PHARMACEUTICAL DOSAGE FORMS

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Tablets

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In Three Volumes

VOLUME 2

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Bioavailability in Tablet Technology

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I. GENERAL CONSIDERATIONS

Drugs are administered locally for protective action, antisepsis, local anesthetic, and antibiotic effects, and they are given systemically for action on the cells and organs of the body or to counter the effects of invading organisms. A number of physiological and chemical factors are important in the absorption, distribution, and elimination of drugs in the body. Some of the properties of the buccal cavity, stomach, and small and large intestines that influence drug therapy are found in Tables 1 and 2.

When drugs are given for systemic action, a number of routes are available, including oral, rectal, parenteral, sublingual, and inhalation. After absorption into the body, a drug is distributed by the blood and lymphatic system and passes into the extracellular fluids of various tissues. The drug molecules may enter cells immediately and exert their pharmacological action in this way or be stored as a reservoir in muscle and fatty tissue for prolonged action. The drug may also be bound to albumin and other components of the plasma, altering tissue distribution and elimination from the body.

Drugs are metabolized by enzyme systems of the body, and this process is given the general term "biotransformation." The net effect may be inactivation or detoxification of the compound, or the drug may be converted from an inactive or prodrug form into the pharmacologically active species. For example, the azo dye Prontosil is reduced in the body to sulfanilamide, and the discovery of this conversion led to the development and use of sulfonamides as medicinal agents. Biotransformation is mainly handled in the liver, but the process also occurs in the kidneys, intestines, muscles, and blood.

Table 1 Physiological and Chemical Characteristics of Gastrointestinal Fluids in the Gastrointestinal Tract

Factors	Stomach	Small intestine
Properties of fluids[a]		
pH value	1–3	5–8
Volume of fluid available (ml)	50–250	25–125
Surface tension (dyn cm^{-1})	35–50	32–45
Viscosity (cP)	0.8–2.5	0.7–1.2
Buffer capacity[b]		
β (NaOH)	30–60	4–8
β (HCl)	600	8–16
Δt (°C)	0.3–0.8	0.62
Density	1.01	1.01
Water (%)	98	98
Juice secretion (liter day^{-1})	2–4	0.2–0.8
Water circulation (liter day^{-1})	1–5	1.5–5
Enzymes and electrolytes	Variable	Variable

[a]Fasting subjects, temperature 37°C.

[b]= mmol NaOH or HCl/liter × ΔpH × pH (stomach fluid 1.5 ± 0.1).
Source: Modified from Ref. 121.

Table 2 Buccal, Gastric, and Intestinal Fluids

	Daily volume (ml)	pH
Saliva	1200	6.0–7.0
Gastric secretion	2000	1.0–3.5
Pancreatic secretion	1200	8.0–8.3
Bile	700	7.8
Succus entericus	2000	7.8–8.0
Brunner's gland secretion	50	8.0–8.9
Larger intestinal secretion	60	7.5–8.0

Source: Modified from Ref. 121.

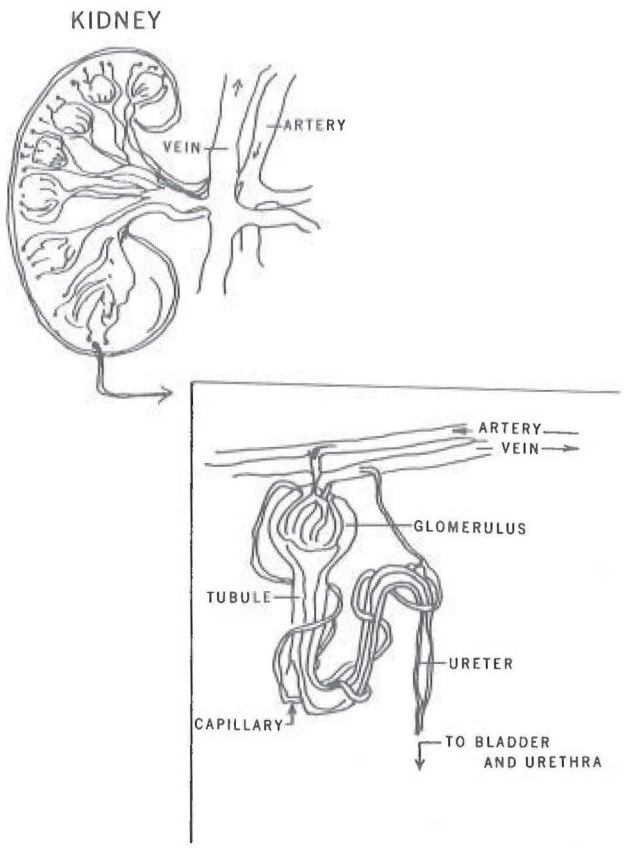

Figure 1 Schematic diagram of kidney. A glomerulus and associated structure are enlarged and shown in the insert.

Whether biotransformed or not, the drug molecules must finally be eliminated from the body. The kidneys are the principal organs of excretion, but foreign compounds may be eliminated from the lungs or in bile, saliva, and sweat. The kidney (Fig. 1) is composed of millions of units consisting of a filtering capsule or glomerulus. The liquid, which contains soluble excrement that is filtered through the glomerulus into the kidney tubules, is referred to as the glomerular filtrate. The tubules are surrounded by capillaries, and some solute molecules present in the glomerular filtrate may be reabsorbed and returned to the bloodstream. Molecules of physiological importance, such as glucose, water, chloride, potassium, and sodium sions, are reabsorbed at various segments of the tubules. Some drugs, such as penicillin G, do not pass through the glomerular apparatus but rather are actively transported from capillaries directly to the tubules where they are excreted in the urine. Thus, owing to pH, active transport mechanisms, solubility, and ionic characteristics, drug molecules may be eliminated in the glomerular filtrate or directly absorbed into the tubules and excreted, and may be reabsorbed

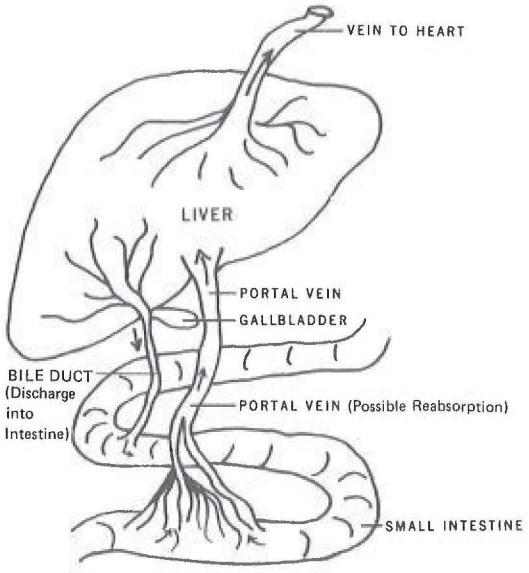

Figure 2 Liver, portal vein, gallbladder, and bile duct in relation to small intestine.

from the tubules into the systemic circulation for recycling through the body. The excretion of weakly acidic and basic drugs is influenced by the pH of the urine, and elimination of these compounds may be altered by controlling urinary pH. Alkalinizing the urine results in reabsorption of quinidine and may result in sufficiently high plasma levels of the drug as to manifest toxicity.

Biliary excretion has been found to be an important elimination route for some drugs (see Fig. 2). The drug present in the bile is discharged into the intestines and may be eliminated in the feces or into the vascular system from a region lower in the intestinal tract. Thus, as in kidney excretion, biliary passage may involve a cycle of elimination and reabsorption. This process, which occurs with morphine, penicillins, and a number of dyes, is called enterohepatic circulation; the process tends to promote higher and prolonged concentrations of the drug and its metabolites in the body than would be expected were the recycling process not involved. However, repeated passage through the liver may lead to significant metabolism of the drug.

The processes of diffusion and the partitioning of drug molecules across membranes as a function of pH, pK_a, and other factors will be discussed in later sections of this chapter.

The kinetics of absorption, distribution, and excretion of drugs following administration was first set forth by Teorell [1] in 1937 (Fig. 3).

A. Biopharmaceutics and Pharmacokinetics

A drug administered as a tablet or another dosage form must be released and reach its site of action in an active state before it can exert a

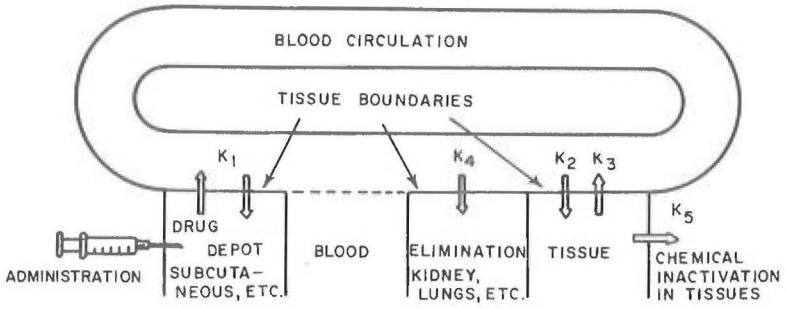

Figure 3 Schematic description of drug absorption, distribution, and elimination. (After Ref. 1.)

pharmacological response. The physical chemical properties of the drug, the characteristics of the dosage form in which the drug is administered, and the physiological factors controlling absorption, distribution, metabolism, and elimination of the drug must all be considered in order to formulate and manufacture effective and safe therapeutic agents. This wide range of considerations comprise the subject called biopharmaceutics.

Pharmacokinetics is a branch of biopharmaceutics and encompasses in a quantitative way the kinetics of absorption, distribution, metabolism, and excretion, often called ADME, of therapeutic agents and related chemical substances. The time course of passage of intact drugs and metabolites in various body tissues and fluids and the models constructed to interpret these data, which comprise the subjects of pharmacokinetics, will be introduced as elementary mathamatical expressions and graphs of data early in the chapter and elaborated upon in later sections. The final part of the chapter considers the elements of pharmacokinetics in some detail. The subject is presented step by step with worked examples, so that a reader with a background in pharmacy, chemistry, or biology but minimal grounding in mathematics can follow the treatment with relative ease.

At the beginning it is well to define some of the terms to be used throughout the chapter, particularly the words biopharmaceutics, pharmacokinetics, and bioequivalency. Some of the terms, as defined in the 1977 Bioequivalence Requirements and In Vitro Bioavailability Procedures of the FDA [2], are found in Table 3.

B. Bioequivalence

The forces that have led in the last decade to the concept of bioavailability were principally those due to the generic equivalence (bioequivalence) issue. Specifically, it is of the utmost importance to be assured that chemically equivalent drug products from different manufacturers result in essentially the same degree of therapeutic action.

An examination of the definitions as outlined in Table 3 shows that emphasis has been placed on the ability of two or more drug products to produce essentially identical blood levels in the same individual. The dosage form is a drug delivery system; it can be a good one or a poor one in its role of releasing the drug efficiently for absorption into the systemic circulation or site of action. Thus, appropriate testing of generic products

Table 3 Definition of Terms Dealing with Bioavailability and Bioequivalence

Drug	Active therapeutic moiety.
Drug product	Delivery system, tablet, capsule, suspension (e.g., containing the therpeutic moiety), generally but not necessarily in association with inactive ingredients.
Bioavailability	The rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and beocmes available at the site of action.
Bioequivalent drug products	Pharmaceutical equivalents or alternatives whose rate and extent of absorption are not significantly different when administered to humans at the same molar dose under similar conditions.
Pharmaceutical equivalents	Drug products identical in amount of active drug ingredient and dosage form, and meeting compendial or other standards for identity, strength, quality, and purity. They may not be identical in terms of inactive ingredients. An example is erythromycin stearate tablets (Brand X and Brand Y).
Pharmaceutical alternatives	Drug products that contain the identical therapeutic moiety or its precursor but not necessarily in the same amount or dosage form and not necessarily as the same salt or ester. Examples are erythromycin stearate versus erythromycin ester; chlorpheniramine maleate chewable tablets versus chlorpheniramine maleate capsules.
Bioequivalence requirements	A requirement imposed by the FDA for in vitro and/or in vivo testing of specified drug products which must be satisfied as a condition of marketing.

must be conducted. These tests are not done only through clinical trials of efficacy since it is ordinarily not the drug that is in question but the dosage form; the latter primarily influencing the absorption step. It is the absorption process or factors connected with the delivery system that must be studied to assure proper bioavailability of the drug and bioequivalence of products from one manufacturer to another and from batch to batch.

A number of studies of marketed drug products containing the same chemical ingredient have revealed differences in bioavailability. Examples of problems with chemically equivalent drug products include tetracycline [3,4], chloramphenicol [5], digoxin [6,7], phenylbutazone [8,9], and oxytetracycline [10,11]. In addition, variations in bioavailability of different batches of digoxin from the same company have been demonstrated [7]. In one report, a thyroid preparation that met compendial standards was found to be therapeutically inactive [12]. Since lack of bioequivalence in these examples involves marketed products, it can be concluded that neither

standards for testing the finished product nor specifications for materials, manufacturing processes, and controls are presently adequate to ensure that drug products are bioequivalent. Good Laboratory Practice and Good Manufacturing Practice regulations promulgated by the U.S. Food and Drug Administration (FDA) will assist in correcting the problem, but specific actions regarding bioavailability must be taken to assure equivalent marketed products. In most instances it is concluded that therapeutic inequivalence is a result of variations in the bioavailability of drug products.

