8

Bioavailability

The time course of a drug in the body depends on how the drug is given. Blood levels are likely to be different after a single oral dose compared with the same dose given by rapid intravenous injection. There are two reasons for this difference: one is related to the completeness of absorption and the other to the rate of absorption of the drug. These two characteristics of drug absorption are called the *bioavailability* of the drug.

In most cases, we are particularly concerned with the fraction of the oral dose that actually reaches the bloodstream, because this amount is the *effective dose* of a drug. In some cases, notably those involving drugs used as a single dose for acute purposes, such as sedation or pain, we are also concerned with the rate of absorption of the drug.

Many drugs are not completely available after oral administration. Some drugs have low permeability and are slowly absorbed even when given in solution; examples include cromolyn, neomycin, and riboflavin. Since the residence of a drug at absorption sites in the gastrointestinal tract is limited by motility, there may be insufficient time for complete absorption. The availability of these compounds may be increased by administering them with food or with drugs that decrease motility, or by developing more lipid-soluble prodrugs.

Other drugs are so poorly water soluble that dissolution may be incomplete during the period of time available for absorption; some examples are phenytoin, griseofulvin, and isotretinoin. The availability of these drugs may be increased, in some cases dramatically, by dosage form changes, such as particle size reduction, or by means of water-soluble prodrugs.

A large number of drugs demonstrate incomplete bioavailability because of chemical degradation in the stomach (e.g., penicillin G), preabsorptive metabolism by enzymes in the proximal small intestine (e.g., aspirin) or bacteria in the distal small intestine and colon (e.g., digoxin), or presystemic metabolism in the gut wall (e.g., isoproterenol) or liver (e.g., propranolol) during absorption. A drug subject to presystemic metabolism may be completely absorbed but incompletely available, because part of the dose is metabolized to other products during the drug's passage from the gut lumen to the systemic circulation.

The availability of drugs subject to acid hydrolysis in the stomach may be improved by the use of enteric-coated dosage forms. Few strategies are available to improve the availability of drugs subject to preabsorptive or presystemic metabolism.

ESTIMATING THE BIOAVAILABILITY OF A DRUG

The fraction or percent of an administered dose that actually reaches the systemic circulation is called the *absolute* or *systemic bioavailability* of a drug. Systemic bioavailability is determined from blood level or urinary excretion data after oral administration, with reference to similar data after intravenous administration.

The total area under the drug level in blood or plasma versus time curve (AUC), after a single dose, reflects the amount of drug reaching the bloodstream. For most drugs, if we double the amount injected intravenously, we double the AUC. It follows that if we compare the AUC after oral administration with that obtained after intravenous administration, we can determine the fraction (F) of the oral dose available to the systemic circulation. In other words,

$$F = (AUC)_{oral}/(AUC)_{iv} \qquad (8\text{–}1)$$

If only 60% of an oral dose reaches the bloodstream, then F = 0.60; if the entire dose is available, then F = 1.0

As noted in Chapter 1, the usual bioavailability study is terminated before drug concentrations in blood return to negligible levels. The AUC beyond the last concentration data point (C* at t*) is estimated from the equation:

$$\text{Area from } t^* \text{ to } \infty = C^*/k \qquad (8\text{–}2)$$

where k is the first-order elimination rate constant. This partial area is added to the area from t = 0 to t = t*, calculated by means of the trapezoidal rule (see Appendix I), to determine AUC.

We sometimes recognize, from preliminary data, that the intravenous dose must be smaller than the oral dose to achieve comparable blood levels. In this case, for purposes of safety, different oral and intravenous doses are used for estimating systemic availability. Under these conditions,

$$F = (AUC)_{oral}D_{iv}/(AUC)_{iv}D_{oral} \qquad (8\text{–}3)$$

where D refers to the dose.

For some drugs, urinary excretion data can also be used to estimate availability. After intravenous administration of a drug, a fraction of the dose is excreted unchanged in the urine; the rest of the dose is subject to nonrenal elimination. In some cases, this fraction is so small as to represent a negligible amount or an amount too small to measure with precision. Under these conditions, urinary excretion data will not be useful. On the other hand, there are drugs for which evaluation of urinary excretion data is the method of choice for estimating availability. The thiazide class of diuretics is an example.[1]

For most drugs, the same fraction of the dose is excreted in the urine regardless of the size of the intravenous dose. Accordingly, by comparing the total amount of drug excreted unchanged (A_u) after a single oral and intravenous dose of a drug, we can determine the fraction (F) of the oral dose available to the bloodstream. In other words,

$$F = (A_u)_{oral}/(A_u)_{iv} \qquad (8\text{–}4)$$

When different oral and intravenous doses are used, the following equation applies:

$$F = (A_u)_{oral}D_{iv}/(A_u)_{iv}D_{oral} \qquad (8\text{–}5)$$

Absolute bioavailability has been determined for comparatively few drugs. The principal reason for this lack of information is that most drugs are not approved for intravenous use. Intramuscular administration may be an alternative absolute standard, particularly for soluble drugs, but again, relatively few drugs are approved for intramuscular administration. Because of this, the bioavailability of a drug is usually determined against a relative standard, one that does not assure complete availability.

A commonly used relative standard is an aqueous oral solution of the drug. Blood levels or urinary excretion data are compared after a single dose of the drug administered as the test product or the oral solution. To determine the availability of the drug from the test dosage form *relative* to that from the standard dosage form (F_{rel}), the following equations apply:

$$F_{rel} = (AUC)_{test}/(AUC)_{standard} \qquad (8\text{–}6)$$

and

$$F_{rel} = (A_u)_{test}/(A_u)_{standard} \qquad (8\text{–}7)$$

It can be debated that the maximum availability of a drug from an oral dosage form can never exceed that found from an aqueous oral solution. This is probably true in most instances; however, it may not be true for drugs that are poorly soluble in acid and precipitate in the form of coarse crystals in the stomach on swallowing the aqueous solution, or for drugs that are subject to acid hydrolysis and for which the test dosage form provides protection not afforded by the solution. In these cases, F_{rel} may exceed unity.

Some drugs defy formulation as aqueous solutions and one must resort to other relative standards; these include nonaqueous oral solutions, oral suspensions, or other solid oral dosage forms.

The physicochemical basis for using a nonaqueous solution of a drug as a bioavailability standard has been considered by Serajuddin et al.,[2] who studied the absorption of an investigational drug coded REV 5901. The drug existed in both solid and metastable liquid forms, had a pK_a of 3.7, and low water solubility (0.002 mg/ml at 37°). Appreciable solubility was observed only at pH values of 2 or less. Dissolution rate at pH >3 was practically zero.

REV 5901 was quite soluble in several nonaqueous solvents approved for oral use. The bioavailability of some of these nonaqueous solutions as well as an aqueous suspension was compared. Bioavailability was 76% after administration of a solution in polysorbate 80 (Tween 80), 61% when

given as a solution in peanut oil, and 35% as an aqueous suspension, relative to an oral solution of polyethylene glycol 400 (PEG 400), which provided the highest blood levels.

The investigators observed that on dilution of the water-miscible organic solutions (PEG 400 and Tween 80) with aqueous media, the drug immediately formed saturated solutions and the excess drug separated as emulsified oily globules. The dispersability of the globules improved when surfactants were present in the aqueous media. The average globule size was 1.6 μM, compared with a particle size of 5 to 10 μM when the drug was suspended in water. Therefore, a considerably larger surface area was available when the drug was ingested as a solution in Tween or PEG 400, rather than as an aqueous suspension.

Although the investigational drug was practically insoluble at the pH of the small intestine, its solubility was increased dramatically when bile salts and lecithin were added to the aqueous media. Serajuddin et al. concluded that the large surface area of the drug separating from organic solutions would facilitate dissolution in the presence of biological surfactants and increase bioavailability.

The innovator's dosage form, regardless of its availability, is often used as a relative standard, because presumably its efficacy is established. When a relative standard, other than an aqueous oral solution, is used, it is not uncommon to find that $F_{rel} > 1.0$.

Figure 8–1 shows blood levels of the antihypertensive drug prazosin after oral administration of 5 mg by capsule or hydroalcoholic solution.[3] The mean AUC for the test capsule was 174 ng/hr per ml whereas that for the solution was 199.0 ng/hr per ml. According to Equation 8–6, the relative availability of prazosin from the capsule is 0.87 or 87%.

A relative availability of 1.0 does not imply complete availability; we can only conclude from this information that the availability of drug from the test dosage form is equal to that from the standard. Propoxyphene gives almost the same blood levels after oral administration of commercial capsules or aqueous solution,[4] but the systemic availability of the drug after either dosage form is only about 20% because of presystemic metabolism.[5]

Most bioavailability studies are carried out by giving a single dose of drug to ambulatory, healthy subjects, after an overnight fast. There is concern that, in some instances, this kind of study does not reflect the general use of the drug and may provide misleading information. This concern is particularly evident for the evaluation of prolonged-release dosage forms. We have learned enough about drug absorption to recognize that, in some cases, food, activity (sleeping vs awake), and disease may have differential effects on drug availability from oral dosage forms. Two dosage forms that differ in their release rates of drug may show equivalent AUC values in normal subjects but different values in a population with above average gastrointestinal motility. Differences between fed and fasted populations may also occur.

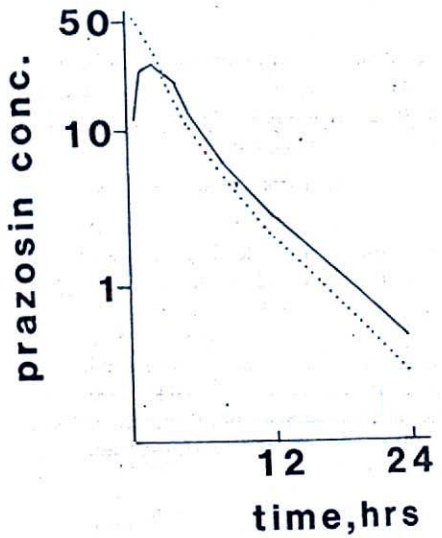

Fig. 8–1. Semilogarithmic plot of prazosin concentrations in plasma (ng/ml) following a 5-mg oral dose by capsule (—) or solution (···). (Data from Hobbs, D.C., Twomey, T.M., and Palmer, R.F.[3])

Oral administration of two 0.25 mg digoxin tablets and two 0.2 mg digoxin capsules containing a water-miscible solution of the drug yields similar values for AUC, indicating bioequivalence. The area under the curve following the tablets is 103% relative to the capsules. When either dosage form is given with propantheline, an anticholinergic that slows stomach emptying and decreases gastrointestinal motility, there is an increase in AUC but the change is larger for the tablets than for the capsules—24% versus 13%. Consequently, under conditions of hypomotility, digoxin AUC after administration of the tablets is 113% relative to the capsules.[6]

The oral absorption of digoxin in tablet form has been reported to be reduced after cancer chemotherapy and radiation therapy. Bjornsson et al.[7] studied possible differences in the effect of high-dose cancer chemotherapy on the relative bioavailability of digoxin given in tablet form and in solution-in-capsule form. Each subject received a single oral dose of either 0.5 mg tablets or 0.4 mg capsules before and after chemotherapy.

Before chemotherapy, the AUC following the tablets was 104% relative to the capsules. Chemotherapy reduced the average AUC after tablet administration by nearly 50%, compared with a reduction of only 15% with the capsules. Consequently, after chemotherapy, digoxin AUC following the tablets was only 74% relative to the capsules.

These concerns have led to increasing interest in steady-state studies for the evaluation of relative availability. When a constant dose of a drug is given at constant dosing intervals (e.g., 150 mg every 12 hr), the AUC during a single dosing interval at steady state (AUC_{ss}) is equal to the total AUC after a single dose (AUC). It follows from Equation 8–6 that:

$$F_{rel} = (AUC_{ss})_{test}/(AUC_{ss})_{standard} \qquad (8–8)$$

We can also show that:

$$F_{rel} = (A_{u,ss})_{test}/(A_{u,ss})_{standard} \qquad (8–9)$$

where $A_{u,ss}$ is the amount of drug excreted unchanged in the urine during a single dosing interval at steady state. Since the average drug concentration in blood or plasma at steady state, $\overline{C}_{ss}$, is equal to the ratio of AUC_{ss} to the dosing interval, τ, it follows that:

$$F_{rel} = (\overline{C}_{ss})_{test}/(\overline{C}_{ss})_{standard} \qquad (8–10)$$

By obtaining blood levels or urinary excretion data at steady state for a relatively short period of time (one dosing interval), we can determine the relative availability of a drug. Moreover, this assessment takes into account the general conditions of use of the drug, particularly when patients rather than healthy subjects are studied.

