BIOAVAILABILITY OF DRUGS FROM FORMULATIONS AFTER ORAL ADMINISTRATION

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An administered drug can elicit only the pharmacologic response for which it was developed, provided that sufficient concentrations of drug reach and are available to the receptors. Determination of the likely availability of the active drug to the receptors is the basis of bioavailability testing. Drugs that are chemically equivalent may not be therapeutically equivalent because of differences in dosage form. Certainly, the more potent the pharmacologic action of the drug, the more imperative is the need for bioavailability testing (1), but only recently has such testing gained acceptance as a worthwhile and necessary adjunct (2–4) to the gamut of tests to which new and existing drugs are subjected.

Among the multiplicity of terms coined in recent years, that of bioavailability has been the subject of much discussion and considerable misunderstanding. Bioavailability, or biologic availability, has been usefully reviewed or discussed by several authors (5–16). Confusion has arisen, however, over interchange of the terms biologic availability (bioavailability), physiologic availability, generic equivalence, and therapeutic equivalence, all of which have been used to define essentially the same events. Bioavailability, which in this decade has become the preferred term, describes the extent to which and the rate at which the active drug reaches the systemic circulation, and ultimately the receptors or sites of action at concentrations that are effective, and thereby defines the efficiency of the dosage formulation as an extravascular drug delivery system. Because it is generally impossible to measure receptor drug concentrations, these are measured in the circulation, venous or arterial, from which the receptors receive their supply. Alternatively, urinary concentrations of the active drug or a characteristic metabolite can be measured (13, 17, 18). There is no guarantee, however, that a drug reaching the systemic circulation will also reach the receptors in adequate concentrations. Sometimes the response of the receptors to the drug may be quantified in controlled clinical trials, for example, the

lowering of blood sugar by hypoglycemics (19, 20), the excretion of electrolytes after administration of diuretics (21), or the anticoagulant effects of certain coumarins (22), but it is important to know whether the intensity of the pharmacologic effect in a particular case is a function of drug concentration in the body.

VARIATIONS IN BIOAVAILABILITY

In reality, because the drug has to cross several membranes, exist in numerous physiologic environments, and be subjected to tissue uptake, biotransformation, and excretion (18, 23–26), much of an administered dose never reaches the receptors. So that patients are provided with drug formulations that are physically and chemically stable, pharmaceutically reliable, and aesthetically acceptable, drugs are prepared in various physical forms with a number of other ingredients which may influence their bioavailability. To be absorbed from the gastrointestinal tract, the drug must be presented in a soluble form to the site of absorption; for example, an administered tablet must disintegrate and the particles must dissolve in the gastrointestinal milieu before absorption can occur. Different dosage forms of drugs may thus provide varying amounts of the drug for absorption and thereby cause differences in the onset, extent, and duration of pharmacologic effect. These differences may derive from physiologically modified bioavailability and be due to the physiology or pathlogy of the patient and/or his genetic makeup (27), or alternatively from dosage form-modified bioavailability and be due to the methods of manufacture or to the physicochemical properties of the drug (13) (Table 1). This review is mainly concerned with the latter category. For these reasons (Table 1), in vitro tests which do not take into account some of these factors cannot be presumed to predict in vivo drug availability. The in vitro system must be compared against the in vivo case (28) for every formulation type, and in vitro systems are generally only useful for quality control or for the selection of suitable formulations for in vivo testing.

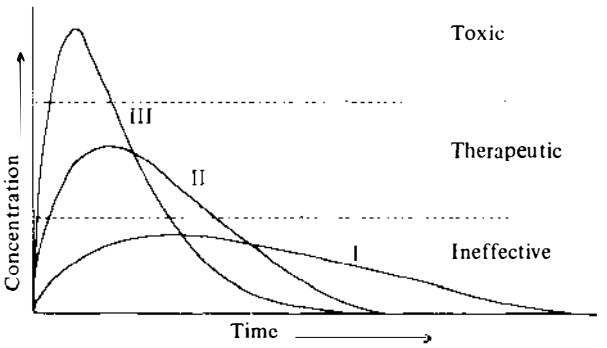
Table 1 Factors affecting bioavailability (13)

