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*REVIEW ARTICLE*

# Pharmacokinetics and Molecular Modification: Implications in Drug Design and Evaluation

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**Keyphrases** ☐ Pharmacokinetics and molecular modifications—review of application to drug design, consideration of absorption, drug–receptor interactions, pH–partition effects, prodrugs, interpretation of parameters ☐ Drug design—implications of pharmacokinetics and molecular modification, review of absorption processes and kinetics, drug–receptor interactions, pH–partition effects, prodrugs, interpretation of parameters ☐ Absorption processes and kinetics—review of application to drug design ☐ Drug–receptor interactions—review of application to drug design ☐ Antibiotics (penicillins and tetracyclines)—molecular modifications, optimization of pharmacokinetic parameters, review of implications in drug design ☐ pH–partition effects—optimizing oral absorption by chemical modification, implications in drug design, review ☐ Prodrugs and bioavailability—interpretation of pharmacokinetic parameters, application to drug design, review

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## INTRODUCTION

This is a review (and in some cases an analysis) of selected pharmacokinetic studies on drugs with closely related chemical structures. It is not an attempt to survey all of the work that has been reported in the field of biopharmaceutics and pharmacokinetics, nor is it a summary of all studies involving alteration of drug absorption, distribution, and excretion through molecular modification. Several recent publications collectively serve that purpose, and these are cited within the text. This review is an attempt to stress the significance of pharmacokinetics in drug design and to illustrate the main points using examples selected from the literature.

The concept of pharmacokinetics and compartmental analysis is not a new one, although the level of sophistication and interest in this subject has rapidly increased during the last decade. As early as 1920, Widmark and Tanberg (1) and Widmark (2) studied the kinetics of drug elimination and drug plasma levels after multiple doses. Compartmental analysis was first introduced by Teorell (3, 4) in 1937 when he reported the kinetics of the distribution of substances in the

body. Marshall *et al.* (5, 6) made early contributions to what is now called pharmacokinetic analysis with their work on the elimination of sulfanilamide, as did Stewart *et al.* (7).

The first review of pharmacokinetics appeared in 1961 when Nelson (8) surveyed the literature on the kinetics of drug absorption, distribution, metabolism, and excretion. Also in 1961 Wagner (9) published a review of the fundamental principles involved in the absorption aspects of biopharmaceutics. A very fine text was written by Rescigno and Segré in 1961 covering the mathematical aspects of modeling and compartmental analysis. The English edition appeared in 1966 (10). Several texts are available (11, 12) which discuss the fundamentals of drug metabolism, disposition, and evaluation using pharmacokinetic principles, and guidelines have been published for the evaluation of bioavailability of drugs (13). The principles and methods used in biopharmaceutics and pharmacokinetics are the subjects of several texts (14–19) for the beginning and advanced student. Pharmacokinetics have been applied to the evaluation of oral timed-release drugs and used to determine the effects of dissolution rate, particle size, inert tablet ingredients, and urinary pH on the absorption and excretion of drugs. The pharmacokinetic parameters involved in drug–receptor interactions, binding to nonreceptor tissues, passive and active transport, metabolism, and excretion have been the subject of papers too numerous to list here.

**Absorption Aspects**—Although the use of compartmental analysis of drugs in the body is quite common and well publicized, relatively little use has been made of *a priori* considerations of pharmacokinetic parameters in drug design. Most of what has been reported applies primarily to GI absorption and may be described as attempts to:

1. Maximize the absorption rate by increasing the dissolution rate (as in micronization, salts of acids or bases, buffers, amorphous or metastable polymorphs, *etc.*).
2. Extend duration by decreasing the release rate from the dosage form (timed release, repository injections of slowly soluble salts, macrocrystals, free acid or base instead of salt, *etc.*).
3. Decrease loss to degradation in the stomach (acid-insoluble esters or salts, chemically stable derivatives, enteric coating, *etc.*).
4. Decrease loss due to complexation with foods (chemical modification, *etc.*).

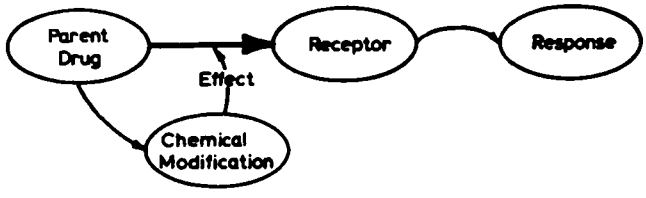
Although modification in the formulation (or of the dosage forms themselves) has received the most attention, many of the above alterations may be considered as chemical changes. While these modifications usually occurred in response to a particular problem with the parent compound, it is imperative that this aspect of drug design move (and no doubt it will) from an era of "corrective" research into one of "predictive" and "preventative" research. Obviously, the evaluation and thus the control of pharmacokinetic parameters must be introduced in the early phases of drug design to optimize all considerations that will become significant when the drug is in clinical use.

**Need for Pharmacokinetics in Drug Design**—Very few studies have been reported wherein the goal was to define substituent group effects on the total drug–organism interaction, including not only biological responses but also correlations between the observed pharmacological effects and the absorption, distribution, and excretion of the derivatives. Generally, examples illustrating such effects must be found in retrospect by examining classes of drugs where both pharmacokinetic studies and biological responses have been reported and then deducing, where possible, the connection between them. While this is not an easy task, the results do point up three important facts:

1. Pharmacokinetic parameters do influence biological response, and they are indeed critical in drug design.
2. Pharmacokinetic parameters can be modified by rather subtle structural changes.
3. There is a great need for basic studies in this field to arrive at the level of sophistication necessary to make *a priori* judgments practical.

The ultimate goal is to design a drug molecule having a desired pharmacological effect resulting from the proper balance of absorption, distribution, intrinsic activity, metabolism, and excretion without resorting to the costly and time-consuming process of screening large numbers of analogs.

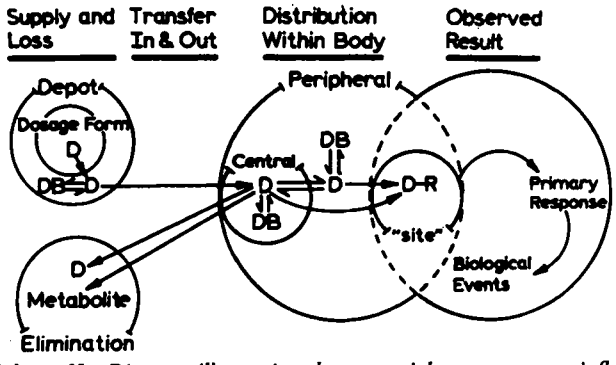
**Drug–Receptor Interactions**—It is common practice to consider the effects of substituent groups in a series of molecules upon the "drug–receptor" interaction (Scheme I). Typically, some assumptions are made




*Scheme I—Diagram of molecular modification effects at the drug–receptor interaction*

regarding the interaction between parent compound and receptor. Molecular modifications are made, and the basic assumptions are tested. Generally, abnormalities (unexpected results) are explained by modifying the concept of the receptor and occasionally even by modifying the concept of the drug structure, as in the case where the observed activity is explained by arguing for a particular conformation being the preferred one for that molecule *only* when it is in the vicinity of *that* receptor. In many cases, the conclusions are based upon dose–response curves and the dose is assumed to be responsible for the magnitude of the response. However, it is well recognized (albeit seldom evaluated) that the time course for a drug at the receptor must also be considered. The onset, duration, and intensity of effect may be considered as a function of at least two factors: (*a*) transport processes affecting the time course at the receptor site: delivery and removal from the site, absorption, distribution, excretion, metabolism, binding, *etc.*, and (*b*) interaction between drug and receptor after arrival at the site.

**Time Course for Drug–Receptor Interactions**—Scheme II illustrates how modification of a parent structure



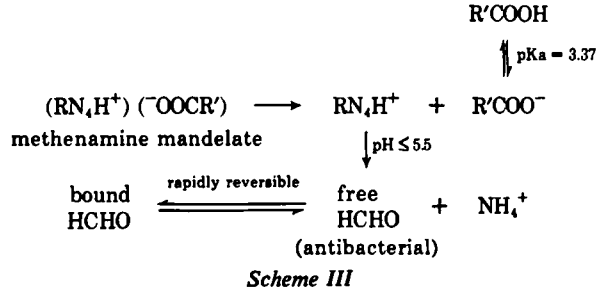

*Scheme II—Diagram illustrating the potential rate processes influencing the time course for a drug at the receptor. The drug–receptor interaction is indicated as* D–R *and dosage form is placed in depot. See text for further definition of symbols.*

can influence the drug available to the receptor site. The following processes in Scheme II can be altered by changing a substituent group on a drug (*D*):

1. Supply and loss
   (*a*) Release from dosage form (rate and/or amount)
   (*b*) Stability in depot
   (*c*) Binding in depot (*DB*)
   (*d*) Transfer from depot to central compartment (rate and/or amount)
   (*e*) Elimination rate from central compartment
2. Distribution
   (*a*) Binding in central compartment (*DB*)
   (*b*) Binding in peripheral compartment (*DB*)
   (*c*) Rate and volume of distribution
   (*d*) Transfer to receptor site
3. Drug–receptor interaction

An immediate problem challenging those who would consider and "optimize" all of these factors is physically locating the receptor site and defining an ideal time course for the drug–receptor interaction, *e.g.*, "hit and run," sustained effect, *etc.* Although quite arbitrary, it has been stated (14) that an *ideal drug* should: (*a*) reach the site of action, (*b*) arrive rapidly in sufficient quantity, (*c*) remain for a sufficient duration, (*d*) be excluded from other sites, and (*e*) be removed from the site when appropriate.

Consider a relatively simple problem and a well-known example: the design of a urinary tract anti-infective. For the simplest case, the site of infection will be regarded as the urinary tract itself. The example chosen is the prodrug, methenamine. In acid pH, methenamine is converted to formaldehyde, which acts as the antibacterial agent as illustrated in Scheme III. Tablets of methenamine are often enteric coated to prevent conversion to formaldehyde in the stomach




*Scheme III*

(20). Thus, absorption occurs from the intestine into the bloodstream where the pH is too alkaline for conversion in both cases. Methenamine is cleared intact in the urine where it becomes converted to formaldehyde if the pH is less than 5.5. The rate of conversion can be controlled by management of urinary pH.