Dickerson and co-workers[8] determined the steady-state levels of pseudoephedrine after multiple dosing of two prolonged-release capsules given every 12 hr; one capsule (A) contained 120-mg pseudoephedrine and the other (B) contained 150 mg of the drug. The mean steady-state concentrations, $\overline{C}_{ss}$, were 447 ng/ml for capsule A and 510 ng/ml for capsule B. Adjusting these data for the difference in dose (120 mg vs 150 mg), we can calculate that the bioavailability of pseudoephedrine from capsule A relative to capsule B is 110%. Therefore, the dosage forms are nearly bioequivalent.

An advantage of steady state over single dose evaluation of availability is evident in the results of studies with the anticonvulsant drug carbamazepine.[9] Figure 8–2 shows serum concentrations of carbamazepine after single 200-mg doses of two different commercial tablets. It is difficult to determine from these data whether the higher serum levels resulting from product A are the result of greater availability of carbamazepine or merely faster absorption. Steady-state concentrations, shown in Figure 8–3, resulting from multiple dosing of each product at equal daily doses in each patient, clearly indicate that the products are bioequivalent.

Bioavailability studies are typically of a cross-over design; each person in a panel of subjects receives each treatment. This design avoids the problem of intersubject variability in drug elimination, which could obscure comparisons of AUC or A_u; all dosage forms are compared in each individual. The cross-over design, however, does not account for intrasubject variability (i.e., variability in drug elimination in the same subject from one administration to another). Drugs that show a high degree of intrasubject variability require large panels of subjects to differentiate dosage forms or to conclude that dosage forms are bioequivalent with an adequate degree of certainty.

When two products are given to the same individual on separate occasions and result in different AUC values, the dissimilarity may either be due to different bioavailability characteristics or to variability in drug clearance from one occasion to the other. In a two-period crossover study, we may incorrectly interpret the variation in clearance as reflecting a difference in bioavailability. Therefore, we would like to correct for the variability in clearance to improve our evaluation of bioavailability.

Some investigators have suggested that if half-lives are different between two treatments, this might reflect a difference in clearance. The equation for this correction is as follows:

$$F = (AUC)_{test}(t_{1_2})_{standard}/(AUC)_{standard}(t_{1_2})_{test} \qquad (8–10a)$$

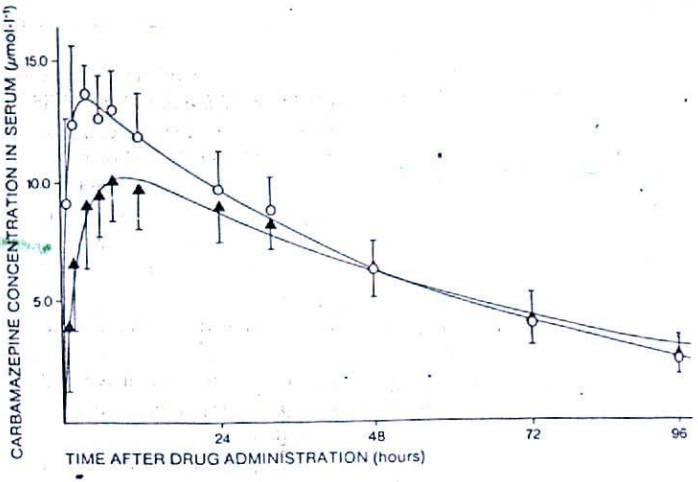

Fig. 8–2. Carbamazepine concentrations in serum after single 200-mg oral doses in 2 different tablet products. (Data from Anttila, M., et al.[9])

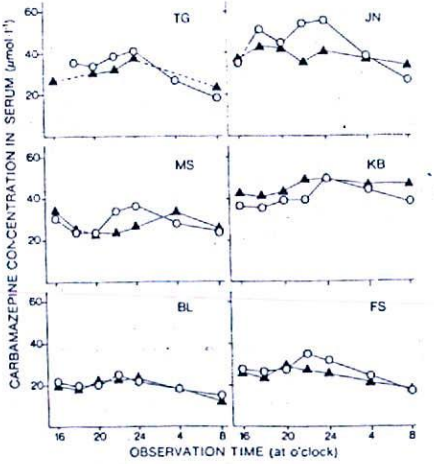

Fig. 8–3. Carbamazepine concentrations in serum at steady state in different subjects after repetitive oral dosing with 2 different tablet products. (Data from Anttila, M., et al.[9])

If half-life estimates are randomly distributed for test and reference treatments, then half-life correction is warranted, if the variance of the corrected bioavailability (F) value is less than for uncorrected values. In those situations where half-life estimates are not randomly distributed across treatments (i.e., the half-life for one treatment is consistently larger than for another), then prolonged absorption of the drug rather than variation in clearance may be causing the apparent half-life change. In this circumstance, half-life correction is not appropriate.

A more rigorous correction can be applied by administering simultaneously the oral dosage form and an intravenous solution of labeled drug. In this manner, clearance can be calculated independently for each leg of the study. Alternatively, an oral solution containing labeled drug can be given at the same time as the test dosage form. Interest in reducing the effect of intrasubject variability on bioavailability studies by correcting for differences in clearance has been stimulated by increased availability of stable isotopes (e.g., drug molecules containing ^{2}H or ^{13}C atoms), which are considered safer than radioactive isotopes, and the advances in gas chromatography-mass spectrometry (GC-MS).[10]

One report describing the use of stable isotopes was concerned with the bioavailability of maprotiline, a tetracyclic antidepressant.[11] Six subjects were given simultaneous single 50-mg oral doses of tablets containing maprotiline HCl and an aqueous solution containing trideuterated maprotiline HCl. The mean AUC values for the solution and tablet had coefficients of variation (CVs) of about 65%, whereas the mean value for relative bioavailability (AUC_{tab}/AUC_{soln}) had a CV of only 5%.

More recently, Shinohara et al.[12] used stable isotopes to determine the bioavailability of methyl-

testosterone (MT) tablets in 8 subjects. The study was carried out in a crossover manner in order to compare the stable isotope method with the conventional crossover method. Each subject was given a 10-mg MT tablet with a reference solution containing 10 mg trideuterated methyltestosterone (MT3D) on one occasion, and a solution containing 10 mg MT with the MT3D reference solution on another. Serum samples were analyzed for MT and MT3D by GC-MS.

When the tablet and reference solution were given at the same time, the peak concentration of MT3D (reference solution) was almost twice as great as that for MT (tablet), but the average AUC values were nearly identical. Mean relative bioavailability for the tablet was 101%. The mean AUCs for the reference solution and tablet had CVs of 42% and 45%, respectively. The mean relative bioavailability had a coefficient of variation of only 18%.

Relative bioavailability was also determined from AUC values for MT after administration of tablet and solution on separate occasions. The mean was 97%, similar to the results in the stable-isotope study, but the coefficient of variation was 38%, more than twice that observed in the isotope study. The investigators concluded that the assumption of a constant clearance in individual subjects on different occasions may be a poor one, certainly for methyltestosterone and probably for most drugs.

Shinohara et al. also made theoretical calculations to estimate the number of subjects required to detect (with a probability of 0.8) a difference of 20% between the tablet and solution. They estimated that 40 subjects were required for a conventional crossover study, whereas only 12 subjects would be needed for the stable-isotope method.

In 1979, investigators from the FDA and other laboratories reported a new approach to comparative bioavailability testing.[13] They proposed the usual crossover design but added that each formulation would be taken with a solution containing a stable isotope of the drug. They used this approach to compare the bioavailability of two brands of imipramine tablets.

A solution containing 25 mg dideuterated imipramine (IMP2D) was taken each time an imipramine (IMP) tablet was administered. Blood samples were collected after drug administration and plasma was analyzed for IMP and IMP2D. Crossover studies were run 1 week apart.

The data were analyzed in the conventional way by comparing the AUC resulting from each tablet, as well as in a new way by comparing relative parameters. The AUC for IMP from tablet *A* relative to the AUC for IMP2D from the reference solution given at the same time was compared with the corresponding values for tablet *B* relative to its reference solution.

Although both methods of comparison suggested that the two imipramine tablets were bioequivalent, statistical power differed remarkably. This is readily seen when the data set is used to calculate the number of subjects needed to detect (with a probability of 0.8) a difference in AUC of 20% between the two tablets. The conventional crossover study was found to require 20 subjects, whereas the relative crossover study (using a stable isotope as an internal standard) would require only 4 subjects.

ESTIMATING THE ABSORPTION RATE OF A DRUG

Rigorous methods are available to evaluate the kinetics of drug absorption after administration of a test dosage form, but these methods require concentration-time data after rapid intravenous injection of the drug in the same individual.[14] Unfortunately, an intravenous reference curve is not available for most drugs.

At this time there are no completely satisfactory methods to evaluate absorption kinetics solely from data obtained after oral administration. Despite the limited methodology, there is keen interest in some quarters for comparative absorption rate data. Regulatory agencies often ask for a quantitative evaluation of absorption kinetics as part of the pharmacokinetic characterization of new drugs; this is considered particularly important for those drugs where rapid absorption is needed for clinical response and for drugs in prolonged-release dosage forms.

The pharmaceutical industry has an additional interest in the evaluation of absorption rate, to establish in vivo-in vitro correlations. Quantitative correlations between gastrointestinal absorption and in vitro dissolution rates may permit rapid screening of new dosage forms and serve as a quality control tool to quickly assess the potential effects of small changes in processing or composition

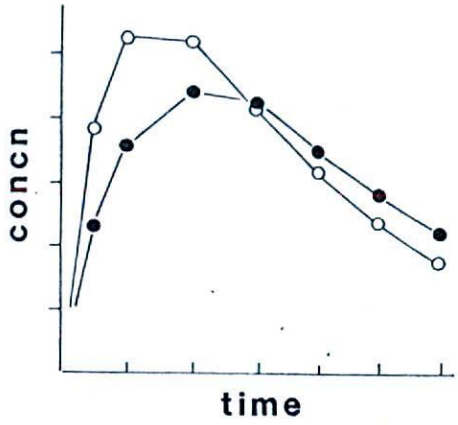

Fig. 8–4. Effect of absorption rate on the time course of drug in the plasma after a single oral dose. The faster the absorption, the higher is the peak concentration and the shorter is the time to peak.

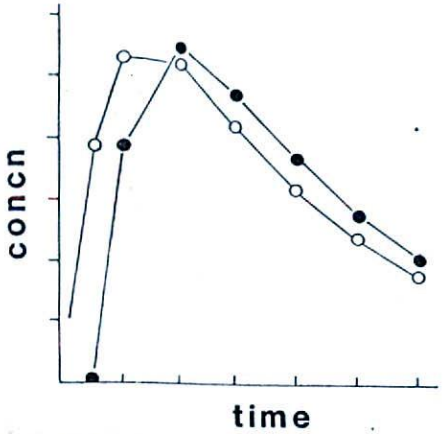

Fig. 8–5. Effect of a delay in gastric emptying or drug release from the dosage form on the time course of drug in the plasma after a single oral dose. The peak concentrations after each dose are similar but there is a difference in the time to peak.

or of product age on the bioavailability of drug from the dosage form.

For clinical purposes, most investigators find it sufficient to compare peak concentrations of drug in blood or plasma and the time required to reach the peak after a single dose of the drug in different dosage forms. The faster the absorption of a drug, the larger is the peak concentration, and the shorter is the time to peak (Fig. 8–4). Sometimes, one may find two dosage forms that release drug at about the same rate but differ in their dependence on gastric emptying or in the time for onset of drug release. The latter may be observed when a film-coated tablet is compared with an uncoated tablet. When this occurs, the peak concentrations will be about the same, but the time to peak will differ, because of the difference in lag time before absorption begins (Fig. 8–5).

Precise definition of the time to peak is often difficult because of limited opportunities to take blood samples. Ronfeld and Benet have shown that, with normal biological and experimental variability, it may be impossible to differentiate, on the basis of peak times, two dosage forms that differ in their release rates of drug by a factor of two.[15] Accordingly, this method for comparing absorption rates may be insufficiently sensitive for some needs. Furthermore, estimates of relative times to peak or peak concentrations are of little use in the evaluation of prolonged release dosage forms, which may produce no well defined peak concentration.

The statistical moments theory offers an attractive alternative for the evaluation of absorption data. As noted in Chapter 2, the difference between the mean residence time (MRT) after administration of a test dosage form (MRT_{test}) and the MRT after rapid intravenous injection (MRT_{iv}) is the mean absorption time (MAT):

$$MAT = MRT_{test} - MRT_{iv} \qquad (8\text{–}11)$$

If absorption is first-order, then:

$$MAT = 1/k_a \qquad (8\text{–}12)$$

where k_a is the first-order absorption rate constant.