C. Relative Bioavailability and Drug Performance

If a drug is administered at a dosage level that does not greatly exceed the minimum effective blood concentration (MEC) required, the availability of the drug from the dosage form may greatly influence the drug's performance. Figure 4 schematically illustrates this case. In curve I the product formulation causes the drug to have a good therapeutic response. In curve II, because of a delayed rate of absorption, the effective response is more transient. The slow absorption process of curve III leads to a lack of pharmacological response, even through the amount absorbed as determined by the total area under the curve is equal to the other two. This example illustrates that although the amount of drug absorbed may not differ, the rate of absorption of three products may be quite different, leading to variations in therapeutic action. A real example is presented in Figure 5, illustrating the work by Sullivan et al. [13]. It shows average

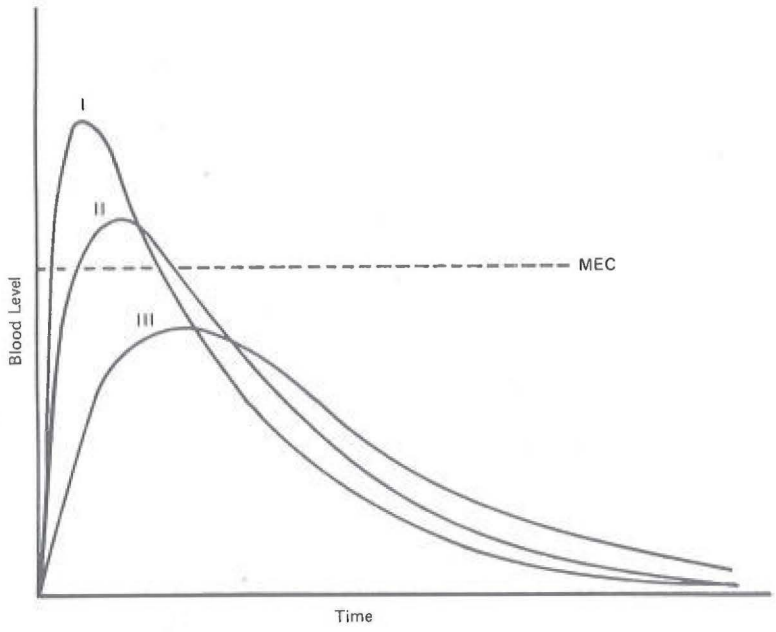

Figure 4 Blood levels from three products, illustrating differences in the rate of absorption but not in the total amount of drug absorbed.

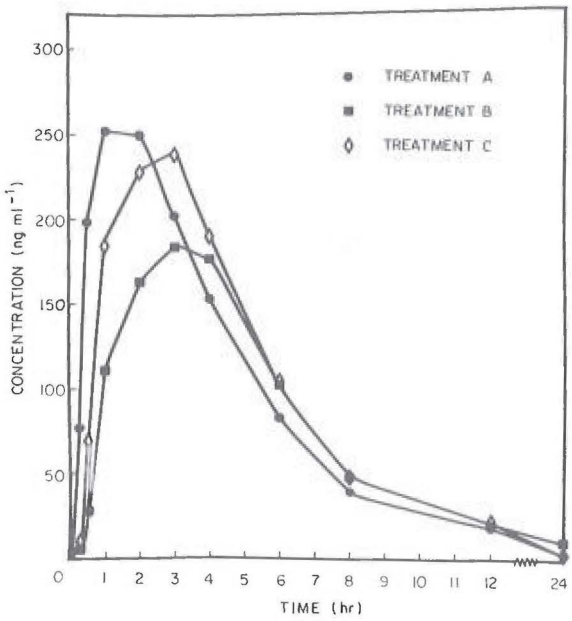

Figure 5 Average plasma levels of prednisone in nine adult volunteers following oral administration of 10 mg of prednisone (as two 5-mg tablets). (From Ref. 13.)

plasma levels of prednisone obtained in nine adult volunteers in a three-way crossover study when a 10-mg dose of prednisone was administered as two 5-mg tablets made by three different manufacturers. Treatment A gave the fastest absorption and highest plasma levels. Treatments B and C were two generic prednisone tablets that had a history of clinical failure and did not pass the USP tablet dissolution test. Treatment A passed all compendial tests in the laboratories of the FDA. In this case the rate of appearance of prednisone in plasma was different for the three tablets, although the average areas under the plasma concentration-time curves of individual subjects did not differ significantly. This is a case in which documented evidence of clinical failure with generic tablets can be related to differences in rates of absorption.

Figure 6 illustrates the results obtained by Glazko et al. [5] when testing four capsules of chloramphenicol in human subjects. Here the principal difference, as indicated by the area under the curve, is that the four products differ in the total amount of chloramphenicol absorbed. Product A gave an excellent blood level curve in subjects, whereas the other three formulations gave poor plasma levels. Because of these data, the FDA had products B, C, and D recalled and reformulated, and then instituted requirements that have brought the problem of chloramphenicol products under control. The significance of this finding is that all the products were chemically equivalent. That is, they contained the correct amount of chloramphenicol, and the particle size of the drug in each of the products was similar. Figure 7 illustrates the results obtained by

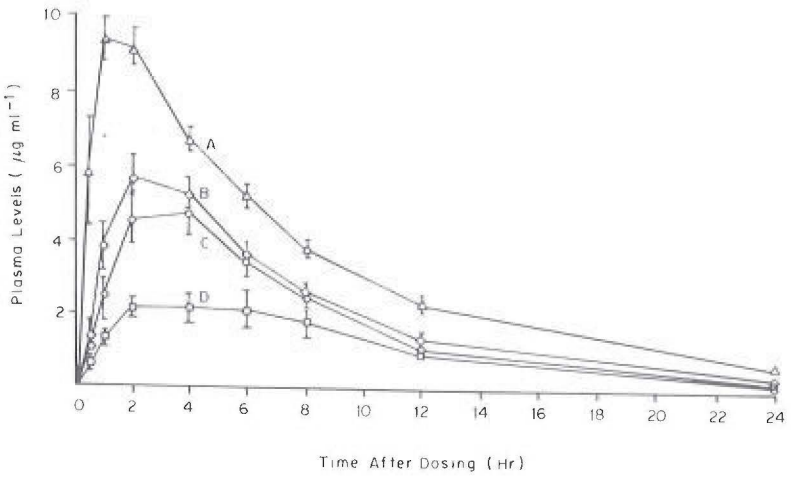

Figure 6 Mean plasma levels for groups of 10 human subjects receiving single 0.5-g oral doses of chloramphenicol capsules. Vertical lines represent one standard error on either side of the mean. Capsule A, Δ; capsule B, ◇; capsule C, ○; capsule D, □. (From Ref. 5.)

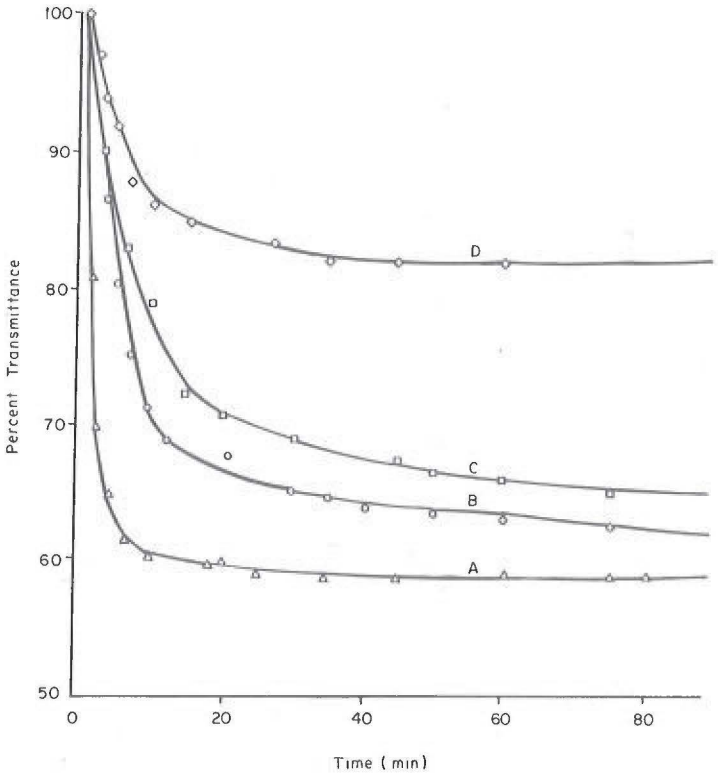

Figure 7 Relative disintegration rates of chloramphenicol capsules in simulated gastric fluid. Capsule A, Δ; capsule B, ○; capsule C, □; capsule D, ◇. (From Ref. 14.)

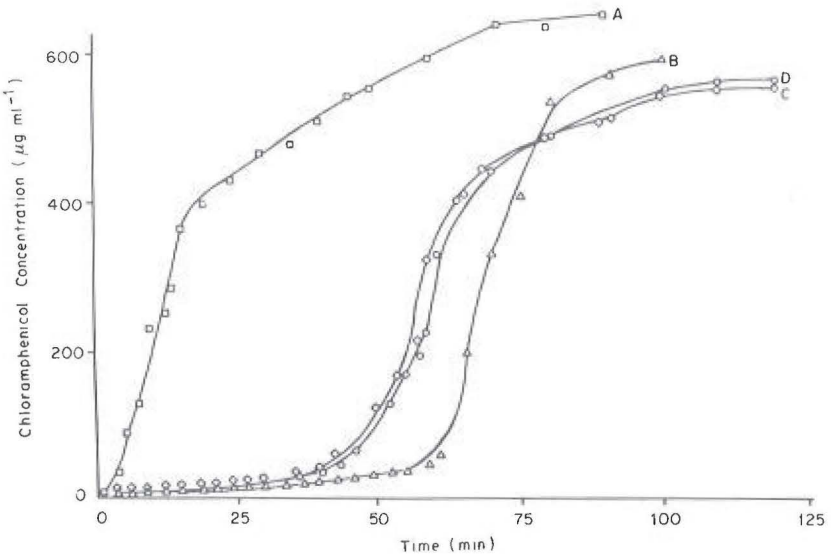

Figure 8 Dissolution rates of chloramphenicol capsules in simulated gastric fluid. Capsule A, □; capsule B, Δ; capsule C, ◇; capsule D, ○. (From Ref. 14.)

Aguiar, showing that the disintegration rates of the four products differed greatly [14]. Product A, the one that produced the highest plasma level in the study by Glazko, had excellent disintegration characteristics, whereas products B and C showed poor rates, and product D, the product having the poorest plasma level in patients, exhibited the poorest disintegration rate. In fact, product D had such poor disintegration properties that the powder mass maintained its capsule shape in simulated gastric fluid after the gelatin capsule had dissolved. The results in Figure 8 demonstrate the performance of the four products in a dissolution rate test. When product A was placed in simulated gastric fluid, it dissolved rapidly, whereas products B, C, and D showed greater lag times prior to dissolution, principally because of the time required for deaggregation [14].

Dissolution tests and disintegration tests in some cases have been shown to correlate well with human bioavailability tests, as evidenced by the previous example. Lindenbaum's work on digoxin is another example of in vivo/in vitro correlations [7].

The FDA, in its bioequivalence preamble, stated [2]:

> Advances in pharmaceutical technology have made bioequivalence a most precise and reproducible method for determining drug product variability. These bioequivalence techniques are not inadequately defined or reachless concepts. They are scientifically valid methods of comparing different drug products as well as different batches of the same drug products.

It is indeed fortunate that dissolution methodology frequently provides a measure of variability among drug dosage forms. The conclusion that can

be drawn from the bioequivalence requirements and in vivo bioavailability procedures of the FDA, published in the *Federal Register* of January 1977, is that dissolution testing will more than likely become the most frequently used means of testing and assuring bioequivalence [2]. Although it will probably not be the only criterion for obtaining marketing approval, the FDA report states that "a dissolution test may constitute a proper element in reaching the decision to approve an NDA or supplemental application for a drug product with a bioequivalence problem." In another section of this chapter we discuss some of the methodology presently available for dissolution testing.

The digoxin tablet problem is an example of a situation that led to establishment by the FDA of a dissolution rate certification requirement and adoption of a dissolution specification by the USP. Digoxin is a drug in which the effective blood level is close to the toxic concentration. Product formulation may greatly influence the rate and extent of absorption, and consequently the therapeutic activity and toxicity of digoxin.

D. Federal Regulations Covering Bioavailability and Bioequivalence

In the *Federal Register* of January 1973 [15], the FDA published proposed bioavailability requirements for new drugs and for generic drug products. These proposals became regulations as published in the *Federal Register* of January 1977 [2], with appropriate modifications as suggested by various individuals and groups. The regulations clearly establish that studies must be undertaken with new drugs or new dosage forms to assure that optimal absorption characteristics are achieved.

The selection of the reference material (standard drug sample) as stated by the FDA is an important consideration. It depends upon the scientific questions to be answered, the data needed to establish comparability with a currently marketed drug product, and the data needed to establish dosage regimens. The reference material should be taken from a current batch of a drug product that is the subject of an approved new drug application and that contains the same active drug ingredient or therapeutic moiety. Thus, tetracycline hydrochloride cannot be the reference product for tetracycline phosphate; salts cannot be compared against esters, capsules cannot be compared with tablets.

In the report of the Office of Technology Assessment (OTA), Drug Bioequivalence Study Panel [16], it was concluded that studies on bioavailability are neither feasible nor desirable for all drugs or drug products. According to OTA, certain classes of drug should be identified for which evidence of bioequivalence is critical. Selection of these classes would be based on clinical importance, ratio of therapeutic to toxic concentration in blood, and certain pharmaceutical characteristics. The panel believed, however, that bioavailability studies should be required for products if the active ingredient in the product had not yet been introduced into the market.

