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Keyphrases

Drug binding by plasma proteins—review
Experimental methods
Protein binding—drug distribution
Plasma binding—competitive inhibition
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Research Articles

New Method for Calculating the Intrinsic Absorption Rate of Drugs

By J. C. K. LOO and S. RIEGELMAN

If conceiving the body to be a single compartment is correct, calculations based on this presumption should result in an exact estimate of the rate of appearance of a drug into the blood when administered intravenously at a precisely known rate. In order to test this hypothesis selected drugs were administered intravenously at known logarithmic and linear rates of infusion, thereby mimicking first- and zero-order absorption conditions. It is shown that methods based on the single-compartment concept do not result in acceptable estimates of the absorption rates. Not only do these methods lead to an incorrect rate constant but occasionally allow incorrect assignment of the order of the process. A new equation is presented, presuming the drug distributes between a central and one peripheral compartment, which allows one to calculate the rate of absorption. The equation results in an accurate estimate of the known rates of infusion (absorption) for the drugs studied to date.

PROBABLY THE oldest published method for estimation of the rate of absorption of a drug into the blood was published by Dominguez and Pomerene (1). Their method was based on a presumption that the body may be treated as a single-compartmental reservoir from which the drug is eliminated by first-order processes. The calculation of the absorption by their method required estimation of the apparent volume of distribution of the drug in this single-compart-

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ment, the slope of the blood level time curve at time, t, and the apparent elimination rate constant of the drug from the volume of distribution which is assumed to be constant and independent of time and dose administered. A number of other methods based on the same model have been proposed. These include the blood level maximum and time of maximum methods of Dost (2), and the ordinate intercept and total area methods of Krüger-Thiemer and Diller (3). Each of these methods requires one to make a basic presumption that the drug is being absorbed by a first-order process starting immediately after the drug is administered.

In 1960 Nelson (4) proposed a method utilizing the first and second derivatives of the urinary excretion data as a function of time. Later, in 1963, Wagner and Nelson (5) published what has become the most commonly used methods for estimating the apparent absorption rate of drugs utilizing either urinary or blood data. These methods have gained wide acceptance since they do not require prior estimate of the volume of distribution and they place no limitations on the order of the absorption rate process.

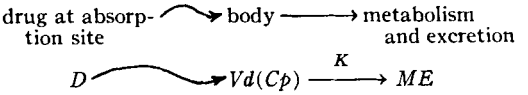
Several methods have been proposed by different workers which utilize the complete i.v. curve in conjunction with the oral absorption curve. The method of Scholer and Code (6) published in 1954 is an involved and extensive numerical estimation. The method of Silverman and Burgen published in 1961 (7) involves a curve follower and analog computer to deconvolute the blood data relative to a known i.v. curve. Recently Rescigno and Segre (8) have published a numerical rather than a computer method of deconvoluting the blood data. In 1965 Stelmach *et al.* (9) proposed an analog computer program which included the release of the drug from a dosage form prior to the absorption step. Their program initially was presented on a single-compartmental model. Their procedure was based on the presumption that one had previously defined the elimination rate constant, and the absorption rate constant of the drug administrated orally in solution.

In spite of all the available methods and the large number of estimates of the apparent absorption rate of different drugs by a variety of available methods, the literature[1] apparently contains no comparisons of these estimates with known input rates obtained from individual test subjects or animals.

The purpose of this paper is to present some experimental blood level data following infusion at known input rates. It will then be shown that the methods based on the single-compartmental model result in grossly inaccurate estimates of the known input rates and can lead to error and occasionally false interpretations of the results. Further, a new equation will be derived which allows one to use rather simple numerical procedures to estimate the true input rates.

THEORY

The Single-Compartmental Model—The single-compartmental model is based on the assumption that the dose absorbed is distributed into what is conceived to be a single compartment and is eliminated from it by first-order processes of metabolism and excretion. This is represented schematically below:



D is the amount of the original dose remaining at the absorption site, Cp is the concentration of the drug in the blood, Vd is the apparent volume of distribution[2] based on the presumption that the body behaves as a single compartment, ME represents the combined amount of drug metabolized and excreted by simultaneous first-order processes, K is the first-order rate constant for the elimination of the drug from the body and is equal to the sum of the individual rates of metabolism and excretion. On the basis of material balance, the amount of the intact drug reaching the fluids of distribution is taken to be the amount of drug absorbed at any time $(A)_t$. This is in effect the *net* influx of intact drug which has transferred into the blood stream. This is set equal to the amount of the drug in the body compartment plus the amount eliminated by all processes. Wagner and Nelson derived their absorption equations on the basis of this material balance. Since the rates of metabolism and excretion are often found to be directly proportional to the blood level, they proposed that the amount of the drug eliminated up to time t_n could be evaluated by $KV\int_{t_0}^{t_n} Cpdt$. Therefore, integration of the differential mass balance equation between the limits of t_0 and t_n, resulted in what has become known as the Wagner-Nelson (W-N) absorption equation for blood data:

$$(A/Vd)_{t_n} = Cp_{t_n} + K\int_{t_0}^{t_n} Cpdt \quad \text{(Eq. 1)}$$

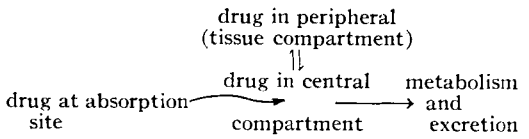
With Eq. 1, one attempts to evaluate the amount absorbed (expressed in concentration units) as being the sum of the concentration in the body as represented by the plasma concentration plus the second terms which is equal to the amount eliminated (expressed in concentration units). When all of the drug is absorbed, the value of $(A/Vd)_\infty$ will approach

[1] Wagner (10) utilized averaged data for 24 subjects obtained from Fig. 1 of Jopikii and Turpeinen's (11) data on the administration of i.v. infusions of glucose. Although a good fit was apparently obtained, the original data of Jopikii from these 24 subjects had been corrected for a varying basal glucose level. Further, the postinfusion elimination constant was different from the elimination constant during the infusion.