Even in the absence of intravenous data, MAT is useful. For example, the relative ranking of MRT values following several dosage forms mirrors the relative ranking of the dosage forms with respect to drug release and absorption.

Riegelman and Collier proposed that the difference in MRT after a test oral dosage form and an aqueous solution, (MRT_{soln}) is equivalent to the mean dissolution time (MDT) or mean release rate of drug from the dosage form in the gastrointestinal tract:[16]

$$MDT = MRT_{test} - MRT_{soln} \qquad (8\text{–}13)$$

This approach has the potential to be a useful tool in the biopharmaceutic evaluation of dosage forms.

The absorption of furosemide has been studied by means of moment analysis.[17] The mean residence time after an intravenous bolus of furosemide, MRT_{iv}, was 51 min. After oral administration of a furosemide tablet to fasting subjects MRT was 135 min. The difference (MAT) is 84 min. The mean absorption time for oral furosemide was significantly greater than MRT_{iv}, indicating absorption rate-limited elimination kinetics.

The mean absorption time for furosemide tablets given immediately after a meal was 144 min, considerably longer than the mean value calculated when the tablets were given to fasting subjects. The difference in MAT values for the tablet given to fasted and fed subjects, 60 min in this case, is a representation of the delay in absorption resulting from the meal. It might be looked upon as the mean increase in gastric emptying time.

When an oral solution of furosemide was given after a meal, MAT was 109 min. The difference between MAT for the tablet and solution given after a meal was 35 min, representing the mean postprandial dissolution time for furosemide tablets.

PREABSORPTIVE HYDROLYSIS AND METABOLISM

The principal sites of chemical or biochemical (metabolic) conversion of a drug in the gut lumen are the stomach (acid), small intestine (esterases and other enzymes), and distal small intestine and colon (gut bacteria). These conversions can take place in parallel with or precede drug absorption and result in reduced availability.

Some drugs are not chemically stable at the low pH of the stomach; examples include penicillin G, methicillin, erythromycin, and digoxin. After oral administration, they are subject to acid hydrolysis in the stomach to form inactive products; less than 100% of the administered dose is available for absorption. This problem can usually be predicted from in vitro chemical stability studies.

The availability of drugs subject to acid hydrolysis in the stomach is a function of the rate of dissolution and the residence time of the drug in the stomach. Minimizing the dissolution of the drug in the stomach leads to increased availability. Factors that promote gastric emptying or increase gastric pH also result in improved bioavailability.

The importance of enzymatic hydrolysis in the fluids of the small intestine in determining the availability of drugs is unknown. Esterases are certainly ubiquitous in the body and could, in principle, degrade drugs like aspirin or ester prodrugs like pivampicillin or chloramphenicol palmitate before or in competition with the absorption process. In general, however, the gut wall is likely to be a more important site for the enzymatic hydrolysis of esters than is the gut lumen. If pivampicillin, for example, is subject to hydrolysis in the fluids of the small intestine, this surely must represent only a small fraction of the dose because the blood levels of ampicillin are much higher after a dose of the prodrug than after an equivalent dose of ampicillin. This means that a significant fraction of the pivampicillin dose must be absorbed (penetrate the gut wall) as such and thereby evade preabsorptive metabolism.

Many different kinds of microorganisms are normal residents of the lower intestine. These bacteria, which constitute the intestinal microflora, can carry out a variety of metabolic processes, but they are particularly adept at reduction, including the reduction of double bonds, azo groups, aldehydes, ketones, and alcohols.[18]

Most drugs are absorbed before reaching the ileum and are not subject to metabolism by intestinal microorganisms. On the other hand, a substantial fraction of an oral dose of a slowly absorbed drug or a drug given in a prolonged-release dosage form may reach the lower intestines. When this occurs, preabsorptive metabolism by the intestinal microflora may affect the availability of the drug. This situation applies to digoxin.

In certain patients, about 10% of the population taking the drug, the availability of digoxin is unusually low. These patients also excrete large amounts of digoxin reduction products or DRPs in the urine. Moreover, there is a tendency in the general population to greater excretion of DRPs when poorly absorbed preparations are taken (Fig. 8–6). There is convincing evidence that digoxin is extensively inactivated by intestinal microorganisms in a minority of those receiving the drug and that this problem is more widespread with slowly absorbed preparations of the drug.[19,20]

The proposition that metabolism by intestinal microflora is more important for slowly-absorbed than for rapidly-absorbed drug products was tested by determining the effect of metoclopramide on digoxin absorption after a 0.5-mg dose of digoxin tablets or a 0.4-mg dose of a digoxin solution encapsulated in soft gelatin.[21] Digoxin is more rapidly and more completely absorbed from the soft gelatin capsules than from the tablets. Metoclopramide de-

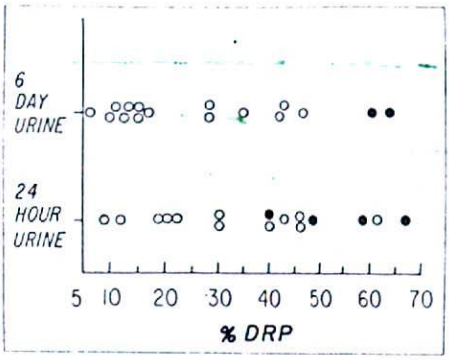

Fig. 8–6. Percent of drug-related material in the urine present as digoxin reduction products after a single oral dose of digoxin. ○: solutions or tablets with high dissolution rates. ●: tablets with low dissolution rates. (From Lindenbaum, J., et al.[23])

creased the bioavailability of digoxin tablets by about 25%, on the average, but had no effect on the bioavailability of digoxin following administration of soft gelatin capsules.

Another example is seen with acenocoumarol, an oral anticoagulant used outside the U.S. Acenocoumarol is converted by gut flora in vitro to amino and amido metabolites. Under typical clinical conditions, however, bacterial metabolism is of little importance because acenocoumarol is rapidly absorbed from its dosage form. Studies with commercial tablets indicate no measurable levels of reduced metabolites in plasma and less than 1% of the oral dose is excreted in urine as reduced metabolites. Administration of slowly-dissolving capsules containing relative coarse, crystalline acenocoumarol produced measurable plasma levels of both the amido and amino metabolites. Urinary recovery of reduced metabolites accounted for 6 to 12% of the dose.[22]

Certain oral antibiotics, including tetracycline and erythromycin alter the bacterial flora and decrease the inactivation of digoxin. Steady-state serum levels of digoxin in some patients have been found to increase 2-fold during oral antibiotic treatment, presenting the risk of toxicity.[24]

Other reports indicate that changes in gut bacteria as a result of treatment with antibiotics affect the disposition of sulfasalazine and oral contraceptives. Bacterial metabolism reduces the azo linkage in sulfasalazine to liberate sulfapyridine and 5-aminosalicylic acid (mesalamine) in the lower bowel. A 5-day course of oral ampicillin, 250 mg 4 times daily, significantly reduced gut bacteria-mediated conversion of sulfasalazine to sulfapyridine. AUC values for sulfapyridine after a single oral dose of sulfasalazine decreased from 370 μg-hr/ml under control conditions to 239 μg-hr/ml after ampicillin.[23]

PRESYSTEMIC METABOLISM

After oral administration, a drug must pass sequentially from the gastrointestinal lumen, through the gut wall, then through the liver before reaching the systemic circulation (Fig. 8–7). This sequence is an anatomic requirement because blood perfusing the entire length of the gastrointestinal tract, with the exception of the buccal cavity and lower rectum, drains into the liver by way of the hepatic portal vein. Since the gut wall and liver are sites of drug metabolism, a fraction of the amount absorbed may be eliminated (metabolized) before reaching the bloodstream. Therefore, an oral dose of a drug may be completely absorbed but incompletely available to the systemic circulation because of presystemic or *first-pass* metabolism in the gut wall or liver.

Criteria have been developed to identify and quantify the extent of presystemic metabolism and to indicate where it is occurring. Its detection requires only that systemic availability is less than the fraction of the dose absorbed. The fraction absorbed may be determined from the urinary excretion of drug and metabolites, usually as total radioactivity, after oral administration of the drug (in a radiolabeled form), relative to that after intravenous administration. Many drugs undergoing presystemic metabolism in man have been identified on the basis of this type of information. Differentiation of the gut wall and liver as the site of presystemic metabolism is relatively simple in animals, but more difficult in man.

The theory and our understanding of hepatic presystemic metabolism is relatively advanced; our knowledge of gut wall metabolism is less well developed. Because an understanding of the hepatic first-pass effect is often useful in differentiating the sites of presystemic elimination, we will first consider the liver as the site of presystemic metabolism.

Hepatic Presystemic Metabolism

The liver is the most important site of presystemic elimination because of its high level of drug

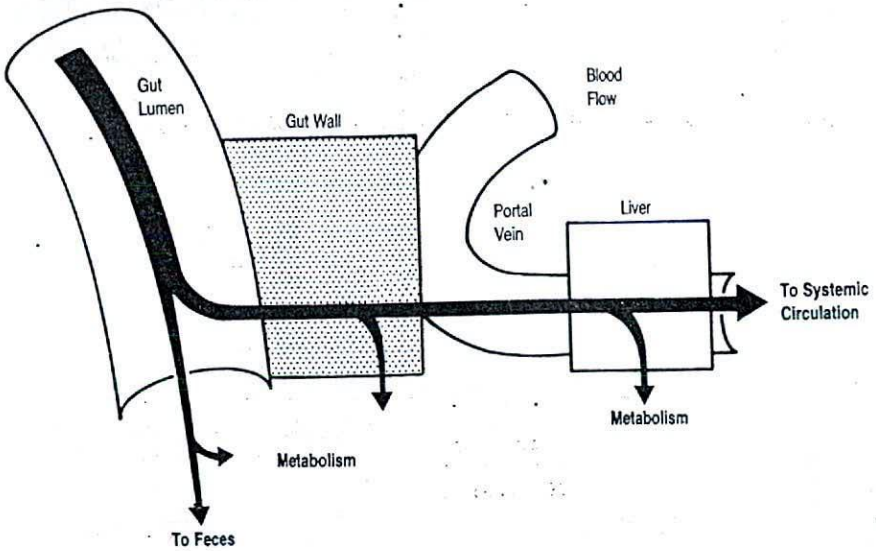

Fig. 8–7. After oral administration, a drug must pass sequentially from the gut lumen through the gut wall, then through the liver, before reaching the systemic circulation. Metabolism may occur in the lumen before absorption, in the gut wall during absorption, and/or in the liver after absorption but before reaching the systemic circulation. (From Rowland, M., and Tozer, T.N.[24])

metabolizing enzymes, its ability to rapidly metabolize many different kinds of drug molecules, and its unique anatomic location. A large number of drugs are subject to considerable hepatic first-pass metabolism; examples include β-blockers (propranolol and metoprolol), analgesics (propoxyphene, meperidine, and pentazocine), antidepressants (imipramine and nortriptyline), and antiarrhythmics (lidocaine and verapamil).

Hepatic presystemic metabolism is most easily understood when the liver is the sole organ of drug elimination. Under these conditions, the clearance of a drug, as determined after intravenous administration from the ratio of dose to area (AUC), is equal to hepatic clearance (Cl_H), which is given by:

$$Cl_H = Q_H ER_H \qquad (8\text{–}14)$$

where Q_H is hepatic blood flow and ER_H is the hepatic extraction ratio (see Chap. 2). Hepatic blood flow in man ranges from about 1.1 to 1.8 L/min, with an average of about 1.5 L/min. Hepatic extraction ratio may range from 0 to 1, depending on the liver's ability to metabolize the drug. The maximum clearance of a drug eliminated exclusively by hepatic metabolism is equal to hepatic blood flow; this occurs when $ER_H = 1.0$.