A large number of drug products are available on the market, and for only a few of these are there adequate data documenting bioavailability in humans. Thus, many bioavailability studies would be required; this involves large numbers of human volunteers and the expense of clinical investigators and other scientific personnel. Consequently, it is not feasible

and justifiable to carry out studies of bioavailability in humans for all drug products. In asserting that studies of bioavailability will not be required for all drug products, it becomes important to set general criteria to guide the selection of those products whose bioavailability should be documented by testing in humans, those requiring no testing, and those few in which in vitro methodology would be deemed adequate. The report of the OTA study panel concluded that for drugs with a wide therapeutic range, moderate differences in drug blood levels, owing to differences in bioavailability of chemically equivalent products, would be tolerated. Conversely, drugs that have a relatively narrow therapeutic range would be candidates for testing of bioavailability on human subjects. Examples of drugs that fall into this category include cardioactive agents (digitalis glycosides), anticonvulsants (diphenylhydantoin), some corticosteroids, and certain antibiotics (chloramphenicol and cephalosporins). In summary, drug products will be considered candidates for human bioavailability studies if they:

Are used for treatment or prevention of serious illness
Have steep dose-response curves or unfavorable therapeutic indices
Contain active ingredients that are relatively insoluble or are converted to insoluble forms in the gastrointestinal fluids

In the *Federal Register* of January 1977, the FDA [2] published criteria for waiver of evidence of bioavailability. The requirement for submission of in vivo bioavailability data will be waived if:

1. The drug product meets both of the following conditions:
   a. It is a solution intended solely for intravenous administration.
   b. It contains an active drug ingredient or therapeutic moiety in the same solvent and concentration as an intravenous solution that is the subject of an approved full new drug application.
2. The drug product is a topically applied preparation (e.g., a cream, ointment, or gel) intended for local therapeutic effect.
3. The drug product is an oral dosage form that is not intended to be absorbed (e.g., an antacid or a radiopaque medium).
4. The drug product meets both of the following conditions:
   a. It is an oral solution, elixir, syrup, tincture, or similar other solubilized form.
   b. It contains an active drug ingredient or therapeutic moiety in the same concentration as a drug product that is the subject of an approved full new drug application.
   c. It contains no inactive ingredient that is known to significantly affect absorption of the active drug ingredient or therapeutic moiety.

The regulations proceed to list drugs for which in vivo bioavailability data of solid oral dosage forms need not be submitted to the FDA.

For certain drug products, bioavailability may be demonstrated by evidence obtained in vitro in lieu of in vivo data. The FDA waives the requirements for the submission of evidence obtained in vivo demonstrating the bioavailability of the drug product if the drug product meets one of the following criteria:

1. The drug product is subjected to the bioequivalence requirement established by the Food and Drug Administration under Subpart C of this part that specifies only an in vitro testing requirement.
2. The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product made by the same manufacturer and the following conditions are met:
   a. The bioavailability of this other drug product has been demonstrated.
   b. Both drug products meet an appropriate in vitro test approved by the Food and Drug Administration.
   c. The applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients.
3. The drug product is, on the basis of scientific evidence submitted in the application, shown to meet an in vitro test that assures bioavailability (i.e., an in vitro test that has been correlated with in vivo data).
4. The drug product is a reformulated product that is identical, except for color, flavor, or preservative, to another drug product made by the same manufacturer and both of the following conditions are met:
   a. The bioavailability of the other product has been demonstrated.
   b. Both drug products meet an appropriate in vitro test approved by the Food and Drug Administration.
5. The drug product contains the same active drug ingredient or therapeutic moiety and is in the same strength and dosage form as a drug product that is the subject of an approved full or abbreviated new drug application, and both drug products meet an appropriate in vitro test that has been approved by the Food and Drug Administration.

The FDA, for good cause, may defer or waive a requirement for the submission of evidence of in vivo bioavailability if deferral or waiver is compatible with the protection of the public health.

In the 1970s the FDA developed what is now known as the Approved Drug Products with Therapeutic Evaluation Publication [17] (referred to in text as the List). The List was distributed as a proposal in January 1979. It included only currently marketed prescription drug products approved by FDA through new drug applications (NDAs) or abbreviated new drug applications (ANDAs) under the provisions of Section 505 or 507 of the Federal Food, Drug, and Cosmetic Act (the Act). The therapeutic equivalence evaluations in the List reflect FDA's application of specific criteria to the approved multisource prescription drug products on the List. These evaluations are presented in the form of code letters that indicate the basis for the evaluation made.

A complete discussion of the background and basis of FDA's therapeutic equivalence evaluation policy was published in the *Federal Register* on January 12, 1979 (44 FR 2932). The final rule, which includes FDA's responses to the public comments on the proposal, was published in the

Federal Register on October 31, 1980 (45 FR 72582). The first publication, October 1980, of the final version of the List incorporated appropriate corrections and additions. Each subsequent edition has included the latest approvals and data changes.

On September 24, 1984, the President signed into law the Drug Price Competition and Patent Term Restoration Act (1984 Amendments). The 1984 Amendments require that FDA, among other things, make publicly available a list of approved drug products with monthly supplements. The *Approved Drug Products with Therapeutic Equivalence Evaluations* publication and its monthly Cumulative Supplements satisfy this requirement.

The main criterion for the inclusion of any product in the List, is that the product is the subject of an approved application that has not been withdrawn for safety or efficacy reasons. Inclusion of products on the List is independent of any current regulatory action through administrative or legal means against a drug product. In addition, the List contains therapeutic equivalence evaluations for approved multisource prescription drug products.

The List is composed of four parts: approved prescription drug products with therapeutic equivalence evaluations, over-the-counter (OTC) drug products that require approved applications as a condition of marketing, drug products in the Division of Blood and Blood Products approved under Section 505 of the Act, and products discontinued from marketing or products which have had their approval withdrawn for other than safety or efficacy reasons. This publication also includes indices of prescription and OTC drug products by trade or established name and by applicant name (holder of the approved application), and a list of applicants' abbreviated name designations. In addition, a list of uniform terms is provided. An Addendum contains appropriate drug patent and exclusivity information for the Prescription, OTC, and Drug Products in the Division of Blood and Blood Products Approved Under Section 505 of the Act lists.

E. In Vitro Indexes of Bioavailability

In view of these regulations, additional research aimed at improving the assessment and prediction of bioequivalence is needed. It is important that this research include efforts to develop in vitro tests that will be valid predictors of bioavailability in humans. If in vitro dissolution properties for a drug are found to serve as a useful index of in vivo absorption, the time, expense, and difficulties of clinical studies may be reduced or eliminated. Levy et al. [18] and Wagner [19] have shown in several cases that correlations exist between in vitro testing and in vivo absorption.

In vitro dissolution rate screening has been used [20] as a sensitive quality control measure to show changes in drug release for products undergoing variable storage conditions. It is also used to warn of poor bioavailability of drugs from dosage forms that show erratic release patterns in comparative studies.

An early investigation by Nelson [21] demonstrated a correlation between in vitro dissolution rate and the speed at which tetracycline in four different dosage forms was excreted in vivo. Levy [22] showed a linear correlation between salicylate excretion following oral administration of two aspirin tablets and rate of in vitro dissolution. Levy went on to demonstrate a correlation between percent of drug absorbed and rate of

dissolution. MacDonald et al. [23] found differences in in vivo availability among tetracycline capsules from various sources and attempted to correlate these differences by an in vitro method using an automated dissolution apparatus. The authors reported that they were partially successful in their goal. Bergan et al. [24] determined that in vitro dissolution rates of two tetracycline and seven oxytetracycline preparations and compared them with absorption characteristics as obtained by a crossover study on 10 volunteers. The products showed marked variations in bioavailability. The rate of dissolution was found to correlate well with absorption characteristics for some products but not for others.

Other workers [25,26] have found good correlation between dissolution rate and drug plasma levels. However, correlations cannot always be expected between in vivo and in vitro results. The failure of good correlation is the result of a number of factors, including improper in vitro stirring rate, variable gastric emptying time, rapid versus slow absorbers, failure of dissolution to be the rate-limiting step in vivo, and other problems.

F. Methodology for Conducting Bioavailability Studies

In 1972, the Academy of Pharmaceutical Sciences [27] published Guidelines for Biopharmaceutical Studies in Man. This monograph presents a systematic approach to the conduct of bioavailability studies based on analytical determination of drug in blood and/or urine.

In January 1977, the FDA [2] published in the *Federal Register* the Characteristics of good analytical methodology for an in vivo bioavailability study. They stated that the method for

> metabolite(s), in body fluids or excretory products, or the method used to measure an acute pharmacological effect shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate precision, the actual concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), achieved in the body.

In addition, when the analytical method is not sensitive enough to measure accurately the concentration of the active drug ingredient or therapeutic moiety, or its "metabolite(s), in body fluids or excretory products produced by a single dose of the test product, two or more single doses may be given together to produce higher concentration."

It is interesting that the regulations were not more specific in their analytical requirements. For example, no distinction is made between chemical, radioactive, microbiological, and other methods. Radioactive methods are used extensively today in drug development, and the investigators must be careful to verify that the measured radioactivity is contained in the intact compound separated from its metabolites. It is also important to recognize that the dosage form containing the radioactive drug to be tested possess physical and chemical properties identical to those of the unlabeled dosage form.

The 1977 monograph of the FDA [2] listed the following general approaches for determining bioavailability:

1. Bioavailability is usually determined by measurement of:
   a. The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in biological fluids as a function of time; or
   b. The urinary excretion of the therapeutic moiety or its metabolite(s) as a function of time; or
   c. An appropriate acute pharmacological effect.
2. Bioavailability may be determined by several direct or indirect in vivo methods, generally involving testing in humans. The selection of the method depends upon the purpose of the study, the analytical method available, and the nature of the drug product. These limitations affect the degree to which precise pharmacokinetic studies can be applied and, in some cases, necessitate the use of other methods. Bioavailability testing shall be conducted using the most accurate, sensitive, and reproducible approach available among those set forth in paragraph (c) of this section.
3. The following in vivo approaches, in descending order of accuracy, sensititivity, and reproducibility, are acceptable for determining the bioavailability of a drug product:
   a. In vivo testing in humans in which the concentration of the active drug ingredient or therapeutic moiety or its metabolite(s), in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time, or in which the urinary excretion of the therapeutic moiety, or its metabolite(s), is measured as a function of time. This approach is particularly applicable to dosage forms intended to deliver the active drug ingredient or therapeutic moiety to the bloodstream for systemic distribution within the body (i.e., injectable drugs, most oral dosage forms, most suppositories, certain drugs administered by inhalation, and some drugs administered by local application to mucous membranes).
   b. In vivo testing in humans in which an appropriate acute pharmacological effect of the active drug ingredient or therapeutic moiety, or metabolite(s), is measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility. This approach is applicable when appropriate methods are not available for measurement of the concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in biological fluids or excretory products but a method is available for the measurement of an appropriate acute pharmacological effect. This approach is applicable to the same dosage forms listed in paragraph (3, a) of this section.
   c. Well-controlled clinical trials in humans that establish the safety and effectiveness of the drug product. This approach is the least accurate, sensitive, and reproducible of the general approaches for determining in vivo bioavailability in humans. For dosage forms intended to deliver the active drug ingredient or therapeutic moiety to the bloodstream for systemic distribution within the body, this approach shall be considered as providing a sufficiently accurate estimate of in vivo bioavailability only when analytical methods are not available to permit use of one of the approaches outlined in paragraph (3,

a and b) of this section. This approach shall also be considered as sufficiently accurate for determining the bioavailability of dosage forms intended to deliver the therapeutic moiety locally (e.g., topical preparations for the skin, eye, ear, musous membranes); oral dosage forms not intended to be absorbed (e.g., an antacid or a radiopaque medium); and bronchodilators administered by inhalation if the onset and duration of pharmacological activity are defined.

d. Any other in vivo approach approved by the Food and Drug Administration intended for special situations should include those circumstances where the in vivo bioavailability of a drug product might be determined in a suitable animal model rather than in humans or by using a radioactive or nonradioactive isotopically labeled drug product.

When a drug dosage form is administered to humans and/or animals and serial blood samples are obtained and quantified for drug content, data are obtained as a function of time. This enables one to graphically represent the results as illustrated in Figure 9. The curve can be mathematically analyzed and pharmacokinetic parameters obtained as discussed in Section IV. However, it should be noted that there are three important parameters necessary for the interpretation of bioavailability studies. These include the peak height concentration, the time of the peak concentration, and the area under the plasma concentration-time curve. The peak height is important because it gives an indication of the intensity,

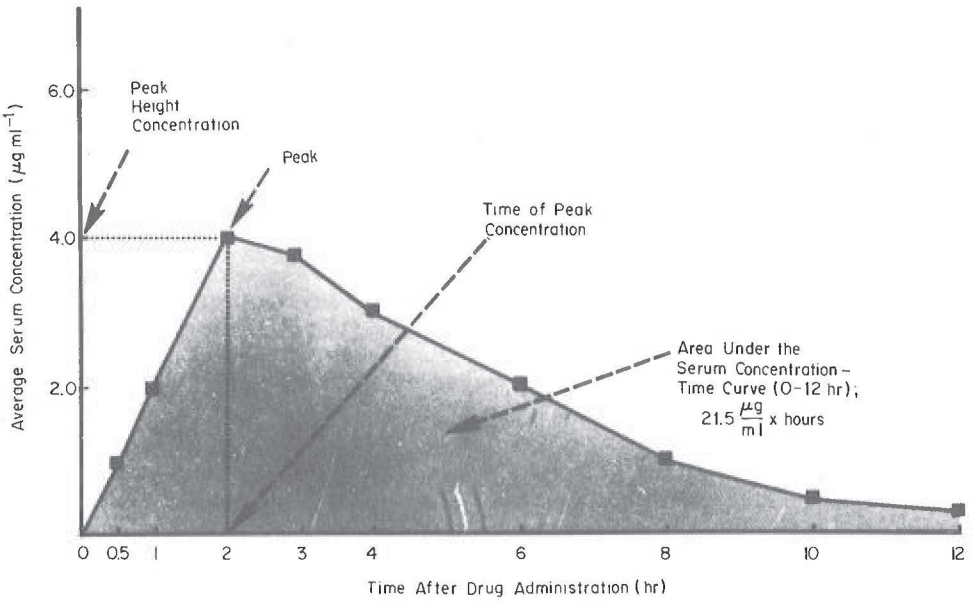

Figure 9 Serum concentration-time curve following a single dose of a drug that shows an absorption phase and an elimination phase. (From Ref. 27.)

and in conjunction with the minimum effective concentration (MEC), provides an indication of duration of action. The time for the peak to occur is important because it is related to the rate of absorption of the drug from dosage form after oral administration. It should only be considered as a simple measurement of rate of absorption. The area under the curve is perhaps one of the most important parameters. It represents, in this case, the amount of drug absorbed following a single administration of the drug.