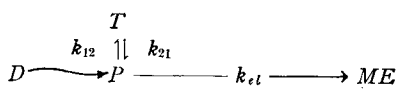
[2] In the nomenclature of the earlier papers of this series, this apparent volume of distribution is more correctly specified as Vd_{area}(11).

an asymptotic value representing the estimated total amount of the drug absorbed. Since the apparent volume of distribution may not be known, it is necessary to express the data in terms of the percent remaining to be absorbed. These data are frequently interpreted in terms of pseudo-zero and pseudo-first-order rates. Occasionally more complicated interpretation of the results is attempted (10).

The Two-Compartmental Open-System Model—Riegelman, Loo, and Rowland have recently proposed that it is mathematically and physiologically more acceptable to conceive the body to be a two-compartmental open system (12). Such a model is shown in the following schematic:



or in appropriate symbols:



P is the amount of the intact drug in the central compartment, which includes the blood, fluids, and tissues which, in effect, spontaneously equilibrate with the blood; T is the amount of intact drug in the peripheral (tissue) compartment; Vp is the volume of the central compartment; k_{12} and k_{21} are the first-order distribution rate constants out of and back into the central compartment from the peripheral compartment, and k_{el} is the sum of the (presumed) first-order rate constants for the simultaneous processes of metabolism and excretion. The above two-compartmental model results in the following general equation describing the blood level time curve after a single i.v. injection:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 2)}$$

where at $t = 0$, $Cp^{\circ} = A + B$, and α and β are hybrid rate constants each influenced by *all* the individual rate processes. Because of this, Riegelman *et al.* (12) proposed that α and β be called the *fast and slow disposition* rate constants, respectively. The relationships between these disposition rate constants and the individual rate constants of this model have been presented in earlier publications. (12, 13). In the following presentation, it will be presumed that the accurate data have been obtained at appropriat : times and analyzed for the rate constants, k_{12}, k_{21}, and k_{el}.

It is convenient to convert all of the drug amounts represented in the model to concentration terms by dividing by Vp, whereupon they become Cp, Ct, and Cme, respectively. The amount metabolized and excreted up to time t_n, expressed in concentration units becomes:

$$(Cme)_{t_n} = k_{el} \int_{t_0}^{t_n} Cpdt \qquad \text{(Eq. 3)}$$

Integration of the differential mass balance equation between the limits of t_0 and t_n results in:

$$(A/Vp)_{t_n} = Cp_{t_n} + k_{el} \int_{t_0}^{t_n} Cpdt + Ct_{t_n} \qquad \text{(Eq. 4)}$$

This is in effect a modified Wagner-Nelson equation for the two-compartmental model; however, before Eq. 4 can be used to estimate the absorption rate of a drug, it is necessary for the tissue compartment concentration term to be expressed in measurable quantities. The differential equation representing the rate of change of this compartment with time is:

$$dCt/dt = -k_{21}Ct + k_{12}Cp \qquad \text{(Eq. 5)}$$

Since k_{12} and k_{21} can be estimated from the blood data, if Cp could be expressed as a function of time, the solution of Eq. 5 would be feasible. If one assumes that between the two data point times t_n and t_{n-1} the blood level data can be approximated by a straight-line segment, it is possible to substitute for Cp_{t_n} on the basis of the equation for a straight line:

$$Cp_{t_n} = Cp_{t_{n-1}} + M \cdot \Delta t \qquad \text{(Eq. 6)}$$

where

$$\Delta t = t_n - t_{n-1}\,; \quad \Delta Cp = Cp_{t_n} - Cp_{t_{n-1}}$$

and

$$M = \Delta Cp/\Delta t$$

whereupon we may substitute for Cp_{t_n} in Eq. 5 the equation can be integrated by Laplace transforms and the second term simplified by a two-term Taylor approximation:[3]

$$Ct_{t_n} = \frac{k_{12}}{k_{21}} Cp_{t_{n-1}}[1 - e^{-k_{21}\Delta t}] + k_{12}\frac{\Delta Cp\ \Delta t}{2} + Ct_{t_{n-1}}e^{-k_{21}\Delta t} \qquad \text{(Eq. 7)}$$

The total absorption equation based on the two-compartmental model can be obtained by substituting for $(Ct)_{t_n}$ in Eq. 4.

$$(A/Vp)_{t_n} = Cp_{t_n} + k_{el} \int_{t_0}^{t_n} Cpdt + \frac{k_{12}}{k_{21}} Cp_{t_{n-1}}[1 - e^{-k_{21}\Delta t}] + \frac{k_{12}\Delta Cp\Delta t}{2} + Ct_{t_{n-1}}\, e^{-k_{21}\Delta t} \qquad \text{(Eq. 8)}$$

As with the W-N equation, Eq. 1, the second term including the integral is evaluated by progressive summing of the area under the plasma concentration time curve from t_0 to t_n, usually using the trapezoidal rule for estimating the areas. Since the concentration of drug in the tissues is usually zero at the start of the study, when evaluating the tissue concentration at t_1 it is appropriate to set the last term in Eq. 8 equal to zero.[4] At each subsequent time, t_n, the tissue term must be solved by a method of successive substitution of the tissue concentration from the previous time interval.[5]

EXPERIMENTAL

The *N*-methylglucamine salt of acetylsalicylic acid (ASA) was prepared by mixing equimolar amounts of the base and acid. A 650-mg. dose of ASA was prepared in 10 ml. of solution. The solution was bacteriologically filtered and injected into

[3] A detailed derivation of Eq. 7 is given in the appendix.
[4] As with the use of Eq. 1, it may be necessary to make a time shift to correct for a delay in absorption.
[5] In order to facilitate the use of this complete absorption equation, a detailed analysis of sample data is included in Tables I and II under *Appendix*.

the antecubital vein by rapid i.v. injection. Blood samples were taken from the opposite arm at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180 min., and at hourly time periods for 8 hr. The samples were assayed for ASA by the specific gas chromatographic method of Rowland and Riegelman (13). Several weeks later the experiment was repeated using a Harvard infusion pump (model 975). Since this pump has a series of gears at constant ratio to one another, it was possible to establish a logarithmic rate of infusion by shifting the gears at constant time intervals. *In vitro* experiments showed the pump to produce logarithmic results through 97% of the injection, whereupon the final 3% were injected without further gear change. Different rates of infusion could be generated by merely modifying the time intervals for the gear changes. In order to keep the sampling as near as possible to the true log curve, the blood samples were taken at or near to the time of the gear changes. All experiments reported herein were done on male tests subjects.