The fraction of drug eliminated from portal blood during absorption is given by the hepatic extraction ratio, ER_H; the remainder $(1 - ER_H)$ escapes into the systemic circulation, and is then cleared from the circulation by the liver, according to Equation 8–14. If a fraction (f) of the oral dose (D_o) is absorbed and then subjected to hepatic presystemic metabolism, the AUC after oral administration (AUC_o) is given by:

$$AUC_o = fD_o(1 - ER_H)/Q_H ER_H \qquad (8\text{–}15)$$

Since $Q_H ER_H$ is equal to hepatic clearance, which, under these conditions, is given by the ratio of intravenous dose (D_{iv}) to area (AUC_{iv}), we may rewrite Equation 8–15 as follows:

$$AUC_o/AUC_{iv} = fD_o(1 - ER_H)/D_{iv} \qquad (8\text{–}16)$$

The ratio of areas after oral and intravenous administration of equal doses of a drug is equal to its systemic availability (F). If we also assume that absorption is complete (f = 1), then:

$$F = (1 - ER_H) \qquad (8\text{–}17)$$

Equation 8–17 shows that systemic availability

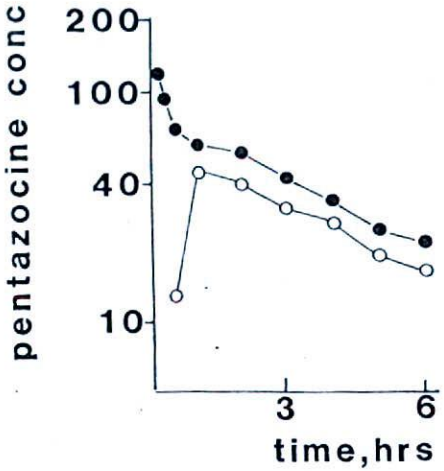

Fig. 8–8. Pentazocine concentrations in plasma (ng/ml) after administration of 100 mg orally (○) or 30 mg intravenously (●). (Data from Ehrnebo, M., Boréus, L.O., and Lönroth, U.[29])

depends on the hepatic extraction ratio. Drugs with low extraction ratios, such as antipyrine, warfarin, and tolbutamide, undergo little presystemic metabolism.

An estimate of the hepatic extraction ratio may be made by determining the clearance of the drug after intravenous administration and comparing this value to a mean value for liver blood flow, according to a rearrangement of Equation 8–14:

$$ER_H = Cl_H/Q_H \qquad (8–18)$$

The intravenous clearance of propranolol is about 1.05 L/min in man. Assuming an average liver blood flow of 1.5 L/min, we can calculate that $ER_H = 0.7$ and $F = 0.3$. Although propranolol is well absorbed, only 30% of an oral dose is available to the systemic circulation. This kind of information, in conjunction with experimental estimates of F, has been used to substantiate the predominantly hepatic presystemic elimination of several drugs, including propranolol,[25] lidocaine,[26] imipramine,[27] papaverine,[28] and pentazocine.[29]

Plasma concentrations of pentazocine after administration of 100 mg orally and 30 mg intravenously are shown in Figure 8–8. Although the intravenous dose is smaller, it results in higher plasma levels. The systemic availability of pentazocine after oral administration, calculated after taking into account the difference between intravenous and oral doses in 5 subjects, varied from 11 to 32%, with a mean value of 18%. This low systemic availability of pentazocine is consistent with its high hepatic clearance, in the order of 1.2 L/min.[29]

Table 8–1. Relationship Between Steady-State Concentration of Alprenolol on 200 mg Twice a Day and Single Dose Data After Oral or Intravenous Administration*

Rank No.	Steady-state concn. (ng/ml)	Bioavailability (oral)	Clearance (iv)
1	37.0	0.15	0.71
2	32.1	0.13	0.52
3	14.1	–	1.37
4	13.2	0.07	0.94
5	12.0	0.05	0.78
6	3.9	0.03	0.41
7	2.7	0.01	2.03

*Data from Alván, G., et al.[30]

With many drugs, presystemic metabolism and systemic availability vary markedly from one person to another. The variability contributes to the interindividual differences in steady-state concentrations of the drug. Studies with the β-blocker alprenolol show a 14-fold range in steady-state concentrations in healthy subjects taking oral doses of 200 mg twice a day. Intravenous studies in the same subjects indicate only a 4-fold range of clearance values.

Additional studies reveal that the rank order for individual steady-state plasma concentrations of alprenolol is the same as that for the relative bioavailability of the 200-mg oral dose; no correlation is found between steady-state levels and individual clearance values (Table 8–1). These results demonstrate that differences in first-pass metabolism contribute substantially to interindividual variability in steady-state plasma concentrations of a drug with a high hepatic extraction ratio.[30]

Presystemic metabolism after oral administration of a drug results in the formation of a bolus of metabolites during the drug's first pass through the liver. Accordingly, we would expect to see higher peak levels of metabolites after oral administration of a drug with a high hepatic extraction ratio than after parenteral administration. Figure 8–9 shows mean plasma concentrations of nortriptyline (NT) and its 10-hydroxy metabolite after oral and intramuscular administration of the same dose of NT. Lower concentrations of NT occur after oral than after intramuscular administration. In contrast, initial plasma concentrations of the metabolite (up to 10 hr) are much higher after oral than after intramuscular doses.[31]

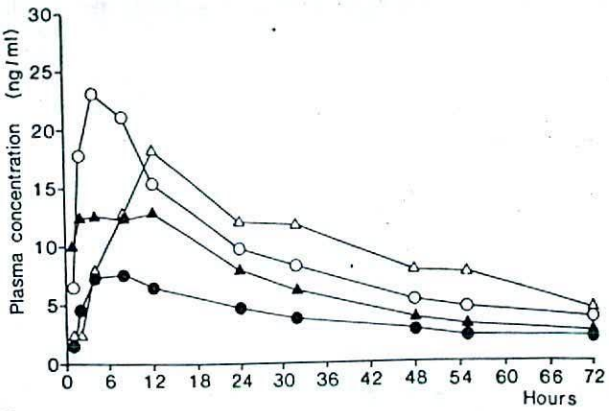

Fig. 8–9. Nortriptyline (▲,●) and 10-hydroxynortriptyline (△,○) concentrations in plasma after oral (●,○) and intramuscular injection (▲,△) of a 40-mg dose of nortriptyline. (From Alván, G., et al.[31])

Gut Wall Presystemic Metabolism

Presystemic metabolism in the gut wall and liver can be differentiated in animals by comparing drug concentration after oral and intraportal administrations to assess the contribution of the gut wall, and after intraportal and intravenous administrations to assess the contribution of the liver.

Glucuronidation of morphine, naloxone, and buprenorphine by the liver and intestine has been compared in rats.[32] The drugs were given by peripheral intravenous (iv) and hepatic portal vein (hpv) injection, and instilled into the duodenum (id). AUC decreased in the following order: iv > hpv > id. The results suggest that these related compounds are subject to presystemic metabolism, in both the gastrointestinal wall and the liver. For each drug, hepatic extraction was more efficient than intestinal extraction.

Another experimental model was developed to determine the site of first-pass metabolism of midazolam, a benzodiazepine with high presystemic extraction after oral administration.[33] Domestic pigs received single intravenous and oral doses of the drug. Multiple blood samples were simultaneously drawn from the portal vein and from a systemic vein during the first 8 hr after the dose. Differences in AUC at the two sampling sites after oral administration indicate hepatic extraction; differences after iv administration indicate gut wall extraction.

After iv administration, midazolam had a high systemic clearance value, suggesting the likelihood of first-pass metabolism. AUC values for systemic vs portal sites were nearly identical, suggesting little, if any, metabolism in the gut wall. After oral administration the systemic/portal AUC ratio averaged only 0.15, suggesting a high degree of hepatic extraction. The portal AUC after oral administration was similar to the systemic AUC after iv administration, again suggesting little gut wall metabolism. The investigators concluded that the extensive presystemic extraction of oral midazolam is largely the result of hepatic biotransformation rather than metabolism either within the gastrointestinal tract or during absorption into the portal circulation.

Despite the importance of understanding the site of presystemic extraction of drugs, human studies are limited by the necessarily invasive experimental techniques. Sampling of portal blood is generally possible only in patients in whom portal catheterization is otherwise clinically indicated.

An example is found in a report on the concentrations of phenacetin and its metabolite, acetaminophen, in portal and hepatic venous blood after intragastric or intraduodenal administration of phenacetin to patients with portal hypertension.[34] The concentration ratio of metabolite to drug in portal blood soon after drug administration was low, ranging from 0.01 to 0.11. Furthermore, at each sampling time, the concentration ratio in the portal vein was much lower than in the hepatic vein or in peripheral blood. The hepatic extraction ratio of phenacetin was estimated to be about 0.6 to 0.8, consistent with the low bioavailability of the drug.[35]

These results indicate that O-dealkylation of phenacetin occurs mainly in the liver and only to a limited extent in the gut wall.

A similar study in patients with portal hypertension was carried out with flurazepam.[36] High concentrations of the mono- and didesethyl metabolites of flurazepam were found in portal vein blood soon after intraduodenal administration of the drug, consistent with intestinal wall metabolism. Efficient hepatic extraction of both flurazepam and its metabolites, however, was also observed. The results suggest that presystemic metabolism of flurazepam in man occurs in the gut wall as well as in the liver.

More direct evidence of gut wall metabolism in man is found in a report on the concentrations of ethinyl estradiol and its conjugated metabolite in portal and peripheral vein blood following oral administration to postsurgical patients.[37] In each patient, for about 40 to 50 min after administration, the concentration of conjugated ethinyl estradiol in the portal vein was considerably higher than in the peripheral vein. Back and co-workers calculated that about 44% of the absorbed dose undergoes presystemic metabolism in the gut wall;[37] an additional 25% of the dose is subjected to hepatic first-pass metabolism.

In vitro studies show that ethinyl estradiol is extensively metabolized by human jejunal mucosa, obtained by biopsy from healthy subjects, to form the sulfate conjugate.[38] The degree of conjugation of mestranol and levonorgestrel, two other contraceptive steroids, was much lower than for ethinyl estradiol. The results with levonorgestrol are consistent with the high systemic availability of the steroid.[39]

Changes in metabolite excretion patterns may provide indirect evidence for gut wall metabolism. Intravenous isoproterenol is excreted largely unchanged in man. On the other hand, the sulfate conjugate accounts for 80% of the drug in the urine after oral administration. No sulfate conjugate is found after intravenous administration. The results suggest that the presystemic metabolism of isoproterenol in man is confined to the mucosal surface of the gut wall.[40]

Albuterol (salbutamol), a potent beta-adrenergic agonist used widely in the treatment of bronchial asthma, is subject to substantial presystemic metabolism after oral administration. Morgan et al.[41] studied the kinetics of albuterol and its sulfate conjugate metabolite, in plasma and urine, after intravenous and oral administration.

After iv administration, total plasma clearance was 480 ml/min and the elimination half-life was about 4 hr. Urinary excretion of unchanged albuterol accounted for 64% of the dose and the sulfate metabolite accounted for 12%. After oral administration, systemic availability was only 50%, and urinary excretion of unchanged drug and metabolite accounted for 32% and 48% of the dose, respectively.

Total urinary recovery of drug-related material was similar after each route of administration, indicating that although oral albuterol has a low bioavailability, it is well absorbed from the gastrointestinal tract. The data also indicate that the fraction of the dose of albuterol eliminated on the first pass could be accounted for entirely as sulfate conjugate formed, presumably, in the gut wall.

Commonly, the existence of gut wall metabolism is inferred when the degree of presystemic metabolism of drug exceeds the hepatic extraction ratio. For example, the hepatic extraction ratio of terbutaline, determined after intravenous administration, is only about 0.08. This means that if the entire oral dose were absorbed, a systemic availability of 92% should result. In fact, the availability of terbutaline is only 10%. Determination of free terbutaline in the feces suggests that only 55% of the drug is absorbed. Under these conditions, we expect a systemic availability of 0.55×0.92 or 51%. Clearly, incomplete absorption and hepatic presystemic metabolism cannot account for the low systemic availability of terbutaline. We must conclude that a large fraction of the dose of terbutaline is metabolized by another presystemic route, most likely the gut wall.[42]

REGULATORY AND CLINICAL CONSIDERATIONS

Both biopharmaceutic and metabolic factors influence the bioavailability of drugs. Although there is usually little we can do to alter unfavorable metabolic characteristics, this is not true for biopharmaceutic factors that limit the availability of a drug. During the last decade there has been a heightened awareness of the role of the dosage form on the bioavailability and clinical efficacy of drugs; the general result has been better dosage forms.

For some time now, the U.S. Food and Drug Administration has required some degree of characterization of bioavailability for all new drugs intended for oral use. Some attention has also been given to dosage forms intended for other routes of

administration. These requirements have established a standard of performance.

More recently, the FDA has required secondary (or generic) manufacturers who are interested in marketing a drug after a patent or period of exclusive-use has lapsed to demonstrate bioequivalence (comparable bioavailability) with the innovator's dosage form before approval to market is granted. The Congress directed the FDA to apply these criteria to generic products through the passage of the Drug Price Competition and Patent Restoration Act in 1984. Before this landmark legislation, the only way a secondary manufacturer could market a drug was to carry out clinical trials demonstrating comparable efficacy to the innovator's product.

A bioequivalence trial generally consists of a comparison of the area under the drug concentration-time curve, peak concentration, and time to peak concentration after a single dose of the generic and "standard" product using a randomized, two-way crossover design. Urinary excretion data may also be useful, particularly for drugs that are substantially excreted unchanged. The FDA bioequivalence guidance for hydrochlorothiazide recommends a urinary excretion study.