Several integration techniques may be used to determine the area under the curve, and the accuracy of the value obtained will vary depending on the integration technique selected. The following integration methods (in decreasing order of accuracy) have been employed [28]:

1. Milne fifth-order predictor-corrector
2. Range-Kutta fourth order
3. Adams second order
4. Simpson's rule
5. Trapezoidal rule
6. Rectangular rule

The trapezoid rule has gained popularity, although it is not the most accurate method. It is described in a later section, where the trapezoidal rule is used in a sample calculation.

Let us assume that a bioavailability study is conducted with the purpose of comparing two different formulations containing the same therapeutic moiety. The results of these studies are graphically illustrated in Figure 10. It can be concentration and time to peak. The area under the

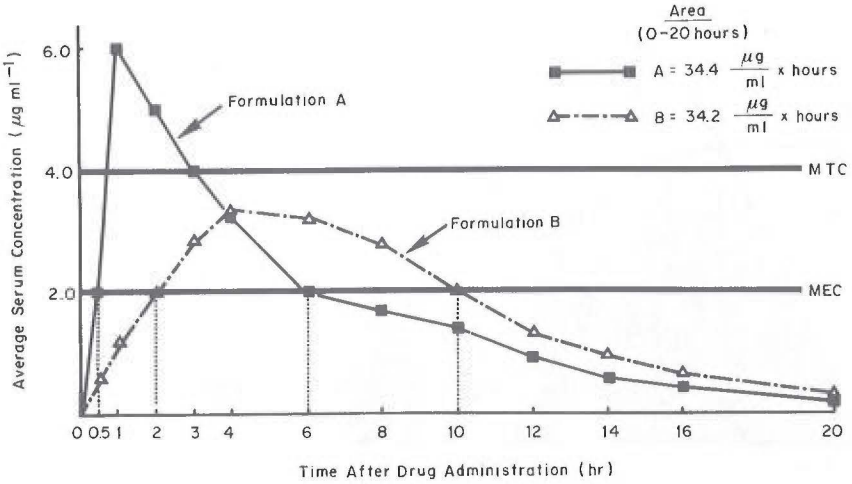

Figure 10 Serum concentration-time curves obtained for two different formulations of the same drug given at the same dose. The relationship of the curves to the minimum toxic concentration (MTC) and minimum effective concentration (MEC) is shown. (From Ref. 27.)

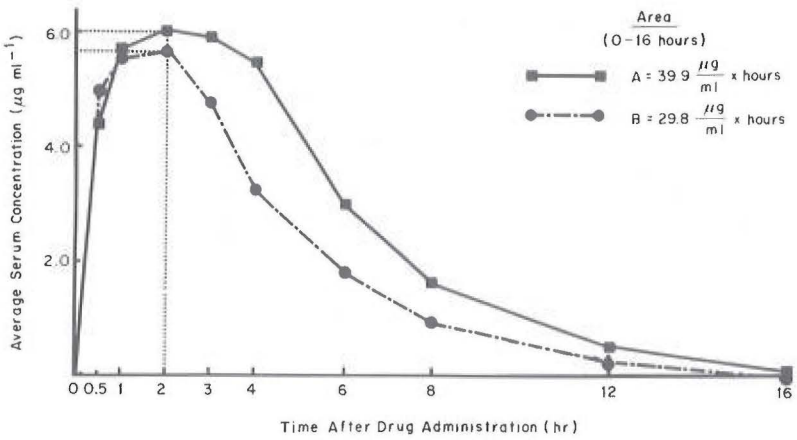

Figure 11 Serum concentration-time curves obtained for two formulations of the same drug given at the same dose, showing similar peak heights and times, but significantly different areas under the curve. (From Ref. 27.)

concentration-time curve for each formulation is the same, indicating that the extent (amount) of drug absorption is the same for both formulations. However, since disparities exist with regard to peak height and time to peak, these formulations cannot be considered to be bioequivalent. Consequently, they may not perform equivalently in terms of efficacy and toxicity. It should be emphasized that differences between formulations are reflected in rate of absorption and not in extent of obsorption. Figure 11 illustrates an example in which the rate of absorption is the same for both formulations, as observed by the same time to peak and peak concentration, but the extent of drug absorption as reflected by the areas under the respective concentration curves is different. In neither of the cases can the formulations be considered bioequivalent; these are clear examples of inequivalence in bioavailability. Wagner [29] summarized the different types of comparative bioavailability studies.

Figures 12 to 14 depict methods of estimating bioavailability based on studies in humans where blood levels, urinary excretion, or acute pharmacological response are measured. The methods can be classified depending on the measurement that is made: Figure 12 considers measurement of unchanged drug, Figure 13 a metabolite of a drug, and Figure 14, the total drug, that is, metabolite(s) plus unchanged drug. The symbol τ represents the dosing interval.

The quantitative aspects of biopharmaceutics and pharmacokinetics and associated methods are presented in later sections of the chapter. But before we can study the absorption kinetics of a drug, we should become familiar with the preparation of protocols for comparative bioavailability testing, and then examine various factors, both physiological and physical chemical, which influence the release of a drug from its dosage form and absorption into the systemic circulation.

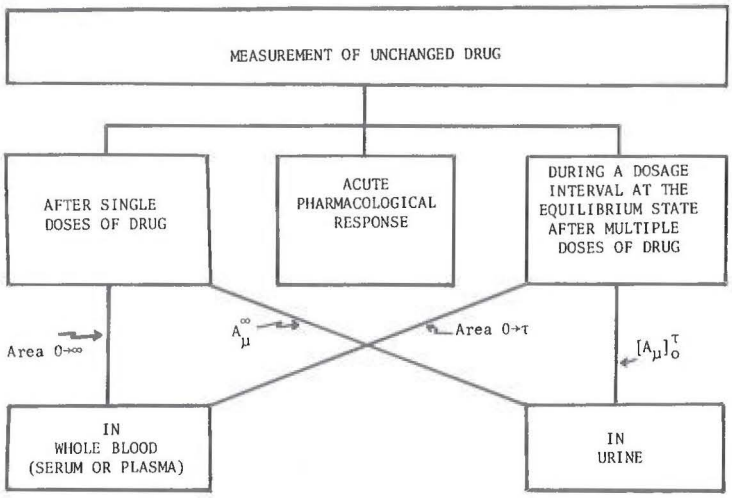

Figure 12 Measurement of unchanged drug for the estimation of bioavailability. Area $0 \rightarrow \infty$ = area under concentration curve from zero to infinite time. Area $0 \rightarrow \tau$ = area under concentration curve within a dosage interval τ. A_μ^∞ = amount of unchanged drug excreted in the urine in infinite time after a single dose. $[A_\mu]_o^\tau$ = amount of unchanged drug excreted in the urine during a dosage interval τ. (From Ref. 29.)

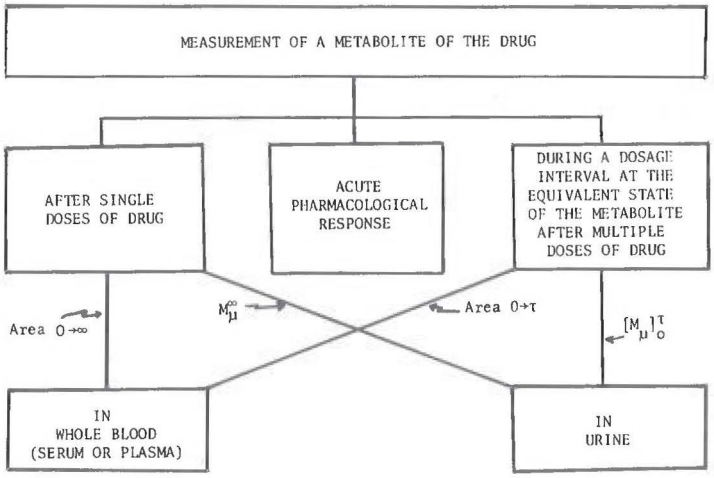

Figure 13 Measurement of a drug metabolite for the estimation of bioavailability. M_μ^∞ = the amount of a metabolite excreted in the urine in infinite time. $[M_\mu]_o^\tau$ = the amount of metabolite excreted in the urine in a dosage interval τ. (From Ref. 29.)

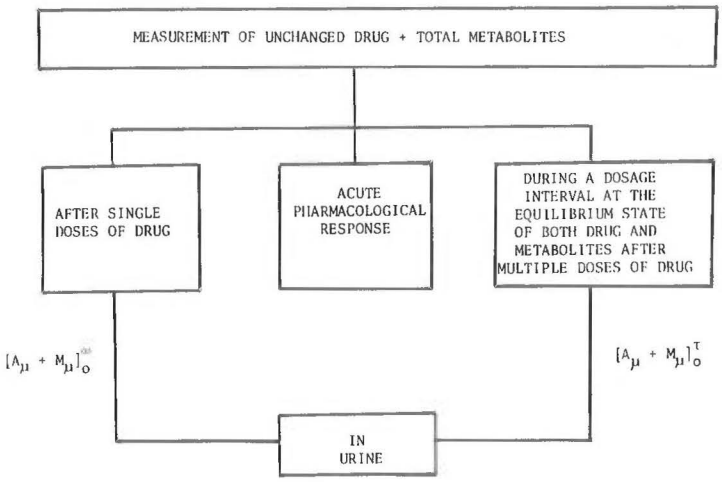

Figure 14 Measurement of unchanged drug and total metabolites for the estimation of bioavailability. $[A_\mu + M_\mu]_o^\infty$ = total amount of unchanged drug and all metabolites excreted in the urine in infinite time. $[A_\mu + M_\mu]_o^\tau$ = total amount of unchanged drug and all metabolites excreted in the urine during a dosage interval τ. (From Ref. 29.)

G. Protocols for Comparative Bioavailability Trials

The success of any experiment lies in its design. For this reason, protocols for bioavailability studies are of extreme importance. At the 13th Annual International Industrial Pharmacy Conference at Lakeway, Texas, Skelly [30] discussed the elements of a good protocol. His intent was to make it easier for the FDA and the applicant to recognize and resolve differences of opinion before a study got under way rather than after, in order to expedite the review of drugs where bioavailability is a critical requirement for approval.

The protocol guidelines for ANDA and NDA submissions as suggested by Skelly [30] include information on the drug and its clinical use; clinical facilities to be used and investigators responsible for the study; a plan of experimentation including reference to subjects involved; drugs to be administered; the treatment plan; sample collection; chemical, pharmacological, and/or clinical end points; assay methodology; and data analysis. Appendices to the protocol should include the consent form, precautions for the subject regarding possible adverse reactions, subject instruction sheet, clinical chemistry form, and an insert describing the characteristics of the drug. The reader should refer to the original paper [30] for a detailed outline of the proposed protocol.

H. Statistical Considerations of Bioavailability/ Bioequivalence Studies

Donald Schuirmann [31], in a very elegant way, discussed the statistical issue associated with the analysis of bioavailability/bioequivalence studies.

Briefly, the issue that has received the most attention in the pharmaceutical and statistical literature is the question of statistical methods for determining whether two formulations of a drug have been shown to be equivalent with respect to average bioavailability in the population. Bioavailability, in this context, is to be characterized by one or more blood concentration profile variables, such as area under the blood concentration-time curve (AUC), maximum concentration (C_{max}), etc., and possibly by urinary excretion variables as well.

Hauck and Anderson [32], in an article in which they proposed a new approach to this problem, gave a clear explanation of why the null hypothesis of *no difference* between the two averages, as tested by the "treatments" F test from the analysis of variance of a two-treatment (formulation) study, is the wrong statistical hypothesis for assessing the evidence in favor of a conclusion of equivalence. And yet, as Hauck and Anderson note, the test of the hypothesis of no difference is still utilized by many who seek to demonstrate equivalence of two formulations. In most cases those who utilize the test of the hypothesis of no difference supplement it with some assessment of what the power of the test would have been if the averages had been different enough to be considered inequivalent. This Power Approach, as it will be called, has been a standard method in bioequivalence testing, in spite of the fact that it is based on the test of an inappropriate statistical hypothesis.

