tions containing more than 40% DMSO does not prevent damage to erythrocytes and/or the precipitation of blood components. It appears that aqueous solutions containing more than 40% DMSO would be unsafe as a vehicle for intravenous preparations.

To determine the effect of a ternary solvent system on erythrocytes, hemolysis experiments were run at 37° in solutions containing 20% DMSO, 0.9% sodium chloride, and various amounts of either propylene glycol or polyethylene glycol 300 (PEG 300). Solutions containing 0.0 to 30% propylene glycol did not hemolyze red blood cells, however, complete hemolysis with slight discoloration took place in those solutions containing more than 30% propylene glycol. Hemolysis did not take place in solutions containing 0.0 to 20% PEG 300; however, when blood was placed in solutions containing 25% or more PEG 300, the solutions became green-brown, and a brown precipitate formed. In these ternary solvent systems, the addition of DMSO did not alter the critical concentrations (in 0.9% saline) (2, 3) at which propylene glycol and PEG 300 have been reported to hemolyze red blood cells. The damaging effect of the glycols appeared to be solely dependent on their concentration in solution, and there was no additive effect contributed by the DMSO present.

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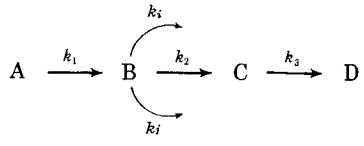
Fallacy in Concluding There are Zero-Order Kinetics from Blood Level and Urinary Excretion Data

By JOHN G. WAGNER

During the past several years there have been a number of reports in which the authors concluded their data proved zero-order formation of a metabolite or zero-order absorption of a drug in the animal or human body. For two of these reviewed in this paper—namely, the conjugation of benzoate with glycine in the rabbit following doses of 500 mg./Kg. of benzoic acid and the conversion of salicylate to salicyluarate in rats at doses above 20 mg./Kg.—the evidence is very convincing. However, the principal evidence in the remainder of the reports is the apparent linearity of a segment of a cumulative urinary excretion curve and/or the curvature of semilogarithmic plots of drug blood levels or of amounts of drug not excreted against time. Model studies and simulations presented here show that neither of the latter is sufficient evidence for concluding there is zero-order rate of formation of a metabolite or zero-order absorption of a drug. The simulations of pharmacokinetic data have far-reaching implications which go much beyond the zero-order–first-order problem. They show one cannot disregard first-order rate constants of relatively large magnitude in many cases; that graphical fitting of pharmacokinetic, and even chemical kinetic, data may often lead to serious misinterpretations; and that "Lineweaver-Burk" plots, which are artifacts, are produced by plotting the reciprocal of excretion rate of a metabolite against the reciprocal of the amount of metabolite remaining in the body.

To illustrate how one may erroneously conclude that there are parallel zero-order and first order kinetics when, in fact, all kinetics are first order, a model simulating the real situation is presented. Assume there is a catenary chain with branching of parallel paths at one point in the chain and that all rate constants are first-order rate constants. Then we may write the model as shown in Scheme I.



Scheme I

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Let k be the first-order rate constant for over-all loss from the B compartment. Hence, as written, we have $k = k_2 + k_i + k_j$. If there are no parallel paths, then $k = k_2$. If there is an additional parallel path initiating at B, with rate constant k_p, then $k = k_2 + k_i + k_j + k_p$. The kinetics of the catenary chain depend only on the values of k, k_1, k_2, and k_3; hence, it is necessary only to know k and not its components k_i, k_j, k_p, *etc.* The solutions below are applicable in all cases where no two or more of the constants k, k_1, k_2, and k_3 are exactly equal.

In the model, A represents a drug at the absorption site and B represents the drug in blood and other fluids of distribution. In the real world, the A → B process may be quite complicated and the process may only be approximated by first-order kinetics. However, it is well documented (1–7) that the over-all effects of dissolution of solid drug, stomach emptying, and absorption of the drug through the stomach and/or small intestinal barriers may be represented, usually by a first-order rate constant with or without a small lag time. In the case of acetylsalicylic acid (ASA) in tablet form, A would represent ASA in tablets, and B would represent salicylate in blood and other fluids of distribution. The over-all effects of tablet disintegration, dissolution of ASA from the resulting granules, stomach emptying, absorption of ASA from the stomach and small intestine, and hydrolysis of the ASA to salicylate have been shown (2, 5) to be represented by a combination of a first-order rate constant and a small lag time.

In the model, C represents a metabolite which is formed from B, and D represents the cumulated metabolite excreted in the urine. Interpretation of blood level and urinary excretion data has revealed that *usually* the apparent rate constant for absorption (k_1 in the model) and the rate constant for urinary excretion of metabolites (k_3 in the model) are greater in magnitude than the rate constant for over-all loss of drug from the body (k in the model). To simulate the usual kinetic situation the numerical values of k_1 and k_3 have been made larger than the numerical value of k in the examples. The rate constant for formation of metabolite (k_2 in the model) will always be less than k if there are parallel paths, and the ratio k_2/k gives the fraction of the total drug reaching the circulation which is converted to the metabolite. Hence, the ultimate amount in D will be this fraction multiplied by total amount of drug reaching the circulation. It is assumed that the amounts in compartments A, B, C, and D are all in the same mass units and in terms of one of the substances, since the substance in C and D is different than the substance in A and B.