Panels of healthy human subjects are almost always used in bioequivalence studies. The FDA recognizes the possibility that some conditions found only in special populations (patients, elderly, etc.) could affect bioavailability and is prepared to modify its guideline calling for the use of normal subjects if the need is adequately documented for a given drug.

The Agency also requires the determination of metabolite kinetics if the drug is metabolized to a clinically important biotransformation product. This requirement is controversial. Some scientists believe that a metabolite should be followed only as an alternative when it is difficult to measure unchanged drug in the plasma.

Can dissolution testing assure bioequivalence? This question has been widely debated. The FDA and most pharmaceutical scientists believe that there is not yet evidence to show that a dissolution test will assure bioequivalence. Dissolution testing is important in assuring lot-to-lot uniformity of a drug product and supporting minor changes (e.g., a change in color) in the product. Also, it is FDA policy that if a product meets in vivo bioequivalence requirements at one dosage strength and the formulations of other strengths are proportional to the strength tested and meet dissolution requirements, then no further in vivo studies are needed for approval.

The usual criteria for bioequivalence calls for the mean AUC and C_{max} values for the two products to be within 20%, but the FDA also applies a 90% confidence interval test based on the two one-sided t-test approach,[43] one test to verify that the bioavailability of the test product is not too low, and the other to show that it is not too high. The entire 90% confidence interval must also lie within the limits of plus-or-minus 20%.

This confidence interval requirement ensures that the difference in mean values for AUC and C_{max} will be much less than 20%. The experience to date in reviewing bioequivalence studies with generic products indicates that 80% of the approvals had AUC values within 5% of the reference product. In view of this experience, some scientists believe that the FDA should be more stringent, requiring the mean values for AUC to be within 10% rather than 20%. On the other hand, some believe that the current requirements for C_{max} values are too stringent, considering the difficulty in accurately estimating this value, and the typical finding for most products (generic or brand name) that C_{max} values are more variable than AUC values.

The approval process for generic products has worked remarkably well for conventional oral dosage forms. Almost no documented examples of clinically important differences between generic and original products have been reported. The one class of drugs that continues to be put forward (often with scant evidence) as a challenge to the sufficiency of bioequivalence studies to assure the performance of a generic product is the anticonvulsants.

A case for bioinequivalence of a generic drug product has been made in a report concerning a 16-year-old girl with severe cerebral palsy and seizures since birth.[44] During treatment with primidone and other medication, her usual seizure frequency was one to two seizures per week. Serum levels of both primidone and phenobarbital, its metabolite, are frequently monitored in patients receiving primidone.

The patient had been taking the same antiepileptic medication for 9 years. Within 3 weeks of switching her to a generic primidone, there was a rise in seizure frequency and she was switched back to the original dosage form. With this change, the seizure frequency decreased to baseline. Serum

drug concentrations were not measured during this period.

The patient's condition remained stable until 3 months later, when she was admitted to hospital for feeding problems. Before admission, she was taking her usual medication and serum trough levels were 10.8 mg/L for primidone and 19.1 mg/L for phenobarbital. During hospitalization, she was again switched to the primidone product that caused a problem 3 months earlier. After 6 days of receiving this product, morning trough levels were 5.1 mg/L for primidone and 15.9 mg/L for phenobarbital.

On day 6, the daily dose of primidone was increased from 500 to 625 mg, but despite this change, serum levels continued to fall and the patient had more frequent seizures. On day 10, serum primidone was less than 2.0 mg/L and serum phenobarbital was 10.4 mg/L. At this time, the patient was returned once again to the original primidone product. After 6 days of receiving this product at a dose of 500 mg/day, primidone levels were 9.0 mg/L and phenobarbital levels were 12 mg/L, and the patient's seizure frequency returned to baseline.

The evidence is clear that the two primidone products used in this patient were not bioequivalent. This observation raises concern that an initial determination of bioequivalence may change with time because of subtle changes in manufacturing or lot-to-lot variability. This problem seems to call for some stringent dissolution criteria. In any event, the investigators urged that product substitution be cautiously considered in patients who have already been titrated and maintained on an antiepileptic preparation.

Controlled-Release Medication

A basic question in developing a controlled-release product of a drug that has been used in a conventional dosage form is whether a formal clinical evaluation of the new dosage form's safety and efficacy is needed, or whether a pharmacokinetic evaluation will suffice. The FDA's position is that if there is a well-defined relationship between plasma concentration of drug and/or active metabolite and clinical response, it may be possible to rely on plasma concentration data alone as a basis for the approval of a product.

On the other hand, "where the therapeutic effect is indirect, where irreversible toxicity can occur, where there is evidence of functional (pharmacodynamic) tolerance, where peak to trough differences of the immediate release form are very large, or where there is any other reasonable uncertainty concerning the relationship between plasma concentration and therapeutic and adverse effects, it will probably be necessary to carry out clinical studies."[45]

For the development of a controlled-release oral dosage form of a drug marketed in an immediate-release form for which an extensive base of pharmacodynamic-pharmacokinetic data exists, the following pharmacokinetic studies are usually required. A single dose, three-way crossover study where the immediate-release and the controlled-release products are given to fasted subjects, and the controlled-release form is also given after a high fat meal.

The fasting comparison permits an estimation of the extent of absorption from the controlled-release form relative to the immediate-release form. The food study is essentially a drug interaction assessment. If there are no differences in AUC and peak concentration following administration of the controlled-release form to fed and fasted subjects, then no further food studies are needed. If a decrease or an increase in the extent of absorption is found after a meal, it may be necessary to determine the cause of the food effect as well as the effect of time on the food-drug effect (i.e., would absorption be affected if the dosage form were given 1 or 2 hr after a meal rather than with a meal).

The FDA also requires a multiple dose, steady-state, crossover comparison of the controlled-release and immediate-release products as part of the pharmacokinetic evaluation. Ordinarily, the same daily dose is used for each regimen but the immediate-release form is given more frequently than the controlled-release form (e.g., 3 times a day versus once a day). Concentrations over at least one dosing interval should be measured in each leg of the crossover. Some investigators favor measurements over 24 hr in each leg of the study, to account for diurnal variation.

The controlled-release product should produce an AUC equivalent to the immediate release product, and the degree of fluctuation at steady-state [i.e., $(C_{max} - C_{min})/C_{av}$] for the controlled-release product should be similar to, or less than, that for the immediate release form. If appropriate, levels of major active metabolites should also be measured. For racemic drugs, consideration should be given to measurement of individual enantiomers.

Since the passage of the Drug Price Competition and Patent Restoration Act in 1984, attention has

also been given to criteria needed to demonstrate the equivalence of a generic product to an approved controlled-release product. The current position of the FDA on this matter is as follows: "the new generic formulation must be comparable with respect to AUC, C_{max}, and C_{min} in a cross-over steady-state study *vs* the standard controlled-release product using the accepted Agency criteria for equivalence. In some cases, it may also be necessary to match the concentration-time profile of the approved controlled-release dosage form. The food studies described previously are also needed."[45]

SPECIFIC DRUGS

The following discussion is a summary of reports of poor bioavailability or "inequivalences" of marketed products, listed alphabetically by drug. That most of the material has been taken from previous editions of this text and that comparatively few examples of bioinequivalence have been reported in the past five years are encouraging signs, indicative of the attention given to the development of dosage forms today.

Acetazolamide

Most of the reports on differences in bioavailability of marketed products have concerned prolonged-release dosage forms. Clinical studies with acetazolamide, a carbonic anhydrase inhibitor used in treating glaucoma, provide an example.[46] Acetazolamide was only 60% available from a sustained-release capsule, Diamox Sequels, compared to that observed after an aqueous suspension. Consistent with these results, steady-state concentrations of acetazolamide for the prolonged-release capsules were about half the values observed for an immediate-release dosage form. Since Diamox Sequels is considered to be an effective product, the results suggest that lower doses of acetazolamide in rapid-release dosage forms may be useful for treating glaucoma.[47]

Aminosalicylate

Studies in Canada with various dosage forms of aminosalicylic acid (PAS), which is used, usually in combination, in the treatment of pulmonary and extrapulmonary tuberculosis, indicated large differences in drug absorption.[48] The availability of a prolonged-release product, estimated from cumulative urinary recovery of the drug in 8 subjects, was only 42% compared to that observed following administration of a standard capsule containing drug and lactose. The relative availability of PAS from two different lots of an enteric-coated tablet and from a powder containing a polyamine resin complex of the drug was 51%, 64%, and 66%, respectively. Another investigation found no absorption of PAS in 8 subjects after administration of an enteric-coated tablet.[49]

Ampicillin

Concern for differences in bioavailability of the widely used antibiotic ampicillin was stimulated by a report from Canada demonstrating that two brands of ampicillin capsules produced lower serum concentrations than did ampicillin capsules manufactured by a third company.[50] Products B and C were only 78% and 72% as available as product A, based on the area under the serum concentration versus time curves. A second bioavailability study comparing product A with a reformulated product C indicated bioequivalence.[51] The reformulation involved a minor change in the amount of a dispersing agent. The bioavailability monograph on ampicillin published by the American Pharmaceutical Association in 1975 concluded that it is unlikely that possible differences in bioavailability among the current major United States suppliers are of clinical importance.[52] The same holds true today.

Aspirin

Poor bioavailability of aspirin has been reported only with enteric-coated products. Less than 25% of the dose was absorbed in 3 of 4 subjects after administration of a certain brand of enteric-coated aspirin tablets.[53] A clinical study with this enteric-coated product in arthritic patients showed erratic and low concentrations of salicylate, compared to those observed after regular administration of conventional aspirin tablets.[54] This problem has all but disappeared with the materials in use today to provide enteric protection.

Ascorbic Acid

This vitamin has been widely used since the claim in 1970 that daily consumption of large quantities of ascorbic acid may be beneficial for reducing the frequency and duration of the common cold. Ascorbic acid absorption was investigated in 4 subjects who received different oral dosage forms containing 1 g of vitamin C.[55] About 85% of a 1-g intravenous dose was recovered in the urine as as-

corbic acid and its major metabolite. In contrast, only about 30% of the dose was recovered after an oral aqueous solution, a conventional tablet, or a chewable tablet. A still smaller fraction of the dose, about 14%, was recovered after a prolonged-release product. The incomplete availability of ascorbic acid after the solution and tablets reflects the capacity-limited absorption of the vitamin; the same daily dose given in divided doses is absorbed more efficiently. The poor results with the prolonged-release capsule may reflect the site-specific absorption of the vitamin in the proximal intestine or a poorly formulated product.

Carbamazepine

Carbamazepine is gaining wide acceptance as monotherapy for seizures and is increasingly used in children. Two formulations are marketed in the United States: 200 mg tablets and 100 mg chewable tablets. These two forms, from the same manufacturer, were compared in a single-dose randomized crossover study in fasting healthy adults with a four-week washout period between doses.[56] In each leg, a 200-mg dose of carbamazepine was given and blood was collected for 48 hr.

The area under the curve to 48 hr was about 10% larger for the chewable tablet than for the conventional tablet, but this difference was not statistically significant. A significantly higher mean C_{max} value, however, was observed with the chewable tablet (4.6 vs 3.8 mg/L). The investigators concluded that the difference in C_{max} was not clinically relevant and that carbamazepine tablets and chewable tablets could be used interchangeably. Some clinicians might take issue with this conclusion because there is evidence that adverse effects of carbamazepine are related to peak concentration.

Chloramphenicol

Although chloramphenicol is rarely the drug of choice for treating infections, it is still used in certain situations. The absorption characteristics of four different chloramphenicol products were compared in normal adults by means of blood level measurements and urinary excretion of chloramphenicol and its metabolites following single 0.5-g oral doses.[57] Mean plasma levels for groups of 10 subjects are shown in Figure 8–10. Relative bioavailabilities based on cumulative urinary excretion of total nitro compounds were 100%, 71%, 83%, and 39% for products A, B, C, and D, respectively. Similar differences in apparent bioavailability can

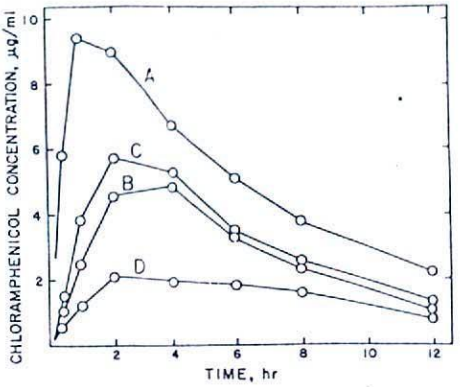

Fig. 8–10. Chloramphenicol concentrations in plasma after a single 500-mg oral dose of 4 different commercial products. (Data from Glazko, A.J., et al.[57])

be calculated from the plasma concentration data. In vitro tests indicated that products B, C, and D dissolved more slowly than did product A.