Schuirmann compares this power approach to another method for assessing the equivalence of two formulations which will be called the *Two One-Sided Tests Procedure*. Then the two one-sided tests procedure was compared to the proposed method of Hauck and Anderson.

Schuirmann concluded that for the specific choice of $\alpha = 0.05$ as the nominal level of the one-sided tests, the two one-sided tests procedure has uniformly superior properties to the power approach in most cases. The only cases where the power approach has superior properties when the true averages are equivalent correspond to cases where the chance of concluding equivalence with the power approach when the true averages are not equivalent exceeds 0.05. With appropriate choice of the nominal level of significance of the one-sided tests, the two one-sided test procedure always has uniformly superior properties to the power approach.

The Two One-Sided Tests Procedure

The Two One-Sided Tests Procedure, as its name implies, consists of decomposing the interval hypothesis H_0 and H_1 into two sets of one-sided hypotheses

$$H_{01}: \quad \mu_T - \mu_R \leq \theta_1$$

$$H_{11}: \quad \mu_T - \mu_R > \theta_1$$

and

$$H_{02}: \quad \mu_T - \mu_R \geq \theta_2$$

$$H_{12}: \quad \mu_T - \mu_R < \theta_2$$

The two one-sided tests procedure consists of rejecting the interval hypothesis H_0, and thus concluding equivalence of μ_T and μ_R, if and only if both H_{01} *and* H_{02} are rejected at a chosen *nominal level of significance* α. The logic underlying the two one-sided tests procedure is that if one may conclude that $\theta_1 < \mu_T - \mu_R$, and may also conclude that $\mu_T - \mu_R \leqslant \theta_2$, then it has in effect been concluded that $\theta_1 < \mu_T - \mu_R < \theta_2$.

Under the normality assumption that has been made, the two sets of one-sided hypothesis will be tested with ordinary one-sided *t* tests. Thus it will be concluded that μ_T and μ_R are equivalent (for a balanced study) if

$$t_1 = \frac{(\bar{X}_T - \bar{X}_{12}) - \theta_1}{s\sqrt{2/n}} \geqslant t_{1-\alpha(v)}$$

and

$$t_2 = \frac{\theta_2 - (\bar{X}_T - \bar{X}_R)}{s\sqrt{2/n}} \geqslant t_{1-\alpha(v)}$$

where, once again, *s* is the square root of the "error" mean square from the crossover design analysis of variance. $t_{1-\alpha(v)}$ is the point that isolates probability α in the upper tail of the Student's *t* distribution with v degrees of freedom associated with the "error" mean square.

The two one-sided test procedure turns out to be operationally identical to the procedure of declaring equivalence only if the ordinary $1 - 2\alpha$ (not $1 - \alpha$) confidence interval for $\mu_T - \mu_R$ is completely contained in the equivalence interval $[\theta_1, \theta_2]$. For this reason, it is sometimes referred to as the confidence interval approach. In this form, it has been recommended by Westlake [33].

Unbalanced Crossover Studies

Schuirmann [31] discussed the statistical procedure for unbalanced studies. The assumption made previously is that the bioavailability/bioequivalence study under consideration was a balanced crossover study, that is

1. There is an equal number of subjects in each treatment-administration sequence.
2. There are no missing observations from any subject.

All of the results cited above concerning the properties of the two one-sided tests procedure and the power approach are equally true for unbalanced crossover studies. If we let *Est.* be the estimator of $\mu_T - \mu_R$, and SE be the standard error of the estimator, then the two one-sided tests procedure utilizes the two test statistics.

$$t_1 = \frac{Est. - \theta_1}{SE} \qquad \text{and} \qquad t_2 = \frac{\theta_2 - Est.}{SE}$$

In the case of balanced studies, the estimator *Est.* is in fact the difference of the *observed* means, $\bar{X}_T - \bar{X}_R$, and therefore the standard error SE is equal to $s\sqrt{2/n}$. In the case of unbalanced studies, the best unbiased (least squares) estimator of $\mu_T - \mu_R$ is *not*, in general, the difference of observed means.

For the special case of a two-treatment, two-period crossover study in which n_1 subjects receive the test formulation in period one and the reference formulation in period two, while n_2 subjects receive the reference formulation in period one and the test formulation in period two, the unbiased estimator is given by

$$Est. = \frac{\bar{X}_{T1} + \bar{X}_{T2}}{2} - \frac{\bar{X}_{R1} + \bar{X}_{R2}}{2}$$

where

$\bar{X}_{T1}$ = The observed mean of the n_1 observations on the test formulation in period one.

$\bar{X}_{T2}$ = The observed mean of the n_2 observations on the test formulation in period two.

$\bar{X}_{R1}$ = The observed mean of the n_2 observations on the reference formulation in period one.

$\bar{X}_{R2}$ = The observed mean of the n_1 observations on the reference formulation in period two.

$$Se = s\sqrt{\frac{1}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

where as before s is the square root of the "error" mean square from the crossover design analysis of variance, based on ν degrees of freedom.

Note that if $n_1 = n_2$, these formulas reduce to *Est.* $\bar{X}_T - \bar{X}_R$ and $s\sqrt{2/n}$ (where n = total number of subjects = $n_1 + n_2$) as before.

In the case of a study with more than two treatments (formulations) and/or periods, the formulas for *Est.* and SE will depend on the particular pattern of unbalance, and can be very complicated. Usually a computer routine will be needed to obtain them. The following example will illustrate the use of the two one-sided t-test.

A two-way crossover beioequivalence study was conducted on 27 subjects. The study is a balance study in the statistical sense. The following data was obtained from the Division of Generic Drugs at the FDA (Personal Communication).

Test product mean = 20.69

Reference product mean = 20.51

Error degrees of freedom = 26

t-value for 26 d_f = 1.7081

Error mean square = 5.41186

Number of subjects N = 27

Thus

$$20.96 - 20.51 \pm 1.708 \times \sqrt{5.41186} \times \sqrt{2/27}$$

$$0.18 \pm 1.080 \text{ or}$$

$$-0.9008 \text{ and } 1.2608$$

Using 100% as the reference value, the lower limit of confidence interval will be

$$-0.9008/20.51 = -0.0439 \text{ or } -4.39\%$$

$$100\% - 4.39\% = 95.61\% \text{ lower limit.}$$

The upper limit of the confidence interval will be

$$1.2608/20.51 = 0.0615 \text{ or } 6.15\%$$

$$100\% + 6.15\% = 106.15\% \text{ upper limit.}$$

In conclusion, the confidence interval will be

$$95.61\% - 106.15\%$$

I. Common Pitfalls in Evaluating Bioavailability Data

Shrikant Dighé [34] has discussed the current Bioavailability and Bioequivalence requirements and regulations. In his discussion he indicated that despite the progress in the conduct of bioequivalence studies the FDA has observed the following five major causes of deficient submissions:

1. Inadequate validation of analytical methodology
2. Insufficient duration of study
3. Insufficient sampling
4. Inadequate number of subjects
5. Deficient experimental design

Validation of Analytical Methodology

Once a drug assay is validated with respect to precision, accuracy, and linearity, subject samples should be measured along with a suitable standard or calibrated curve, and at least two independently prepared control samples. Ideally, controls should be run at three concentrations—low, intermediate, and high. Each should be analyzed in duplicate. The advantage of the multilevel control is that if a systematic error develops, information may be obtained as to whether the error is proportional to concentration or constant over the assay range. The duplicate analyses of controls permit assessment of within-batch imprecision. Loss of precision is often an early warning of problems in an analytical system.

The control samples should be spread evenly throughout the batch. A control sample after every 8–10 subject samples is not unreasonable, and each should be preceded by a subject sample. Good laboratories generate a standard curve daily for analysis of samples. Some laboratories even prepare a new standard curve for analysis of blood samples of each

subject. Whatever the practice, a reliable standard curve and use of controls during analysis are required to assure the validity and reliability of results.

Insufficient Duration of Study

Insufficient duration of a bioavailability study is another deficiency that crops up from time to time in the submissions. In a bioequivalence study, sampling of the biological fluid should normally be conducted for 3 to 5 biological half-lives of the drug which is under investigation. A drug with an elimination of half-life of 15 hr would, thus, entail collecting blood or urine samples for at least 48 hr.

Insufficient Sampling

Insufficient number of samples and inappropriate times of sampling is a deficiency that was quite common a few years ago. However, even today the FDA occasionally see submissions with inadequate sampling times. From an analytical point of view, the blood level curve for an oral dosage form consists of two important portions—the absorptive portion and the elimination portion. It is important that for an acceptable bioavailability study that both the absorption and elimination phases are characterized properly. One can achieve this by collecting enough blood samples at regular intervals. Thus, for a study of 24-hr duration, at least four samples should characterize absorption phase and an equal number of samples, the elimination phase. In view of the fact that three samples are needed to characterize the peak as accurately as possible, the number of samples needed would be actually greater than minimal eight samples for such a study.

Experimental Design

The experimental design for bioequivalence study should preferably be randomized Latin square crossover. Although rather rare now, one does occasionally see studies with parallel or sequential treatment design when a proper design calls for a crossover.

Insufficient number of subjects in a bioequivalence study still plagues some of the studies in submissions. A bioequivalence study should have sufficient power statistically to detect at least 20% difference between the two treatments at α of 0.05 and β of 0.2. A power of 0.8 or better ensures the acceptability of the study statistically. The power of the study depends on the variability observed from subject to subject and hence, on the number of subjects employed in the study. The higher the variability, the greater the number of subjects needed for a bioequivalence study with adequate power.

Minor Deficiencies in Biopharmaceutical Submissions

Dighé [34] also pointed out minor deficiencies encountered in bioequivalence studies. Some of these are:

1. Absence of data on dropouts
2. Lack of reporting of adverse reactions
3. Absence of information of the test product lot employed in bioavailability/dissolution testing
4. Dissolution testing on 6 instead of required 12 dosage units

5. Absence of description of assay method used in dissolution testing
6. Formulations of different strengths of a dosage form not submitted
7. Reformulation Supplements: Composition of old formulation and dissolution data on it not submitted

Dighé [34] discussed in his article that investigators should be aware of the requirement of bioequivalence studies for a dosage form with multiple strengths. New products with multiple strengths subject to bioavailability requirement under ANDAs and paper NDAs will be required to conduct in vivo bioequivalence studies on the low as well as high strengths in order to obtain approval of the two strengths, and the strengths in between. In the case of controlled release products a single dose study and a multi-dose study will have to be conducted on at least one strength, and a single dose study on other strengths.

The FDA is increasingly becoming aware of the role played in therapy of metabolites which exhibit pharmacologic activity similar to that of the parent drug. In light of this information, the Division of Biopharmaceutics will require that active metabolites be also measured in addition to the parent drug in a bioavailability study. As an example, the FDA cited thioridazine hydrochloride tablets. Recently, the agency approved ANDAs from three firms for this drug product on the basis of single dose studies. The two active metabolites of thioridazine are mesoridazine and sulforidazine. The agency requires that in a bioequivalence study for thioridazine HCl tablets, the two active metabolites along the parent drug should also be measured.

Dittert and DiSanto [35] have discussed common pitfalls in evaluating bioavailability data. They indicated that perhaps the single most common error made in interpreting bioavailability data is that of "cross-study comparison." This occurs when the blood concentration-time curve of a drug product in one study is compared with the blood concentration-time curve of that drug product in another study. They stated three reasons why such cross-study comparisons are dangerous and can lead to false conclusions. The following examples used to illustrate the three points are taken from actual bioavailability data.

1. *Different Subject Populations*, Figure 15 shows serum concentration-time curves for the same lot of penicillin tablets in two subject populations. Both studies were performed with the same protocol. Study 1 was done with hospital employees, while study 2 was done with prison volunteers. There is approximately a 25% difference in both peak concentration and area (0 to 6 hr) under the serum concentration-time curve. This apparent difference in the bioavailability of the same lot of tablets conducted with identical protocols and assayed by the same technique can be attributed to the different subjects used in these studies.

2. *Different Study Conditions*. Parameters such as the food intake of the subjects before and after drug administration can have dramatic effects on the absorption of certain drugs. Figure 16 shows the results of a three-way crossover test where the subjects were fasted 12 hr overnight and 2 hr after drug administration of (a) an uncoated tablet, (b) a film-coated tablet, and (c) an enteric-coated tablet of an acid-labile antibiotic. The results of this study suggest that the unprotected tablet is superior to both the film-coated and enteric-coated tablet in terms of blood level performance. These results also suggest that neither film coating nor enteric coating is necessary for optimal blood level performance. Figure 17

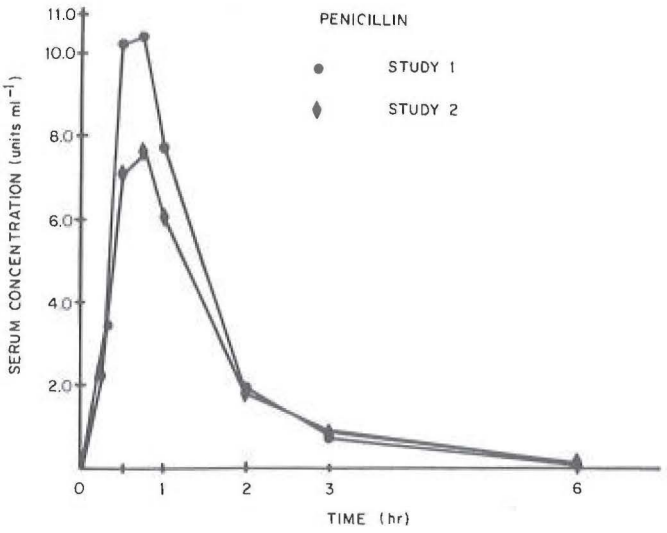

Figure 15 Average serum concentrations obtained after a single oral 500-mg dose of penicillin using the same lot of penicillin tablets in two different subject populations. (From Ref. 27.)