Griseofulvin was injected into adult male volunteers in a specially prepared solution of griseofulvin dissolved in polyethylene glycol 300. The solution was not injected directly but by diluting it into a rapidly flowing normal saline solution, which was being administered by intravenous drip over a 3-min. period. Several weeks later the drug was administered over a 2-hr. constant infusion into the same test subject. The plasma samples were assayed for intact drug by a modification of the method of Bedford *et al.* (14).

An AEI model TR48 analog computer and a Varian Associates 11 × 17-in. X-Y recorder was used to study the models.

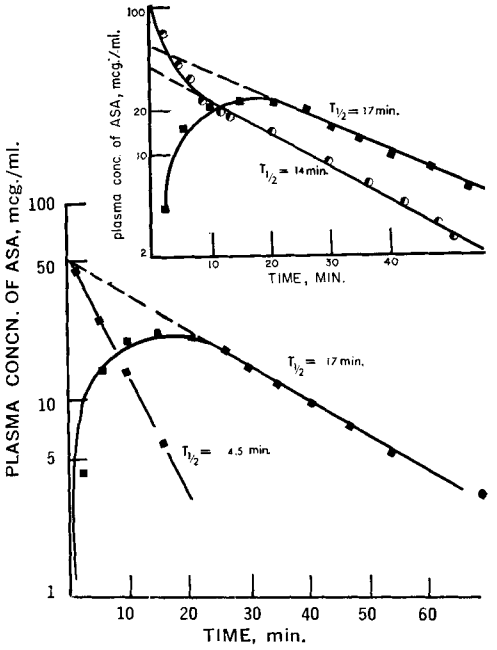


Fig. 1—Blood level of ASA after logarithmic infusion of 650 mg. into subject S at first-order rate of 0.0693 min.$^{-1}$ ($t_{1/2}$ = 10.0 min.) Infusion ceased at 64 min. Insert (Fig. 1a)—The superimposed blood curves after log infusion (■) and single i.v. injection (◐) of 650 mg. ASA.

RESULTS AND DISCUSSION

Figure 1 represents the data obtained when ASA was administered intravenously under logarithmic infusion conditions to a test subject. The data appear to be completely compatible with the concept of the body exhibiting the properties of a single compartment since the up curve could be said to represent the infusion period while the down curve then could represent the disappearance of the drug from the body by a single rate constant process. Indeed, a theoretical curve could be drawn through all the data points, except the 1-min. point, using the following equation where the rate constants were obtained by graphical estimation:

$$Cp = 0.53(e^{-0.041t} - e^{-0.15t})$$

The insert curves show the logarithmic infusion data superimposed on the rapid (10-sec.) i.v. injection data. The logarithmic infusion was continued for 64 min. Therefore, not only is absorption taking place at the same time as the drug is distributing into the peripheral tissue compartment, but it continues well beyond the peak in the blood curve. Due to the relative rates of drug infusion and loss from the body, the apparent disappearance curve changes. The observed disposition half life, $0.693/\beta$, for the i.v. curve is 14 min., while due to the continued influence of the infusion (absorption) process, the disposition half-life from the simulated logarithmic absorption data becomes 17 min. Changes in half-life such as these have been occasionally proposed to be due to changes in the elimination rate during the test period. Wagner (11) has also emphasized that the absorption process continues far beyond $t_{max.}$, the time of the maximum blood level.

Figure 2*a* represents the amount absorbed, divided by the appropriate volume constants *versus* time plots for Eqs. 1 and 8. The convergence on a false early asymptote with Eq. 1 is illustrated. Figure 2*b* includes plot of the log percent remaining to be absorbed *versus* time plot of the same data. The ASA was infused at a rate such that the half-life was precisely 10.0 min. Analysis of the data using Eq. 1 results in an estimate of the half-life for absorption in error by several orders of magnitude. In contrast, calculations based on Eq. 8 almost exactly duplicate the known infusion data. When 3% or less remains to be infused, the infusion pump no longer produces a log infusion. It is in this region that the calculated data diverges from the theoretical curve.

Figure 3 contains data for a second test subject administered ASA by a rapid i.v. and log infusion at a precise rate equal to a half-life of 6.0 min. The i.v. data are for a 650-mg. and a 325-mg. dose of the drug. While there is some small amount of experimental error, if the two curves are normalized, they are superimposable and therefore result in identical distribution and elimination rate constants and volume of distribution. Again there is a slight increase in disposition half-life in the log infusion (absorption curve). Additional assay data beyond the time indicated were obtained out to the limit of the assay method (0.4 mcg./ml.) and all three curves show mono-exponential decay. Figure 4 includes semilog and linear plots of the percent remaining to be absorbed plots. Once again the complete absorp-

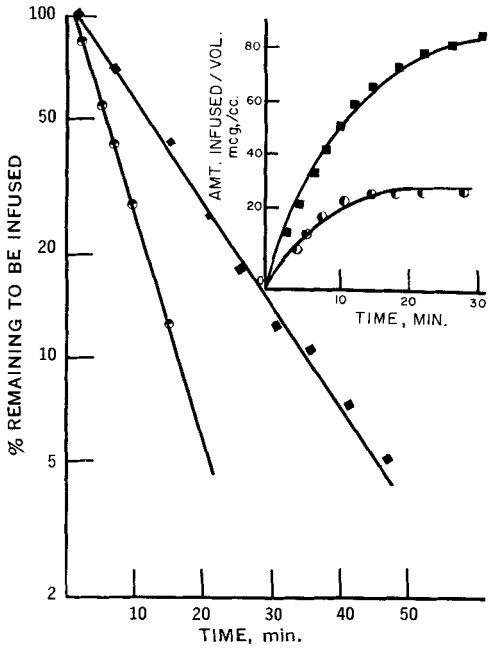


Fig. 2—The percent ASA remaining to be infused as determined by Eq. 1 (◐) and Eq. 8 (■). The latter data exactly coincide with the actual infusion rate ($t_{1/2}$ = 10.0 min.). Insert (Fig. 2a)—Corresponding amount infused divided by the volume constant intrinsic to the model, Eq. 1 (◐) and Eq. 8 (■).