Chlorothiazide

In 1977, the FDA implemented bioequivalence requirements for tablets of chlorothiazide, a widely used diuretic, because of concern about bioavailability differences among marketed products. The availability of chlorothiazide is best determined from urinary excretion data; almost the entire dose is excreted unchanged after intravenous administration. A urinary excretion bioavailability study was conducted in 12 healthy males to evaluate three 250-mg and 500-mg chlorothiazide tablets.[58] Chlorothiazide excretion did not exceed 20% of the dose for any product, reflecting the incomplete absorption of the drug from the gastrointestinal tract. No important differences were found among the 250-mg tablets; availability ranged from 16 to 20% of the dose. Drug recovery in the urine after one of the 500-mg tablets (11% of the dose) was significantly less than that from the other two 500-mg tablets (13% and 16% of the dose).

Chlorpropamide

Chlorpropamide is an antidiabetic agent used in adult-onset diabetes. Studies in England with three marketed products showed that the rate and extent of chlorpropamide absorption were markedly impaired with one product compared to the other two.[59] The results are shown in Figure 8–11. The peak concentration of chlorpropamide after admin-

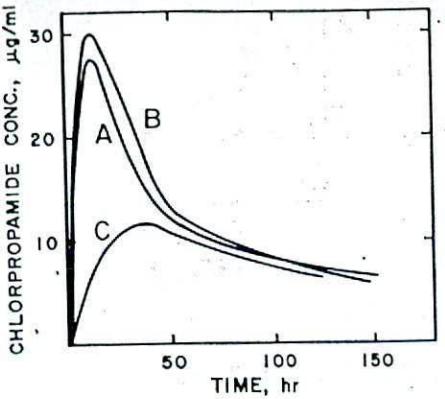

Fig. 8–11. Chlorpropamide concentrations in plasma after a single 250-mg oral dose of 3 different commercial products. (Data from Monro, A.M., and Welling, P.G.[59])

istration of product C was less than half that found after administration of the other formulations.

Diazepam

A report is available from Sweden concerning the plasma concentrations of diazepam and its major metabolite N-desmethyldiazepam after treatment with 5-mg oral doses 3 times a day.[60] A crossover study to evaluate the bioavailability of several tablet products and a marketed suspension was included in the investigation. All three tablets gave similar plasma levels but the suspension showed lower values during steady state, indicating incomplete absorption.

Differences in the rate but not the extent of absorption of marketed diazepam tablets have been reported when the drug was given after treatment with an H_2-blocker. Under these conditions, gastric pH is increased and the dissolution rate of diazepam is decreased. Some products appear to be more affected by this pH change than others. Similar differences between products might also be found in elderly patients, who tend to have elevated gastric pH. It is not likely, however, that small differences in the rate of absorption of diazepam would be of clinical interest.

Digoxin

Perhaps, more bioavailability data have been reported for digoxin than for all other drugs combined. Digoxin is poorly water-soluble and has a low therapeutic index. Relatively small differences in bioavailability of digoxin products may be clinically significant.

The first published comparative bioavailability study for digoxin appeared in 1971.[61] The investigation was prompted by the observation that several patients in a New York City hospital required unusually large maintenance doses of digoxin but had low serum drug concentrations. Four lots of digoxin tablets from three manufacturers were evaluated in healthy subjects. The mean peak serum digoxin levels, which reflect absorption rates, varied sevenfold, with Lanoxin brand of digoxin showing the highest peak. Some of the differences observed in this investigation could have been due to low tablet potency rather than poor bioavailability.

In a later study, Lanoxin and another brand of digoxin tablets, both of which met U.S.P. specifications, were compared.[62] On the basis of areas under the serum level-time curve, the availability of the test product was only 55% of that observed with Lanoxin.

The influence of dissolution rate on the bioavailability of digoxin from commercial tablets has been appreciated since 1972. The more rapidly dissolving of two formulations marketed at different times by the same manufacturer in England resulted in higher peak serum levels.[63,64] Two digoxin products available in Sweden that differed in dissolution rate showed comparable differences in steady-state serum concentrations after chronic administration.[65] A strong correlation between dissolution rate and peak serum digoxin concentration after a single 0.5-mg dose of digoxin in tablets from 12 different lots (Fig. 8–12) and between dissolution rate and mean steady-state serum digoxin levels after 8 to 10 days of 5 different digoxin products has also been reported.[66] The U.S.P. XXI requires that not less than 65% of the labeled amount of drug from digoxin tablets dissolve in 60 min in dilute hydrochloric acid.

An unusual and potentially dangerous situation with digoxin arose in the United Kingdom.[67] The evidence indicates that three different formulations of Lanoxin tablets, the product used by more than half the British patients requiring digoxin, were marketed over a relatively short period of time. The pre-1970 and post-May 1972 tablets gave steady-state levels that were two-thirds higher than those observed after administration of tablets marketed from 1970 to 1972. The first formulation change, made in late 1969, appears to have reduced

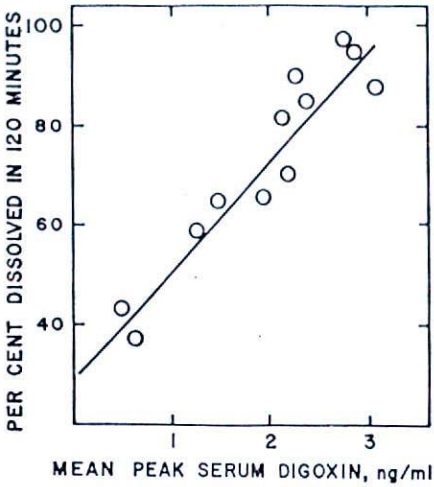

Fig. 8–12. Correlation between dissolution rate and peak serum digoxin level after a single 0.5-mg dose of different tablets. (Data from Lindenbaum, J., et al.[66])

the bioavailability of digoxin, but this was corrected in mid-1972 by a second formulation change. From autumn 1969 to mid-1972, Lanoxin tablets were bioequivalent to most brands of digoxin marketed in England. Since mid-1972, however, there has been a significant bioavailability difference between Lanoxin tablets and the tablets of most other manufacturers.

Differences in digoxin bioavailability from different marketed tablets have also been reported in Finland[68] and Australia.[69] A useful review of digoxin bioavailability, from Sweden, was published in 1977.[70]

An interesting report from Israel, entitled "An outbreak of digoxin intoxication," has also been published.[71] Within a 2-month period between October and December 1975, 15 cases of digoxin intoxication were diagnosed on a medical ward. Almost no cases of digoxin toxicity were noted by the same physicians on the same ward during the previous year. An inquiry disclosed that the local manufacturer, without notice, had modified his formulation of digoxin to improve dissolution. Plasma levels of digoxin following single 0.5-mg doses of the old and new tablets are shown in Figure 8–13. Urinary excretion data showed more than a 2-fold difference between the two tablets in the availability of digoxin.

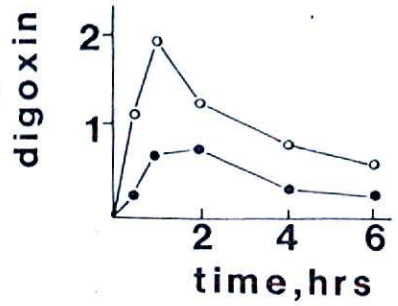

Fig. 8–13. Digoxin concentrations in plasma (ng/ml) after a single 0.5-mg oral dose in old (●) and new (○) tablets. (Data from Danon, A., et al.[71])

Furosemide

In 1979, the FDA issued a nationwide alert to patients taking the diuretic furosemide. Three manufacturers had marketed tablets of the drug without approval; these tablets were believed to be ineffective because of poor bioavailability. Patients who failed to respond to treatment with these tablets recovered when switched to an approved brand of the drug.

Martin et al.[72] compared the relative bioavailability of the brand-name tablet formulation of furosemide available in the U.S. (Lasix) and one of the generic tablets cited above. Furosemide concentrations in plasma and urine were measured after a 40-mg single dose. The bioavailability of the generic tablet was significantly less than that of the brand-name tablet. Peak furosemide levels following administration of the generic tablet were little more than 50% that observed after Lasix; total AUC was about one-third less with the generic product.

On the other hand, there was little difference with respect to 24-hr urine volume or sodium output following each product. Comparison of the effect of the two treatments is a less sensitive measure of bioequivalence and does not excuse the need for a generic product to meet expected bioavailability standards; the findings support the FDA's action against this product.

There continues to be concern about the bioavailability of furosemide tablets, fueled by differences in dissolution rate among marketed products. A recent bioavailability study compared Lasix tablets from two different lots (A, D), a generic product from two different lots (C, E), and another

generic product (B), with a solution of furosemide.[73]

All of the tablets were absorbed at a slower rate (as determined by C_{max} values) and to a lesser extent (as assessed by AUC and amount excreted unchanged in the urine) than the orally administered solution of furosemide. The extent of absorption ranged from 66% for product C to 96% for product D. Variability from one lot of furosemide to another was considerable; the extent of absorption from product A was only 87% that from product D. The data suggest that products A, B, C, and E are bioequivalent but less bioavailable than product D.

Hydrochlorothiazide/Triamterene

Dyazide, a combination product containing hydrochlorothiazide 25 mg and triamterene 50 mg, is a widely prescribed potassium-sparing diuretic/antihypertensive. Since 1968, however, we have been aware that Dyazide has poor bioavailability with respect to both drugs. This was not a serious problem so long as the combination was a single source product. Matters became complicated when the period of exclusivity lapsed and other manufacturers wished to market an equivalent product. Matching precisely the incomplete bioavailability of hydrochlorothiazide and triamterene after administration of Dyazide was a difficult task and success meant the development of a poorly formulated product.

This unusual circumstance prompted the development of Maxzide, a combination product containing hydrochlorothiazide 50 mg and triamterene 75 mg. The bioavailability of both components of Maxzide is comparable to that of a liquid preparation. In fasted subjects, the absorption of both hydrochlorothiazide and triamterene from Dyazide capsules is about half that from Maxzide tablets. Maxzide was approved by the FDA on the basis of clinical studies demonstrating safety and efficacy.

A steady-state study in patients with essential hypertension concluded that the hydrochlorothiazide component of Dyazide was about two-thirds as available as that in Maxzide, while the triamterene component was less than half as bioavailable as that in Maxzide.[74] Williams et al.[75] found that the high-fat, high-calorie breakfast, recommended by the Food and Drug Administration in the evaluation of controlled-release dosage forms, had no effect on the absorption of hydrochlorothiazide or triamterene from Maxzide. On the other hand, the absorption of hydrochlorothiazide was increased by 40% and that of triamterene by 120% when Dyazide was given with the high-fat breakfast.

Levothyroxine

The bioavailability of two brands of levothyroxine, Levothroid and Synthroid, was evaluated in 34 patients who required long-term treatment with the hormone.[76] Half the patients received Levothroid for 1 month, followed by 1 month treatment with Synthroid; the other half had the opposite sequence. When patients were switched from Levothroid to Synthroid, significant decreases occurred in mean serum thyroxine levels; switching from Synthroid to Levothroid resulted in increases in thyroxine levels (Fig. 8–14). Ramos-Gabatin and co-workers concluded that marketed products of thyroxine are therapeutically inequivalent and that patients should be treated consistently with a single brand.[76] Adjustment of the dose may be necessary if the patient is switched from one brand to another.

These findings were confirmed by Sawin et al.[77] Patients with primary hypothyroidism were given oral thyroxine as Levothroid or Synthroid. Serum thyroxine was lower in all 32 patients when taking Synthroid than when taking Levothroid. Direct measurement of thyroxine in the tablets showed that Synthroid tablets contained 20 to 30% less thyroxine than label claim.

In 1984, the U.S. Pharmacopeia adopted a new method of assaying for the hormone content of levothyroxine tablets. The new assay was based on high-pressure liquid chromatography and replaced a less accurate method based on measurement of iodine content. This change required the manufacturers of Synthroid to alter their method of making the product. Synthroid tablets made before reformulation were found to contain less than 80% of labeled value, while Synthroid tablets made after the change contained 100% of the amount stated on the label.[78]

The replacement dose of the new Synthroid tablets was evaluated in 19 patients with hypothyroidism.[79] The dose was titrated monthly until thyrotropin levels become normal. The mean replacement dose was 112 μg per day, much smaller than the mean dose needed when the original product was evaluated—169 μg per day. Based on the average replacement dose, it appears that the levothyroxine content of the original tablets was approximately 70% of label claim.