The principal purpose of the model and the examples is to show that even when k_1 and k_3 are relatively large they become important in the kinetics in those situations when $1 < k_1/k < 100$ and/or $1 < k_3/k < 100$. Also, neither k_1 nor k_3 can be ignored, without appreciable error, when $1 < k_1/k < 10$ and/or $1 < k_3/k < 10$! These statements are valid when k_1 and k_3 are both greater than k. Since k_1 is usually in the range 0.5–2 hr.$^{-1}$, as estimated from actual data (1–7), and k_3 is usually in the range 0.07–2 hr.$^{-1}$ as estimated from actual data (8–11), then k_1 and k_3 cannot be ignored, without appreciable error, with drugs (simulated by B) whose k values range from 0.007 to 2 hr.$^{-1}$ or whose $t_{1/2}$ values ($0.693/k$) range from 0.3 to 99 hr.! This includes the vast majority of drugs which the author has been privileged to investigate and read about during the past 15 years. Another purpose of the examples is to show, as stated above, how one may erroneously conclude there are parallel zero-order and first-order processes when, in fact, all processes are first order. This error can be made readily when $1 < k_1/k < 10$ and $1 < k_3/k < 10$ since, in such cases, plots of both the cumulative amount of B converted to C *versus* time and the cumulative excretion plot are only slightly curved for a considerable period of time and may be erroneously interpreted as being linear. But this interpretation is incorrect. Following the assumption of linearity the conclusion can be made that the formation step, B → C, is obeying zero-order kinetics and, hence, this leads to the conclusion that there are parallel zero-order and first-order kinetics for loss of the drug from the body. Proof of zero-order rate of formation of a metabolite or zero-order rate of absorption of a drug requires, for any given drug, more evidence than just the appearance of the cumulative urinary excretion and semilogarithmic blood level plots. The experiments needed to provide the necessary additional data are usually feasible and have been performed with specific drugs by some investigators.

EXPERIMENTAL

Refer to Scheme I. Let X_A, X_B, X_C, and X_D be the amounts in compartments A, B, C, and D, respectively, at time t. Let X_A^0 be the initial amount in compartment A at time zero. Assume that $X_B = X_C = X_D = 0$ at time zero.

The differential equations and their solutions for the model are as follows:

$$\frac{-dX_A}{dt} = k_1 X_A \qquad \text{(Eq. 1)}$$

and

$$X_A = X_A^0 e^{-k_1 t} \quad \text{(Eq. 2)}$$

$$\frac{dX_B}{dt} = k_1 X_A - k X_B \quad \text{(Eq. 3)}$$

and

$$X_B = X_A^0 \left(\frac{k_1}{k_1 - k}\right)[e^{-kt} - e^{-k_1 t}] \quad \text{(Eq. 4)}$$

$$\frac{dX_C}{dt} = k_2 X_B - k_3 X_C \quad \text{(Eq. 5)}$$

and

$$X_C = X_A^0 \left(\frac{k_2}{k}\right)\left[\frac{-k_1 k e^{-k_1 t}}{(k_1 - k)(k_3 - k_1)} + \frac{k_1 k e^{-kt}}{(k_1 - k)(k_3 - k)} + \frac{k_1 k e^{-k_3 t}}{(k_3 - k_1)(k_3 - k)}\right] \quad \text{(Eq. 6)}$$

$$\frac{dX_D}{dt} = k_3 X_C \quad \text{(Eq. 7)}$$

and

$$X_D = X_A^0 \left(\frac{k_2}{k}\right)\left[\frac{-k k_3 (1 - e^{-k_1 t})}{(k_1 - k)(k_3 - k_1)} + \frac{k_1 k_3 (1 - e^{-kt})}{(k_1 - k)(k_3 - k)} + \frac{k_1 k (1 - e^{-k_3 t})}{(k_3 - k_1)(k_3 - k)}\right] \quad \text{(Eq. 8)}$$

For computational and instructional purposes, it is best to let $t = n/k$. Hence, n is the number of time constants where the time constant is $1/k$. Also, if we let $t_{1/2} = 0.693/k$, then $1/k = 1.44 t_{1/2}$. Since, in both examples below, $k = 0.25$ hr.$^{-1}$, then $t = 4n$ and $6n$ represents 24 hr. All graphs have been drawn with two abscissa scales; one scale is in terms of n, the other in terms of t. With k the smallest of the rate constants appearing in the exponential terms of Eqs. 4, 6, and 8, the n scale indicates it requires about 6 time constants or 8.6 half-lives of the drug to clear essentially all of the drug and metabolite from compartments B and C, respectively, and to reach essentially the asymptotic amount in compartment D.

Levy (11) has implied that the critical ratio is k_3/k_2; that is, the ratio of the excretion rate constant to the formation rate constant of the metabolite. This ratio is *not* critical unless $k_2/k \to 1$, *i.e.*, unless $k_2 \to k$. This can be seen by substituting n/k for t in the exponential e^{-kt} which yields e^{-n}; similar substitutions change $e^{-k_1 t}$ to $e^{-(k_1/k)n}$ and change $e^{-k_3 t}$ to $e^{-(k_3/k)n}$. Hence, the critical ratios are k_1/k and k_3/k, not k_3/k_2. The ratio k_2/k is equivalent to the fraction of B converted to C and which ultimately appears in D. In the case of the salicylurate–salicylate system $k_2/k \simeq 0.6$ and the ratios k_1/k and k_3/k become quite critical due to the order of magnitudes of the rate constants.