tion equation predicts the known infusion rate, while it is difficult to draw a good curve through the W-N plot. When comparing Figs. 2, 4, and 6 it may appear that the magnitude of error in the Wagner-Nelson method is large only because of the infusion rates used and the selection of a rapidly metabolized drug such as ASA. That this is not so is seen where a PACE TR48 analog computer was programmed for distribution and elimination of griseofulvin found in the test subject reported in Figs. 5 and 6. Figure 7 includes curves illustrating the W-N plots which would have been found for this compound at selected first-order absorption rates. The disposition half-life was set at 9.5 hr. while the half-lives of the absorption processes were adjusted from 1.8 to 7 hr. When the input rates are large relative to the disposition rate constants, then the W-N calculation may result in an apparent first-order plot. However, the relative error in the estimated half-life varies with each experimental situation. The intermediate input rates result in concave descending-type curves to which it is difficult to assign a half-life. Usually the initial data points are emphasized in these analyses. However, it is important to note that the curves approach the theoretical half-lives near the end of the absorption process. Unfortunately, this is the portion of the data where the experimental error would be the largest. It would appear that if a given drug were made up as different formulations resulting in different rates of absorption, analysis of the blood data by Eq. 1 would apparently result in a varying error of estimation of their true absorption rates. The data for the theoretical input with a half-life of 3.8 hr. are replotted in Fig. 8 where the method of residuals is utilized to separate out the fast absorption component from the slower step. The appropriate bi-exponential equation for such a plot is:

$$\text{percent unabsorbed} = 0.45e^{-0.77t} + 0.55e^{0.183t}$$

It can be interpreted to indicate 45% of the drug was

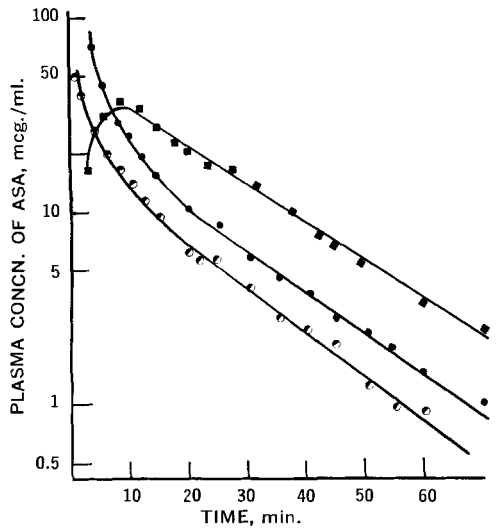


Fig. 3—Blood levels of ASA of subject P after single i.v. injection of 325 mg. (◐) and 650 mg. (●), and after a logarithmic infusion of 650 mg. ASA (■) at 0.115 min.$^{-1}$ ($t^1/_2$ = 6.0 min.).

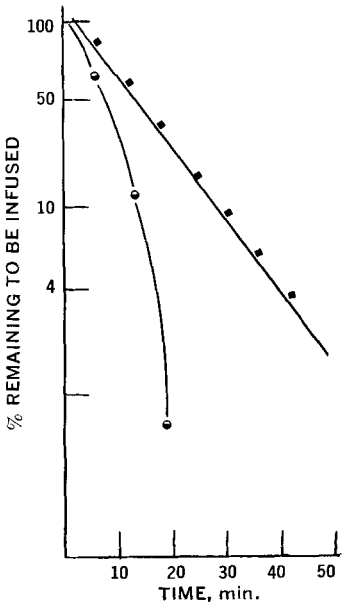


Fig. 4—The calculated percent ASA remaining to be infused as estimated by Eq. 1 (◐) and Eq. 8 (■). The latter is a good estimate of the actual infusion rate ($t_{1/2}$ = 6.0 min.).

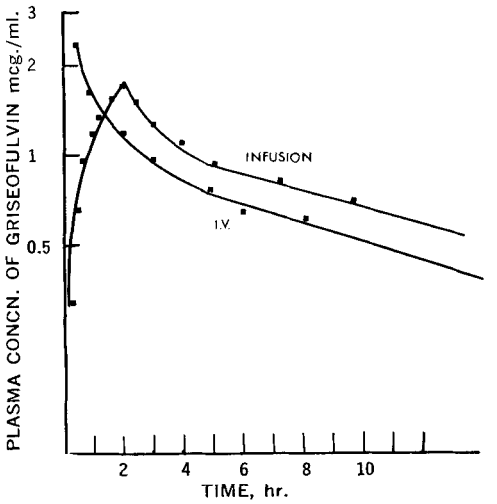


Fig. 5—The blood level of griseofulvin after a 2-hr. constant infusion at a rate of 12.8 mg./min. and after a single intravenous injection of 142 mg.

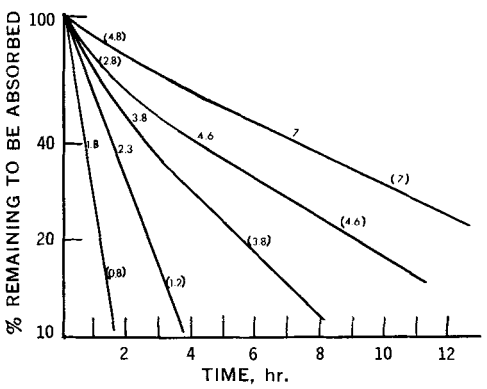


Fig. 7—Analog computer simulated plots of the percent remaining to be absorbed computed in accordance with Eq. 1. The values for the rate constants of the two-compartmental model are: $k_{12} = 0.29$ hr.$^{-1}$; $k_{21} = 0.31$ hr.$^{-1}$; $k_{el} = 0.16$ hr.$^{-1}$. The absorption half-life was changed for each plot and is indicated on each curve. The half-life values in parentheses are those which may be estimated from each appropriate linear segment.

absorbed at a half-life of 0.9 hr. while 55% was absorbed at a half-life of 3.8 hr. These are, of course, figments of the numerical method.

Using other appropriate distribution and elimination constants, the A/V *versus* time curves for the W-N method were obtained. It was noted that when the absorption rate is large relative to the rate of disappearance from the body that some of these curves exhibit a pronounced maxima. In their original publication Wagner and Nelson allude to this problem by indicating that the A/V value may reach a maximum. It appears that both the bi-exponential and the occasional appearance of maxima are artifacts of the numerical method. In Figs. 2*b* and 4, it can be seen from the percent unabsorbed plots that the use of Eq. 1 results in an underestimation of the time at which absorption ceases. This may cause difficulty in defining the release characteristics of prolonged-action dosage forms. It would appear virtually impossible to separate the perturbation of the absorption data caused by the use of the W-N equation from the complexities introduced by the two different absorption rates.

