 25, 28, 32, and 38. Where m_i is an obligatory precursor of m_j, the serial survival of drug and m_i across each tissue must be accounted for in the bioavailability of m_j while eqs. 25, 28, 32 and 38 remain valid for m_i. Where m_i is one of the sources of m_j, or *vice versa*, additional branch points must be included to account for the survival of m_i and m_j across each tissue in addition to their direct descendence from the parent drug. Insofar as their applicability extends to precursor-product relationships, the proposed strategies are not limited to bioavailability assessment but should be generally useful in the *in vivo* evaluation of sites of drug metabolism and metabolite formation.

Most of the analytical expressions of interest involve area comparisons after two or more different treatments. Experimental designs can be made more efficient, therefore, by the concomitant administration of different isotope-labeled drug or metabolite by different routes. Whereas four separate administrations are needed to resolve and estimate F_H, F_G, and F_X by eqs. 6, 10, and 9, respectively, only two treatments would suffice by giving two different labels concomitantly in each. Alternatively, the concomitant administration of an intravenous dose with each of the other three routes not only reduces the total number of treatments but also dispenses with the assumption of constant total body clearance between treatments. In paradigms involving simultaneous measurements in the portal and peripheral circulation, concomitant administration of a labeled dose intravenously ensures a competent estimate of $AUC_{\hat{p}}$ free from assumptions of constant clearance and components thereof. Furthermore, drug and metabolites can be administered simultaneously by one route concomitantly with differently labeled drug and metabolites by another. The number of compounds that can be co-administered by each route and the diversity of the isotope labels needed therein depend on one's ability to distinguish and quantify drug and metabolite(s) unequivocally by source.

While it is desirable to have experimental strategies and procedures in place to effect estimates for the individual components of bioavailability, their routine application *in toto* is seldom indicated. On a given occasion, primary interest is usually limited to one or two elements. The following sequence of events is only intended to be illustrative. An otherwise ideal compound is poorly bioavailable when given orally. By administering a dose to the mesenteric artery, one learns the problem is poor absorption not extensive first-pass elimination. Drug bioavailability remains poor after oral administration of a prodrug. By administering the prodrug intravenously, one learns that the oral bioavailability of the prodrug is excellent but biotransformation to drug is poor. The process continues. By posing questions precisely, each iteration seldom requires more than one or two additional experiments.

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