Example 1—Assignments made were $k_1 = 0.5$ hr.$^{-1}$, $k = 0.25$ hr.$^{-1}$, $k_2 = 0.15$ hr.$^{-1}$, and $k_3 = 0.75$ hr.$^{-1}$. Hence, $k_1/k = 2$, $k_2/k = 0.6$, and $k_3/k = 3$. For these numerical values, Eqs. 2, 4, 6, and 8 are reduced to Eqs. 9, 10, 11, and 12, respectively, if we let $X_A^0 = 100$ units.

$$X_A = 100e^{-2n} \quad \text{(Eq. 9)}$$

$$X_B = 200[e^{-n} - e^{-2n}] \quad \text{(Eq. 10)}$$

$$X_C = 60[e^{-n} - 2e^{-2n} + e^{-3n}] \quad \text{(Eq. 11)}$$

$$X_D = 60[1 - 3e^{-n} + 3e^{-2n} - e^{-3n}] \quad \text{(Eq. 12)}$$

Also, the rate of formation of C is given by

$$k_2 X_B = 30[e^{-n} - e^{-2n}] \quad \text{(Eq. 13)}$$

And the rate of appearance of C in D is given by

$$\frac{dX_D}{dt} = k_3 X_C = 45[e^{-n} - 2e^{-2n} + e^{-3n}] \quad \text{(Eq. 14)}$$

The cumulative amount of B converted to C in time T is given by

$$k_2 \int_0^T X_B \cdot dt = \left[\left(\frac{1}{k_3} \cdot \frac{dX_D}{dn}\right) + X_D\right] \quad \text{(Eq. 15)}$$

It should be clear that in Eqs. 9 through 14 terms with exponent $[-n]$ are associated with k, terms with exponent $[-2n]$ are associated with k_1, and terms with exponent $[-3n]$ are associated with k_3 in this example. The constants 2 and 3 in these terms are just the ratios k_1/k and k_3/k, respectively. As n increases, the order of magnitudes will always be $e^{-n} > e^{-2n} > e^{-3n}$. Also, as t increases, the order of magnitudes will be $e^{-kt} > e^{-(k_1/k)t} > e^{-(k_3/k)t}$ if $k_3 > k_1 > k$, and the order will be $e^{-kt} > e^{-(k_3/k)t} > e^{-(k_1/k)t}$ if $k_1 > k_3 > k$. Hence, the ratios k_1/k and k_3/k are critical as indicated above. If the numerical values of these ratios are very close, there will be very little curvature in a large segment of the X_D, t plot.

Equations 9 through 15 were solved for $n = 0.05$, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.55, 0.6, 0.65, 0.7, 0.8, 0.9, 0.95, 1, 1.125, 1.25, 1.375, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6. In addition, values of $\Delta X_D/\Delta t$ were calculated corresponding to midpoint times of 0.2, 0.6, 1, 1.6, 2.2, 2.6, 3.2, 3.8, 4.5, 5.5, 7, 10, 14, 18, and 22 hr. The latter were intended to simulate the estimation of urinary excretion rates from analysis of fractional urine collections made at $t = 0.4$, 0.8, 1.2, 2, 2.4, 2.8, 3.6, 4, 5, 6, 8, 12, 16, 20, and 24 hr.

Example 2—Assignments made were $k_1 = 1.25$ hr.$^{-1}$, $k = 0.25$ hr.$^{-1}$, $k_2 = 0.15$ hr.$^{-1}$, and $k_3 = 1.0$ hr.$^{-1}$. Hence $k_1/k = 5$, $k_2/k = 0.6$, and $k_3/k = 4$. For these numerical values of the constants, Eqs. 2, 4, 6, and 8 become Eqs. 16, 17, 18, and 19, respectively, if $X_A^0 = 100$ units.

$$X_A = 100e^{-5n} \quad \text{(Eq. 16)}$$

$$X_B = 125[e^{-n} - e^{-5n}] \quad \text{(Eq. 17)}$$

$$X_C = 60[0.416e^{-n} - 1.66e^{-4n} + 1.25e^{-5n}] \quad \text{(Eq. 18)}$$

$$X_D = 60[1 - 1.66e^{-n} + 1.66e^{-4n} - e^{-5n}] \quad \text{(Eq. 19)}$$

RESULTS

Example 1—The time course of the amounts in compartments A, B, C, and D is shown in Fig. 1. The amount, X_A, drops rapidly with increase in time so that after 4 hr. only 13.5% of the initial amount remains in A. The amount, X_B, reaches a peak in the 2.5–3-hr. region then falls off without a great deal of curvature until 12 hr. The X_C curve rises slowly, reaches a peak of about 8.9 units at 4.5 hr., then falls off with little curvature until about 12 hr. The low peak of the X_C curve illustrates why it is often very difficult to measure blood levels of a metabolite. The X_D curve rises very slowly during the first 2 hr., but from 2 to 7 hr. has only *slight* curvature; during the latter period

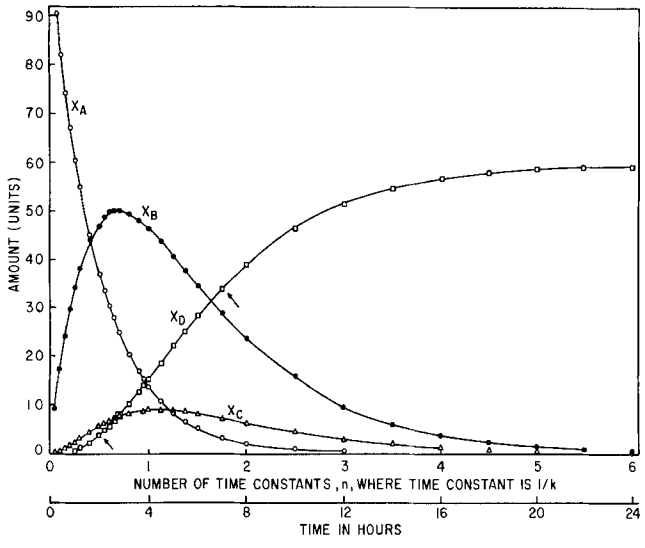


Fig. 1—The time course of the amounts X_A, X_B, X_C, and X_D in compartments A, B, C, and D, respectively, for the model shown as Scheme I and the constants of Example 1.