The absorption process of a drug is undoubtedly

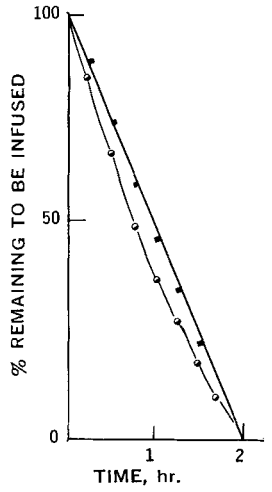


Fig. 6—The calculated percent griseofulvin remaining to be infused after a 2-hr. constant infusion. The curves are based on calculation using Eq. 1 (◐) and Eq. 8 (■); the latter is an excellent fit of the actual infusion rate curve.

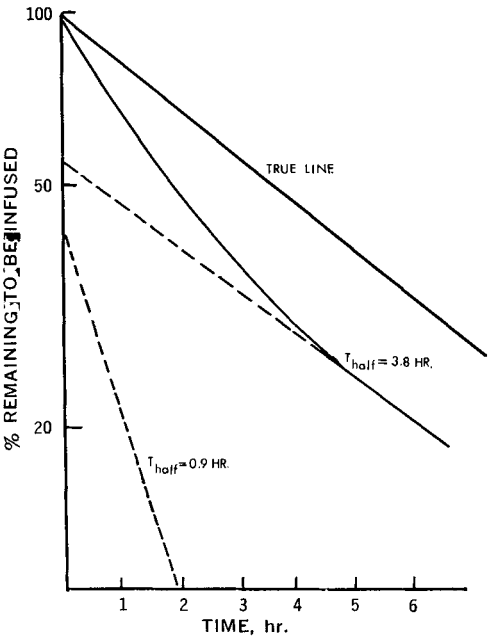


Fig. 8—An enlargement of the data given in Fig. 7 in which the input absorption half-life was set at 3.8 hr. The middle curve represents the data that would be obtained from Eq. 1. The lower curve is obtained from the middle curve by the method of residuals

highly complex. It is probable that each dose differs in its rate of passage down the gastrointestinal tract as well as in the rate at which the drug is released from its dosage form. If the new equation allows us to obtain valid data on such absorption rate processes, as it now appears, then it may allow some interpretation of the influence of the site of absorption on the rate process. This has been proposed as a significant contributing factor to the results obtained from the oral administration of griseofulvin to man. It appears that a significant fraction of the total griseofulvin is absorbed in a short interval of time which may coincide with the transfer of the drug through the duodenum. This is followed by a prolonged slow absorption for many additional hours (15). This small amount of secondary absorption causes a profound change in the apparent rate of disappearance of the drug from the blood. It would have been impossible to make any quantitative analysis of the absorption process of griseofulvin without the availability of i.v. data on this drug (15) along with this new method of analysis. The present authors have emphasized in earlier publications of this series (12, 13) that the plasma concentration time curves for virtually all drugs require utilization of a multi-exponential equation such as represented by Eq. 2. This is well recognized by many workers. However, until an i.v. dose is administered, with blood taken at proper sampling intervals, it is impossible to assess the contribution of the first term, Ae^{-at}, to the early phase of the drug disposition. The W-N equation is derived on the presumption that this first term is negligible. The modified absorption equation does not make this presumption. It is based only on the following two assumptions: (*a*) that the i.v. blood curve can be defined by a set of first-order differential equations, and (*b*) that the values of the rate constants are independent of dosage level and time. The data presented in this study have been shown to comply with those restrictions within the dosage ranges studied.

The two-compartmental model derivation has resulted in three important changes in the W-N equation. First, the very important peripheral tissue compartmental term Ct has been included. Second, the model forces one to distinguish between the rate constant for the disappearance of the compound from the body and the specific elimination rate constant, k_{el}. The former constant is a hybrid constant and is a complex function of the distribution rate constants k_{12} and k_{21} as well as the elimination rate constant, k_{el}. In most drugs the elimination rate constant, k_{el}, calculated on the multicompartmental model is two to fivefold larger than the slowest disposition rate constant. However, with drugs such as digitoxin, it may be as much as 40 times larger than the slowest disposition rate constant. Third, the volume constant used to convert the amounts into concentrations is found to be the volume of the central compartment, Vp, rather than the apparent total volume of distribution Vd_{area} (11). The effect of the first two modifications influences the shape of the A/V *versus* time plot, and results in evidence for a longer period for the absorption before the asymptotic value is reached. The third term merely changes the absolute value of the A/V.

It should be noted that the new absorption equation, derived on a two-compartmental open-system model, can be expanded to include additional peripheral compartments when necessary. For example, if the i.v. data indicates that a tri-exponential equation is required to describe the total curve, it would be necessary to expand the mammillary model to two peripheral compartments. One would then estimate the distribution rate constants for each compartment (13, 16). Finally, during an absorption study of such a drug one would have to estimate the tissue concentration in each peripheral compartment at each data point time by using Eq. 7 written to include the unique distribution rate constants of each compartment.

One criticism which might be made against the new equation is the dependency it places upon the evaluation of the distribution and elimination rate constants from previously administered i.v. dose. Undoubtedly there will be instances when these constants will change with continued use of the drug and the absorption test should be conducted as soon as possible after the i.v. experiment. Further, the effect of possible changes in the elimination rates, such as might be caused by induction of drug metabolizing enzymes can best be evaluated on the basis of the multicompartmental model. It is believed, however, that some of the variation in the disposition rate constant reported by some observers is due to the variable influence of the absorption rate process on the disposition rate constant. This can be seen in Fig. 9 by comparing the i.v. and oral curves which have been generated on theoretical data derived in the appendix and in experimental data shown in Figs. 3 and 4.

Absorption Analysis Based on Urinary Excretion Data—A relatively large number of the analyses of absorption rates published to date have been made using the W-N equation derived for the analysis of urinary data, which is:

$$f(A)_{t_n} = \frac{1}{k}\left(\frac{d\,Ae}{dt}\right)_{t_n} + (Ae)_{t_n} \qquad \text{(Eq. 9)}$$

$(Ae)_{t_n}$ is the cumulative amount excreted up to time t_n and $(dAe/dt)_{t_n}$ is the rate of excretion estimated from the differential data from t_{n-1} to t_n, and f is the fraction of the administered drug excreted intact. The first term on the right-hand side represents the amount of the absorbed dose remaining in the body and the second term is to represent the amount eliminated by all processes.

