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Application of the Loo–Riegelman Absorption Method

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The Loo–Riegelman absorption method provides the correct A_∞/V_1 value and the correct rate constant k_a (if absorption is first order), whether metabolism occurs in compartment 1 only, compartment 2 only, or both compartments 1 and 2 of the two-compartment open model. In cases where there is metabolism in compartment 2, the disposition parameters estimated from intravenous data are only apparent and not the real values. The correct A_∞/V_1 and k_a values are obtained, however, only under conditions not hithertofore specified. These conditions are that there must be essentially no bias in the disposition parameters k_{12}, k_{21}, and k_{el}, and in the C_0 value estimated from the intravenous data, and that in the oral study a large number of interpolated plasma concentrations, as well as the observed plasma concentrations, must be used, especially for drugs with long half-lives. It is shown that application of the Guggenheim method to the initial A_1/V_1, t values frequently provides a better method of estimating A_∞/V_1 and k_a than the classical method. If biased disposition parameters are used in application of the Loo–Riegelman method to oral data, then essentially the correct value of k_a will be estimated, but the estimate of A_∞/V_1 will be approximately equal to the true value of A_∞/V_1 multiplied by the ratio of the biased C_0 value (obtained in fitting the intravenous data) to the true C_0 value of the intravenous data. The above indicates that intravenous data should be fitted by computer until there are no systematic deviations or trends and as small a sum of squared deviations as possible is obtained. The oral data should be fitted by spline or Akima methods, or similar procedures, to produce a function which passes through each observed plasma concentration and at the same time provides a large number of interpolated concentration data.

KEY WORDS: absorption plot; amount absorbed; kinetics of absorption; bias in computer fitting; disposition parameters; interpolation of blood levels.

INTRODUCTION

Loo and Riegelman (1) derived equations 1 and 2:

$$A_{t_n}/V_1 = (C_1)_{t_n} + k_{el}\int_{t_0}^{t_n} C_1\,dt + (C_2)_{t_n} \qquad (1)$$

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$$(C_2)_{t_n} = (C_2)_{t_{n-1}} \cdot e^{-k_{21}\Delta t} + k_{12}/k_{21} \cdot (C_1)_{t_{n-1}}(1 - e^{-k_{21}\Delta t}) + k_{12}\,\Delta C_1\,\Delta t/2 \quad (2)$$

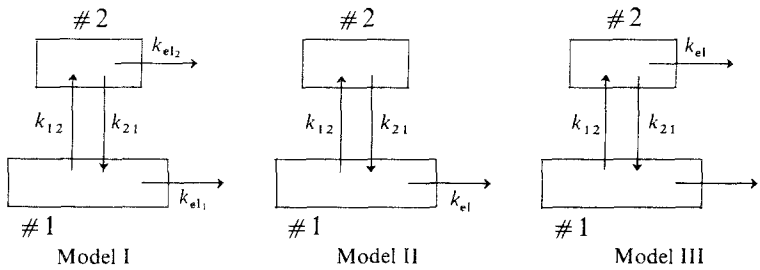
Although not explicitly stated in their article (1), it is implicit that the asymptote of a plot of A_{t_n}/V_1 is A_∞/V_1, which is given by equation 3:

$$A_\infty/V_1 = k_{el}\int_{t_0}^{\infty} C_1\,dt \quad (3)$$

In equations 1–3, A_{t_n}/V_1 is the cumulative amount of drug absorbed to time t_n divided by the volume of the inner (central) compartment of Model II below (if drug is given by intravenous infusion it is the amount which has been infused to time t_n divided by V_1), $(C_1)_{t_n}$ is the concentration of drug in the central compartment (assumed to be the equivalent of the plasma concentration), $(C_2)_{t_n}$ is the amount of drug in the outer so-called tissue compartment at time t_n divided by the volume of the inner compartment (i.e., A_2/V_1), the integral is the area under the C_1,t curve from time t_0 (when absorption commences) to time t_n, and the rate constants k_{12}, k_{21}, and k_{el} refer to those of Model II below. In equation 3, A_∞/V_1 represents the total amount of drug absorbed divided by the volume V_1. Usually t_0 is taken equal to zero since the integral is closely approximated by the trapezoidal rule, and, in such a case, one assumes $(C_1) = 0$ when $t = 0$, where the latter is the time of dosing.