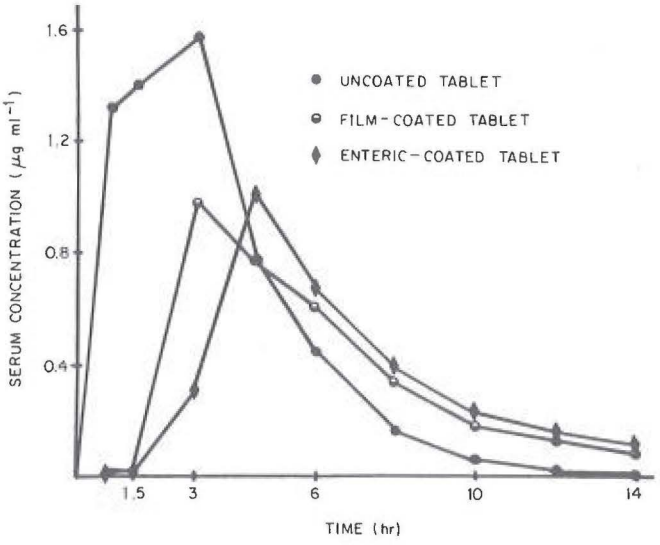

Figure 16 Serum level-time profiles of an acid-labile antibiotic administered in equal doses but as three different tablet dosage forms. The single oral dose consisted of two 250-mg tablets. Results are for 21 normal adults who fasted overnight. (From Ref. 27.)

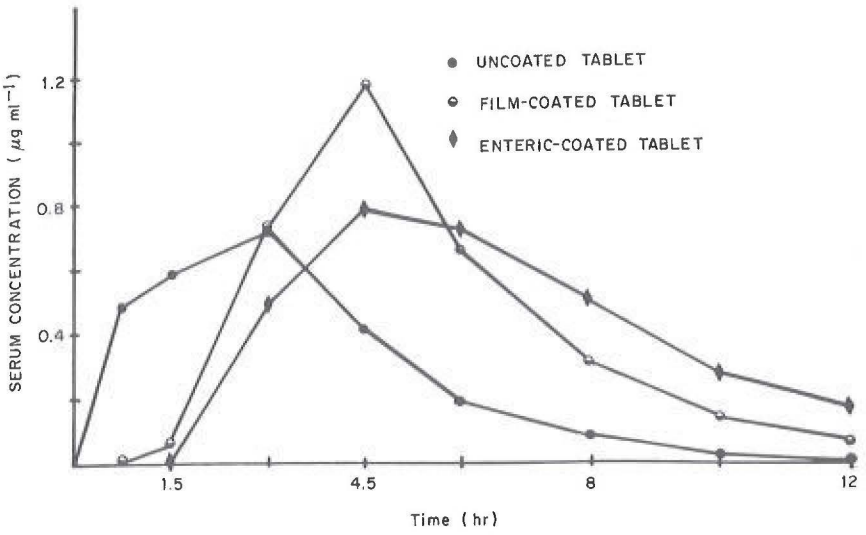

Figure 17 Serum level-time profiles of an acid-labile antibiotic administered in equal doses but as three different tablet dosage forms. The single oral dose consisted of two 250-mg tablets. Results are for 12 normal adults with only a 2-hr preadministration fast. (From Ref. 35.)

shows results with the same tablets when the study conditions were changed to only a 2-hr preadministration fast with 2 hr of fasting preadministration. In this case, the blood levels of the uncoated tablet were markedly depressed, whereas the film-coated and enteric-coated tablets showed relatively little difference in blood levels. From this second study, it might be concluded that film coating appears to impart the same degree of acid stability as does an enteric coating. This might be acceptable if only one dose of the antibiotic were required. However, Figure 18 shows the results of a multiple-dose study in which the enteric-coated tablet and the film-coated tablet were administered four times a day immediately after meals. The results show that the film coating indeed does not impart the degree of acid stability that the enteric coating does when the tablets are administered immediately after food in a typical clinical situation.

3. *Different Assay Methodology.* Depending on the drug under study, more than one assay method may be available. For example, some steroids can be assayed by a radioimmunoassay, competitive protein binding, gas–liquid chromatography, or indirectly by a 17-hydroxycorticosteroid assay. Figure 19 shows the results of a comparison of steroid tablets using a competitive protein binding method and a radioimmunoassay, respectively. Obviously, the wrong conclusion would have been reached if one product had been assayed by one method and the other product by the second method and the results had been compared. Even in cases where only one assay method is employed, there are numerous modifications with respect to technique among laboratories which could make direct comparisons hazardous.

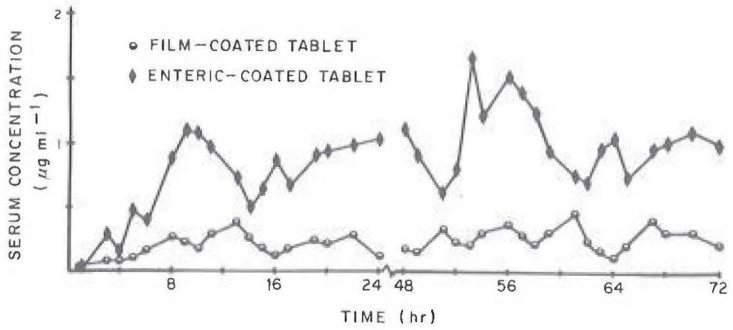

Figure 18 Average serum level-time curves for an acid-labile antibiotic administered in two different tablet dosage forms. In each case the oral tablets were tested in 24 normal adults each of whom received the medication q.i.d., with meals and at bedtime. (From Ref. 35.)

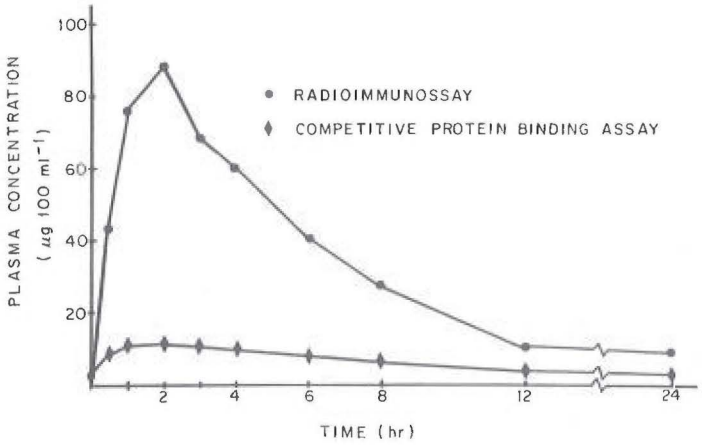

Figure 19 Average plasma level curves for a steroid administered as single oral doses to 24 normal adults. In one case plasma levels were determined by a competitive protein binding assay and in the other case by a radioimmunoassay. (From Ref. 35.)

II. DRUG ABSORPTION

A drug must transverse several biological membranes before it reaches its site of action irrespective of the route of administration. In the oral cavity there are two regions, buccal and sublingual, where the membranes are very thin and have a copious blood supply. Sublingual administration of a drug entails the placing of the drug in its dosage form (e.g., tablet) under the tongue for its ultimate absorption into the systemic circulation. Buccal administration of a drug is ordinarily accomplished by placing the drug between the cheek and the gums.

Drugs administered orally, and probably rectally, pass directly through the liver on their circulation through the body (see Figure 2). If the drug is readily biotransformed in the liver, this initial passage by way of the hepatic route can result in considerable metabolism of the drug before it arrives at the peripheral circulation. This loss of drug on first passage through the liver is called the *first pass effect*. Drugs given by intravenous, intramuscular, subcutaneous, sublingual, and buccal administration, on the other hand, enter the circulation directly and are carried to body tissues before passage through the liver, where they might be broken down. Sublingual and buccal tablets are therefore ideal for potent, low-dose drugs.

Venous drainage from the oral cavity goes directly to the heart, which makes it an excellent route for treating angina with nitroglycerin. On the other hand, toxic drugs such as nicotine also pass directly to the heart without any chance of detoxication. Once widely used as an insecticide, nicotine produced an environmental hazard because of its great toxicity.

The transport or passage of drugs across membranes in various parts of the body depends to a large degree on the selectivity and characteristics of the membrane. Pore size, membrane composition, and the presence of energy-dependent carriers may all affect drug absorption; however, most drugs pass across biological membranes by simple or passive diffusion.

A. Passive Membrane Diffusion

Passive diffusion happens when drug molecules exist in high concentration on one side of a membrane and lower concentration on the other side. Diffusion occurs in an effort to equalize drug concentration on both sides of the membrane, the rate of transport being proportional to the concentration gradient across the membrane.

When the volume of fluids are fixed, the movement of drug across a membrane can be described in terms of Fick's laws. Fick's first law states that the rate of diffusion or transport across a membrane is directly proportional to the surface area of the membrane and to the concentration gradient, and is inversely proportional to the thickness of the membrane. The expression for Fick's first law is

$$\frac{dm}{dt} = -DA\,\frac{dc}{dx} \qquad (1)$$

where m is the quantity of drug or solute diffusing in time t, dm/dt the rate of diffusion, D the diffusion constant, A the cross-sectional area of the membrane, dc the change in concentration, and dx the thickness of the membrane. A change in any of these variables will alter the rate of transport of drug into the blood. The value of the diffusion coefficient is dependent on the chemical nature of the drug and, in particular, its degree of lipophilicity, which can be evaluated approximately from the oil–water partition coefficient. Diffusion can also be influenced by temperature, pressure, and by the nature of the solvent. Faster absorption occurs in the small intestine rather than the stomach because of the high surface area provided by villi and microvilli found in the small intestine. Drugs are rapidly absorbed through very thin membranes (e.g., the alveolar membrane of the lungs). This explains why inhaled medication is more

rapid acting than drugs in oral dosage forms. The driving force for passive diffusion in Eq. (1) is the concentration gradient between drug in the gut and drug in the blood. According to Fick's law, the rate of diffusion is proportional to the concentration gradient. Therefore, the rate of change of concentration is proportional to the concentration, and since the concentration of drug in the blood is negligible compared to that in the gut:

$$\frac{dc}{dt} = -kc \tag{2}$$

The negative sign in this first-order equation indicates that concentration decreases with time.

If the concentration of drug at the absorption site is c_0 at $t = 0$, then at some later time t, the concentration of drug remaining unabsorbed may be designated as c. Integration of Eq. (2),

$$\int_{c_0}^{c} \frac{dc}{c} = -k \int_{0}^{t} dt \tag{3}$$

yields the equation

$$\ln \frac{c}{c_0} = -kt \tag{4}$$

or

$$\log \frac{c}{c_0} = \frac{-kt}{2.303} \tag{5}$$

A plot of c/c_0 on a logarithmic scale against time on a rectangular scale should produce a straight line with a negative slope representing the absorption rate constant k. The half-life for drug absorption is the time required for the concentration of drug in the gut, c, to be reduced to one-half its initial value, and for a first-order process can be calculated by using the expression

$$t_{1/2} = \frac{0.693}{k} \tag{6}$$

B. Active Membrane Transport

Drugs absorbed via an active transport mechanism may pass from areas of lower concentration to areas of higher concentration. The transfer is thought to be mediated by a "carrier," and in contrast to passive diffusion, chemical energy expended by the body is required for active transport to occur. The carrier system may consist of an enzyme or other substance in the gastrointestinal wall. The carrier combines with the drug and accompanies it through the membrane to be discharged on the other side. The carrier-drug complex is considered to have a higher permeability through the membrane of the gut than the drug alone. The process is

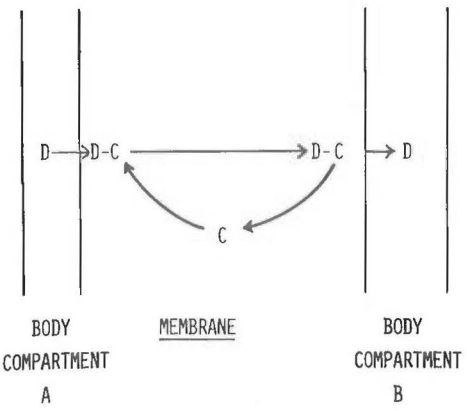

Figure 20 Action of carrier C, which facilitates passage of drug molecule D across a membrane. C combines with the drug at the surface of compartment A and releases the drug at compartment B. Carrier C returns to ferry the next drug molecule across the membrane.

depicted in Figure 20. Active transport of drugs is site-specific, and the greatest absorption occurs in locations of the gastrointestinal tract, where carrier concentration is highest. At low concentrations, the rate of drug absorption by active transport is proportional to drug concentration in the gut. At higher levels of drug, the carrier system eventually becomes saturated and the absorption levels off at a fixed maximum rate. Therefore, the absorption rate for substances absorbed via active transport will not increase as the dose increases, once the carrier mechanism has been saturated. Several body nutrients and vitamins, such as amino acids, thiamine, niacin, and riboflavin, are absorbed via active transport [36, 37]. Where the structure of a drug resembles that of an actively absorbed material, a strong possibility exists for the drug to pass into the blood via active transport. A number of organic compounds, including penicillin and phenol red, are secreted in the proximal renal tubules by active transport. Other examples include the transport of sodium ions from the gut lumen into the blood, and the secretion of hydrogen ions into the stomach [38]. Some antitumor drugs are thought to be transported actively, and these include 5-fluorouracil [39] and the serine and threonine derivatives of nitrogen mustard [40].