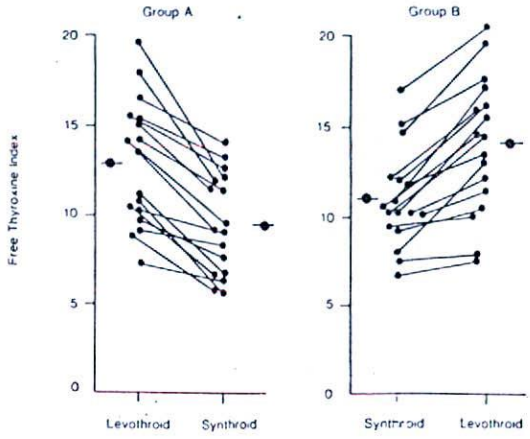

Fig. 8–14. Differences in free thyroxine index in patients switching from a regimen of Levothroid to Synthroid (group A) and in patients switching from Synthroid to Levothroid (group B). (From Ramos-Gabatin, A., Jacobson, J.M., and Young, R.L.: In vivo comparison of levothyroxine preparations. JAMA, 247:203, 1982. Copyright 1982, American Medical Association.)

Nitrofurantoin

In single-dose bioavailability studies involving 14 different marketed products, all of which met U.S.P. specifications, significant differences between products were found in the cumulative urinary excretion of nitrofurantoin.[80] The results with two products suggested that less than 50% of the dose was absorbed. There have been several FDA recalls of nitrofurantoin tablets. Two were for failure to pass U.S.P. disintegration tests and a third was because bioavailability studies indicated poor absorption. In the latter case, urinary recovery of nitrofurantoin ranged from 2 to 14% of the dose compared to the 32% specified in the original New Drug Application.

The interest in reducing the dissolution rate of nitrofurantoin to reduce gastrointestinal upset may have led to these bioavailability problems. This situation was exacerbated by the dissolution requirement for nitrofurantoin tablets in U.S.P. XVIII, which stated that the time required for 60% of the labeled amount of nitrofurantoin to dissolve is *not less than* 1 hr. A tablet from which nitrofurantoin dissolved infinitely slowly would meet this requirement. The U.S.P. XXI requires that *not less than* 25% of the labeled amount of nitrofurantoin is dissolved in 60 min, and not less than 85% is dissolved in 120 min.

Nitrofurantoin tablets from 7 Mexican manufacturers as well as the innovator's tablet (Furadantin) were evaluated for disintegration, dissolution, and bioavailability.[81] The disintegration time for Furadantin was less than 1 minute; disintegration time for three lots from a single Mexican manufacturer and for one lot from another manufacturer exceeded 30 min. The percent dissolved in 60 min was less than 25% for 7 products from 3 different manufacturers. Only three products (each from a different manufacturer) dissolved sufficiently rapidly so that 85% was in solution at 120 min. Tablets from other lots made by the same manufacturers as well as the innovator's product did not meet the upper limit.

Bioavailability studies based on cumulative amount excreted after a single dose indicated that two different lots of tablets from the same manufacturer were only 30% absorbed in 1 case and 60% in the other, relative to Furadantin. For two different lots of tablets from another manufacturer, relative bioavailability was 90% for one but only 45% for the other. A statistically significant correlation ($r = 0.91$) was observed between the cumulative amount of nitrofurantoin excreted and the percent dissolved (in vitro) in 60 min for the 5 products evaluated for both dissolution and bioavailability.

Another product (Macrodantin) contains relatively large particle size nitrofurantoin, dissolves more slowly than Furadantin, and may reduce the

gastrointestinal intolerance associated with rapidly dissolving products without compromising effectiveness. Mason et al.[82] evaluated the bioavailability of three macrocrystalline nitrofurantoin products, available outside the United States, relative to that of Macrodantin, the originally marketed product.

Dissolution studies indicate that at 60 min only 19% of the dose was dissolved from the Macrodantin capsule compared with 30 to 37% from the other products. The maximum rate of urinary excretion of nitrofurantoin (a parameter analogous to C_{max} and indicative of absorption rate) after a single dose of Macrodantin was 5.6 mg/hr. Maximum rates of excretion for the other products ranged from 7.5 to 8.3 mg/hr. The results indicate that some nitrofurantoin products available outside the U.S. that claim to be macrocrystalline are not bioequivalent to Macrodantin.

Oxytetracycline

In 1969, a crossover serum level study in 20 subjects was carried out on 16 lots of FDA-certified oxytetracycline hydrochloride capsules, distributed by 13 suppliers, with a single lot of Terramycin brand of oxytetracycline hydrochloride capsules as the standard in each case. The serum levels produced by capsules from 12 of these lots were significantly lower than those found with Terramycin capsules.[60]

Some time later, oxytetracycline hydrochloride capsules produced by all 11 manufacturers supplying the United States market were compared in a series of two-way crossover studies.[61] The original manufacturer's product (Terramycin) was used as the reference product. Serum concentrations of oxytetracycline after administration of 7 comparison products were more variable and markedly lower (about 50%) than those resulting from administration of the reference product.

In June 1969, the FDA stopped certification of all oxytetracycline capsules except those demonstrating acceptable bioavailability. In the next few months, some 40 million oxytetracycline capsules were recalled. Many of these products were reformulated and returned to the market in a more effective form.

Oxytetracycline bioavailability problems have also been observed in England. Significant differences in bioavailability were noted between four different marketed tablets of oxytetracycline dihydrate.[62] The results of these studies are shown in Figure 8–15. The dihydrate form of oxytetracycline is 1000 times less soluble than the hydrochloride salt and may introduce additional bioavailability problems.

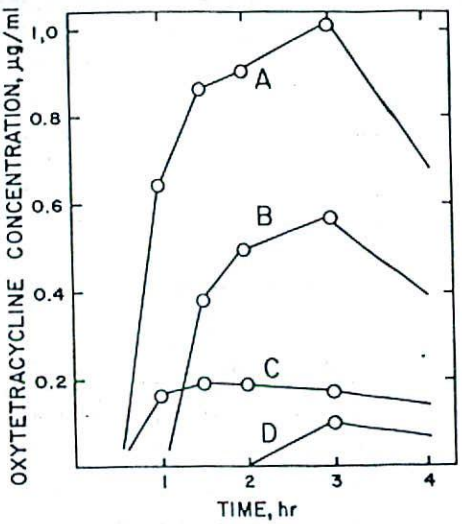

Fig. 8–15. Oxytetracycline concentrations in plasma after oral administration of different commercial tablets each containing 250 mg of oxytetracycline dihydrate. (Data from Barber, H.E., Calvey, T.N., and Muir, K.[85])

Papaverine

Papaverine is used as a vasodilator and antispasmodic in the treatment of peripheral vascular disease. The bioavailability of papaverine from prolonged-release dosage forms, a conventional tablet, and an elixir was compared in healthy human subjects.[86] Plasma level data indicated equal availability of papaverine from the elixir and regular tablet; however, the AUC values for the 9 prolonged-release products ranged from 18 to 64%, relative to that resulting from the elixir. The poor performance of these marketed products may have contributed to the lack of clear-cut efficacy of the drug in various clinical trials.

Phenylbutazone

An initial report from Canada suggested significant differences in the bioavailability of phenylbutazone from different products.[87] This prompted a more comprehensive investigation of phenylbutazone blood levels after administration of 9 different tablets marketed in Canada and of an aqueous solution.[88] In comparison with the control solution, two products produced significantly lower

blood levels of phenylbutazone. The absorption of phenylbutazone from one of these products was estimated to be only 60% that found from solution.

A follow-up study, in 1978, of 23 Canadian formulations of phenylbutazone found that 5 products were at least 20% less available than an oral aqueous solution of the drug.[89] Upon the advice of the Health Protection Branch Advisory Committee on Bioavailability, these products were removed from the market.

Phenytoin

The potential for variable and incomplete absorption of phenytoin from its dosage forms has been cited in many reports. Interchange of phenytoin formulations with different bioavailabilities can lead to therapeutic failure or intoxication.[90]

An unusual incidence of phenytoin intoxication in epileptic patients occurred in Australia in 1968 and 1969, following a change in the diluent of phenytoin sodium capsules from calcium sulfate to lactose. A crossover study showed that phenytoin with calcium sulfate produced lower blood levels than did phenytoin with lactose in 12 of 13 patients.[91] Presumably, the change in diluent led to greater bioavailability and a higher incidence of adverse effects with the reformulated product.

Another report, from Sweden, showed that plasma levels of phenytoin in epileptic patients were significantly higher after treatment with two preparations containing phenytoin sodium than after treatment with a third preparation containing an equivalent dose of the free acid.[92] The higher plasma levels were accompanied by better control of generalized seizures. Single-dose studies in healthy subjects showed the two preparations containing phenytoin sodium to be bioequivalent. The relative availability of phenytoin from the preparation containing the free acid was only 65%.

Substantial differences in the bioavailability of phenytoin from marketed products have also been reported in the United Kingdom[93] and in Finland.[94] The studies in the United Kingdom involved measurement of steady-state plasma phenytoin levels in 60 patients for six weeks. During the trial, the preparation of phenytoin was changed from one brand to another. A significant increase in plasma phenytoin levels was observed following the change. This was accompanied by a decrease in the number of seizures. The results of the studies in Finland are summarized in Table 8–2.

In 1978, the FDA issued new prescribing directions for phenytoin. A slow-release form, extended phenytoin sodium capsules, and a fast-release form, prompt phenytoin sodium capsules, were recognized. Only the slow-release form of the drug is approved for once-a-day dosing. On the average, the bioavailability of phenytoin is lower from the slow-release form than from the fast-release form, but considerable variability is found among patients. Patients who are maintained on one brand of phenytoin should not be switched to another brand, without considering the need for dosage adjustments.

Table 8–2. Average Areas Under the Serum Phenytoin Concentration-Time Curves (AUC) in 6 Volunteers After Administration of a Single Oral Dose of 600 mg of the Drug in 4 Different Tablets and a Reference Suspension*

Dosage form	AUC (mg-hr/l)	Relative availability (%)
Tablet A	327	68
Tablet B	124	26
Tablet C	429	90
Tablet D	283	59
Suspension	480	100

*Data from Pentikäinen, P.J., Neuvonen, P.J., and Elfving, S.M.[94]

Other reports on phenytoin bioavailability have appeared.[95,96] In 1979, Neuvonen published a review article on phenytoin bioavailability, stressing therapeutic implications.[97] For as long as phenytoin is used we must be concerned about bioavailability because this drug presents us with characteristics, including poor water solubility, low therapeutic index, and capacity-limited metabolism, that collectively are unique.

Procainamide

The bioequivalence of two prolonged-release procainamide products, Procan-SR and Pronestyl-SR, was evaluated at steady state in ten patients with cardiac arrhythmias.[98] The dose of procainamide was individualized and ranged from 2 to 3 g per day divided into 6 or 8 hourly intervals. The products were compared on a milligram-equivalent (adjusted) basis, because some patients received different daily doses of the two products.

Steady-state levels of procainamide in plasma were higher with Procan-SR than with Pronestyl-SR in 8 of 10 patients, but differences were not statistically significant. Average drug concentrations at steady state were 3.9 μg/ml for Pronestyl-SR and 4.5 μg/ml for Procan-SR. One patient,

when crossed-over from Procan-SR to Pronestyl-SR, developed frequent episodes of nonsustained ventricular tachycardia. Procainamide levels in this patient were 40% less on Pronestyl-SR than on Procan-SR. These two products are probably inequivalent, but too few patients were studied to make this statement with confidence.

Quinidine

Quinidine is an old drug but still used as an antiarrhythmic agent. In a crossover study, carried out in Sweden, healthy subjects took three different formulations, each containing the same amount of quinidine base, every 12 hr for 4 days.[99] A mean steady-state serum quinidine level of 1.8 μg/ml was produced by rapidly dissolving tablets of quinidine bisulfate. One of the prolonged-release products containing quinidine bisulfate produced a mean steady-state serum level that was 23% lower than that produced by the rapidly dissolving product. A second prolonged-release preparation containing quinidine arabogalactone sulfate gave an average level that was 46% lower.

A later report from Sweden concerned the evaluation of two slow-release preparations of quinidine bisulfate (A and B).[100] The in vitro dissolution of B was unusual in that drug release was considerably faster at low pH than at neutral pH. The dissolution of quinidine from product A was essentially independent of pH. Clinical studies indicated that the availability of quinidine, as determined from AUC measurements, was about 50% greater for product A than for product B.