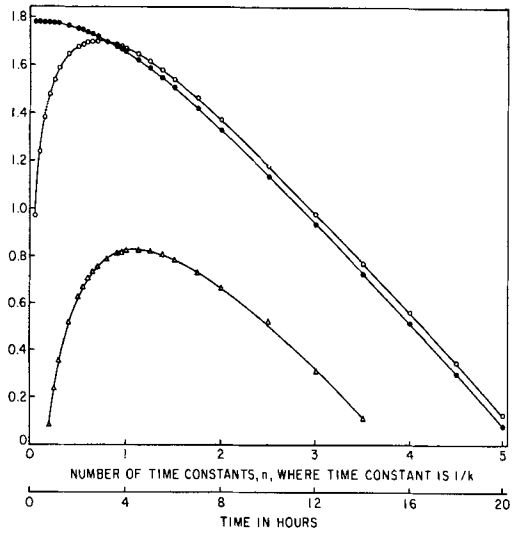


Fig. 2—Plots of $\log_{10}(60-X_D)$, $\log_{10}(X_B)$, and $\log_{10}(k_3 \cdot X_C)$ against time. These correspond to plots of the logarithm of the amount of metabolite not excreted, the logarithm of the plasma concentration of drug, and the logarithm of the excretion rate of metabolite against time. The constants of Example 1 were used. Key: ●, logarithm of amount not excreted = $\log_{10}(60 - X_D)$; ○, logarithm of amount in "B" = $\log_{10}(X_B)$; Δ, logarithm of excretion rate = $\log_{10}(K_3 \cdot X_C)$.

about 30 units or 50% of the C destined for the D compartment is contributed. It is not difficult to see how such a segment of a cumulative urinary excretion curve could be interpreted as being linear, particularly when analytical error, incomplete urine collections due to bladder retention, and other factors are contributing scatter to the points in the case of actual biological data.

Figure 2 is a plot of $\log_{10}(60 - X_D)$, $\log_{10}(X_B)$, and $\log_{10}(k_3X_C)$ *versus* time. These are comparable to plots of the logarithm of the amount of metabolite not excreted, the logarithm of the plasma concentration of drug, and the logarithm of the excretion rate of a metabolite. The curvature of the plots and the "plateau" in the excretion rate plot from about 3 to 6 hr. are noteworthy since proponents of zero-order formation have used these observations to support their claims.

Plots of the cumulative amount of B converted to C and the cumulative amount in D *versus* time are compared in Fig. 3. The first curve mentioned (the formation curve) could be interpreted, erroneously, as being linear in the region from 1 to 6 hr., while the excretion curve could be interpreted, also erroneously, as being linear in the region from 2 to 7 hr. The proper curve to resolve is the formation curve, not the excretion curve. The excretion curve was used by Levy (11), although Eq. 15 was published (12) for a different purpose; namely, to estimate absorption rate from measurement of unchanged drug in the urine and Levy (2) has made use of the equation.

Figure 4 is a plot of k_3X_C (the rate of excretion of metabolite) against k_2X_B (the rate of formation of metabolite). If formation rate were equal to excretion rate, all points would lie on the 45° line drawn through the origin; but, of course this is not true. Cummings and Martin (13) pointed out that in respect to drugs which are excreted predominantly as metabolites, the rate of excretion of a metabolite in urine will only parallel the rate of formation some time after the attainment of the maximum plasma concentration of the metabolite which is about 4 hr. in this example. This time corresponds to the change-over from diamonds to squares on the graph. One can estimate the relationship between rate of formation and rate of excretion if $k < k_3$ since at later times

$$k_2X_B \simeq \left(1 - \frac{k}{k_3}\right) k_2X_C \qquad \text{(Eq. 20)}$$

Substituting the values of k and k_3 for Example 1 into Eq. 20 yields

$$k_2X_B \simeq 0.67k_2X_C \qquad \text{(Eq. 21)}$$

The average ratio, k_2X_B/k_2X_C is 0.686 based on eight pairs of values in the 10–24-hr. interval.

The "mathematical error" involved in fractional urine collections may be gauged by the ratios $(\Delta X_D/\Delta t)/(dX_D/dt)$ where the derivatives are those at the midpoints of the Δt intervals. For the 15 "fractional collections" simulated the average value of this ratio was 0.99 with a range of 0.62 to 1.30. The extremes of the range were those corresponding to the midpoints of 0.4 and 0.2 hr., respectively. For the 13 "fractional collections" made in the 1.2–24-hr. range, the average ratio was 1.0 with a range of 0.95 to 1.17. It is heartening to know that, except at the very early times, $\Delta X_D/\Delta t$ estimates dX_D/dt within a reasonably small error.

Figure 5 is a "Lineweaver-Burk" (14) type plot of the reciprocal of $\Delta X_D/\Delta t$ against the reciprocal of X_B taken at the midpoints of the $\Delta X_D/\Delta t$ intervals. The midpoints used were those at 7, 10, 14, 18, and 22 hr. Levy (15) and Nelson *et al.* (16) interpreted such a plot as indicating a metabolite was being formed according to Michaelis-Menton kinetics (17). But the apparent "$V_{max.}$" and apparent "K_m" one calculates from the intercept and slope in the conventional manner (14) are purely artifacts. From the intercept one estimates an apparent "$V_{max.}$" of 19 units/hr. which is higher than the maximum excretion rate of about 6.67 units/hr. Hence, if one assumes that the maximum excretion rate is "$V_{max.}$," as Levy did, then this type of plot overestimates "$V_{max.}$" exactly as Levy found (15). The reciprocal of the slope of the line in Fig. 5, also equivalent to apparent "$V_{max.}$/apparent 'K_m'," since apparent "K_m" = slope x apparent "$V_{max.}$," is a meaningless number in the context of the model. As a result of the method of plotting and Eq. 21, the value of the reciprocal, 0.238, multiplied by 0.67,