Loo and Riegelman (1) showed that the $A_{t_n}/V_1, t_n$ data resulting from application of equations 1 and 2 could be analyzed to obtain the kinetics of input of drug to the bloodstream. This was shown by infusing an aspirin solution intravenously in man with a logarithmic infusion pump and back-calculating the first-order infusion rate. They also infused griseofulvin at a constant rate and accurately back-calculated the infusion rate. Subsequently, they reported (2) that when the interval between blood samples became too long the linear piecewise integration procedure used to estimate $(C_2)_{t_n}$ resulted in poor estimates, and they proposed a new equation which was based on a logarithmic piecewise procedure which they claimed solved the problem. Another variation in which the value of k_{el} could be adjusted from the intravenous and oral experiments was reported (3).

The Loo–Riegelman method (1–3) is based on Model II below, but in a given practical situation one does not know whether the true model is Model I, Model II, or Model III. Recently, Suzuki and Saitoh (4) showed that the result obtained by application of the Loo–Riegelman method was independent of the ratio of k_{el_1}/k_{el_2} if the true model was Model I. Kaplan (5) showed that for data collected on coumermycin A_1 the absorption plot obtained by the Loo–Riegelman method, based on Model II, and the absorption plot, based on Model III, were identical, but they did not give



the reason for the result. Analogously, Breckenridge and Orme (6) applied both the Loo–Riegelman method, based on Model II, and the corresponding equations of Kaplan (5), based on Model III, to intravenous and oral warfarin plasma concentration data. They reported that the absorption plots obtained by the two methods were identical, but did not give the reason for the result. This report will show why these authors obtained the results that they reported.

In addition, the effect of bias on the estimates of k_{12}, k_{21}, and k_{el} obtained from intravenous data on the result obtained by application of the Loo–Riegelman method to plasma concentrations observed in the same subject following oral administration has not been reported. Bias in such parameter estimates may exist either from (1) computer fitting of intravenous data using initial estimates of the parameters which are too distant from the real values, resulting in convergence at a local minimum, or (2) using graphical estimates of parameters, as has been shown by Wagner (7) and Wagner and Metzler (8).

Since the Loo–Riegelman method assumes that the plasma concentration curve is linear between two adjacent points, then the interval or intervals between concentrations measured become very critical in the result attained with the method. This problem is examined further, and some suggestions are made to eliminate this problem as a major source of error.

EXPERIMENTAL AND RESULTS

Effect of Metabolism in Compartment 2

Define: A_T = amount of drug absorbed to time T (same as A_{t_n} in equation 1).

$C_2 = A_2/V_1$ = amount of drug in compartment 2 at time t/volume of compartment No. 1.

For Model I,

$$A_T/V_1 = (C_1)_T + (C_2)_T + k_{el_1}\int_0^T C_1(t)\,dt + k_{el_2}\int_0^T C_2(t)\,dt \qquad (4)$$

$$A_\infty/V_1 = k_{el_1}\int_0^\infty C_1(t)\,dt + k_{el_2}\int_0^\infty C_2(t)\,dt \tag{5}$$

Since there is no way of knowing k_{el_2} unless drug is measured in compartment 2 as well as in compartment 1, then one cannot apply these equations.

Model II is assumed in applying the method of Loo and Riegelman (1):

$$A_T/V_1 = (C_1)_T + (C_2)_T + k_{el}\int_0^T C_1(t)\,dt \tag{6}$$

If the model is really Model II (i.e., elimination occurs only from compartment 1), then the Loo–Riegelman method provides the correct asymptote (A_∞/V_1), the correct A_T/V_1 plot, and the correct values of k_{12}, k_{21}, and k_{el}. If the model is really Model I and one applies Model II in the form of the Loo–Riegelman method, the method gives the correct asymptote (A_∞/V_1), the correct A_T/V_1 vs. T plot (i.e., the correct kinetics of absorption are obtained), but only apparent values, $(k_{12})_{app}$, $(k_{21})_{app}$, and $(k_{el})_{app}$, of the disposition portion of the model are obtained, and not the real values, k_{12}, k_{21}, and k_{el_1}. The reason for this is as follows.