This example is somewhat simplistic since the site of action has been limited to the urinary tract. Thus, the physiological location for delivery of the active agent can be defined and monitored. The time course for drug at the site can be determined by assay of urine samples for formaldehyde as a function of time. The goal of product design would thus be to achieve the optimum time course of formaldehyde concentration in the urine.

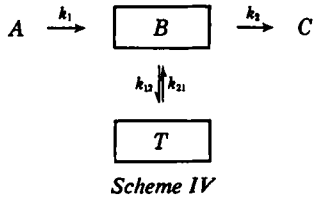
In general, the problem is a more difficult one because the site of action cannot be easily defined or sampled. Pharmacokinetic analysis presents a powerful tool for understanding the entire time course of a drug in the patient by kinetic modeling. The following discussions are intended to illustrate this premise as well as to suggest possible approaches in defining substituent effects.

## DEFINING PHARMACOKINETIC RESULTS OF MOLECULAR MODIFICATION

**Interpretation of Data**—Which parameters have changed? How have they changed? Why? These are the basic questions in the evaluation of substituent effects on pharmacokinetic behavior. However, the more significant question ultimately is: "Does this alteration in pharmacokinetic behavior improve the course of therapy with this drug?" It is difficult to envision a model system that will lend itself to simple assessments of these questions. Indeed, this is the challenge of future structure modification in drug design.

This review is limited to pharmacokinetic parameters associated with compartmental analysis. Several recent texts define the methodology involved in this approach (14–19). Although the terminology employed is fairly standard, the symbols vary somewhat throughout the literature. The most frequently encountered terminology is summarized in Table I, where typical symbols and their references are also listed. The symbols in Column 1, taken from *Reference 14*, are used in this review.

Scheme IV illustrates the model that corresponds to the symbols in Column 1, Table I. The meaning of this scheme is defined in Table I. This model is used to represent both one- and two-compartment systems. A one-compartment system is arbitrarily defined as one that immediately achieves equilibrium ratios of $T/B$ following a rapid intravenous dose. Graphically, this would result in a monoexponential first-order plot for concentration in *B versus* time. When distribution




*Scheme IV*

**Table I**—Survey of Symbols Commonly Used in Pharmacokinetic and Biopharmaceutical Literature (Those Taken from *Reference 14* Are Used throughout This Review)

| Description of Parameter and Its Units | References 14 | 15 | 21 | 16, 22 | 19 |
|---|---|---|---|---|---|
| First-order rate constant for absorption ($time^{-1}$) | $k_1$ | $K$ | $k_a$ | $k_a$ | $k_{ab}$ |
| First-order rate constant for elimination from central compartment[a] ($time^{-1}$) | $k_2$ | $K_2$ | $k_{el}$ | $k_{el}$ | $k_{10}$ |
| First-order rate constant for elimination from central compartment by excretion ($time^{-1}$) | $k_e$ | — | $k_e$ | $k_e$ | — |
| First-order rate constant for elimination from central compartment by metabolism ($time^{-1}$) | $k_m$ | — | $k_m$ | $k_m$ | — |
| First-order rate constant for transfer from central[a] to peripheral[b] compartment ($time^{-1}$) | $k_{12}$ | $K_1$ | $k_{12}$ | $k_{12}$ | $k_{12}$ |
| First-order rate constant for transfer from peripheral[b] to central compartment[a] ($time^{-1}$) | $k_{21}$ | $K_{-1}$ | $k_{21}$ | $k_{21}$ | $k_{21}$ |
| Apparent first-order rate constant for elimination from body ($time^{-1}$) | $\beta$ | $K$ | $\beta$ | $\beta$ | $\beta$ |
| Apparent first-order rate constant for elimination from body by excretion ($time^{-1}$) | $\beta_{ex}$ | $k_r$ | — | — | $k_{ex}$ |
| Apparent first-order rate constant for elimination from body by metabolism ($time^{-1}$) | $\beta_{met}$ | $k_{nr}$ | — | — | $k_{mX}$, $k_{mY}$, *etc.* |
| Apparent volume of distribution (volume) | $Vd$ | $V$ | $Vd$ | $Vd$ | $V$ |
| Apparent volume of central compartment[a] (volume) | $V_p$ | $V_1$ | $V_p$ | $V_c$ | $V_c$ |
| Concentration of drug in bloodstream or plasma (amount/volume, mg. %, *etc.*) | $P$ | $C$ | $Cp$ | $Cp$ | $D_t$ |
| Dose of drug in body (amount) | $D$ | $f \times D$ | — | $X^0$ | $D$ |
| Biological half-life for elimination from body (time) | $t_{1/2}$ | $t_{1/2}$ | $t_{1/2}$ | $t_{1/2}$ | $t_{1/2}$ |
| Renal clearance (ml./min.) | $C$ | $Cl_d$ | — | — | $Cl_r$ |
| Site of administration of drug | $A$ | — | $D$ | — | — |
| Central compartment[a] | $B$ | $C_1$ | $P$ | $C$ | 1 |
| Compartment accounting for total elimination[b] | $C$ | — | $ME$ | — | — |
| Peripheral compartment[c] | $T$ | $C_2$ | $T$ | $T$ | 2 |

[a] The central compartment is often referred to as the blood or plasma although it is recognized that it can in fact be larger in volume (23). [b] See Scheme IV. [c] The peripheral compartment is often referred to as the tissue compartment.

between $T$ and $B$ is slow relative to elimination, Scheme IV represents a two-compartment model. In practice, this is evidenced by a biexponential first-order plot for drug in compartment $B$. In either case the biological half-life is defined as:

$$t_{1/2} = 0.693/\beta \qquad \text{(Eq. 1)}$$

where $\beta$ represents the negative value of the slope of the first-order plot for a one-compartment model or the slope of the terminal portion of the plot for a two-compartment model.

**Table II**—Reported (or Calculated) Values for Selected Pharmacokinetic Parameters[a] for Several Penicillins

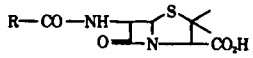
| Penicillin | $t_{1/2}$, hr. | $Vd$, l. | $C$, ml./min.[b] |
|---|---|---|---|
| Ampicillin | 1.0 (41), 0.8 (42), 1.2 (43) | 22 (41), 20[c] (42), 43 (43), 25 (49)[d], 30 (50) | 283 (41), 210 (49)[e], 312 (50) |
| Carbenicillin | 1.0 (42) | 10[c] (42) | 86 (42) |
| Cloxacillin | 0.42 (44), 0.6 (42) | 10[c] (44), 11 (42), 23 (51) | 162 (44), 287 (50) |
| Dicloxacillin | 0.88 (41), 0.71 (44), 0.7 (42) | 13 (41), 10[c] (44), 9.4 (42), 16 (50) | 88 (41), 162 (44), 130 (50) |
| Methicillin | 0.43 (41) | 22 (41) | 350 (41) |
| Nafcillin | 0.55 (45), 1.2 (43) | 21[c] (45), 57 (43) | 160 (45)[e] |
| Oxacillin | 0.70 (41), 0.38 (44), 0.40 (45) | 27 (41), 14[c] (44), 13 (42), 15[c] (45), 26 (50) | 190 (45)[e], 402 (50) |
| Penicillin G | 0.70 (41), 0.5 (42), 0.84–0.93 (46)[f], 0.6–0.99 (47)[g], 0.54 (24), 0.65[h], 0.78 (48) | 26 (41), 22[c] (42), 37–37 (46)[f], 35 (24) | 433 (41), 386 (49)[e], 340–480 (46)[f], 377[h] |
| Phenethicillin | 0.77 (48) | Appears to be $0.6 \times Vd$ of penicillin G (48) | — |
| Phenoxymethyl penicillin | 0.53 (24), 0.43[h], 0.52 (48) | 51 (24), 54[h] | 393[h] |

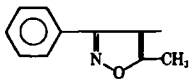
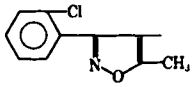
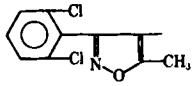
[a] The reference for each value listed is given in parenthesis. [b] Values for renal clearance. See Table I for symbols. [c] Calculated from $Vd = k_a/\beta P_o$ (14, 40) using the 3-hr. constant-infusion data reported in the reference cited. [d] Average value calculated from $P_o$ data using all three $t_{1/2}$ estimates in Column 1 of this table. [e] Units are ml./min./1.73 m.$^2$. [f] Variation due to ambulatory *versus* bed rest. [g] Variation attributed to size of dose. [h] Calculated from data in *Reference 51*.

**Optimizing Parameters: Antibiotics**—Methods for assessing structural effects on pharmacokinetic parameters are illustrated using literature data for penicillin derivatives. As stated earlier, a more basic concern is that of tailoring pharmacokinetic parameters for optimum therapeutic response. It has been emphasized, for example, that steady-state levels of carbenicillin are twice that of ampicillin. These high blood levels of carbenicillin following intravenous administration have been said to be a primary factor for its efficacy in treatment of relatively resistant infections such as pseudomonas (42). However, the reasons for the observed differences in blood levels must be determined to compare the drugs rationally. It is demonstrated in the following section that the reason for this difference is the larger volume of distribution for ampicillin since the elimination rate constants are similar.