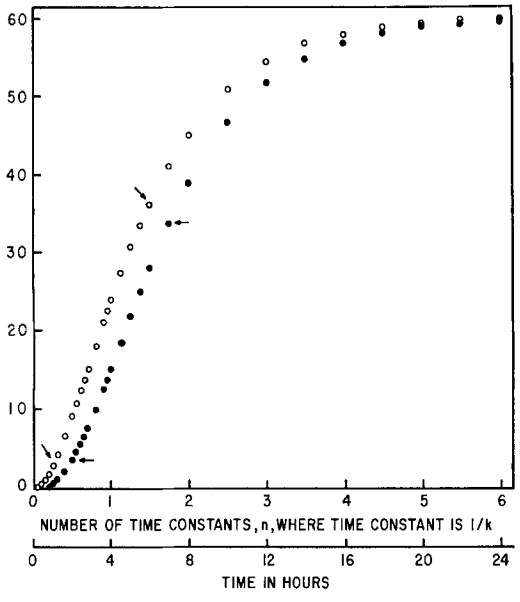


Fig. 3—Plots of the cumulative amount of B converted to C and the cumulative amount in D against time. These correspond to plots of the cumulative amount of drug converted to metabolite and the cumulative urinary excretion of the metabolite against time. The constants of Example 1 were used. Key: ○, cumulative amount of B converted to C; ●, cumulative amount in D.

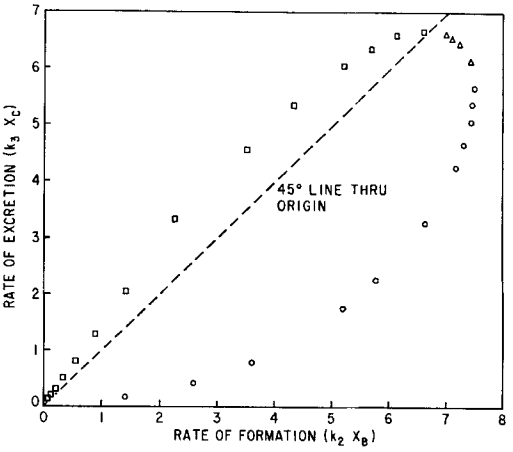


Fig. 4—A plot of the rate of excretion of metabolite (k_3X_C) against the rate of formation of metabolite (k_2X_B). Key: ○, values in the range $0 < n < 0.7$ and $0 < t < 2.8$; Δ, values in the range $0.8 < n < 1$ and $3.2 < t < 4$; □, values in the range $1.125 < n < 6$ and $4.5 < t < 24$. The plot shows that initially excretion rate underestimates formation rate then, beyond the time of occurrence of the peak metabolite concentration in blood, excretion rate overestimates formation rate. If excretion rate equalled formation rate, all points would lie on the 45° line drawn through the origin; but it is obvious this is not the case. The constants of Example 1 were used.

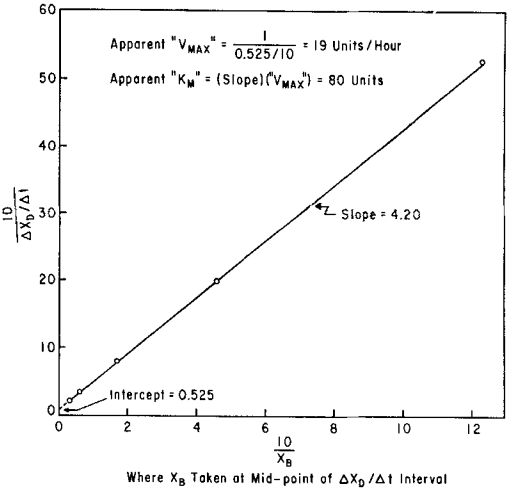


Fig. 5—Showing how a "Lineweaver-Burk" plot, which is an artifact, results from plotting the reciprocal of the excretion rate, $1\ \frac{\Delta X_D}{\Delta t}$, against the reciprocal of the amount of B, $1/X_B$, where X_B is taken at the midpoints of the excretion intervals. The constants of Example 1 were used.

is 0.16; the resultant number is essentially the same as the value of 0.15 obtained for k_2 from the correct plot shown in Fig. 6.

If Michaelis-Menton kinetics apply, one must use the reciprocal of the formation rate, *i.e.*, $1/k_2X_B$, rather than the reciprocal of the excretion rate for making a Lineweaver-Burk plot. From Eq. 20 can be calculated the approximate error in the ordinate value of such a plot if $1/k_2X_C$ is used in place of $1/k_2X_B$. If the kinetics are truly those of Michaelis and Menton (17), use of the reciprocal of the excretion rate causes rotation of the true line, so that the slope of the line drawn from excretion rate data has a slope which is less, and an intercept which is greater, than those of the true line. In such a case $V_{max.}$ is not estimated accurately, but K_m is estimated accurately since K_m = (slope) ($V_{max.}$). However, K_m will not have the same significance as it has usually (18). This is discussed in more detail under *Discussion*.

The proper plot employing $1/k_2X_B$ and $1/X_B$ is shown in Fig. 6. As expected, the reciprocal of the slope is k_2.

Example 2—The time course of the amounts in compartments A, B, C, and D is shown in Fig. 7. Since k_1 = 1.25 hr.$^{-1}$ in this example, compared with 0.5 hr.$^{-1}$ in the first example, X_A drops more rapidly in Example 2 than in Example 1, so that after only 1.6 hr. 13.5% of the initial amount remains in A. Hollister and Levy (19) reported an average k of 0.258 hr.$^{-1}$, an average k_2 of 0.153 hr.$^{-1}$, and Levy (11) reported an average k_3 of 1.1 hr.$^{-1}$ for the salicylate–salicylurate system in man. Hence, except for k_1, which Levy ignored, and the initial value $X_A{}^0$, the values of the constants in this example are very similar to those actually calculated for a specific drug–metabolite system as a result of the analysis of biological samples obtained from man.