Application of the two-compartmental open-system model to urinary data requires insertion of a term for the tissue compartment. The resultant equation is:

$$f(A)_{t_n} = k_{el}\left[\left(\frac{dAe}{dt}\right) + Ae\right]_{t_n} + T_n \qquad \text{(Eq. 10)}$$

where

$$T_n = \frac{1}{k_{el}}\left[\left(\frac{dAe}{dt}\right)_{t_{n-1}} \cdot \frac{k_{12}}{k_{21}}\left(1 - e^{-k_{21}\Delta t}\right)\right] + \frac{1}{k_{el}}\left[\left(\frac{dAe}{dt}\right)_{t_n} - \left(\frac{dAe}{dt}\right)_{t_{n-1}}\right]\frac{k_{12}\Delta t}{2} + T_{n-1}e^{-k_{21}\Delta t}$$

It is immediately apparent that the application of this equation to urinary excretion data will be difficult. With some drugs, such as amines, it is extremely difficult to detect the level of these com-

pounds in the blood without recourse to radioactive tracers while they may be conveniently and more simply followed by urinary excretion methods. Nevertheless, to apply Eq. 10, it will be necessary to define the distribution and elimination rate constants from the urinary data after an i.v. injection. This will require urine samples at short intervals in the first few hours or may at times necessitate collection of the samples by catheterization. If urine samples are taken at sufficiently frequent intervals, under conditions of controlled pH, the urinary excretion data should also result in a bi-exponential curve, which upon being normalized may be analyzed for the appropriate distribution and elimination rate constants.

Alternate Methods for Analyzing Blood Data—The alternative analytical methods for estimating absorption rates utilizing both i.v. and oral data published by earlier authors have not been utilized in pharmacokinetic literature on drug absorption. The method of Scholer and Code was utilized by these authors in studying the oral absorption of D_2O (6) and later by Graham *et al.* (17) in their study of the absorption of ^{28}Mg. This method is a valid, but extremely tedious method of analyzing for the absorption rate of the compound. The method utilizes the multi-exponential equation representing the fate of the compound in the body (obtained in a prior i.v. experiment) to estimate numerically the fate of each increment of dose at each data point. This method, therefore, necessitates a large number of repetitive calculations, *i.e.*, N^2, where N is the number of data points during the interval where absorption is taking place. In contrast, Eq. 9 requires one calculation per data point.

The method of Silverman and Burgen and the method of Recigno and Segre each directly utilize the multi-exponential equation representing the fate of the drug in the body after an i.v. dose. In these methods the absorption data are compared to the curve of the i.v. dose and an amount remaining to be absorbed is obtained by a mathematical procedure of deconvolution of the oral absorption data. The method of Silverman and Burgen utilizes an analog computer while the latter method is a numerical procedure, which also may be carried out by utilizing a digital computer (18).

The method proposed in this paper utilizing Eq. 8 differs in an important characteristic from these alternative methods. While requiring prior information as to the multi-exponential fate of the drug in the body, a two- or three-compartmental open-system model is defined and the distribution and elimination constants are evaluated. The method does not require comparison with the i.v. curve itself but utilizes the distribution and elimination rate constants in the equation. It therefore allows one to carry out this calculation using a simple desk calculator. It also affords an excellent picture of the fate of the drug within the body at all times.

APPENDIX

A Derivation of Eq. 7—As noted in the text, Eq. 6 is the differential equation representing the rate of change of the tissue concentration in terms of the two variables, Ct and Cp. It is necessary to substitute for the Cp at time t_n in terms of the prior blood value Cp at time t_{n-1}. This can be done if we presume that the plasma concentration time curve is linear over this short interval so that the equation for the straight line applies. This is represented as Eq. 6 in the text. Equation 5 can be written as:

$$\frac{dCt}{d\tau} = -k_{21}Ct + k_{12}Cp + k_{12}M\tau \quad \text{(Eq. 1}a\text{)}$$

where $\tau = t_n - t_{n-1}$.

In order to integrate Eq. 1a, we convert to Laplace notation, with $\overline{Ct}$ referring to the Laplace transform of Ct, with the initial conditions set at $(Ct)_{t_{n-1}}$ and S is the Laplace operator notation:

$$\overline{Ct}S - Ct_{t_{n-1}} = -k_{21}\,\overline{Ct} + \frac{k_{12}\,Cp}{S}\,t_{n-1} + \frac{k_{12}M}{S^2} \quad \text{(Eq. 2}a\text{)}$$

Rearranging we have:

$$\overline{Ct}(S + k_{21}) = Ct_{t_{n-1}} + \frac{k_{12}Cp}{S}\,t_{n-1} + \frac{k_{12}M}{S^2} \quad \text{(Eq. 3}a\text{)}$$

or

$$\overline{Ct} = \frac{Ct}{S + k_{21}}\,t_{n-1} + \frac{k_{12}Cp}{S(S + k_{21})}\,t_{n-1} + \frac{k_{21}M}{S^2(S + k_{21})} \quad \text{(Eq. 4}a\text{)}$$

The inverse transform of Eq. 4a is

$$Ct_{t_n} = Ct_{t_{n-1}}e^{-k_{21}\Delta t} + \frac{k_{12}}{k_{21}}\,Cpt_{n-1}(1 - 3^{-k_{21}\Delta t}) + \frac{k_{12}}{(k_{21})^2}\,\frac{\Delta Cp}{\Delta t}\,(e^{-k_{21}\Delta t} + k_{21}\Delta t - 1) \quad \text{(Eq. 5}a\text{)}$$

where Δt is substituted for τ and $\Delta Cp/\Delta t$ for M. The third term of Eq. 5a can be reduced by substituting for the exponential component in terms of a two-term Taylor expansion, namely, $e^{-x} = 1 - x + x^2/2$, or

$$\frac{k_{12}}{(k_{21})^2}\,\frac{\Delta Cp}{\Delta t}\,(e^{-k_{21}\Delta t} + k_{21}\Delta t - 1) = \frac{k_{12}}{k_{21}}\,2\,\frac{\Delta Cp}{\Delta t}\left(1 - k_{21}\Delta t + \frac{(k_{21})^2(\Delta t)^2}{2} + k_{21}\Delta t - 1\right) \quad \text{(Eq. 6}a\text{)}$$

which reduces to $k_{12}\Delta Cp\Delta t/2$.