If all other factors were equal, it might be argued that an increased value for $Vd$ is a clinical advantage. Spitzy and Hitzenberger (24) stated that "bacteria germinate more frequently in the tissues than in the blood," while Pratt (25) stated that "bacteria are more common in other tissues than blood." Several other authors stressed the importance of tissue concentrations (26–28), and Fabre *et al.* (29) stated that an antibiotic's effectiveness depends upon its penetration into tissues, particularly inflamed tissues. Thus, if plasma protein binding is equal, an antibiotic derivative with a larger $Vd$ would appear to be reaching the site of action with better efficiency. Although this is by no means unequivocal, the spectrum of research activity in antibiotic derivatives would imply that the following goals for

Table III—Isoxazolyl Penicillins



| Penicillin | Structure, R | $P_{ss}$, mg./l.[a] | $C$, ml./min.[a] | $Vd$, l.[b] | $\beta$, hr.$^{-1}$[b] | $\beta_{ex}$, hr.$^{-1}$[b] | $\beta_{met}$, hr.$^{-1}$[b] |
|---|---|---|---|---|---|---|---|
| Oxacillin |  | 9.7 | 226 | 14 | 1.83 | 1.02 | 0.81 |
| Cloxacillin |  | 15 | 162 | 10 | 1.65 | 1.02 | 0.63 |
| Dicloxacillin |  | 25 | 113 | 10 | 0.98 | 0.72 | 0.26 |

[a] Taken from *Reference 44.* [b] Calculated from *Reference 44.*

molecular modification are generally pursued: (*a*) increased distribution to tissues; (*b*) increased $t_{1/2}$ to maintain higher postdistribution body levels and to decrease the dosage frequency; (*c*) decreased binding capacity to foods and also to plasma protein; (*d*) increased oral absorption through increased solubility, dissolution rate, GI stability, and partition coefficient; and (*e*) decreased minimum inhibitory concentration (MIC) with resultant decrease in the required dose.

It is interesting (and perhaps amusing) to note that these goals often appear more obvious in promotional literature for antibiotic derivatives than in the scientific literature itself. While this list is not all inclusive, it can serve as the basis for a pharmacokinetic comparison.

*Penicillins*—Antibiotics probably represent the principal group wherein minor molecular modifications have been made on parent molecules with resultant alterations in pharmacokinetic parameters. Since penicillin marked the beginning of antibiotic chemotherapy, there have been a great number of studies on these derivatives. Schwartz and Buckwalter (30) discussed the relative gastric stability of penicillins as the primary factor in determining bioavailability. Hou and Poole (28) reviewed the relationship of penicillin structure to biological activity. Structures as well as typical MIC values for the penicillins discussed here may be found in their excellent review. A number of other reviews on penicillins and cephalosporins are also available (31–39).

The reported values for three pharmacokinetic parameters ($t_{1/2}$, $Vd$, and $C$) are summarized in Table II together with their reference sources. The $t_{1/2}$ values, reported by the various investigators for a given penicillin, appear to be in good agreement. Wagner (15) suggested that the values for the $t_{1/2}$ of a drug would be expected to be distributed normally or log-normally within a total population of subjects. Somewhat surprising, perhaps, is the observation that generally all of the penicillins in Table II have half-lives in the rather narrow range of 0.5–1 hr.

There appears to be a much greater variation in the reported values for the $Vd$ of a given penicillin. Biological or subject-to-subject variation surely accounts for some of these observed differences. This is especially true where absolute values are compared, as they are in Table II where the distribution volumes are given in liters. It is probably more rational to state these on a relative basis such as percent of body weight or milliliters per gram, as illustrated in *References 24* and *47*. Levy (46) illustrated that the $Vd$ of penicillin G is increased during bed rest (47 l.) compared to that of ambulatory patients (37 l.). The rate constant for metabolism of penicillin G, $\beta_{met}$, was also significantly decreased ($p < 0.05$) during bed rest. Studies comparing drug derivatives must be designed to control factors such as biological variation and patient activity for a meaningful experimental design.

There is a more basic problem in evaluating the $Vd$ of a drug. If a drug is truly distributed according to a one-compartment open model, the calculated value for $Vd$ may be expected to be independent of the method used to determine it (52) since equilibrium ratios of $T/P$ will be achieved. However, if the drug is distributed according to a multicompartment model, the value for $Vd$ is dependent upon the kinetics. For example, the value obtained by the extrapolation method will not agree with that obtained by the steady-state method (52). The influence of the elimination rate constant, $k_2$, on the calculated value for $Vd$ was illustrated by Gibaldi and Perrier (53) and Jusko and Gibaldi (54). By using constant intravenous infusion to obtain a steady-state plasma level, the $T$ and $B$ compartments (Scheme IV) can be brought to equilibrium. The steady-state distribution volume is then defined as:

$$Vd_{ss} = \frac{k_{12} + k_{21}}{k_{21}} Vp \qquad \text{(Eq. 2)}$$

which is independent of $k_2$. This offers one approach to comparing distribution of analogs using a method that should produce more reliable results than one that is influenced by elimination.

The isoxazolyl penicillins represent a group of three closely related penicillins with varied pharmacokinetic behavior (Table III). Their structural differences are limited to the number of chlorine atoms. Early studies attributed higher dicloxacillin serum levels to increased oral absorption. The ratios of serum levels following oral administration in one study were roughly 2:1 for dicloxacillin–cloxacillin (55). In another study, similar ratios of 2.3:1.4:1 for dicloxacillin–cloxacillin–oxacillin were obtained for both the oral and intravenous routes

of administration (50). This precludes the possibility of absorption being the sole factor for different plasma profiles. The percent absorption was also calculated from:

$$\% \text{ absorption} = 100\% \, (\text{area}_{\text{oral}}/\text{area}_{\text{i.v.}}) \qquad \text{(Eq. 3)}$$

using the areas under the curves. Some improvement in absorption with chlorine substituents was detected; 80% of dicloxacillin, 77% of cloxacillin, and 67% of oxacillin were absorbed. However, the differences are not sufficient to explain the dramatic contrast in plasma levels following oral administration. Such curves cannot be compared directly. In spite of the seemingly minor structural differences in the isoxazolyl penicillins, each must be regarded pharmacokinetically as a different drug. Dittert *et al.* (41) demonstrated that penicillins are distributed according to a two-compartment open model as represented by Scheme IV. Therefore, the values for all four constants ($k_1$, $k_{12}$, $k_{21}$, and $k_2$) may change with the addition of chlorine atoms, and their individual contributions to the time course must therefore be considered.

The data of Rosenblatt *et al.* (44) provide a means for illustrating how pharmacokinetic substituent effects might be assessed. The steady-state plasma levels following 250 mg./hr. intravenous infusions, as reported in their studies, are given in Table III. The ratio of these steady-state levels is quite similar to the previously discussed results; the dicloxacillin–cloxacillin–oxacillin ratio is 2.6:1.7:1. Why are these plasma levels different? There are three parameters that may be examined: $Vd$, $\beta_{ex}$, and $\beta_{met}$. The values for $Vd$ have been calculated from the equation (14, 40):

$$Vd = k_0/\beta P_{ss} \qquad \text{(Eq. 4)}$$

and results are given in Table III. Now it can be concluded that the dicloxacillin–cloxacillin steady-state plasma ratio of 1.7 cannot be attributed to differences in distribution since the $Vd$ values are the same. The values for $\beta$ have been calculated using Eq. 1. It can now be concluded from the values for $P_{ss}$, $Vd$, and $\beta$ that plasma level differences and their reasons are: dicloxacillin > cloxacillin due to decreased elimination; cloxacillin > oxacillin due to decreased $Vd$; dicloxacillin > oxacillin due to both decreased elimination and $Vd$.

It is generally agreed that extending the duration of penicillin blood levels represents a clinical advantage, especially since these antibiotics have such short $t_{1/2}$ values (42, 44). Dicloxacillin has the longest $t_{1/2}$ (smallest $\beta$) of the isoxazolyl penicillins in Table III. The addition of two chlorine atoms to oxacillin has thus extended its duration. But why has this occurred? It is necessary to determine the mechanism responsible for the change if one is to develop a rational approach to further molecular modification.

The total elimination constant, $\beta$, can be divided into renal, $\beta_{ex}$, and nonrenal, $\beta_{met}$, components using the data for the fraction excreted in the urine (44). The value for $\beta_{met}$ appears to decrease with the addition of chlorine atoms to oxacillin (Table III). A similar trend can be observed with the values for renal clearance. Both renal and nonrenal excretion decrease with increasing lipophilicity. Nayler (56) suggested that active transport systems for organic anions in the kidney tubules and the liver represent the most important pathways for elimination of penicillins. Since both tubular secretion and inactivation involve passage across lipid membranes, there should be an optimum partition coefficient (56). The isoxazolyl penicillins have the most hydrophobic side chains of all penicillins in clinical use and presumably have partition coefficients in excess of the optimum (56). These observations are somewhat supported by the fact that penicillin G has a renal clearance value near 400 ml./min., which approaches the renal plasma flow rate of 650 ml./min. Addition of chlorine atoms to the already lipophilic oxacillin further depresses the clearance value from 226 to 113, which is roughly the value for glomerular filtration (14).

The decrease in metabolism of cloxacillin as compared to oxacillin might have been overlooked if a comparison of $t_{1/2}$ (or $\beta$) were made. The single chlorine atom appears to have no effect on excretion when $t_{1/2}$ is used as the sole criterion. Yet the ratio of renal clearance values is 226:162, indicating that oxacillin is cleared 1.4 times faster than cloxacillin by the kidney. The $t_{1/2}$ values are the same, since this parameter represents the time to remove half of the drug from the entire body, and the ratio of the $Vd$ of oxacillin to cloxacillin is 14:10 or 1.4 times larger so that the difference in renal clearance is offset by the increase in $Vd$. From a structural point of view, the additional chlorine atom has markedly depressed renal clearance.

Similar analyses can be extended to other penicillins. Differences in steady-state plasma levels of related penicillins are frequently attributed solely to their rates of elimination (42, 44, 49, 56). Table IV summarizes the observed steady-state plasma levels following constant intravenous infusion of 500 mg./hr. The values for the elimination rate constant, $\beta$, are also given, together with the predicted plasma level rank order if only elimination is considered. Since infusion rate is constant, the lowest elimination rate would result in the highest plasma level, *etc.* A similar ranking is given using only the $Vd$ as a criterion. Here it is assumed that larger volumes will give more dilute blood samples and thus lower levels. Neither of these approaches is sufficient to account for the observed ranking. A few simple comparisons can be made. For example, the rank order of carbenicillin > dicloxacillin > cloxacillin can be attributed solely to elimination differences. Conversely, cloxacillin levels are higher than oxacillin due to distribution. The correct ranking for all penicillins in Table IV can be obtained by calculating values for $P_{ss}$ using Eq. 4. Results are in good agreement with observed values, illustrating that both $Vd$ and $\beta$ must be considered to compare blood levels of analogs.