Plots of the cumulative amount of B converted to C and the cumulative amount in D *versus* time are compared in Fig. 8. It should be noticed that the effect of increasing k_1 and k_3, but keeping k constant, is to cause the curves to rise more steeply and to become almost linear at an earlier time. One could interpret the formation curve as being linear in the 0.4 to 4.0-hr. period and the excretion curve similarly in the 1.2 to 5-hr. period. As in the previous example, during the latter period about 50% of the total metabolite is excreted.

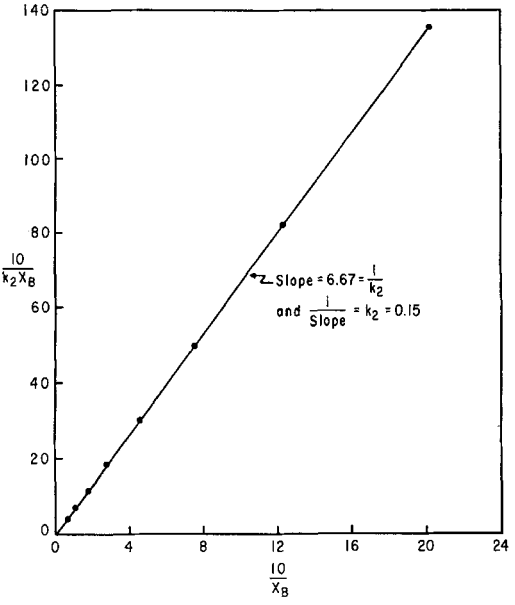


Fig. 6—A plot of the reciprocal of the formation rate, $1/k_2X_B$, against the reciprocal of the amount of B, $1/X_B$. The constants of Example 1 were used.

DISCUSSION

The implications of the simulations and equations presented above are as follows.

(*a*) The conclusion of Cummings *et al.* (20), Levy (11, 15), Nelson *et al.* (16), and Elliott (8) that the formation of salicylurate from salicylate becomes a zero-order process for some period of time following administration of either single oral doses of 1.5 or 2 Gm., or multiple oral doses of about 1 Gm., of acetylsalicylic acid to *adult humans* is most

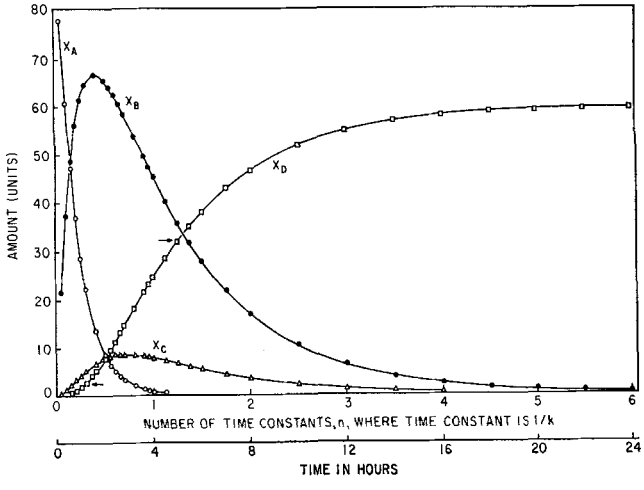


Fig. 7—The time course of the amounts X_A, X_B, X_C, and X_D in compartments A, B, C, and D, respectively, for the model shown as Scheme I and the constants of Example 2.

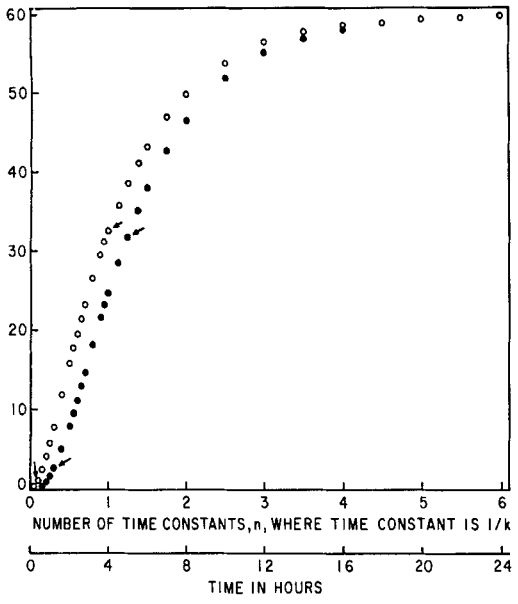


Fig. 8—Plots of the cumulative amount of B converted to C and the cumulative amount in D against time. These correspond to plots of the cumulative amount of drug converted to metabolite and the cumulative urinary excretion of the metabolite against time. The constants of Example 2 were used. Key: ○, cumulative amount of B converted to C; ●, cumulative amount in D.

probably in error. Most probably only first-order rate constants are involved at all times. The data of Levy (11) are fit quite well by Eq. 8 based on Scheme I.

(*b*) The conclusion of Nelson (21) that the absorption of tetracycline becomes a zero-order process for some period of time following oral administration of tetracycline mucate in a special dosage form is most probably in error.

(*c*) Large errors can be made in estimating the half-life of a drug (*i.e.*, $0.693/k$) if the estimation is made in a time period when more than one exponential term is making a significant contribution. The wide variability of the reported half-life of salicylate (11) is explained better on this basis than the explanations presented by Levy (11) and Cummings *et al.* (20).