Dosage form	Physiologic
Particle size, Polymorphic form, Solvation, Hydration, Chemical form, pH, Solubility characteristics, Formulation adjuvants, Manufacturing method	Age, Sex, Physical state of patient, Time of administration, Stomach emptying, Intestinal motility, Food, Other drugs, Disease

PARAMETERS OF BIOAVAILABILITY

The bioavailability of a drug is characterized by two important parameters: the area under the blood concentration-time relationship and the peak height of this relationship together with its time of occurrence. Figure 1 illustrates the plasma concentration-time relationships for a hypothetical drug which needs to attain a minimum

concentration in the plasma to be pharmacologically active. Above the maximum safe concentration, a drug such as digoxin (29) causes toxicity. Inspection of the relationships shows that a formulation producing curve I is ineffective, that producing curve II is active and the preferred dosage form, and that producing curve III is active but also leads to toxicity. Similarity of the areas under all three curves in Figure 1 does not necessarily indicate that the drug will be therapeutically effective in all cases. As a criteria of bioavailability, therefore, both parameters should be considered. Rates of bioavailability are likely to be important for drugs with a low therapeutic index, sparingly soluble drugs, drugs that are destroyed in the gastrointestinal tract or are actively absorbed, or when adequate drug concentrations are required rapidly, as with antibiotics, analgesics, coronary vasodilators, and hypoglycemics. Differences in bioavailability are, however, equivalent to differences in dosage. Suitable reduction in dosage for formulation III and increase in dosage for formulation I should produce a therapeutic and nontoxic response (Figure 1). If bioavailability is estimated from urinary excretion data, suitable parameters are the cumulative excretion of drug (or metabolite) in the urine and the maximum excretion rate and time of its occurrence.



ESTIMATION OF BIOAVAILABILITY

Earlier methods of estimating bioavailability were qualitative, such as monitoring the disintegration of formulations in the gastrointestinal tract (30–32). Disintegration of a formulation or indeed the dissolution of its contents does not provide absolute proof of absorption. The concept of bioavailability was introduced in 1945 (33) during studies of the relative absorption of vitamins from pharmaceutical preparations and was estimated by comparing the fraction of a dose from a test formulation, and that from an aqueous solution, excreted in the urine during a fixed time. An aqueous solution was considered to present the drug in an ideal form for absorption. More generally, bioavailability may be measured as the ratio

$$\text{Bioavailability} = \frac{\text{amount of drug absorbed from test formulation}}{\text{amount of drug absorbed from reference formulation}} \times 100\%$$

where the reference formulation is one from which the drug is readily absorbed, or, preferably, is known to be clinically effective. So measured, bioavailability is a statement of *relative* absorption, not of amount absorbed.

For bioavailability studies, healthy volunteers are preferred to patients because disease states may influence drug bioavailability (34) or elimination (35). Subjects should be selected on the basis of a satisfactory medical examination, normal renal and hepatic function, and freedom from a history of renal, hepatic, gastrointestinal, and endocrine disorders or from a known sensitivity to drugs. Female subjects should be selected only if they are unlikely to be pregnant during or for some time after the studies. The very thin or obese should be excluded so that wide intersubject variations in apparent volumes of distribution are avoided. The use of subjects aged between 18 and 50 years reduces anomalous age-dependent responses (36, 37). Since large intra- and intersubject variations in absorption commonly occur, a sufficient number of subjects, usually 6 to 20, should be used to permit a satisfactory statistical analysis of the data (18, 38–40), and to demonstrate equivalence, a larger number may be necessary. The subjects should give their informed consent and should not be taking other drugs. Equal doses of test and reference formulations should be administered, as plasma concentrations or clearances may not be linearly related to the dose (41, 42). Experimental designs (43) are commonly of a complete crossover type, where every subject receives each formulation according to a random treatment schedule (38). The intensity of the pharmacologic effect of a drug is often nonlinearly related to the logarithm of the administered dose (23) and the therapeutic consequences of changes in dose due to modification of bioavailability may be more serious at lower doses. For this reason acceptable limits of bioavailability must be established for each drug at or near the expected therapeutic dose (13).