One problem that continues to confuse the issue when comparing various antibiotic derivatives is their difference in protein binding. The binding of drugs to plasma protein was discussed in an excellent review by Meyer and Guttman (57) and will not be repeated here. It should be emphasized, however, that the significance of protein binding (as well as tissue binding) in relation to therapeutic efficacy remains unclear for

Table IV—Observed and Predicted Rank Orders (Highest to Lowest) for Plasma Steady-State Concentration of Various Penicillins during Constant Intravenous Infusion of 500 mg./hr. (See Text for Explanation)

| Penicillin | $\beta$, hr.$^{-1}$ | Rank | *Vd*, l. | Rank | $P_{ss}$, mg./l. Calc.[a] | $P_{ss}$, mg./l. Obs. | Rank | Reference |
|---|---|---|---|---|---|---|---|---|
| Carbenicillin | 0.69 | 1 | 10 | 1 | 72 | 73 | 1 | 42 |
| Dicloxacillin | 0.98 | 3 | 10 | 1 | 51 | 51 | 2 | 44[b] |
| Cloxacillin | 1.65 | 6 | 10 | 1 | 30 | 30 | 3 | 44[b] |
| Ampicillin | 0.71 | 2 | 25 | 4 | 28 | 29 | 3 | 49 |
| Oxacillin | 1.78[c] | 7 | 14[c] | 2 | 19 | 19 | 4 | 44,45[b] |
| Nafcillin | 1.26 | 5 | 21 | 3 | 19 | 18 | 4 | 45 |
| Penicillin G | 1.13 | 4 | 24[d] | 4 | 18 | 16 | 5 | 49 |

[a] $P_{ss}$ = (500 mg./hr.) ÷ ($\beta$)(*Vd*). [b] Observed $P_{ss}$ × 2 to correct for infusion rate of 250 mg./hr. [c] Average value from *References 44* and *45*. [d] Average value from *References 41* and *42*.

many antibiotics. Warren (27), in his thorough review of that subject, points out that readily reversible binding to serum or tissues can serve as a pool of active antibiotic to prolong therapeutic levels. In contrast, several authors who hold the position that penicillins bound to proteins lose their activity are cited in that review. The ultimate criteria in comparing serum levels of derivatives must always be based on how much is required to treat the disease in question. A derivative producing a plasma level twice as high as the original drug is insignificant if 10 times the original is required. Warren contended that absorption, distribution, and inactivation may be more important than binding in determining penicillin therapy, presumably due to reversibility.

Kunin (58) demonstrated that the MIC of eight penicillins in human serum is the same as that in broth when corrected for the bound fraction. That report stresses a decrease in bioavailability of penicillins by inactivation through protein binding. Methicillin, the least active in broth, not only required the smallest concentration to kill more than 99% of inoculum in serum but it was also more rapid than any other penicillin. A decrease in distribution of penicillins to tissues was also predicted as an expected result of the inability of bound penicillin to diffuse from the plasma. The work of Dittert *et al.* (41) and Doluisio *et al.* (59) does not support the latter hypothesis, since similar values for *Vd* were obtained for four penicillins whose percent binding in plasma varied from 22 to 94 (58). The fraction of the dose in the peripheral compartment was considered similar for all five penicillins studied (41, 59). One report suggests that the antibacterial activity of three long-acting sulfonamides is independent of their protein binding but that this binding determines their duration (60).

While the significance of protein binding of penicillins is still in dispute, there appears to be some agreement that reversibility of bound drug is the key factor. It has been noted and documented by authors with seemingly opposing views that reversible binding can serve to prolong drug action (27, 58) whereas only irreversible binding removes drug from the biophase. It has been suggested that plasma protein binding will not affect the $t_{1/2}$ if a drug is cleared by secretion in kidney tubules, but increased binding may prolong the $t_{1/2}$ of drug excreted solely by glomerular filtration (61). It would appear, from present evidence, that the significance is probably dependent upon the strength of binding. It was previously calculated that protein binding will affect drug distribution only if the binding constant exceeds $10^4$ (57).

*Tetracyclines*—Development of tetracycline analogs may be considered as illustrative of research aimed at achieving the goals listed previously under *Optimizing Parameters*. It became apparent soon after the introduction of chlortetracycline in 1948 that concurrent administration of aluminum hydroxide gel resulted in decreased bacteriological activity for the antibiotic (62). Decreased GI absorption due to complexation with divalent and trivalent cations such as calcium, aluminum, and magnesium precluded coadministration with milk or antacids to reduce the known potential for anorexia, nausea, vomiting, *etc.*, observed during tetracycline therapy. Development of tetracyclines with a decreased propensity for complexation thus became a clinically significant research goal. Some measure of success has been realized with the newer tetracyclines. Demethylchlortetracycline (demeclocycline) blood levels, for example, are extremely sensitive to milk and aluminum hydroxide gel (63). By using those data, it was calculated that, relative to equal doses in the fasting state, only 13% oral absorption takes place when the antibiotic is administered with 240 ml. (8 oz.) of milk and 22% oral absorption takes place with 20 ml. of aluminum hydroxide gel (14). The data of Rosenblatt *et al.* (64) can be treated in a similar fashion, with the results showing that doxycycline absorption with food and milk is reduced to about 50% whereas demethylchlortetracycline is absorbed about 25% when both are compared to the fasting state during an 8-hr. period following ingestion of 100 and 300 mg., respectively. This comparison may reflect initial absorption since elimination was not completed. Ingestion of antacids containing divalent and trivalent cations resulted in negligible absorption for both drugs.

A similar result, although more pronounced, was noted by Schach von Wittenau and Twomey (65) in their discussion of unpublished work. When doxycycline was administered orally to humans together with aluminum hydroxide gel, the observed plasma concentrations (at an unspecified time) were reduced to 10% of normal. In another report, doxycycline plasma levels following administration with milk were not notably different from those in either the fasting state or when administered with food (66). However, comparisons were made only at 1, 3, and 12 hr. on Day

1 and at 0 and 12 hr. on Day 5 of a twice-a-day regimen. Thus, insufficient sampling could mask differences in absorption. Adequate data should include several values before and after the plasma maximum so that areas under the curves could be compared to establish relative bioavailability in each case.

The extension of biological $t_{1/2}$ to increase duration and decrease frequency of administration was reviewed by Fabre *et al.* (67) as follows. Chlortetracycline first appeared in 1948 with $t_{1/2}$ of 5.6 hr. and a dosage interval of 6 hr. Oxytetracycline and tetracycline soon followed with half-lives of 8.2 and 9.2 hr., respectively, and thus slightly longer durations. In 1958, demethylchlortetracycline ($t_{1/2}$ of 11.8 hr.) and later methacycline were introduced as useful on a 12-hr. regimen. Doxycycline ($t_{1/2}$ of 18–22 hr.) marked a new stage in this evolution with the advent of once-a-day therapy.

This discussion was published in 1967 and was most encouraging to those interested in improvement of tetracyclines by molecular modification. However, the $t_{1/2}$ values employed for the discussion do not appear to be unequivocal. Doluisio and Dittert (68) redetermined the $t_{1/2}$ values for several tetracyclines and compared their results with those previously reported (Table V). Comparison of the $t_{1/2}$ values following a single oral dose to those determined during steady-state plasma levels within the same study indicates that the $t_{1/2}$ of each of the four antibiotics increased significantly during the 6-day study. It is also apparent that the $t_{1/2}$ values for the four tetracyclines do not vary as much as indicated in the earlier review (67). The range of the four $t_{1/2}$ values in the steady state, for example, is only 14 ± 3 hr. Therapy with an antibiotic drug involves the administration of multiple doses and not just a single dose. The values obtained during multiple dosing would therefore appear to be more significant in clinical use of the drugs. Doluisio and Dittert demonstrated that $t_{1/2}$ values from single-dose studies do not predict steady-state serum levels of tetracyclines during multiple dosing whereas the steady-state $t_{1/2}$ values adequately describe their data.

Many factors can alter the $t_{1/2}$ value for a given drug and these were reviewed elsewhere (69, 70). The simplest equation that defines the value for $\beta$ in a manner independent of the kinetic model may be written:

$$\beta = f_c k_2 \qquad \text{(Eq. 5)}$$

where $f_c$ is the fraction of drug in the central compartment under postdistributive conditions (71). The $t_{1/2}$ value for a drug distributed according to a one-compartment model would be expected to be independent of the mode of administration since $f_c$ is constant and equal to $1/(1 + K)$, so that:

$$t_{1/2} = 0.693/\beta = 0.693(K + 1)/k_2 \qquad \text{(Eq. 6)}$$

where:

$$K = k_{12}/k_{21} = T/P \qquad \text{(Eq. 7)}$$

Conversely, a two-compartment drug does not achieve preequilibrium following rapid intravenous injection and its $t_{1/2}$ cannot, therefore, be defined by a simple equation employing the equilibrium constant, $K$. In this case the ratio of drug in the tissues to that in the central compartment during the $\beta$-phase is larger than that predicted by assuming the equilibrium condition described in Eq. 7 (14). Under steady-state conditions imposed during constant-rate intravenous infusion, the tissue–plasma ratio again becomes defined by Eq. 7. Thus the fraction, $f_c$, can be shown to be larger during steady-state conditions than it is during the $\beta$-phase of a single intravenous dose of drug whose distribution is not instantaneous (71). Gibaldi (72) estimated that the $T/P$ ratio for a single dose of oxacillin would be about twice that achieved during infusion equilibrium. Since $\beta$ is directly proportional to $f_c$ (Eq. 5), the value for $\beta_{ss}$ would be larger than the value obtained from a single dose of a two-compartment drug. During steady-state plasma levels, the value of $f_c$ becomes constant and so does $t_{1/2_{ss}}$, which is then defined by Eq. 6. Thus, the $t_{1/2}$ that is operative during the steady-state would be expected to be shorter than that observed following a single dose. Therefore, the increased values for $t_{1/2}$ observed during continuous dosing (*Reference 68* also cites two similar previous reports for doxycycline) cannot be explained from the standpoint of approaching steady-state equilibrium.