(*d*) When analyzing such pharmacokinetic data by graphical methods using semilogarithmic graph paper one tends to underestimate the over-all rate constant for loss from the body (k) if it is the smallest rate constant appearing in Eqs. 7, 9, and 11 or, in the case of other models, in equations similar to these. This in turn means one tends to overestimate the half-life of the drug. This is due to the fact that the semilogarithmic plots are actually gently curving when one assumes they are linear. Hence, when residuals are taken by extrapolating the terminal line drawn, one also obtains biased residuals. This ultimately results in estimates of the other rate constants, like k_1 and k_3, which are different from their real or "best" values. The best approach is to treat the graphical estimates of the rate parameters as "purely estimates" which are used as input data to a digital computer; with the proper iterative procedure programmed into the computer, the data may be fit by the method of least squares so that "least squares" estimates of all the rate constants may be obtained simultaneously. Such sets of rate constants are readily shown to be better estimates than the graphical ones since, in almost all cases, there is a significant reduction in the variance associated with the fit.

(*e*) The method of subtracting amounts of unchanged drug and metabolite excreted to 4 hr. postadministration from the total amounts excreted and elaborating a pharmacokinetic model from the residuals, as practiced by Nelson and O'Reilly (9), may introduce significant errors into the estimates of the rate constants from some, but not necessarily all, subjects' data. The magnitude of the errors depends upon the effective first-order rate constant for absorption (k_1), and this will vary widely from subject to subject (2, 3, 6).

(*f*) The conclusion of Levy (15) and Nelson *et al.* (16) that a plot of the reciprocal of the excretion rate against the reciprocal of the amount of total drug left in the body is a "Lineweaver-Burk" plot, in the accepted sense, is spurious. It has been shown above that when formation rate of the metabolite is first order, such a plot yields an intercept and a slope, but the plot is an artifact in the Michaelis-Menton sense. If the disappearance of B is truly describable by the extended Michaelis-Menton equation (18), namely

$$\frac{-dC_B}{dt} = \frac{V_{max.} \cdot C_B}{K_m + C_B} \qquad \text{(Eq. 22)}$$

where C_B is the concentration in compartment B at time t, and the system is as in Scheme II, where

$$E + B \underset{K_2}{\overset{K_1}{\rightleftarrows}} EB \xrightarrow{K_3} E + C \xrightarrow{K_4} D$$

Scheme II

E denotes the enzyme, EB the enzyme–drug complex, and K_i ($i = 1, 2, 3, 4$) are first order-rate constants, then the K_m obtained would have a different significance than in the classical case. In the classical case, the system is as shown in Scheme III,

$$E + B \underset{K_2}{\overset{K_1}{\rightleftarrows}} EB \xrightarrow{K_3} E + C$$

Scheme III

and the "extended Michaelis constant," $K_m = (K_2 + K_3)/K_1$ (18). The application of the Michaelis-Menton equation to metabolite data derived from the intact animal or human will always be complicated by the C → D step unless $K_4 > 100K_3$ in Scheme II; in addition, there may be additional errors if the application is made in a time period when B is being supplied from a reservoir such as the absorption site or from a precursor.

A Critique of History—Bray *et al.* (22) showed that in the conjugation of benzoic acid with glycine to form hippuric acid the availability of glycine for conjugation controlled the rate of hippuric acid formation and that formation of hippuric acid apparently proceeded at a constant rate when rabbits were given relatively high doses of 500 mg./Kg. This was a significant contribution. However, these authors made the following statement: "Hippuric acid formation takes place at a constant rate and *the acid is excreted immediately*

up to a limiting rate which appears to vary considerably between different rabbits." In their mathematical treatment they completely ignored the rate constant for urinary excretion of hippuric acid. This reference, which has been cited extensively, may be the source of the problem. Some scientists seem to have reached the conclusion that if a rate constant is relatively large, say 1.0 hr.$^{-1}$, it can be ignored. The presentation in this paper should dispel this idea.

Nelson *et al.* (16) showed that following 20, 40, and 60 mg./Kg. doses of salicylic acid *to rats*, the rate of excretion of the metabolite, salicyluric acid, was the same, within error, at all three doses. In addition, they showed that salicyluric acid, administered intraperitoneally at doses of 200 mg./Kg. to rats, was rapidly excreted; the excretion obeyed first-order kinetics with an excretion rate constant of 1.15 hr.$^{-1}$ which is essentially the same as Levy (11) reported for one human given 2 doses of salicyluric acid, but considerably higher than the average value of 0.654 hr.$^{-1}$ reported by Elliott (8) for five humans. The additional rat study showed that saturation of the active secretory pathway in the kidney could not have occurred for salicylurate in the rats dosed with sodium salicylate. Hence, their evidence is very convincing that saturation of the enzyme system which converts salicylate to salicylurate occurred *in rats* at the dose of 20 mg./Kg. and above. One should not conclude, however, that a comparable dose, *i.e.*, about 1.4 Gm. of salicylic acid in a 70-Kg. man, will produce the same effect!

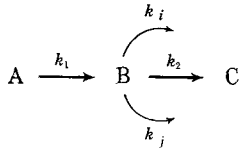
In the same paper (16) the conclusion was reached that the cumulative salicylurate excretion plots were linear following doses 1.5 and 2.0 Gm. of acetylsalicylic acid administered to two humans. Careful analysis of these plots reveals that the data points form a *curve;* the points start below the straight line drawn through them, then the points go above the line, then they go below the line again as time increases. The same result would be obtained if one were to draw a straight line through the points of the apparently linear segments of the X_D, t curves of Figs. 1 and 7! Levy (11) had previously drawn the conclusion that salicylurate was being formed at a constant rate from the same sets of experimental data. Later he (15) constructed "Lineweaver-Burk" plots from the same sets of data. He utilized the reciprocals of the salicylurate *excretion* rates, not the formation rates, in the time period 15 to 27 hr. postadministration. In light of the above presentation it appears that there is convincing proof of zero-order formation of salicylurate from salicylate in the rat, but the same type of proof has not been presented for man. It is obvious that there must be some critical dose level of acetylsalicylic acid or salicylate for man above which there will be zero-order formation of salicylurate for some time postadministration; such levels, for example, could be reached in poisoning cases when children ingest large quantities of acetylsalicylic acid. However, the administration of doses of up to 4 Gm. of sodium salicylate to adult subjects provided no data which supported zero-order formation of salicylurate from salicylate (23). Unfortunately, Levy (11) completely misinterpreted the data of Schachter and Manis (23).