Bioavailability is usually estimated from a statistical comparison of either average drug concentrations in the blood (18) or areas to infinite time under the drug concentration-time relationships in the blood after administration of single doses of both test and reference formulations. The duration of sampling is relatively short and improvements in methodology allow accurate determinations of very low drug concentrations. In single-dose studies, sufficient blood samples should be withdrawn to describe adequately the critical phases of the concentration-time relationship: (*a*) absorption which allows at least a qualitative comparison of the rates of availability, (*b*) time of occurrence of maximal concentrations, and (*c*) the decline of concentrations during the elimination phase. During the latter phase, drug concentrations may fall to very low levels, and inadequate analytical procedures could introduce errors into the calculation of areas to infinite time. The precision of the analytical method should be known and the level of sensitivity should exceed the expected peak blood concentration by at least twentyfold. Total areas under the concentration-time curves are usually measured by the trapezoidal rule (44) up to the last sampling time, and the remaining area to infinite time is calculated from the concentration at that time and the observed rate constant for drug elimination from plasma (45). The calculated areas may be normalized (13, 18, 45, 46) to correct for intra- and intersubject variations in dose, body weight, and the apparent elimina-

tion (biological) half-life of the drug. This allows bioavailabilities estimated in studies performed at different times with different subject panels to be validly compared.

Under some circumstances it may be preferable to estimate bioavailability during a sequence of multiple doses, so that experimental conditions resemble the clinical situation (13, 18). After multiple dosing, blood concentrations are greater and more easily measured, but experimental control is more complex. In multiple dose studies, biovailability can be estimated, after attainment of steady state conditions, by comparison of the areas under the blood concentration-time curves during a complete dosage interval or by comparison of maximal and minimal concentrations reached during the dosage interval. This obviates the need for calculation of areas to infinite time, which may be a prime source of error in single-dose studies.

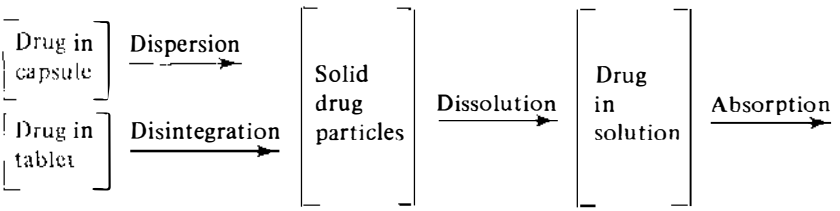
Estimates of bioavailability from urinary excretion data require complete collection of the urine for at least seven drug half-lives, and control of urinary pH may be necessary for certain drugs, such as weak bases (47). Loss of a single sample could invalidate the estimation of bioavailability from measurement of cumulative excretion data, and rates of urinary excretion may not correspond to rates of gastrointestinal absorption. This method is advantageous because the subjects need not undergo numerous venepunctures for blood withdrawal, and drug analysis is simpler, but it should not be used when the drug is extensively biotransformed and less than 20% is excreted in the urine unchanged or as a characteristic metabolite.

All the experimental data obtained should be analyzed by the appropriate statistical procedures (40) with due regard for the methodology used. It should be estimated what differences need to occur between formulations before these are statistically significant.

Seven methods of estimating bioavailability have been described by Wagner & Nelson (48), some of which differ only in the mathematical treatment of the experimental data. Wagner (18) has critically appraised the assumptions involved.

FACTORS AFFECTING BIOAVAILABILITY

Since absorption occurs only after the drug is in solution, orally administered drugs in solid form must first dissolve in the gastrointestinal fluids. The rate at which dissolution occurs is an important determinant of bioavailability and is dependent on several factors. Drugs administered in solid form as capsules or tablets need to disaggregate so that dissolution may occur more readily.



Particle Size

The greater the surface area of drug in contact with the gastrointestinal fluids, the more rapid the dissolution rate. Thus with decrease in particle size, there is an increase in dissolution rate (49, 50). The bioavailabilities of spironolactone (51) and phenacetin (52) are improved by a reduction in particle size. However, particle size reduction provides more opportunity for particle interaction, which may sometimes lead to aggregation. Nitrofurantoin, in high concentrations, causes gastric irritation and nausea when taken orally and it is thus preferable to present this drug to the gastrointestinal tract as larger, slower-dissolving crystals (53, 54).

Diffusion from the Dosage Form

The rate of bioavailability of a drug is enhanced if the rate of diffusion from the dosage form is increased either by use of a more soluble drug form or by alteration of the microenvironment surrounding the drug particle (8, 12). Administration of soluble salts of penicillin V resulted in higher blood levels of antibiotic than were obtained with the less soluble free acid (55, 56). The rates of absorption of different aspirin formulations correlated with their solubility characteristics (57).