Table V—Literature Values for Apparent Biological Half-Lives (in Hours) for Tetracyclines[a]

| Drug | Single Dose | Repeated Dosing: Days 5–6 | Steady State |
|---|---|---|---|
| Tetracycline | 6.3[b], 5.6–9.3, 8.2, 8, 7.2, 7.3 | 10[b], 9.5, 11 | 10.8[b] |
| Demethylchlortetracycline (Demeclocycline) | 9.0[b], 6.3–13.3, 12.6, 10, 11, 12.7 | 14.7[b], 14.7, 15 | 13.6[b] |
| Methacycline | 7.0[b], 14.3, 8.5, 9.6, 7.7 | 11.0[b], 10.5, 11.5 | 14.3[b] |
| Doxycycline | 8.3[b], 11.7, 15, 15.1 | 14.5[b], 22[c] | 16.6[b] |

[a] Taken from *Reference 68.* [b] Determined in *Reference 68.* [c] This value appears to be incorrect since predicted steady-state serum levels using $t_{1/2}$ = 22 hr. do not agree with experimentally observed values.

It has been suggested that the increased $t_{1/2}$ values might arise from a failure to obtain an accurate assessment of the terminal (or $\beta$) slope following a single oral dose (73). Inclusion of part of the distribution phase could tend to make the $t_{1/2}$ appear smaller with a single dose whereas this problem is eliminated during the steady state. This behavior was illustrated using a hypothetical three-compartment model (73). However, it would also apply to a two-compartment case which might be appropriate for assessing tetracyclines (74).

A significant observation is the fact that all four tetracyclines have reasonably similar steady-state $t_{1/2}$ values (Table V). Since the sizes of the doses were varied, it was observed that all four drugs produced comparable serum levels when administered every 12 hr. This is easily understood in terms of what may now be considered classical approaches to dosage regimen development. Schumacher (75) reviewed the methodology employed in calculating a dosage regimen in an applied paper that illustrates the use of the techniques on tetracyclines and penicillins. Five approaches are outlined, one of which may be described as using a fixed dosage interval equal to the $t_{1/2}$ and varying the dosage size to achieve the desired plasma concentrations during the steady state. In the study under consideration (68), the doses of the analogs were varied and the dosage

Table VI—Comparison of Reported Plasma Levels for Tetracyclines

| | Dose[a], mg. | $P_{max}$[a], mcg./ml. | Dosage Regimen[b], mg./Δhr. | $P$[c,d], mcg./ml. Max.[c,d] | Min.[c,d] | Max.[e,f] | Max.[e,g] |
|---|---|---|---|---|---|---|---|
| Tetracycline | 500 | 3.25[h] | 500/12 | 3.5 | 1.9 | 4.0 | 1.9 |
| Demethylchlortetracycline (Demeclocycline) | 300 | 1.74[h] | 300/12 | 2.8 | 1.5 | 2.7 | 1.2 |
| Methacycline | 300 | 2.40[h] | 300/12 | 2.2 | 1.2 | 2.3 | 0.7 |
| Doxycycline | 150 | 1.49[i] | 100/12 (Day 1) then 50/12 | 2.1 | 1.2 | 2.51 | 1.5 |

[a] Taken from *Reference 77*. [b] Administered during a 4-day period. [c] Taken from *Reference 68*. [d] Steady-state levels. [e] Taken from *Reference 76*. [f] Determined at peak. [g] Highest value obtained during first 12 hr. [h] Determined 4 hr. after single dose. May not be true maximum. [i] First determination at 2 hr. is highest.

interval was held constant at 12 hr. It was fortuitous that this interval also approximated the values determined for the steady-state half-lives. Thus, the regimen may be considered as one of a fixed interval equal to the $t_{1/2}$ with variable dose. Statistical analysis showed that levels of tetracycline were higher than doxycycline and methacycline but similar to demethylchlortetracycline after 4 days of therapy. The authors concluded that all four drugs produced comparable levels of antibiotic activity when administered every 12 hr. in their recommended doses.

A similar protocol was employed in a comparison of plasma concentrations which did not employ any pharmacokinetic analyses. The maximum observed plasma values in that study (76) are in excellent agreement with those previously reported (68). However, the maximum observed values obtained during the first 12 hr. appear to be lower than those reported for a single oral dose (77), except for the case of doxycycline which gave equal maximum plasma levels despite a decreased dose (Table VI). Minocycline was reported to yield higher plasma levels than the other tetracyclines (77), but the fact that the fraction of the dose recovered in the urine is smaller than the others attests to the necessity of examining the effect of nonabsorption factors when comparing plasma levels of different chemical entities.

Considerable effort has been applied to examining the distribution of various tetracyclines into body tissues (78–83). The ratio of total tetracycline in muscle to free concentration in serum in dogs has been correlated with chloroform–water partition coefficients (78, 79). Doxycycline had both the highest distribution and partition coefficient of the marketed tetracyclines. The order of values of the tetracyclines studied was demethyldeoxychlortetracycline ≫ doxycycline ≫ tetracycline > demethylchlortetracycline = methacycline ≫ oxytetracycline. Concentrations of tetracycline, demethylchlortetracycline, and chlortetracycline were compared in no less than 47 organs, tissues, or fluids in dogs using radiolabeled compounds (80). Results indicate that all nonfat tissues are penetrated by the antibiotics within 4.5 hr. after intravenous injection, with tissue distribution of tetracycline being generally less than that of chlortetracycline and demethylchlortetracycline. Increased lipophilicity of doxycycline allegedly increased oral absorption (66). Computer analysis showed an absorption $t_{1/2}$ of 28 min., with 80% of the administered dose absorbed within 1 hr.

Doxycycline content in 12 organs sampled operatively in 81 patients was determined with concomitant serum levels, and the tissue to serum ratio was determined for each organ (81). These ratios varied from approximately 2 for kidneys, lungs, and bladder to less than 0.7 for appendix and adipose tissue. When corrected for binding, these ratios became 13.4 for kidneys, 12.2 for lungs, 6.3 for muscle, and 3.9 for lymph node. The concentration in all organs (usually 2–4 mcg./g. and always above 1 mcg./g.) was considered to be amply bacteriostatic. Other reports on distribution of tetracyclines in human organs were indicative of good tissue penetration in general, with ratios for tissue to plasma of 1 (oxytetracycline in gallbladder, methacycline in muscles and lungs) and 0.77 (demeclocycline in uterus) (81). Fat solubility was claimed to play a major role in influencing distribution of tetracyclines into tissues, and serum protein binding appeared to be less of a factor. Tetracyclines permeate both extracellular fluid and intracellular fluid. Calculated values for the apparent volume of distribution in man exceed the volume of total body water (84) and probably reflect protein binding. For example, the following values were reported (results in percent v/w as given in *References 24* and *83*, respectively): chlortetracycline, 92, 148; demethylchlortetracycline, —, 179; oxytetracycline, 90, 189; and tetracycline, 95, 159.

Tetracyclines tend to accumulate during renal insufficiency. Chlortetracycline and doxycycline are notable exceptions; chlortetracycline is excreted primarily in the bile. However, doxycycline urinary clearance decreases during renal insufficiency without a concurrent increase in hepatic excretion despite a relatively constant $t_{1/2}$ in normal and anuric patients. It was suggested that renal insufficiency is accompanied by a compensatory increase in inactivation of doxycycline microbial activity (81). More recent work negated this possibility (65). Studies into the extent of enzymatic degradation (or metabolism) of doxycycline in man and dog were conducted in response to the question of why doxycycline does not appear to accumulate during renal insufficiency. More than 90% of intact drug was recovered from urine and feces in both species. It was concluded that excretion of intact doxycycline in feces probably compensates for decreased renal excretion in uremic patients.

The $t_{1/2}$ appears to be inversely related to protein binding only for those drugs that are primarily cleared by glomerular filtration. In this case the concentration of drug in the filtrate would be expected to be equal to the concentration of free drug in the blood. However, a drug that is excreted by tubular secretion does not appear to be influenced by protein binding, as illus-

trated previously when penicillins were discussed. Penicillins have very high clearance values reflecting tubular secretion; for that reason, their $t_{1/2}$ values are relatively insensitive to protein binding. Conversely, it has been stated that the renal clearance of tetracyclines decreases with increased protein binding (81). Urinary clearance is greatest for oxytetracycline (73% free) and least for doxycycline (18% free), with chlortetracycline and minocycline being considered as exceptions since they are mainly excreted in the bile. Data suggest that tetracyclines are primarily excreted by glomerular filtration rather than tubular secretion, although the lack of precise serum protein binding measurements makes it difficult to calculate individual contributions.

## OPTIMIZING ORAL ABSORPTION BY CHEMICAL MODIFICATION

**pH–Partition Effects**—The pH–partition hypothesis for GI drug absorption was first proposed in 1957 (85, 86). The basic principles are now discussed in many texts and are not reviewed here. A few early reviews summarize the pertinent data (87–89).

Basically this hypothesis predicts that absorption of drugs occurs in the unionized form and that increased lipophilicity increases absorption. Undoubtedly, many compounds behave according to this hypothesis, but many exceptions have been reported within the last decade. Levine (90) reviewed some of the exceptions. For example, phenobarbital and pentobarbital, acids of pKa 7.2 and 8.1, respectively, would be predicted to be absorbed more rapidly from the stomach than the intestines. Instead, 2–3 times as much is absorbed from the intestines during 10 min. than is absorbed from the stomach in 1 hr.

Rate constants for absorption of both ionized and unionized species of sulfaethidole and barbital were calculated using the *in situ* rat stomach and rat small intestinal lumen (91). The ratio of the first-order rate constant for the neutral species to that of the ionized form was roughly 5 for sulfonamide and 3 for barbital. Consequently, at pH 6.1, 42% of the absorption occurs as the ionized form.

**Linear Relationships**—Quantitative relationships that predict structural effects on GI absorption would be most valuable for *a priori* "tailoring" of physicochemical properties by the medicinal chemist. While this is not yet a reality, considerable progress has been made in the form of linear relationships that are predictive for a given series.