When drug is administered intravenously as a bolus of dose D, the plasma concentration–time curve is described by the equation

$$C_1(t) = A\,e^{-\alpha t} + B\,e^{-\beta t} \tag{7}$$

Formulas used to obtain estimates of microscopic rate constants give the following results:

$$(k_{21})_{app} = \{A\beta + B\alpha\}/\{A + B\} \tag{8}$$

$$(k_{el})_{app} = \alpha\beta/(k_{21})_{app} = \{k_{21}k_{el_1} + k_{12}k_{el_2} + k_{el_1}k_{el_2}\}/\{k_{21} + k_{el_2}\} \tag{9}$$

Hence

$$\begin{aligned} A_\infty/V_1 &= (k_{el})_{app}\int_0^\infty C_1(t)\,dt \\ &= [\{k_{21}k_{el_1} + k_{12}k_{el_2} + k_{el_1}k_{el_2}\}/\{k_{21} + k_{el_2}\}]\int_0^\infty C_1(t)\,dt \end{aligned} \tag{10}$$

For Model I, working in amounts (A)

$$A_1 = D[(E_2 - \beta)\,e^{-\beta t} - (E_2 - \alpha)\,e^{-\alpha t}]/(\alpha - \beta) \tag{11}$$

where

$$E_2 = k_{21} + k_{el_2} \tag{12}$$

$$\int_0^\infty A_1(t)\,dt = E_2 D/\alpha\beta \tag{13}$$

$$A_2 = k_{12}D[e^{-\beta t} - e^{-\alpha t}]/(\alpha - \beta) \tag{14}$$

$$\int_0^{\infty} A_2(t)\,dt = k_{12}D/\alpha\beta \tag{15}$$

$$\int_0^{\infty} A_2(t)\,dt \Big/ \int_0^{\infty} A_1(t)\,dt = \int_0^{\infty} C_2(t)\,dt \Big/ \int_0^{\infty} C_1(t)\,dt = k_{12}/E_2 = k_{12}/\{k_{21} + k_{el_2}\} \tag{16}$$

And

$$\int_0^{\infty} C_2(t)\,dt = \{k_{12}/(k_{21} + k_{el_2})\} \int_0^{\infty} C_1(t)\,dt \tag{17}$$

Substituting from equation 17 into equation 5 gives

$$\begin{aligned} A_\infty/V_1 &= [k_{el_1} + \{k_{12}k_{el_2}/(k_{21} + k_{el_2})\}] \int_0^{\infty} C_1(t)\,dt \\ &= [\{k_{21}k_{el_1} + k_{12} + k_{el_2} + k_{el_1}k_{el_2}\} \Big/ \{k_{21} + k_{el_2}\}] \int_0^{\infty} C_1(t)\,dt \end{aligned} \tag{18}$$

Since the right-hand sides of equations 10 and 18 are the same, the Loo–Riegelman method gives the correct amount absorbed even if the model is Model I. Simulations have shown that it also gives the correct kinetics of absorption. An example is given below.

Simulation Example 1. For the intravenous data, let $k_{12} = 0.37$, $k_{21} = 0.23$, $k_{el_1} = 0.08$, $k_{el_2} = 0.08$, $C_0 = D/V_1 = 25$.
For Model I, we have

$$C_1(t) = C_0[(k_{21} + k_{el} - \beta)\,e^{-\beta t} - (k_{21} + k_{el_2} - \alpha)\,e^{-\alpha t}]/(\alpha - \beta) \tag{19}$$

$$C_1(t) = A\,e^{-\alpha t} + B\,e^{-\beta t} \tag{20}$$

$$C_1(t) = 15.417\,e^{-0.68t} + 9.583\,e^{-0.08t} \tag{21}$$

For Model II, we calculate

$$(k_{21})_{app} = (A\beta + B\alpha)/(A + B) = k_{21} + k_{el_2} = 0.31 \tag{22}$$

$$(k_{el})_{app} = \alpha\beta/(k_{21})_{app} = (0.68)(0.08)/0.31 = 0.1755 \tag{23}$$

$$\begin{aligned} (k_{12})_{app} &= \alpha + \beta - (k_{21})_{app} - (k_{el})_{app} = 0.68 + 0.08 \\ &\quad - 0.31 - 0.1755 = 0.2745 \end{aligned} \tag{24}$$