Crystalline Form

Some drugs such as barbiturates (58) exist in several crystalline forms of differing solubilities and other physical properties (59, 60) with resultant differences in bioavailability (61). The amorphous is more soluble than the crystalline form.

Hydration

The hydration state of a drug influences its solubility, and the anhydrous form is usually more soluble. Anhydrous ampicillin has a greater extent of bioavailability in dogs and man than the less soluble trihydrate (62).

Formulation Ingredients

Some of the agents added to formulations which can influence the bioavailability of the drug include fillers, binders, disintegration aids, lubricants, surfactants, and suspending agents. Hydrophobic lubricants such as magnesium stearate prevented adequate contact between the gastrointestinal fluids and the drug solids, thereby slowing dissolution, whereas the hydrophilic sodium laurylsulfate produced the opposite effect (63). Surfactants may alter the rate and extent of absorption of certain drugs (64). Absorption of phenacetin was apparently enhanced by Tween 80 (52).

Pharmaceutical or Dosage Form

The compressed tablet provides a low surface area for dissolution and must first disintegrate (65). Coated tablets, particularly enteric coated types, may release the drug unevenly. The rate of dispersion of drug particles from a capsule influences the bioavailability of the drug, but in general a capsule is considered a reliable dosage form. Drugs with a short biological half-life are sometimes formulated as sustained- or timed-release products (44), but care is necessary since failure of the formulation

could result in toxic levels of drug (66). The expected relative bioavailabilities from various dosage forms are shown in Table 2.

Table 2 Dosage forms for oral administration (8)

Increasing release rate of drug ↑	Aqueous solutions and syrups
	Suspensions
	Powders
	Capsules
	Tablets
	Coated tablets

Compressed tablets are the most widely used form of oral medication, and an accepted laboratory standard measurement of drug release from the tablet has been the disintegration test which merely measures physical breakup of the tablet. This test does not predict drug bioavailability in vivo (65, 67, 68) although it can be assumed that a tablet formulation failing to disintegrate within about 30 min would provide only slowly available drug.

The dissolution rate test provides a means of ranking various solid dosage forms in vitro and, although the results may correlate with in vivo bioavailability (18, 69–72), measured dissolution rates can be affected by test conditions (73, 74). Levy (67) compared various commercial aspirin tablets and found that absorption from the gastrointestinal tract correlated with dissolution but not disintegration rate data. In vitro techniques must always be suspect because they do not compensate for the nervous and circulatory systems that charactize the biological case.

Gastrointestinal Conditions

Absorption of drugs may be affected by the physiologic status (disease, pH, peristalsis) of the gastrointestinal tract. Administration of laxatives, such as $MgSO_4$, may cause dilution of the intestinal contents and enhance intestinal motility, thereby reducing the time available for drug absorption. A pH-dependent dissolution stage precedes tetracycline absorption in man (75), and simultaneous administration with antacids or milk reduces absorption, because these contain divalent metal ions which form a poorly absorbed chelate with tetracycline (76–78). Administration of drugs with food usually reduces or delays absorption (79). Aspirin (80), dicloxacillin (81), and penicillin V (56) were absorbed best by fasting subjects. However, absorption of griseofulvin was least in fasting subjects and was enhanced by meals with a high fat content (82). Dietary components influence the absorption of paracetamol (83) and acetaminophen (84). Drugs that are unstable in acid media are formulated for safe passage through the stomach with an enteric coating (18, 85). Such coatings are pH sensitive and may prematurely disintegrate in the stomach if antacids are taken simultaneously. The action of sustained- or timed-release products formulated with organic solvent-sensitive coatings may be undesirably enhanced by concomitant intake of alcoholic beverages (12).

Drug Interactions

Drug interactions occurring in the gastrointestinal tract, such as lincomycin and kaolin-pectin (38), may be considered as pharmaceutical incompatabilities and avoided by consideration of the relevant physicochemical properties of the formulation ingredients. Drug interactions in the body or during absorption, however, occur by modification of absorption, distribution, biotransformation, excretion, and action at receptor sites (86–92) and are a consequence of polypharmacy. Examples include displacement of protein-bound drugs by other drugs (12, 25, 93–97) and enzyme induction (88, 98, 99). Age, nutrition, and pathological states are other important determinants of drug interactions (100).