Facilitated diffusion is a special form of carrier transport (Fig. 20) that has many of the characteristics of active transport, but the substrate does not move against a concentration gradient. The uptake of glucose by cells is an example of facilitated diffusion. As with active transport, facilitated diffusion is mediated via a carrier molecule in the mucosa, is selective, saturable, and can be "poisoned" or inhibited by certain electrolytes (e.g., fluoride, and organic dinitrophenols). The main difference between active transport and facilitated diffusion is that there is no energy expenditure by the body for the latter process to occur.

Other mechanisms of absorption include pinocytosis and ion-pair absorption. Pinocytosis literally means "cell drinking" and is a process whereby the cell surface invaginates and takes in a small vacuole of liquid

containing the solute or drug. It is an important transport mechanism for proteins, but its significance in drug absorption is not entirely clear. The ion-pair absorption mechanism was postulated by Higuchi [41] to explain the absorption of large ionized compounds (e.g., quaternary amines and sulfonic acids), where absorption cannot be explained by the pH/partition theory. The authors postulated that the organic ion combined with a large ion of opposite charge to form an nonionized species. The increased lipoidal nature of the resulting molecule would account for rapid passage through the mucous membranes of the gastrointestinal tract.

In a recent paper by Boroujerdi [42], the kinetic relationships of the formation of the ion pair as a function of the ion-pair agent and the type of the biological membrane were discussed and analyzed. The author developed criteria for distinguishing the following two cases: (1) when the absorption is the rate limiting step in the process of permeation of the ion-pair, and (2) when the ion-pair crosses the membrane as through it were a fine sieve. Boroujerdi also extensively reviewed several studies where ion-pair formation improved the partition coefficient and diffusion across synthetic and biological membranes.

C. The pH-Partition Hypothesis

Passive diffusion through a membrane is probably the most common mechanism of drug absorption in the body regardless of the location of the membrane. Therefore, the greater the lipid solubility of the nonionized moiety, the faster and easier a drug will pass through the membrane. The pH-partition hypothesis was developed by Brodie et al. [43–46] to explain the absorption of ionized and nonionized drugs. The pK_a and oil/water partition ratio are two important parameters involved in the pH-partition theory of drug absorption. For a particular pH in the gastrointestinal tract, these parameters dictate the degree of ionization and lipoid solubility of a drug, which, in turn, determine the rate of absorption of drugs and transport through cellular membranes in the body. The pH of the human stomach varies from 1 to 3.5, although higher values have been recorded. Disease and the presence of food or antacids may drastically increase gastric pH. The duodenal pH is generally in the range 5 to 6 and the lower ileum may approach a pH of 8. Theoretical calculations and predictions as to the amounts and sites of absorption of weakly acidic and basic drugs have been made using the Henderson-Hasselbalch equations and the pH-partition principle.

For acids:

$$pH = pK_a + \log \frac{[\text{ionized form}]}{[\text{nonionized acid}]} \tag{7}$$

For bases:

$$pH = pK_a + \log \frac{[\text{nonionized base}]}{[\text{ionized form}]} \tag{8}$$

From the equations, one might expect acidic drugs or very weakly basic drugs to be absorbed predominantly from the stomach and basic drugs or very weakly acidic drugs to be absorbed from regions of higher pH in the

intestines. These expectations are based on the premise, following the pH partition theory, that the primary mode of drug absorption is via passive diffusion of the uncharged species. Thus, the Henderson-Hasselbalch equation can be used to calculate the relative amounts of charged and neutral forms from the pK_a of the drug and the pH of the environment.

The percent ionized is given by

$$\% \text{ Ionized} = \frac{I \times 100}{I + U} \tag{9}$$

where I is the concentration of a species in the ionized conjugate form and U the concentration of unionized species. Equation (7) may be rearranged to give

$$\frac{U}{I} = \text{antilog } (pK_a - pH) \tag{10}$$

for a weak acid so that Eq. (9) becomes

$$\% \text{ Ionized} = \frac{100}{1 + \text{antilog } (pK_a - pH)} \tag{11}$$

where pK_a is the dissociation constant for the neutral or nonionized acid. For a weak base, such as atropine or morphine, the comparable expression is

$$\% \text{ Ionized} = \frac{100}{1 + \text{antilog } (pH - pK_a)} \tag{12}$$

where pK_a is the dissociation constant for the cationic acid conjugate to the molecular base.

As an example, if one considers morphine, in which the pK_a of its cationic acid form $(\text{morphine H})^+$ is 7.87 at 25% C, the percentage ionization of morphine at a pH of 7.50 can be calculated using Eq. (12):

$$\% \text{ Ionized} = \frac{100}{1 + \text{antilog } (7.50 - 7.87)} + \frac{100}{1 + \text{antilog } (-0.37)}$$

$$= \frac{100}{1 + 0.427} = 70.10$$

The percentages of ionic (I) and molecular (U) forms of morphine ($pK_a = 7.87$) at various pH values are shown in Table 4. As observed in Figure 21, nonionized drug will distribute across the membrane so that the concentration of nonionized in fluid A will equal nonionized in fluid B. It is assumed that the ionized form of the drug cannot pass through the membrane. The pH of the fluids and the pK_a of the drug will then determine the ratio of nonionized to ionized drug in the two fluids, as seen in Figure 22. The relationship between pK_a and pH at the absorption site has been demonstrated for a variety of acids and bases. Table 5 shows the absorption of drugs from the rat stomach. The acidity of the stomach ensures that weak acids are but slightly ionized. The exceptions are

Table 4 Percentages of Morphine in Ionized (I) and Unionized (U) Forms at Various pH Values

pH	Percent I	Percent U
2.0	100	0.00
4.0	99.99	0.01
6.0	98.67	1.33
7.0	88.11	11.89
7.5	70.10	29.90
8.0	42.57	57.43
8.5	19.00	81.00
9.0	6.90	93.10
10.0	0.74	99.26
13.0	0.00	100.00

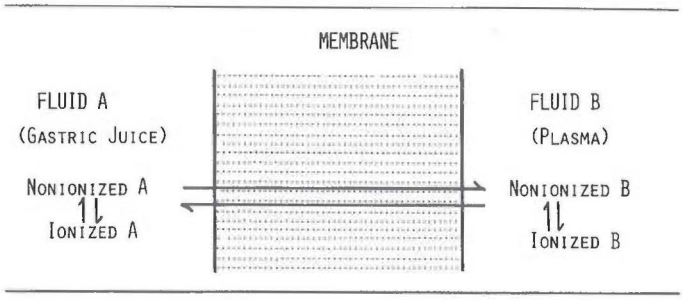

Figure 21 Partitioning of drug between gastric fluid and plasma.

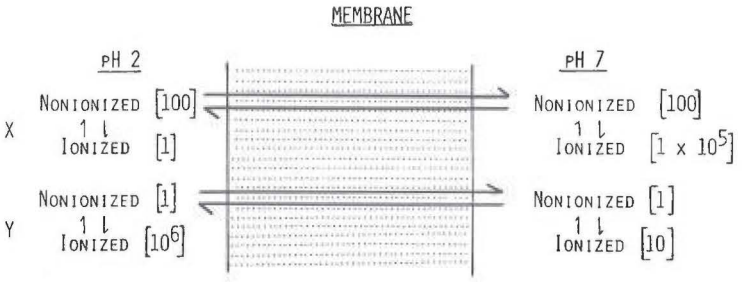

Figure 22 X, Nonionized and ionized concentrations of a weakly acidic drug with a pK_a of 4 in fluids with a pH of 2 and 7 separated by a membrane; Y, nonionized and ionized concentrations of a weakly basic drug with a pK_a of 8, under the same conditions as X.

Table 5 Absorption of Drugs from the Rat Stomach

Drug		pK	Absorption (%)
Acid	5-Sulphosalicylic	Strong	0
	Phenol red	Strong	2
	Salicylic	3.0	61
	Thiopental	7.6	46
	Barbital	7.8	4
	Quinalbarbitone	7.9	30
	Phenol	9.9	40
Base	Acetanilide	0.3	36
	Caffeine	0.8	24
	Aniline	4.6	6
	Dextromethorphan	9.2	0
	Mecamylamine	11.2	0
	Mepipophenidol	Strong	0
	Tetraethylammonium	Strong	0

Source: From Ref. 45.

relatively strong acids, such as phenol red, which is highly ionized even at pH 1.

The poor absorption of barbital illustrates that pK_a is not the only limiting factor, the lipid solubility of the nonionized form also being important. Very weak bases such as caffeine are well absorbed in the stomach, since these bases exist predominately in a nonionized form, even at acid pH. The varying degrees of absorption of three barbiturates have been shown by Schanker [47] to be related to the lipid–water partition coefficient, as shown in Table 6. The three barbiturates have very similar pK_a values but differ in lipid solubility, which apparently controls the degree of absorption.

The absorption of weak acids and weak bases from the rat intestine at varying pHs is shown in Table 7. As the pH of the solution in the intestinal lumen increased, the absorption of weak acids was found to decrease. The opposite was seen for weak bases: as the pH increased, the percent drug absorbed increased. It is important to realize that information in Table 4 was obtained from experiments conducted on animals under special conditions and that, as such, the data are highly idealized. Hogben et al. [44] postulated that the distribution across the gut with weak acids or bases is dependent on the "virtual" pH of the mucosal solution (i.e., the pH of a narrow microclimate adjacent to the mucosal surface rather than the pH of the bulk solution in the lumen of the gut). They

Table 6 Gastric Absorption of Barbiturates Compared with Their pK_a Values and Lipid–Water Partition Coefficients

Barbiturate	pK_a	Absorption (%)	$K = \frac{[CHCl_3]}{[H_2O]}$
Barbital	7.8	4	0.7
Secobarbital	7.9	30	23.3
Thiopental	7.6	46	100.0

Source: From Ref. 45.

pointed out that such a microclimate of fluid with a low pH calculated to be 5.3 would lead to a relatively high concentration of nonionized acid next to the mucosa, as compared to the concentration of nonionized species in the bulk mucosal solution at a higher pH, normally 6.6. The high concentration of the un-ionized species would lead to increased mucosal to serosal movement of the acid by a passive diffusion of the nonionic form. Values greater than 1 for the steady-state concentration ratio C_{plasma}/C_{gut} of a weak acid could be explained by this mechanism without postulating specific active transport.

Table 7 Comparison of Intestinal Absorption in the Rat at Several pH Values

Substance			Percent absorbed at:			
		pK_a	pH 4	pH 5	pH 7	pH 8
Acid	5-Nitrosalicylic	2.3	40	27	0	0
	Salicylic	3.0	64	35	30	10
	Acetylsalicylic	3.5	41	27	–	–
	Benzoic	4.2	62	36	35	5
Base	Aniline	4.6	40	48	58	61
	Amidopyrine	5.0	21	35	48	52
	p-Toluidine	5.3	30	42	65	64
	Quinine	8.4	9	11	41	54

Source: From Ref. 48.

For weak acids, ratios of C_{plasma}/C_{gut} are much higher for the stomach than for the intestine, which accounts for the generalization that weak acids are best absorbed from the stomach. The steady-state or equilibrium conditions employed by Brodie are not present in the intact animals and do not take into account the rate of absorption, which is a better determinant for the optimal absorption site than is the equilibrium ratio of C_{plasma}/C_{gut}. For weak acids the large surface area of the intestines results in a higher rate of absorption than in the stomach, and this is more important than the less favorable pH of the intestine.

Suzuki et al. [49, 50] have used theoretical models to study drug transport and absorption phenomena and have shown that pH-partition theory is a special limiting case of a more general approach. These investigators tested their models using the experimental data of Kakemi et al. [51–54]. These data included the in situ results in rats for intestinal, gastric, and rectal absorption of sulfonamides and barbituric acid derivatives. The correlations of in situ data using the models proved to be generally satisfactory, and pointed out the various diffusional coefficients and constants that should be accounted for in the study of absorption of drugs through living membranes.

D. Gastric Emptying Rate: Influence of Food and Other Factors

When a medicinal agent is preferentially absorbed in the stomach or intestine, and when absorption is site-specific in the gastrointestinal tract, the presence of food can have a profound influence on drug bioavailability. Food will slow tablet disintegration, decrease the dissolution rate of the active ingredient, and decrease intestinal absorption by reducing gastric emptying. An increase in the gut residual of certain drugs may result in several toxic side effects to the patient. Alteration in the normal microbiologic flora of the stomach by broad-spectrum antibiotics results in malabsorption of essential nutrients. The change in flora may cause infections in the tract because of the presence of foreign organisms that are usually kept under control by the natural flora. The ulcerogenic potential of potassium chloride and antiinflammatory agents, both steroidal and nonsteroidal, may be increased by lengthening gut residence time.

Among the several factors [55] that influence gastric emptying are the types of food ingested, volume, pH, temperature, osmotic pressure, and viscosity of stomach contents. The age, health, and position of the patient are also important. Davenport [55] reported that cold meals increase and hot meals decrease the emptying time of gastric contents. Levy and Jusko [56] have shown that an increase in the viscosity of the gastrointestinal fluids can decrease the absorption rate of certain drugs by retarding the diffusion of drug molecules to the absorbing membrane. However, Okuda [57] showed that a delay in gastric emptying due to high viscosity results in enhanced absorption of certain drugs (e.g., vitamin B_{12} and other vitamins).