Substituting into Eq. 5a we arrive at the form given as Eq. 7 in the above text:

$$Ct_{t_n} = Ct_{t_{n-1}}e^{-k_{21}\Delta t} + \frac{k_{21}}{k_{12}}\,Cpt_{n-1}\,(1 - e^{-k_{21}\Delta t}) + \frac{k_{12}\Delta Cp\Delta t}{2} \quad \text{(Eq. 7)}$$

Sample Calculation Using Eq. 7—The following model will be utilized:

$$D \xrightarrow[k_a = 0.41\ \text{hr.}^{-1}]{} P \xrightarrow[k_{el} = 0.16\ \text{hr.}^{-1}]{} ME$$

$$P \underset{k_{21} = 0.31\ \text{hr.}^{-1}}{\overset{k_{12} = 0.29\ \text{hr.}^{-1}}{\rightleftarrows}} T$$

This results in a disposition rate constant, β, of 0.0725 hr.$^{-1}$ ($t_{1/2}$ = 9.5 hr.) after sufficient time for the absorption process to be complete. However, Table I includes data points for Cp at various times up to 15 hr., generated with the use of the TR-48 analog computer. These data are plotted in Fig. 9.

TABLE I—CALCULATIONS OF *Ct* VALUES[a]

1	2	3	4	5	6	7	8	9	10	11	12
Cp_{t_n}	$(t)_{t_n}$	ΔCp	Δt	$(k_{12}\Delta Cp\,\Delta t)/2$	$Cp_{t_{n-1}}$	$k_{12}/k_{21}(1-e^{-k_{21}\Delta t})$	$Cp_{t_{n-1}}k_{12}/k_{21}\times(1-e^{-k_{21}\Delta t})$	$Ct_{t_{n-1}}$	$e^{-k_{21}\Delta t}$	$Ct_{t_{n-1}}e^{-k_{21}\Delta t}$	$Ct_n = 5+8+11$
3.00	0.5	3.0	0.5	0.218	0	0.134	0	0	0.857	0	0.218
5.20	1.0	2.2	0.5	0.160	3.00	0.134	0.402	0.218	0.857	0.187	0.749
6.50	1.5	1.3	0.5	0.094	5.20	0.134	0.697	0.749	0.857	0.642	1.433
7.30	2.0	0.8	0.5	0.058	6.50	0.134	0.871	1.433	0.857	1.228	2.157
7.60	2.5	0.3	0.5	0.022	7.30	0.134	0.978	2.157	0.857	1.849	2.849
7.75	3.0	0.15	0.5	0.011	7.60	0.134	1.018	2.849	0.857	2.442	3.471
7.70	3.5	0.05	0.5	0.004	7.75	0.134	1.039	3.471	0.857	2.976	4.019
7.60	4.0	−0.10	0.5	−0.007	7.70	0.134	1.032	4.019	0.857	3.444	4.469
7.10	5.0	−0.50	1.0	−0.073	7.60	0.250	1.900	4.469	0.733	3.276	5.103
6.60	6.0	−0.50	1.0	−0.073	7.10	0.250	1.775	5.103	0.733	3.740	5.442
6.00	7.0	−0.60	1.0	−0.087	6.60	0.250	1.650	5.442	0.733	3.989	5.552
5.10	9.0	−0.90	2.0	−0.261	6.00	0.432	2.592	5.552	0.538	2.987	5.318
4.40	11.0	−0.70	2.0	−0.203	5.10	0.432	2.203	5.318	0.538	2.861	4.861
3.30	15.0	−1.10	4.0	−0.638	4.40	0.720	3.168	4.861	0.280	1.361	3.891

[a] Calculated using the following rate constants: $k_{12} = 0.29$ hr.$^{-1}$; $k_{21} = 0.31$ hr.$^{-1}$; $k_{el} = 0.16$ hr.$^{-1}$; $\beta = .0725$ hr.$^{-1}$; $Ct_n = k_{12}\Delta Cp\,\Delta t/2 + k_{12}/k_{21}Cp_{t_{n-1}}(1-e^{-k_{21}\Delta t}) + Ct_{t_{n-1}}e^{-k_{21}\Delta t}$.

Fig. 9—Simulated data for a theoretical drug with distribution and elimination characteristics given in Fig. 7 and used in calculation for Tables I and II. Note the slight divergence of the slopes of the i.v. and the oral curves. The straight line indicates the log percent remaining to be absorbed ($t_{1/2}$ = *1.8 hr.*).

It is to be noted that the data points beyond the fourth hour appear to lie on a straight line even though 20% of the drug remains to be absorbed ($t_{1/2}$ for the absorption = 1.8 hr.). One would usually define this slope as the elimination rate constant; more appropriately in the context of the nomenclature of this paper it is called the "apparent" disposition rate constant with a half-life of 8.9 hr. (β = 0.078 hr.$^{-1}$). It is to be noted that the value is not identical with the half-life for the true disposition rate process, *i.e.*, 9.5 hr. since the absorption and the fast disposition component, α, are still contributing to the observed value. Columns 3 through 12 include the steps required in the numerical calculation of the values for Ct_{t_n} utilizing Eq. 7. The accuracy of the estimates of *Ct* can be checked in this instance since the analog computer can generate the tissue concentration curve. The solid line in Fig. 10 increases the computer generated curve and the superimposed open circles indicate the calculated values as listed in Table I, Column 12. The excellent fit is readily apparent.

The estimation of the absorption rate constant from the data utilizing Eq. 7 is given in Table II. The appropriate steps are those used in the original W-N equation, in which the area under the blood curve between each set of data points is estimated by the trapezoidal rule. These are listed in Column 3. These values are progressively summed from t_o to t_n in Column 4, and the cumulative sum of these estimates are multiplied by the known elimination rate constant k_{el}. The A/Vp values are then calculated from the sum of Columns 2 + 5 + 6. These data can be converted into the percent unabsorbed as indicated.