Beckett and coworkers applied their buccal absorption techniques to the evaluation of amines (92), amphetamines (93), alkyl-substituted acids (94), series of amines and acids (95), substituted phenylacetic acids (96), and a series of carboxylic acids (97). Several linear relationships were reported. Studies designed to evaluate structural effects on partition coefficients and absorption determined the buccal absorption of nine aliphatic acids of chain lengths from butyric to dodecanoic as a function of pH. Absorption was shown to be passive and increased by low pH and increasing chain length (94). Rate constants for buccal absorption of 10 carboxylic acids from solutions at pH 4 were determined in a single subject. Rate constants, expressed as clearance values, correlated (correlation coefficient = 0.89) with previously determined *n*-heptane–1 *N* HCl partition coefficient values (97). Substituted benzoic acids were subject to decreased absorption when pKa decreased. A linear relationship was found between log partition coefficient and the buccal absorption of a series of amines and acids under conditions of 1 and 10% ionization. The plot of alkyl chain length and either log partition coefficient or buccal absorption was also linear for amphetamines and 10 fluramines at 1% unionized conditions (95, 96). Stehle (98) had earlier calculated that the log transfer rate through a lipoidal barrier would be expected to increase linearly with log partition coefficient until a maximum constant value was approached.

Ho and Higuchi (99) used the previously reported data (92–94) to test a diffusional model for buccal absorption considering both the pKa and partition coefficient. They determined a factor of 2.3 per methylene group upon the incremental partition constant for the buccal lipoidal membrane–aqueous environment. Later this same model was satisfactorily tested on data for buccal absorption of nonionized species, thus providing further evidence for the applicability of the diffusion model proposed earlier (99) and for the significance of the diffusion layer in buccal transport (100).

Hansch and Dunn (101) recently reviewed the methods of linear free energy calculations (generally bearing Hansch's name) for correlating physicochemical properties with biological response[1]. The partition coefficient of drug derivatives can be calculated by the Hansch treatment of $\pi$ additivity values for the *n*-octanol–water distribution. Flynn (102) showed that $\pi$ values for a given substituent on a steroid are position dependent but can be used successfully once the contributions are known for the position in question. The log of the water solubility of 156 liquid organic compounds was shown to be inversely related to the log partition coefficient (103). Linear plots were obtained for log solubility *versus* $\pi$ values using various alkyl esters of testosterones (104), substituted phenothiazines (105), and *p*-hydroxybenzoate esters (106). The slopes of the lines for these solids varied from approximately 2.4 to 3.1 per methylene group.

In an excellent study and discussion, Flynn and Yalkowsky (107) examined the effect of alkyl chain length for a series of *p*-aminobenzoate esters on physicochemical properties and their resultant ability to penetrate inert barriers. Their model, which is based on solubility-limited diffusion through a membrane diffusion layer composite barrier, predicts a parabolic dependency as the homologous series is ascended, and both the controllable and uncontrollable factors in designing optimum chain length for absorption are discussed. A linear relationship of unit slope was found between log of intrinsic partition coefficient values and extraction constants of ion-pairs for protonated chlorides of *p*-alkylpyridines (108). Methylene increments were concluded to be similar for the free base and the

[1] The parabolic case has now been reviewed by C. Hansch and J. M. Clayton, *J. Pharm. Sci.*, **62**, 1(1973).

ion-pair due to the distance of the alkyl substituent from the polar portion of the molecule.

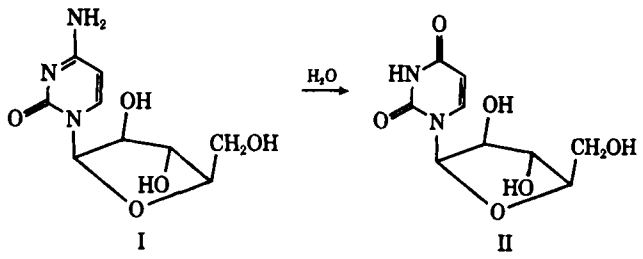
In studies involving more than 50 chemical compounds, Nogami *et al.* (109, 110) examined relationships between chemical structure and absorption rate. Their results indicate that the absorption rate constant can be predicted from an additivity rule and that the values for a given combination of interacting groups are constant and referred to as the "intramolecular interaction constant." Recently, the absorption of pralidoxime methanesulfonate was compared to pralidoxime hydrochloride following oral administration to healthy young men (111). Plasma concentrations were considered equivalent on a molar basis.

Morozowich (112) suggested that an increasing partition coefficient can affect not only absorption characteristics but also distribution in lipids and ultimately the biological $t_{1/2}$. He cited as an example the 3-cyclopentyl ether of 17$\alpha$-ethinyl estradiol which has been reported to exert an estrogenic effect which lasts for 3 months after oral or intramuscular administration of 5 mg. (113).

## PRODRUGS: BIOREVERSIBLE DERIVATIVES

**Nucleosides**—The first report describing a chemical modification of a nucleoside that dramatically increased oral absorption in humans appeared in 1961 (114). Psicofuranine was not significantly absorbed from the GI tract in humans. Acetate esters of the sugar hydroxyls were prepared, and the tetraacetate was chosen for human trials based upon observed efficacy equivalent to the parent compound compared subcutaneously in mice. Although no detectable levels could be determined after oral administration of psicofuranine in humans, its tetraacetate was well absorbed. Assays with and without preliminary hydrolysis indicated that the tetraacetate was rapidly converted to free psicofuranine in the blood. The outstanding difference in physicochemical properties between the drug and its prodrug appears to be the enhanced lipophilicity in the ester. The water–chloroform partition coefficient was 992 for psicofuranine and 0.041 for the tetraacetate. This increased lipophilicity was deemed responsible for the increased absorption.

It is quite common to associate the term bioavailability with absorbability from an oral dosage form. This is probably a natural result of the overwhelming research activity in that area. However, three additional rate processes in Scheme IV can be involved in limiting bioavailability. These are indicated by the constants $k_{12}$, $k_{21}$, and $k_2$. This may be said of any drug since its degree of success is dependent upon its ability to reach the site of action and the resultant time course at that site. This point was illustrated by Skipper *et al.* (115) using animal tumor systems *in vivo*. In the case of arabinosylcytosine, its bioavailability may be said to be limited by $k_2$ (in this case, metabolism) which, in turn, limits its effectiveness. After pharmacokinetic studies successfully defined this problem, therapy was improved by appropriate dosage regimens and derivatives. The details of this research are summarized here as an illustrative example of the potential significance of pharmacokinetics in drug design.




*Scheme V*

1-$\beta$-D-Arabinofuranosylcytosine (I, Scheme V), a cytotoxic agent, exerts antitumor and antiviral activity in a variety of animal and human neoplasms. It is highly effective for the treatment of acute myelogenous leukemia in man (116, 117). The greatest response in a single disease was observed in acute granulocytic leukemia where 35 of 144 adequately treated patients (or 24%) achieved complete or partial remissions (117, 118) and, more recently, 21 out of 49 patients (43%) attained complete remission status through rapid drug injection followed by continuous drug infusion to maintain steady-state therapy for 4 hr./day (119).

This somewhat encouraging remission rate is rather astonishing when one considers the brief duration of bioavailability of this drug. When injected intravenously in human subjects, the resulting blood level data exhibit biphasic first-order plots (120) characteristic of a two-compartment open model. The initial loss from the blood following rapid intravenous injection is extremely fast, has a $t_{1/2}$ of approximately 12 min. (120–122), and may be attributed to simultaneous distribution and elimination. At the end of the initial phase (or $\alpha$-phase), over 80% of the drug remaining in blood and urine is in the form of a single inactive metabolite, 1-$\beta$-D-arabinofuranosyluracil (II, Scheme V), indicating both rapid and extensive deamination during the distribution phase (120).

It is well known that cytosine nucleosides undergo hydrolytic deamination to their corresponding uracils and this may be catalyzed enzymatically (123, 124) or chemically (125–131). The elimination phase (or $\beta$-phase) has a half-life of approximately 111 min. and results in nearly complete conversion of I to II (120) *via* catalysis by pyrimidine nucleoside deaminase (123). Recovery of the metabolite in the urine was 86–96% of the total 48-hr. recovery in one study (122) and 90% of the total 24-hr. recovery (representing 80% of the total dose) in another (120). Uptake of I by red blood cells is rapid and within 5 min. cells attained 60% of the concentration of the plasma (120). However, the half-life within the cell is only 2–3 min. Thus, I exhibits a relatively short duration of bioavailability and its loss is almost entirely due to deamination to II, which is apparently inactive as an inhibitor of cell growth (132).

It is reasonable to expect that an increase in the biological duration of I might lead to increased remission rates. Evidence for this hypothesis already exists in the work of Baguley and Falkenhaug (133) who measured plasma $t_{1/2}$ of I following 30-min. intravenous infusions in leukemic patients. Patients who experienced complete remissions had significantly longer $t_{1/2}$ values than those who did not respond to therapy, and this

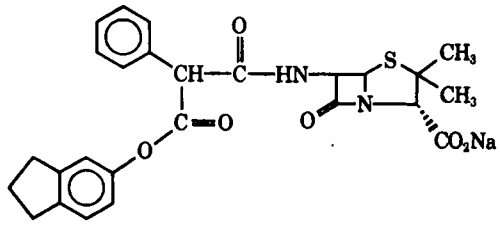
variation was presumably due to variations in deaminase activity. These results suggest that degradation of I may cause failure of the drug to produce hematologic remission in certain patients (133). Further support for this proposal may be taken from the demonstration that the dosage regimen markedly affects its therapeutic index. For example, superior results were obtained using either constant intravenous infusion or injections every 8 hr. compared to single daily injections (134–136). The most effective present schedule appears to be 200 mg./m.²/day given by continuous intravenous infusion (116), which provides steady-state plasma levels of approximately 0.15 mcg./ml. (120).