A surfactant may also exert a specific pharmacologic effect on the gastrointestinal tract which influences drug absorption. Lish [58] reported that dioctyl sodium sulfosuccinate inhibits the propulation of a test

meal in the rat, owing to the formation of an inhibitory compound when the surface-active agent came in contact with the intestinal mucosa. Gastric motility in the dog was also found to be inhibited following the introduction of certain detergents into the gastric pouch [59].

The coadministration of a number of drugs, such as anticholinergics [60], narcotic analgesics [61], nonnarcotic analgesics [62], and certain tricyclic antidepressants [63], also cause delayed stomach emptying. Studies in humans have been reported correlating drug availability and stomach emptying with L-dopa [64] and digitoxin [65].

Jaffee et al. [66] demonstrated that the type of food can influence absorption. In studies with acetaminophen, carbohydrates were found to decrease absorption, whereas proteins did not. A report [67] related to the effects of food on nitrofurantoin absorption in humans indicated that the presence of food in the stomach appreciably delayed gastric emptying. A marked enhancement in the bioavailability of both macro- and microcrystalline nitrofurantoin from commercial solid dosage forms in nonfasting as compared to fasting subjects was also observed. These findings are consistent with the argument that a significant fraction of drug from both dosage forms dissolves in the stomach prior to being emptied into the duodenal region of the small intestine, where absorption is optimal [68]. Penicillin [69], lincomycin [70], tetracycline [71], erythromycin [72], and theophylline [73] have all shown reduced absorption efficiency when given with meals. Meals containing a high content of fats have been shown to increase the absorption of griseofulvin [74].

Drug absorption and excretion in some instances may be influenced by the quantity of fluid intake. Nogami et al. [75] followed the disintegration in vivo of calcium p-aminosalicylate tablets by X-ray. The tablets disintegrated more rapidly when ingested with water than without water. Human studies with digoxin have employed volumes of 240 ml [6] and 100 ml [7] of water administered to the patient. The effect of such gross differences in the quality of water utilized in bioavailability studies has not been extensively studied. The influence of the type of beverage administered with drugs has been studied by Levy et al. [76–78]. Theophylline absorption in the rat [76] was enhanced when administered in hydroalcoholic solutions containing 5 or 15% ethanol. Absorption was significantly decreased when theophylline was administered in 20% ethanolic solutions, as seen in Figure 23. When tested in several normal subjects, there was no significant difference in the average plasma concentrations of theophylline produced by these two solutions [77]. However, three subjects (all female) experienced nausea after taking the aqueous solution, whereas none became nauseous after taking theophylline in the hydroalcoholic solution.

A study [79] in humans showed that caffeine contained in a proprietary carbonated beverage (Coca-Cola) was absorbed much more slowly from this beverage than from coffee or tea. The sucrose and phosphoric acid contained in carbonated beverages have been shown to inhibit gastric emptying [80, 81]. On the other hand, a more recent paper by Houston and Levy [78] reported that compared to water, the carbonated beverage increased the bioavailability of riboflavin-5'-phosphate and significantly altered the metabolic rate of salicylamide when administered to healthy human adults. Sodium and calcium cyclamates have been reported to interfere markedly with the absorption of lincomycin [82]. The interference occurs both when the sweetening agent was mixed in solution and then

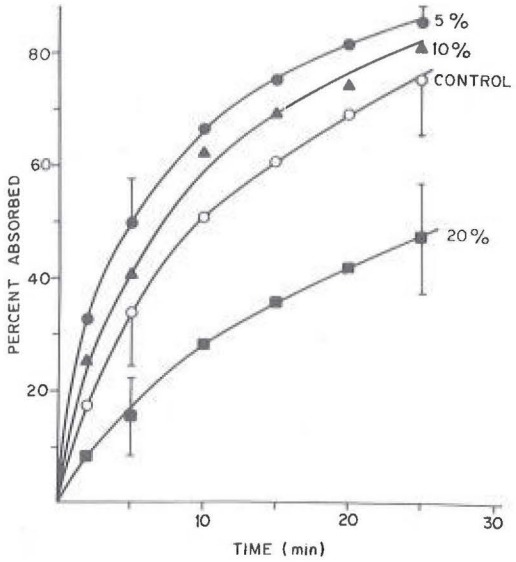

Figure 23 Effect of 0, 5, 10, and 20% (v/v) ethanol on the absorption of theophylline from a 50 mg% solution instilled into a cannulated segment of the small intestine of anesthetized rats (average of six animals per group). Vertical bars indicate one standard deviation in each direction. (From Ref. 76.)

ingested, and when the antibiotic was ingested and the sweetening agent was coadministered as a diet beverage.

Wilson and Washington [83] found that the major complication when studying the dissolution of dosage forms in vivo was the presence of food within the gastrointestinal tract. Food not only affects the rate at which the dosage form travels through the tract, but also influences the distribution of the formulation in the various segments. The size and shape of the dosage form plus the amount and type of food present at the time of administration, all influence the residence time of the dosage form in the stomach. Since food influences the gastric pH, the possibility for physical and chemical interactions between the drug and the food are possible. In addition, the food also changes the viscosity of the gastrointestinal fluid in which the drug is presented to the absorbing mucosa. Wilson and Washington [83] extensively reviewed the applications of gamma scintigraphy to study the dissolution and disintegration of tablets in vivo and drug distribution in the body. Gamma scintigraphy allows the passage of the formulation throughout the gastrointestinal tract to be monitored and stasis of the formulation can usually be detected. The position of a formulation and the degree of dispersion within the gastrointestinal tract can be related to the simultaneous plasma concentration for the drug. Since the majority of drugs are absorbed from the intestine, factors that influence the delivery of a dosage form to this region, e.g., food, can be studied using a dual isotope technique.

Thebault and co-workers [84] studied the influence of food on the bioavailability of theophylline from a slow release hydrophilic matrix tablet.

The release of drug from the tablet was independent of pH and followed zero order kinetics. A bioavailability study was conducted in volunteers who received the drug while fasting, or with a standard low fat, or high fat meal. Although several workers have previously reported the influence of food on the bioavailability of theophylline, Thebault and co-workers concluded from this study that the slight food/drug interaction which was seen with these slow release theophylline tablets seemed to be of no clinical significance.

E. Drug Interactions with Components of the Gastrointestinal Tract

Bile salts, present in the biliary secretions of the small intestine, act as surface-active agents. The influence of orally administered bile salts on the enhancement of drug absorption has been reviewed by Gibaldi and Feldmann [85]. It was suggested that the wetting action of these salts would promote the dissolution of hydrophobic and poorly soluble drugs, resulting in a faster rate of absorption. The dissolution rate of griseofulvin and hexestrol was, in fact, increased in solutions of bile salts [86]. Since the administration of a fatty meal stimulates bile production in the body, the high blood levels of griseofulvin in Figure 24 following such a meal can readily be explained [71].

However, other investigators have shown that bile salts do not enhance drug efficacy in all cases. The formation of insoluble nonabsorbable complexes have been demonstrated with neomycin and kanamycin [87]. More recent studies by Thompson et al. [88] demonstrated by analyses of aspirated intestinal content that ionized fatty acids, bile salts, and labeled cholesterol administered in a test meal were precipitated by neomycin. The precipitation of micellar lipids by the polymeric antibiotic provides an explanation for the hypocholesterolemia induced by this compound.

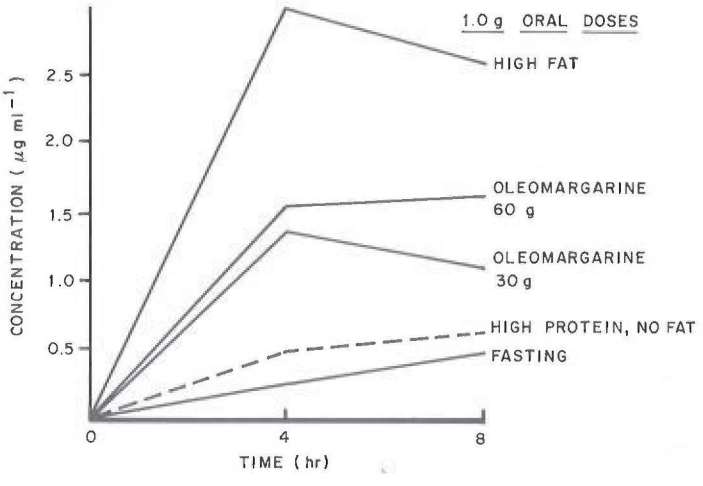

Figure 24 Effects of different types of food intake on the serum griseofulvin levels following a 1-g oral dose. (From Ref. 74.)

Neomycin had no effect on the lipase concentration in the mixed pancrease and lipases, which is called pancreatin. Neomycin was found to have no effect on the pH of intestinal contents. The loss of activity of antibacterial and antimycotic agents has been reported by Scheirson and Amsterdam [89].

Other components in the gastrointestinal tract that may influence drug activity include enzymes and proteins plus the mucopolysaccharide material called mucin, which lines the mucosal surfaces of the stomach and intestine. Enzymes found in the gastrointestinal tract transform the inactive drug moieties, chloramphenicol palmitate [90] and the acetoxymethyl ester of benzylpenicillin [91] to their active parent compounds. The binding of large quantities of dihydrostreptomycin and streptomycin [92] and quaternary ammonium compounds [93] to mycin has been suggested to explain the poor absorption seen with these compounds.

F. Absorption Enhancing Agents

In recent years, significant progress has been made in identifying agents that significantly increase the absorption of drugs that are poorly absorbed when administered alone. In an excellent review by Fix, published in 1987, compounds which have been identified as effective absorption-enhancing agents were reviewed [94]. Since many drugs are characterized by poor or highly variable absorption when administered to the GI tract, several approaches have been employed to increase drug absorption from the GI tract. These approaches include the optimization of drug release, site specific drug administration, prodrugs, modification of GI drug absorption, and vehicle optimization. Fix pointed out that all of these approaches tend to be drug specific and offer little in terms of providing general utility for increasing drug absorption. Absorption enhancing agents, which increase the absorption of coadministered drugs, provide a potential means of increasing mucosal membrane permeability in a more general manner and hence provide greater utility with a variety of compounds. Although a considerable effort has been directed towards identifying safe and effective absorption enhancing agents, little progress however, has been made in defining a mechanism of action for these compounds.

G. Cigarette Smoking and Drug Absorption

Recent studies have shown that the clinical efficacy and toxicity of benzodiazepine [95], propoxyphene [96], and chlorpromazine [97] may be influenced by cigarette smoking. An increase in the metabolism of these drugs by stimulated microsomal systems has been postulated as a possible mechanism. An increase in the rate of biotransformation of pentazocaine [98], phenacetin [99], and nicotine [100] has been demonstrated, and the effect of smoking on the disposition of theophylline was recently examined [101]. The results showed that the plasma half-life of theophylline (administered as aminophylline) in smokers was nearly half (mean value 4.3 hr) that found in nonsmokers (mean value 7.0 hr) and that the apparent volume of distribution of theophylline was larger in smokers than in nonsmokers. The authors suggested that the increase in theophylline clearance caused by smoking was probably the result of induction of drug-metabolizing enzymes, and that these enzymes do not normalize after cessation of smoking for 3 months.

H. Patient Characteristics

Considerable difference in patterns of drug absorption for some drugs have been found. Riegelman [102] and Levy [103] have reported that bioavailabilities of a drug from the same product can differ in the same person from one day to another or with the time of day. In addition, the age, posture, activity, stress, temperature, gastrointestinal pH, mobility, mucosal perfusion, gut flora, and disease state may all influence drug absorption. Drug-induced changes in portal blood flow or in hepatic function would alter the degree of biotransformation of a drug during its passage through the liver in the portal venous blood. Achlorhydria, biliary disorders, malabsorption syndromes [104], and reconstructive gastrointestinal surgery [105] can appreciably impair drug bioavailability. The drugs most influenced by patient factors are those whose bioavailability is quite incomplete under the best of circumstances.

III. PHYSICOCHEMICAL PROPERTIES OF DRUG AND DOSAGE FORM

Of prime concern to the pharmaceutical scientist involved in preformulation and dosage form design is a knowledge of the various properties of dosage forms that influence the biological effectiveness of medicinal agents.

A. Release of Drug from its Dosage Form

When a drug is administered orally in tablet dosage form, the rate of absorption is often controlled by the slowest step in the sequence [106] shown in Figure 25.

Drug must be released from the tablet into the gastrointestinal fluids for absorption to occur. The tableting of a medicinal substance allows the introduction of several variables during the manufacture of the dosage form. Process and formulation variables can be adjusted to assure

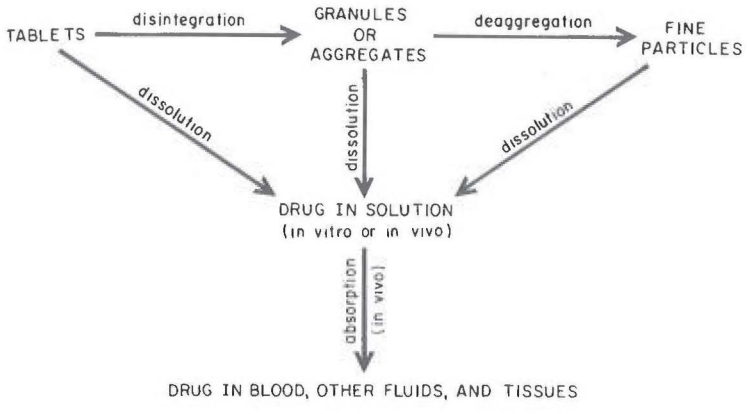

Figure 25 Drug dissolution from a tablet dosage form followed by absorption into the bloodstream. (From Ref. 106.)