Table II also includes the calculation of the

TABLE II—CALCULATIONS OF PERCENTAGE UNABSORBED VALUES[a]

1	2	3	4	5	6	7	8	9	10	11	12	13
Time, hr.	Cp_{t_n}	$\int_{n-1}^{t_n} Cpdt$	$\int_0^{t_n} Cpdt$	$k_{el}\int_0^{t_n} Cpdt$	Clt_n	$A/Vp = 2+5+6$	% A/Vp	100% − A/Vp%	$\beta\int_0^{t_n} Cpdt$	$A/Vd = 2+10$	% A/Vd	100 − % A/Vd
0.5	3.00	0.750	0.750	0.120	0.218	3.338	16.6	83.4	0.054	3.045	32.5	67.5
1.0	5.20	2.050	2.800	0.448	0.749	6.397	31.8	68.2	0.203	5.403	57.5	42.5
1.5	6.50	2.925	5.725	0.916	1.433	8.849	44.0	56.0	0.415	6.915	73.6	26.4
2.0	7.30	3.450	9.175	1.468	2.157	10.925	54.3	45.7	0.665	7.965	84.7	15.3
2.5	7.60	3.725	12.900	2.064	2.849	12.513	62.2	37.8	0.935	8.535	90.8	9.2
3.0	7.75	3.838	16.738	2.678	3.471	13.889	69.1	30.9	1.214	8.964	95.4	4.4
3.5	7.70	3.863	20.601	3.296	4.019	15.015	74.6	25.4	1.494	9.194	97.8	2.2
4.0	7.60	3.825	24.426	3.908	4.469	15.977	79.4	20.6	1.771	9.371	99.7	0.3
5.0	7.10	7.350	31.726	5.084	5.103	17.287	85.9	14.1	2.300	9.400	100.0	0
6.0	6.60	6.850	38.626	6.180	5.442	18.222	90.6	9.4	2.800	9.400		
7.0	6.00	6.300	44.926	7.188	5.552	18.740	93.1	6.9	3.257	9.257		
9.0	5.10	11.100	56.026	8.964	5.318	19.382	96.3	3.7	4.062	9.162		
11.0	4.40	9.500	65.526	10.484	4.861	19.745	98.1	1.9	4.751	9.151		
15.0	3.30	15.400	80.926	12.948	3.891	20.139	100.0	0	5.867	9.167		

[a] Columns 1-9: $A/Vp = Cp_{t_n} + k_{el}\int_0^{t_n} Cpdt + Clt_n$; $Clt_n = k_{12}\Delta Cp\Delta t/2 + k_{12}/k_{21}\, Cp_{t_{n-1}}(1 - e^{-k_{21}\Delta t}) + Clt_{n-1}e^{-k_{21}\Delta t}$. Columns 10-13: $A/Vd = Cp_{t_n} + \beta\int_0^{t_n} Cpdt$.

Fig. 10—Simulated plots of data taken from Table II simulating the blood level (Column 2; ●), tissue level (Column 6; ○), and drug elimination (Column 5; ■). The solid lines are the computer simulated curves. The insert includes the percent remaining to be absorbed plots (Column 9; ●) calculated using Eq. 8 and Eq. 1 (Column 13; □).

"apparent" percent unabsorbed according to the W-N equation. This is listed in Columns 10 through 13. Figure 10 includes the semilog plots of the percent unabsorbed, according to the two methods of calculation, *i.e.*, Columns 9 *versus* 13. It is noted that the calculations based on Eq. 8 (Column 9) result in an exact prediction of the known absorption half-life of 1.8 hr. while the W-N equation (Column 13) leads to a prediction that the half-life for the absorption process is approximately 0.8 hr.

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Keyphrases

Absorption rate, intrinsic—drugs
Drug absorption, distribution, elimination—models
Body considered—one, two compartments
Compartments, two *vs.* one—drug absorption
Kinetic equations—drug absorption
Acetylsalicylic acid salt—test compound
Griseofulvin—test compound

Effect of Topical Vehicle Composition on the *In Vitro* Release of Fluocinolone Acetonide and its Acetate Ester

By B. J. POULSEN, E. YOUNG, V. COQUILLA, and M. KATZ

A model was developed to test certain concepts regarding the *in vitro* release of steroids from topical vehicles. The steroids studied were fluocinolone acetonide and fluocinolone acetonide acetate. The vehicles selected for the study were propylene glycol–water mixtures gelled with Carbopol 934. Isopropyl myristate was used as the receptor phase for the diffusing steroids. Assay of the quantity of the steroids in the receptor phase was simplified by the use of radioactive compounds. The study indicated that the important factors influencing the release of steroid into the receptor phase were the solubility in the vehicle and the partition coefficient of the steroid between the vehicle and the receptor phase. Maximum release was achieved by altering the propylene glycol content of the vehicles.

OCCLUSIVE DRESSINGS have been successfully used to extend the therapeutic effectiveness of topical corticosteroids (1–3). This enhanced activity has been attributed to improved penetration of the skin by the drug (1). This indicates that, in certain instances, the development of formulations that increase drug penetration would be desirable.

There are two general approaches to the problem. One is to include in the vehicle agents which affect the barrier function of the epidermis so as to promote penetration of the therapeutic compound (4–7). The other approach is to alter the physical characteristics of the vehicle and thus affect the diffusion of the drug from the vehicle into the skin. As a preliminary to evaluating this latter effect of vehicles on skin penetration, the *in vitro* release of steroids from model vehicles was studied.

Higuchi (8) suggested the following equivalent relationships could be used to approximate the penetration of the barrier phase of the skin by a drug dissolved in a topical vehicle.

$$\frac{dQ}{dt} = (P.C.)C_v\frac{DA}{L} \qquad \text{(Eq. 1)}$$

$$\frac{dQ}{dt} = \frac{a_v}{\gamma_s}\frac{DA}{L} \qquad \text{(Eq. 2)}$$

The terms in these equations are defined as follows:

dQ/dt = steady rate of penetration
$(P.C.)$ = the effective partition coefficient of drug between skin barrier and vehicle
C_v = concentration of drug in the vehicle
D = the effective average diffusivity of the drug in the skin barrier
A = cross-sectional area of the application site
L = effective thickness of the skin barrier
a_v = thermodynamic activity of the drug in the vehicle

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