Another approach to increasing duration is that of slow release of I from a poorly soluble prodrug. A single dose of 1-β-D-arabinofuranosylcytosine-5′-adamantoate was nearly as effective as an optimum schedule of I given every 3 hr. by injection to L-1210 leukemic mice. The duration of cytotoxic plasma levels of I was greatly increased by administration of the prodrug (137). Gray *et al.* (138) showed that several 5′-acylates were equal to or better than the activity of the 5′-adamantoate. These derivatives appear to be more stable to pyrimidine nucleoside deaminase and capable of yielding I upon hydrolysis in the blood.

Since pharmacokinetic studies indicate rapid loss of I to inactive metabolite *via* enzymatic deamination, the design of new antimetabolites which are more stable and potentially more effective has evolved. Cyclocytidine, effective against L-1210 leukemia (139), was synthesized from cytidine (140, 141). It appeared less toxic and more effective than I administered once daily for 5 days in L-1210 leukemic mice (141). If hydrolyzed *in vivo*, it would yield I and act as a prodrug. It is reportedly more resistant to deaminase.

Additional studies have been carried out on nonbioreversible derivatives with the intent of establishing increased stability to deamination. For example, Panzica *et al.* (142) determined that 1-β-D-arabinofuranosylcytosine-3-*N*-oxide is a potential inhibitor of lymphoid leukemia L-1210. Although it is designed to resist deamination, no enzyme stability studies have been reported as yet.

Studies on the effects of substituent groups on the chemical stability of cytosines to hydrolytic deamination and the possibility that chemical reactivity might be related to the enzyme-catalyzed transformations have been initiated (125, 126, 131). These investigations are designed to provide a systematic study of substituent group effects on the chemical hydrolytic deamination of cytosines and to apply these findings to enzymatic studies with the ultimate goal of extending the biological duration of I or one of its analogs. The effect of the sugar on deamination was previously studied by comparing the deamination kinetics of three cytosines (126). A major difference in the case of I is that of intramolecular attack by the 2′-hydroxyl of the sugar on the C-6 position in the pyrimidine ring. However, support for a common deamination pathway is evidenced by the nucleophilic addition of bisulfite $HSO_3^-$ to C-6 of various cytosine nucleosides (129). Since addition at the C-6 position is involved in the chemical catalysis of deamination, appropriate substitution at



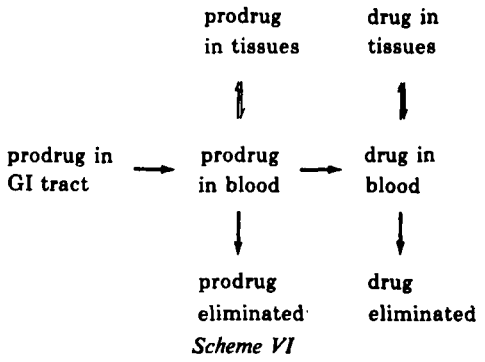
III

that position should hinder catalysis and thereby decrease the deamination rate. To test this hypothesis, three derivatives (1-β-D-arabinofuranosyl-6-methylcytosine, 1-β-D-ribofuranosyl-6-methylcytosine, and 6-methylcytosine) were synthesized and the kinetics of their transformations in aqueous buffer were compared to the unsubstituted compounds (131). Results of the comparisons were only partly as predicted. While 6-methylcytosine was more stable to deamination than cytosine, the substituted nucleosides were found to undergo competing reactions which were not observed for the parent compounds that underwent deamination under identical conditions. The net result is an increased stability for 6-methylcytosine but an increased rate of loss for the 6-methylnucleosides. Relative stability to deaminase has not yet been reported, and biological testing has not been carried out.

**Antibiotic Prodrugs**—There are many possible reasons for developing prodrugs, and several successful examples of antibiotic prodrugs may be cited to illustrate some advantages. Carbenicillin was initially marketed only as an injectable due to its poor oral bioavailability resulting from poor absorption. This was presumably due to either its high polarity (it is a dicarboxylic acid) or acid instability or both. Recently, studies have indicated that the prodrug carbenicillin indanyl sodium (III) is orally absorbed and active (143–147) and it is being marketed as such.

Hetacillin is a prodrug of ampicillin, which may be considered as a condensation product of acetone and ampicillin. Once absorbed, hetacillin is rapidly converted to ampicillin (148). Both are marketed in oral dosage forms as well as in dry powder form for reconstitution before parenteral use. Solutions of hetacillin have been shown to be more stable (149). Six hours was required for 10% loss of activity at room temperature, whereas ampicillin itself showed 10% loss in 1 hr. under similar conditions. There is an advantage of extended shelflife to the user of reconstituted parenteral hetacillin. The oral absorption of hetacillin may be somewhat greater than that of ampicillin based on urinary recovery data (150, 151), although one study reported decreased urinary recovery with the prodrug (152). There have been a number of comparisons based on the plasma concentration time-profiles, but the meaning of these results is equivocal since assays often failed to differentiate between drug and prodrug. This problem will be discussed later in the section dealing with potential pitfalls in data interpretation.

One reason for prodrug formation is to increase stability in the GI tract and thereby increase bioavailability. Erythromycin is rapidly destroyed at pH values less than 4, and numerous esters have been synthesized



*Scheme VI*

with the goal of increasing stability and, in turn, oral absorption. Lee *et al.* (153) examined the absorption of a series of erythromycin esters following oral administration in rats. Based on areas under the plasma curves, the acetate and propionate appeared to be better absorbed than the parent drug while the butyrate was absorbed markedly less. However, these curves may not be indicative of absorption differences alone since intact prodrug was present in significant amounts. Scheme VI illustrates the several rate processes that potentially affect the time course of drug and prodrug in plasma. If blood level curves represent total erythromycin (by hydrolysis of ester to erythromycin before assay), one cannot attribute differences to absorption without further data.

Erythromycin base has a pKa of approximately 8.9. It would be expected to be ionized throughout the entire GI tract, and intestinal absorption would be expected to be better than absorption from the stomach. In rats, erythromycin was most rapidly absorbed in the upper small intestines (153). Two prodrugs, designed to bypass the stomach, are in common use. Erythromycin stearate resists dissolution in the stomach but dissociates rapidly in the intestines to yield free erythromycin. Erythromycin estolate, by contrast, is probably absorbed largely as intact ester. Once in the blood the ester hydrolyzes to yield free erythromycin. The $t_{1/2}$ for hydrolysis in human serum was reported to be 93 min. (154).

The advantages of high serum levels of the propionyl ester prodrug have been a matter of controversy. In a four-way crossover study (fasting and nonfasting), blood levels obtained after single and multiple doses of erythromycin estolate and erythromycin stearate were compared (155). The estolate achieved higher blood levels at a faster rate both with and without food. However, the assay used was probably capable of hydrolyzing estolate esters, since the $t_{1/2}$ for *in vitro* hydrolysis has been reported to vary from 0.5 hr. at pH 8 to 5.0 hr. at pH 5 (156). Stephens *et al.* (157) reported 20–35% free base and 65–80% ester after the fifth dose of ester in humans, so that the average free base was higher than that obtained by administration of the salt.

Esters of lincomycin and clindamycin have been prepared in an effort to mask undesirable taste and maintain (or improve) oral absorption. Four lincomycin monoesters were compared for activity by subcutaneous and oral routes in mice (158). Earlier, 15 esters were compared in a similar manner (159). Sinkula *et al.* (160) reported comparative activity in mice for 16 clindamycin esters and their areas under plasma level–time profiles following oral and intramuscular injections in dogs. The 2-hexanoate, 2-laurate, and 2-palmitate appear to be absorbed as well or better than clindamycin itself when all are administered as hydrochloride salts at doses of 25 mg./kg. equivalent to clindamycin base. In contrast to the erythromycin estolate case already discussed, these esters apparently hydrolyze prior to absorption or immediately thereafter so that intact ester does not present a problem in comparing plasma level–time profiles. At least one product has been marketed from these efforts in the form of clindamycin palmitate hydrochloride flavored granules for oral suspension.

A pharmacokinetic study involving clindamycin and its palmitate in 52 children showed that mean serum levels of clindamycin were lower when administered as the palmitate in a single dose (161). It was concluded that rapid predictable absorption occurred using the palmitate, and neither drug accumulation nor increase in metabolism due to induction occurred. The 8–16-mg./kg./day doses were deemed adequate for the age group.

**Related Publications**—The publications on prodrugs and drug derivatives are too numerous to discuss completely in this review. Examples have been chosen (admittedly arbitrarily) to support the author's various theses. Several reviews consider, at least in part, the effects of molecular modification on pharmacokinetic properties (162–169). In this article, an attempt has been made to avoid repetition of discussions found in the previously published reviews.

In an excellent review of prodrugs and their properties, Sinkula (170) outlined some pharmaceutical reasons for drug modification. He listed the properties which may be altered through derivative formation as follows: (*a*) mask the taste and/or odor of bitter or obnoxious drugs, (*b*) increase or decrease aqueous solubility, (*c*) improve formulatability of drugs difficult to formulate, (*d*) increase stability, (*e*) decrease gastric and intestinal irritation, (*f*) decrease pain on injection, (*g*) improve depot action, (*h*) improve drug absorption, and (*i*) facilitate transport of drug to site of action.

Except for those cases where derivatives revert back to the parent drug before or immediately after absorption, the modification must be considered as potentially capable of affecting the absorption, distribution, and elimination kinetics in addition to the primary mission. Sinkula (170) surveyed a number of marketed drug derivatives and their stated reasons for the modification. Some of these are listed in Table VII.

## PITFALLS IN DATA INTERPRETATION

**Meaning of Parameters**—Although it is imperative that the field of drug design and evaluation involves consideration of pharmacokinetic effects, it is equally imperative that the meaning and limitation of each parameter under comparison be understood. Earlier the values for *Vd* were compared at steady-state concentrations for three isoxazolyl penicillins. The steady state was chosen to avoid the effect of $k_2$ upon the values for *Vd*.