Influence of Route of Administration

Most orally administered drugs are absorbed into the hepatic portal system where extensive biotransformation may result in only a small proportion of the original drug reaching the peripheral circulation. Attempts to estimate the extent of absorption by comparison of the areas under plasma concentration-time curves obtained after oral and intravenous administration are thus invalidated by this "pass effect" of the liver (101–104), as plasma clearances vary according to the route of administration. Extensive biotransformation during passage through the gastrointestinal epithelium may also reduce the bioavailability of an orally administered drug (105). Despite some intersubject variations, an increase in dosage in the ratio 1:1.5: 2–3:3–4:3–5 was thought necessary to obtain similar blood concentrations of pentazocine after intravenous, intramuscular, oral (solution), rectal, and oral (tablet) administration respectively (106). Plasma concentrations were erratic after rectal administration of aspirin (107) when absorption would be limited by the rate of drug diffusion through a viscous medium. The nature of the suppository base would strongly influence bioavailability.

GENERIC PRODUCTS

Nonproprietary preparations are chemically equivalent if they contain the same active drug and they would be therapeutically equivalent if they produced the same biological response. Chemically equivalent formulations from different manufacturers, referred to as generic products, often differ widely in their methods of manufacture and content of pharmacologically inert ingredients. In recent years, controversy has arisen over the possible extent to which such generic products may or may not be therapeutically equivalent. The problem is one of bioavailability (5, 11, 16, 18). Controlled studies in man on twelve commercial drug products showed inequivalence in ten cases (18) in either the rate or extent of bioavailability. Differences in the therapeutic equivalence of some formulations has been noted in clinical practice (11, 108, 109), but it is not entirely clear that formulation effects are alone responsible, especially in cases where therapeutic efficacy is not directly related to drug concentrations in the blood (110) or where the clinical response is subjective. Prescott & Nimmo (11) ranked normal individuals as fast and slow absorbers of paracetamol and showed that plasma concentrations were similar after ingestion of

a suspension, effervescent tablet, and plain tablet by the former but not the latter subjects, which suggests that the inequivalence of some products may only arise in certain subjects.

Maximal plasma concentrations and rates of absorption of chloramphenicol differed after administrations of four different formulations to human subjects (111). Less pronounced differences were observed in another study (112) when fourteen different formulations as capsules, tablets, and suspensions were administered orally. These differences were unlikely to have been caused by intersubject variations in rates of absorption or biotransformation. In both studies the rate of bioavailability was related to the in vitro dissolution time; those preparations providing the lowest plasma concentrations showed the slowest dissolution rates. Differences in either rate or extent of bioavailability of generic products have been shown for digoxin (113, 114), ampicillin (115), phenylbutazone (116), warfarin (117), a combination of trimethoprim-sulfamethoxazole (118), sulfonamides (119, 120), and tetracyclines (121–124), although no differences were found between several products containing phenylbutazone (125) and sulfamethizole (126). Six preparations of isoniazid were shown to be therapeutically equivalent (127) and in vivo bioavailability paralleled in vitro tests. The bioavailability of triple sulfa from 20 generic products was similar (128), although the drugs were more slowly absorbed from the formulations than from aqueous solutions, and there was no in vivo correlation with widely varying dissolution rates. Of generic products, digoxin formulations continue to attract much interest (136–139).

While in vitro tests provide a means of ranking formulations, the available evidence (18) challenges the usefulness of tests in vitro to predict bioavailability in vivo (73, 129–132). Efforts are being made to design in vitro tests capable of predicting the in vivo performance of generic products (73, 133–135), but in vivo studies are currently the only reliable way of ascertaining whether drugs are available from their formulations for absorption and production of a therapeutic response. Although drug formulation has been studied extensively in vitro, the extent of generic inequivalence and its clinical significance is at present unknown but may be expected to have more relevance to drugs of poor water solubility, as known examples of inequivalence seem to reflect the dissolution rather than the disintegration rates of the products. The relative importance of equivalence is also linked to the disease being treated. A criticism of most bioavailability studies that detect differences between generic products is that these studies do not define the extent (if any) to which a particular inequivalence endangers the well being of the patient.

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