Bioavailability problems with prolonged-release quinidine products have also been reported in the U.S. One study compared a relatively new prolonged-release product with a widely used slow-release formulation, Quinaglute Duratabs.[101] Both products contain quinidine gluconate equivalent to 202-mg quinidine base. The extent of absorption of quinidine from the newly marketed product was only 50% that of the older product (Fig. 8–16). These findings resulted in a recall of the poorly available product by the FDA. The FDA concluded that there was a reasonable probability of serious adverse health consequences that could result from the use of this product.

Tetracycline

The first report of potential bioavailability problems with marketed dosage forms of tetracycline hydrochloride was published in 1969.[102] The absorption of tetracycline was studied after administration of four different products, including the innovator's capsule, which was used as the standard. Serum levels of tetracycline after administration of the three test products were significantly lower than those produced by the reference product.

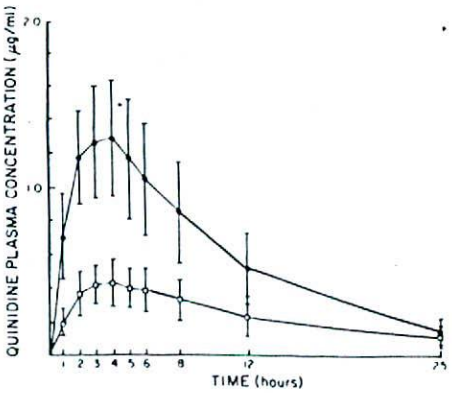

Fig. 8–16. Quinidine concentrations in plasma after single doses of quinidine gluconate tablets from 2 different manufacturers. (From Meyer, M.C., et al.[101])

In another study, 9 brands of tetracycline hydrochloride, marketed in Canada, were compared with an aqueous solution of the drug. Of the 9 products, 7 had relative bioavailabilities of 70 to 100%, but two products showed relative bioavailabilities of only 20 to 30%.[103] Several other reports have been summarized in a monograph.[104]

In a study reported in 1975, serum concentrations of tetracycline were compared in adults who received two different brands of tetracycline tablets.[105] Although both products passed batch certification tests of the FDA, the bioavailability of one product was only 26% that of the other.

Theophylline

The strong interest in prolonged-release theophylline for the treatment of chronic asthma has prompted several bioavailability studies that suggest clinically important differences among marketed products. One study examined several formulations in adults.[106] Absorption of theophylline from a solution or from uncoated tablets was rapid and complete. Of six prolonged-release products, three were slowly but completely and consistently absorbed. Theophylline absorption from the three other prolonged-release formulations was erratic

and incomplete. One product was only 65% available relative to the solution.

Another study, comparing six prolonged-release preparations of theophylline with an elixir, found that two slow-release formulations were substantially less available than the elixir.[107] Availability of theophylline was only 48% from one slow-release product and 59% from the other.

In a recent study, 10 subjects with asthma were given the same dose of four different slow-release theophylline products twice daily for 2-week periods, to determine whether clinically important changes in serum theophylline levels occur when patients switch brands.[108] A randomized, double-blinded, crossover design was employed.

The investigators reported that on at least one occasion in every subject, switching brands was responsible for raising the serum theophylline concentrations outside the accepted therapeutic range (10 to 20 μg/ml); this was associated with adverse effects in 5 subjects. Worsening pulmonary functions were observed in 2 subjects when switching brands resulted in decreased theophylline levels. Many of the seemingly product-related changes in serum theophylline appeared to be idiosyncratic and could not be predicted by bioavailability differences between the products.

Baker et al.[108] oppose the free substitution of these formulations. They suggest that if patients are switched between different brands of slow-release theophylline, their serum theophylline concentrations need to be monitored. This is probably good advice. It is disappointing that the investigators failed to repeatedly administer a single formulation at a fixed dose for four 2-week periods to determine how frequently theophylline levels drift out of the therapeutic range when no switching occurs.

In another recent study, the relative bioavailability of two slow-release theophylline products, Slo-bid and Theo-Dur Sprinkle, was determined from saliva in preschool asthmatic children.[109] A rapidly absorbed theophylline product, Slo-Phyllin Gyrocaps was used as the bioavailability standard.

The extent of absorption was significantly less than the reference product for Theo-Dur Sprinkle but not for Slo-bid. Relative bioavailability was 66% for the Sprinkle and 109% for Slo-bid. Although Theo-Dur Sprinkle is completely absorbed in fasting subjects, under actual conditions of use a bioavailability problem is seen, probably because food decreases the extent of absorption of theophylline from this product.

The investigators pointed out that information regarding incomplete absorption of theophylline from Theo-Dur Sprinkle is not available in the package insert. "Since substitution of more completely absorbed formulations can then inadvertently result in substantially higher serum concentrations, the availability of theophylline formulations with incomplete absorption presents a potential hazard of theophylline treatment."[109]

Tolbutamide

Tolbutamide has been identified as a drug whose clinical efficacy may be compromised by poor bioavailability. Olson et al.[110] have demonstrated that two formulations of tolbutamide, bioequivalent when newly manufactured, change differentially under certain conditions of storage.

Tablets aged by exposure to 98% relative humidity for 3 days show a decrease in dissolution rate but the effect is much greater with a generic tolbutamide product than with Orinase, the innovator's product. Before aging, 93% of the dose of Orinase was dissolved in 10 min and 100% at 30 min, compared with corresponding values of 26% and 83% for the generic tablet. Exposure of the tablets to high relative humidity decreased tolbutamide dissolution at 10 min and 30 min to 47% and 95%, respectively, for Orinase, and to 8% and 24% for the generic product.

Differences were also observed when the aged tablets were given to healthy human subjects. A single 500-mg oral dose produced a peak concentration of 52.5 μg/ml when Orinase was given, compared with a peak of 38.4 μg/ml when the generic tablet was administered. Total AUC, on the other hand, was only 10% greater after Orinase. These kinetic differences were not sufficient to significantly influence glucose concentration response.

Olson et al. also demonstrated that two tolbutamide products may be bioequivalent in fasted subjects but not when given after a meal. They administered newly-obtained tablets of Orinase and a generic tolbutamide to healthy human subjects after a standard breakfast.[110] Peak concentration was about 20% larger after Orinase; the mean time to peak concentration occurred at 2.4 hr after Orinase and 4.1 hr after the generic product. This delay in absorption resulted in a small but signif-

icant difference in glucose response at 36 min after administration, but not before or after.

The findings with tolbutamide are of interest to regulatory agencies in their effort to develop standards for demonstrating bioequivalence between products. Given the relative safety of tolbutamide, however, the changes described are of little clinical significance.

NONORAL MEDICATION

Almost all bioavailability studies reported to date have concerned oral products. This emphasis should not be construed to mean that bioavailability is not of concern with other kinds of dosage forms.

Intravenous injections, ordinarily, are free of bioavailability problems. This is not true when the injected material is a prodrug that must be hydrolyzed to parent drug. The availability of chloramphenicol after intravenous injection of chloramphenicol succinate varies considerably as a function of the patient's ability to hydrolyze the ester prodrug.

Rejection episodes in transplant patients are often treated with high doses of steroids. The aqueous solubility of prednisolone, however, is low. Intravenous injection requires the use of a soluble prodrug of prednisolone. In Switzerland, prednisolone is given intravenously as prednisolone disodium phosphate or as prednisolone sodium tetrahydrophthalate.

Patients treated for acute rejection were given on three occasions oral prednisone, iv prednisolone phosphate, and iv prednisolone phthalate, in equimolar doses.[111] Oral prednisone is biotransformed in the liver to prednisolone, the active agent. In all patients, the hydrolysis of the phosphate ester was faster than that of the phthalate ester. Mean peak concentrations of prednisolone were 18.5 μg/ml after prednisolone phosphate, 2.9 μg/ml after the phthalate, and 3.1 μg/ml after oral prednisone. Assigning a value of 100% to the AUC of prednisolone following administration of the phosphate ester, relative bioavailability was 52% for the phthalate ester and 68% for oral prednisone. The investigators concluded that "therapeutic inequivalence must be expected whenever patients are treated with equimolar doses of these three prodrugs."

Intramuscular injections of suspended material or solutions that precipitate at the injection site can also present bioavailability problems. Patients stabilized on oral phenytoin often require larger doses, at least for a period of time, when switched to the intramuscular preparation, because of the slow dissolution and absorption of crystalline phenytoin from the muscle depot.

Drug availability from rectal suppositories may be incomplete if release from the dosage form is slower than the retention time of the product. This problem has resulted in a dramatic decline in the use of theophylline suppositories.

The bioavailability of tamoxifen from rectal suppositories containing 40 mg of the drug was compared with that of oral tablets containing 20 mg in healthy male subjects.[112] Tamoxifen is widely used in the management of breast cancer. The tablets were taken with water; the suppositories were inserted after evacuation of the bowel. No defecation occurred within 6 hours after administration of a suppository.

The mean relative bioavailability from the suppositories was only 28%; the addition of a surface-active agent reduced bioavailability to 13%. The investigators concluded that rectal administration of tamoxifen leads to lower bioavailability than that found after oral administration and therefore cannot be recommended. This study, as well as others, demonstrates not only that the bioavailability of rectal tamoxifen is less than that of oral tamoxifen, but that important differences may be seen using different rectal preparations of the same drug.

Bioavailability of Topical Medication

How does one measure the bioavailability of a drug in a topical preparation? The literature concerned with this question was reviewed by Guy et al.[113] in 1986. Some investigators have applied conventional bioavailability methods and determined drug levels in plasma or urine. Usually, however, drug levels are so low that radiolabeled material is needed. Other investigators have concentrated on measuring the loss of drug from the site of application and/or the amount of drug that has penetrated the skin, using solvent washes or skin stripping with cellophane tape. Ordinarily, these methods also require labeled drug. Still others have relied on in vitro methods, measuring drug release from the ointment base into a reservoir or into or across excised animal or human skin.

For the evaluation of topical glucocorticoid preparations, most investigators have favored the so-called vasoconstrictor assay developed more than 25 years ago.[114] Application of corticosteroids to normal intact human skin results in vasoconstric-

tion and blanching. The degree of vasoconstriction is assumed to be related to the potency of the drug; blanching is rated using a 4-point scale ranging from 0 (no vasoconstriction) to 3 (severe vasoconstriction).

Several clinical studies have generally confirmed the value of the vasoconstrictor assay, but the degree of blanching for any given product may vary widely from one person to another. The most important validation study was reported in 1985.[115] These investigators demonstrated that in 20 of 23 different comparisons the results of the vasoconstriction assay correlated with the clinical assessment of the drug.

Among the successful correlations were the following: betamethasone dipropionate 0.05% in an optimized ointment vehicle was more effective in the blanching test and in the treatment of psoriasis than was betamethasone dipropionate 0.05% in a conventional vehicle; hydrocortisone valerate cream 0.2% was more effective than hydrocortisone cream 1% in both the vasoconstrictor assay and in the clinical study; there was no difference between 1% and 2.5% hydrocortisone cream in either vasoconstriction or clinical efficacy.

For the most part, the vasoconstrictor assay has been used to predict clinical potency during the development of a new drug. More recently, it has been used to evaluate the bioequivalence of products containing the same drug, at the same strength, but differing in vehicle and/or method of preparation.[116]

The results of these comparative bioavailability studies suggest potentially important differences in clinical effects between products that are assumed to be equivalent. For example, Kenalog cream 0.1% was more potent than 5 generic creams containing triamcinolone acetonide 0.1%, Aristocort A ointment 0.1% was more potent than 2 generic ointments containing triamcinolone acetonide 0.1%, and Valisone cream 0.1% was more potent than 5 generic creams containing betamethasone valerate 0.1%. Surprisingly, no difference in the degree of blanching was noted after application of Kenalog cream 0.025%, 0.1%, or 0.5%.

These differences in marketed products that are widely assumed to be equivalent are troubling. Some will point to the failure of generic medication, but differences between generic and brand-name topical steroid products cut both ways. Some investigators have demonstrated that certain generic products produce more vasoconstriction than the "equivalent" brand-name product.[117]

An authoritative medical newsletter has observed that "different formulations of the same topical corticosteroid in the same concentration may vary in their effect on the vasoconstrictor assay and possibly in treating disease." It also notes that "some brand-name formulations appear to be more potent than their generic counterparts, but generics may also be more potent than some brand-name products. Lower concentrations of some topical corticosteroid brands may have the same effect in vasoconstrictor assays as much higher concentration of the same product."[117]

Although the results of the vasoconstrictor assay are not synonymous with clinical efficacy, there is some relationship. It is clearly imprudent to switch a patient responding to one topical corticosteroid preparation to another product. The U.S. Food and Drug Administration is working on this problem, but it is essential that the issue be resolved in a timely manner because the lack of standardization surely undermines confidence in the drug approval process.

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