The calculation of *Vd* for a two-compartment model has been the subject of considerable confusion in the

Table I—Delivery Volume as a Function of Pressure

| Liquid | Rate, ml./p.s.i. ($10^3$) | Total Volume Delivered, ml. 1400 p.s.i. | 2100 p.s.i. | 2800 p.s.i. |
|---|---|---|---|---|
| Calcium carbonate, 5% | 6.7 | 10 | 14.7 | 19 |
| Syrup USP | 7.1 | 10 | 15 | 20 |
| Water | 6.8 | 12 | 16.8 | 21.5 |
| Alcohol USP | 7.1 | 13 | 18 | 23 |

## EXPERIMENTAL

**Materials and Equipment**—The liquids used in this study were: distilled water; syrup USP; shellac, 2.7 kg. (6 lb.) cut; 5, 15, and 35% (w/v) aqueous suspensions of calcium carbonate; and a 2% (w/v) solution of methylcellulose[1], 500 cps.

The equipment utilized to evaluate the factors involved in an automated tablet-coating system was a self-programming automated tablet-coating system with two automatic spray guns[2] as previously described (4).

**Fluid Delivery Studies**—Fluid delivery experiments were conducted to ascertain the influence of pressure, spray tip orifice size, and type of liquid on the volume of spray delivered.

The volume of fluid delivery in milliliters was determined from 1400 to 2800 p.s.i. nozzle pressure in 140 p.s.i. pressure increments. The tip orifice sizes varied from 0.023 cm. (0.009 in.) to 0.053 cm. (0.021 in.) in diameter. All experiments were conducted in an air conditioned room at approximately 25° and 30–40% relative humidity.

Three-second fluid spray delivery cycles were used, and five replicate samples of each liquid under study were collected, at each pressure and nozzle tip size, in a 100-ml. graduated cylinder; the resulting volumes were recorded.

**Spray Pattern Characteristics**—Spray patterns were determined for the liquids studied, each containing 1% (w/v) FD&C Red No. 2 dye for contrast. The spray from the activated gun of the automated tablet-coating system was directed at a specially designed target which consisted of a 61-cm. square pane of glass with five concentric circles painted 10 cm. apart. The tip of the spray gun was positioned 30 cm. from the glass surface and at the center point of the target. The spray cycle was set for a 0.25-sec. solution delivery. The patterns were subsequently observed at 1400-, 1960-, and 2800-p.s.i. nozzle pressure (the lower, middle, and upper pressure range, respectively) for all tip sizes ranging from 0.023 cm. (0.009 in.) to 0.053 cm. (0.021 in.) in orifice diameter. After each spray, the pattern dimensions were taken and pattern uniformity was noted.

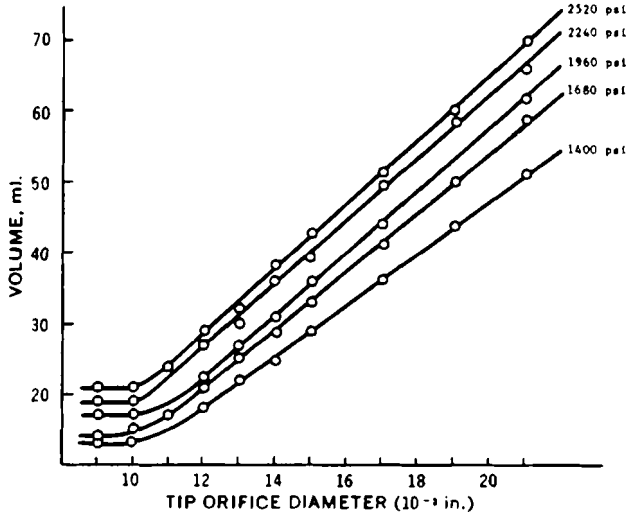



Figure 3—*Change in delivery with respect to change in tip orifice size using water.*

[1] Methocel MC, Dow Chemical Co., Midland, Mich.
[2] Graco Hydro-Spray Unit, Gray Co., Minneapolis, Minn.

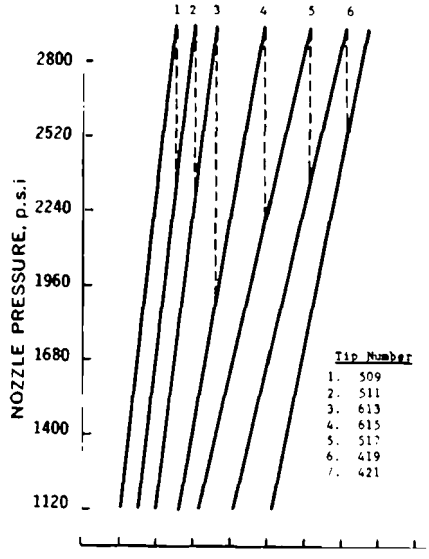



Figure 4—*Fluid delivery curves, 3-sec. cycle.*

## RESULTS AND DISCUSSION

**Fluid Delivery**—The pressure–volume curves in Fig. 1, constructed from fluid delivery data obtained with a 0.023-cm. (0.009-in.) spray tip and a 3-sec. delivery, show that the change in fluid volume delivered as a function of pressure was constant. The curves are representative of some of the liquids studied, and each shows excellent linearity. The rate of change of delivery (Table I) for the liquids represented in Fig. 1 was essentially the same.

A 3–4-ml. difference in total volume delivered at each pressure increment was found between the four liquids studied. These small volume differences do not appear to be significant, but the data show a trend toward a decrease in volume delivered with an increase in fluid viscosity and solids content when comparing syrup and calcium carbonate with water and alcohol. A significant difference would be expected at a much lower or at atmospheric pressure. The data, therefore, indicate that fluid volume delivery is essentially independent of the physical properties of the fluid at very high pressures.

Fluid delivery curves for the three calcium carbonate suspensions, which varied from a "watery" to an "ointment-like" consistency (Fig. 2)[3], are in essence identical and confirm that the elevated pressures used in the study obliterated the effects of the physical properties of the fluids delivered.

In Fig. 3, the volume of water delivered in 3 sec. *versus* tip orifice diameter shows a good linear relationship within the range of 0.030 cm. (0.012 in.)–0.053 cm. (0.021 in.). This finding is unusual because one would expect an orifice–volume relationship based on

Figure 5—*Spray patterns with water. Left: 0.023-cm. (0.009-in.) tip size and 30 × 9-cm. pattern size. Right: 0.053-cm. (0.021-in.) tip size and 40 × 30-cm. pattern size.*

[3] Data points for Fig. 2 were omitted for the purpose of clarity.

of distribution in patients with renal failure as compared to normal patients (53, 176). Also, administration of probenecid, an inhibitor of renal tubular secretion of organic acids, reduces the apparent volume of distribution of penicillin derivatives (43, 176). This observation is not surprising in view of certain facts. Since the *Vd* does not necessarily represent a real volume, observed changes may not reflect any change in the tissues through which the drugs are distributed. The values obtained may depend upon the size of $k_2$ for a two- (or more) compartment drug. Thus, comparisons of *Vd* for derivatives should be carried out at steady-state plasma levels or be interpreted in terms of the effects of $k_2$ on the distribution value.

In addition, equilibrium between nonequivalent compartments involves primarily the unionized drug or unbound species which can pass through the biological membrane. This has important consequences in the calculation of *Vd* since many drugs, such as erythromycin, sulfonylureas, salicylates, and coumarin anticoagulants are bound to plasma protein. The apparent volume of distribution should be calculated on the basis of freely diffusing drug, so a correction must be made for the fraction bound. If this correction is not made, two types of error can occur. When the assay method determines only free drug, the bound drug is counted with drug distributed to the tissues and the *Vd* calculated is too large. On the other hand, if the assay is for total drug, the value of *Vd* is too small. If corrections are not made for protein binding, *Vd* values calculated at different doses for a drug whose extent of binding varies with dose also vary (177).

**Absorption Rate Constants**—The determination of absorption rate constants from blood level data is a common problem when biopharmaceutics and pharmacokinetics are applied to design and evaluation of drugs and their derivatives. Wagner and Nelson (178) developed a method for calculating the absorption rate constant for transfer from an extravascular depot into the body for a drug whose distribution can be described by a one-compartment model. Similarly, Loo and Riegelman (179) reported a method for calculating the absorption rate constant, $k_1$, for the case where the drug is distributed according to a two-compartment model. When simultaneous loss of drug occurs from the site of administration, the competing rate increases the calculated apparent value for the absorption constant (180). For the case where the competing loss of drug is by a first-order process, the apparent rate constant calculated by either method yields the sum of the competing first-order rate constants (180).

The significance of this potential problem is readily apparent. False impressions of rapid absorption could result from rapidly hydrolyzable analogs. The compound with the fastest apparent absorption rate constant in a series may be the most susceptible to biotransformation. The total amount of drug absorbed from the depot should be determined for an accurate interpretation of the calculated rate constants for absorption. If a drug is well absorbed from the depot, the calculated absorption rate constant may be considered to be a good estimate. If, however, the drug is poorly absorbed, the reason for the incomplete absorption must be determined before one can assign a physical meaning to the calculated value of the apparent absorption rate constant.

This problem is not unique to oral dosage forms. Doluisio *et al.* (181) showed that intramuscular injections of sodium dicloxacillin and sodium ampicillin solutions were only 75–78% absorbed. They suggested that the drug may have undergone chemical or enzymatic decomposition at the injection site. If 22–25% of the drug placed into the muscle is indeed lost to some simultaneous first-order rate process, the apparent absorption rate constants would be approximately one-third larger than the actual value.

**Bioavailability of Prodrugs: Hetacillin**—Scheme VI illustrates one potential problem in prodrug evaluation. If conversion of prodrug to drug is not instantaneous, then the distribution, elimination, and conversion kinetics for the prodrug must be determined to describe the system adequately.

A second potential problem may be illustrated by considering hetacillin, a prodrug of ampicillin. Early studies indicated increased ampicillin plasma levels from intravenous administration of hetacillin (182). However, this result was later shown to be due to an artifact of the assay method (183). Because of the long incubation period for the microbiological assay, hetacillin was also converted to ampicillin and thus measured as drug. Thus, plasma levels after hetacillin injection appeared to exceed those obtained by rapid intravenous injection of the drug itself. Application of a differential assay technique showed the areas under the ampicillin curves to be identical from either source (148, 183). This represents a good didactic example since an understanding of pharmacokinetic parameters would dictate *a priori* that no increase in bioavailability would be expected beyond that observed by an intravenous injection of the drug itself.

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