Cummings and Martin (24) made some erroneous statements about metabolite formation which should be corrected. They stated that one could estimate the maximum rate constant for renal excretion of a metabolite from the volume of distribution (V) and the maximum renal clearance (R) employing the equation of Butler (25). But this is not so. The Butler equation, transferred into the terminology of this paper, is

$$R = Vkf = Vk_2 \qquad \text{(Eq. 23)}$$

where f is the fraction of B converted to C (*i.e.*, $f = k_2/k$). Hence, from renal clearance one can only estimate k or k_2 and not k_3. They also stated that the slope of the terminal segment of a plot of the logarithm of the excretion rate of metabolite would yield an estimate of k_2 when $k_2 < k_3$. But this is not true. The constant determined by this method of plotting will be k, providing k is less than k_1 and k_3. This is so because k, and not k_2, appears in the exponents of exponential terms of the solutions of the differential equations. Later, these authors (26) stated that if there was zero-order formation of one metabolite and first-order formation of another metabolite, then a plot of the logarithm of formation rate of total metabolites *versus* time would be a straight line having the same slope as if the latter metabolite were being formed alone. Their equations on which this conclusion is based are in error, and their own plots show this is not true!

The same type of error with respect to linearity of cumulative excretion curves, as discussed above for metabolities, can lead to the conclusion that constant rate (zero-order) absorption is operative, when, in fact, all rate constants are first-order rate constants. If a drug is partly metabolized and partly excreted unchanged in the urine and one measures unchanged drug in the urine, then one pertinent model may be written as in Scheme IV



Scheme IV

where A represents the solid drug administered, B represents the drug in blood and other fluids of distribution, and C represents the drug in urine. If k has the same meaning as for Scheme I, and k_2 is the rate constant for urinary excretion of unchanged drug, then a plot of the cumulative amount of unchanged drug in the urine (*i.e.*, X_C *versus* t) will be apparently linear, but actually slightly curved, during some time period if $1 < k_1/k < 10$. An example is shown in Fig. 9 for the case when $k_1 = 0.5$ hr.$^{-1}$, $k = 0.25$ hr.$^{-1}$, and $k_2 = 0.15$ hr.$^{-1}$. It may be seen that if a smooth line was drawn through the points, the line would be only slightly curved in the period 1.2 to 6 hr. postadministration. This region is marked off by the arrows on the graph. If one assumed linearity of the plot in this time period, one would conclude the A $\rightarrow$ B step was zero order—*i.e.*, that absorption was proceeding at a constant rate. This probably explains the results of Nelson (21) obtained with tetracycline mucate. The writer once made a similar interpretation from data derived following administration of a different drug and lived to regret it. As in Scheme I, the critical ratio for Scheme IV is k_1/k and not k_1/k_2.

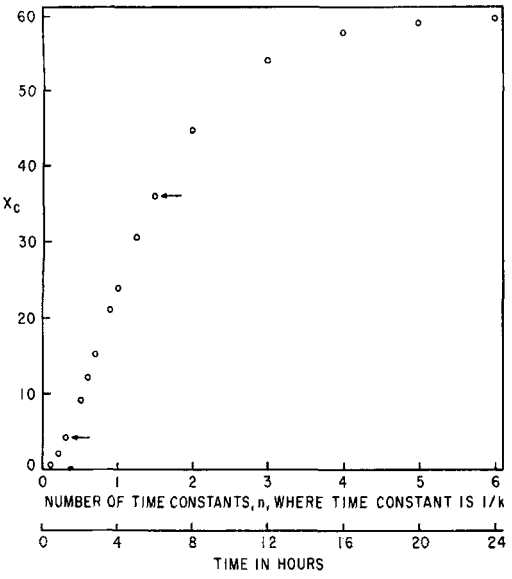


Fig. 9—A plot of the cumulative amount of unchanged drug in the urine, X_C versus time for the model shown as Scheme IV and the constants given in the text.

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Pharmacokinetic Model for Nalidixic Acid in Man III

Effect of Repeated Oral Dosage

By E. W. MCCHESNEY, G. A. PORTMANN, and R. F. KOSS

In studies which involved the administration of 1-Gm. doses of nalidixic acid to human volunteers four times daily for a period of 10 days, it has been shown that the resulting plasma levels of nalidixic and of hydroxynalidixic acid could be predicted reasonably well from a model derived from single-dose studies. More satisfactory blood levels are obtained if the doses are taken at least 1 hr. before meals. Repeated dosage results in no important change in the absorption-excretion-metabolism patterns of the drug.

IN PREVIOUS communications (1, 2) a model describing the absorption and elimination of nalidixic acid[1] (NA) in man was presented. This model was derived from observations in seven subjects following the ingestion of single 1-Gm. doses of NA in several physical forms. There remained, however, the question of how well such a model would describe the situation in which the drug is given as a standard therapeutic course, *i.e.*, 1 Gm. four times daily. In particular there was the problem of whether such a dosage regimen would result in a significant carry-over of the drug and its metabolites from one day to another [it was already known (3) that following single 1-Gm. doses the excretion is usually not quite complete within 24 hr.], and whether the repeated administration of 4 Gm. per day would result in significant changes in the way the body handles

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Previous paper: Portmann, G. A., McChesney, E. W., Stander, H., and Moore, W. E., *J. Pharm. Sci.*, **55**, 72(1966).
[1] Nalidixic acid is 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid. Marketed as NegGram by Winthrop Laboratories, New York, N.Y.