Note that

$$\int_0^{\infty} C_1(t)\,dt = (15.417/0.68) + (9.583/0.08) = 142.5 \tag{25}$$

and

$$(k_{el})_{app} \int_0^\infty C_1(t)\,dt = (0.1755)(142.5) = 25. \tag{26}$$

In the above,

$$\alpha + \beta = k_{12} + k_{21} + k_{el_1} + k_{el_2} = 0.76 \tag{27}$$

$$\alpha\beta = k_{21}k_{el_1} + k_{12}k_{el_2} + k_{el_1}k_{el_2} = E_1E_2 - k_{12}k_{21} = 0.0544 \tag{28}$$

where $E_1 = k_{12} + k_{el_1}$ and $E_2 = k_{21} + k_{el_2}$

$$\alpha = \tfrac{1}{2}[(\alpha + \beta) + \sqrt{(\alpha + \beta)^2 - 4\alpha\beta}] = 0.68 \tag{29}$$

$$\beta = \tfrac{1}{2}[(\alpha + \beta) - \sqrt{(\alpha + \beta)^2 - 4\alpha\beta}] = 0.08 \tag{30}$$

Oral data were generated with the appropriate equation for Model I, namely

$$C_1(t) = k_aC_0[\{(E_2 - \alpha)/(k_a - \alpha)(\beta - \alpha)\}\, e^{-\alpha t} + \{(E_2 - \beta)/(k_a - \beta)(\alpha - \beta)\, e^{-\beta t} + \{(E_2 - k_a)/(\alpha - k_a)(\beta - k_a)\}\, e^{-k_a t}] \tag{31}$$

$$C_1(t) = A_1\, e^{-\alpha t} + A_2\, e^{-\beta t} + A_3\, e^{-k_a t} \tag{32}$$

$$C_1(t) = -23.4105\, e^{-0.68t} + 11.9066\, e^{-0.08t} + 11.5039\, e^{-0.41t} \tag{33}$$

Table I. Simulation Example 1 Showing That Loo–Riegelman Method Gives Correct Result if the Model Is Model I

t_n	$(C_1)_{t_n}$	$\int_0^{t_n} C_1(t)\,dt$	$(k_{el})_{app}\int_0^{t_n} C_1(t)\,dt$	$(\hat{C}_2)t_n$[a]	A_{t_n}/V_1[b]	Guggenheim data t_1	Guggenheim data $\Delta(A_{t_n}/V_1)$
0	0	0	0	0	0	0	4.6152
0.5	4.1485	1.0371	0.1820	0.2847	4.6152	0.5	3.7622
1.0	6.7656	3.7657	0.6609	0.9509	8.3774	1.0	3.0666
1.5	8.3380	7.5416	1.3236	1.7824	11.4440	1.5	2.4995
2.0	9.2042	11.9271	2.0932	2.6461	13.9435	2.0	2.0373
2.5	9.5991	16.6279	2.9182	3.4635	15.9808	2.5	1.6605
3.0	9.6845	21.4488	3.7643	4.1925	17.6413	3.0	1.3532
3.5	9.5714	26.2628	4.6091	4.8140	18.9945	3.5	1.1030
4.0	9.3353	30.9895	5.4387	5.3235	20.0975		
5.0	8.6809	39.9976	7.0196	6.0181	21.7186		
6.0	7.9546	48.3153	8.4793	6.3632	22.7971		
7.0	7.2529	55.9191	9.8138	6.4483	23.5150		
9.0	6.0314	69.2034	12.1452	6.1010	24.2776		
11.0	5.0520	80.2868	14.0903	5.4809	24.6231		
15.0	3.6099	97.6106	17.1307	3.9733	24.7139		
18.0	2.8281	107.2676	18.8255	3.1811	24.8347	Average = 24.7491	
24.0	1.7462	120.9905	21.2338	1.7187	24.6987		

[a]Calculated with equation 2.
[b]Calculated with equation 1.

where $A_1 = -23.4105$, $\alpha = 0.68$, $A_2 = 11.9066$, $\beta = 0.08$, $A_3 = 11.5039$, and $k_a = 0.41$. Data generated are shown in columns 1 and 2 of Table I. The Loo–Riegelman method was applied to these data using the values as follows: $(k_{12})_{app} = 0.2745$, $(k_{21})_{app} = 0.31$, $(k_{el})_{app} = 0.1755$. Results are shown in columns 5 and 6 of Table I.

Application of the Guggenheim Method to Estimate k_a

The data for k_a according to the Guggenheim method are shown in the last two columns of Table I. Linear least-squares regression of $\ln\{\Delta(A_{t_n}/V_1)\}, t_1$ values gave the equation

$$\ln[\Delta(A_{t_n}/V_1)] = 1.5295 - 0.409t_1 \tag{34}$$

whence

$$\Delta(A_{t_n}/V_1) = 4.6158\, e^{-0.409t_1} \tag{35}$$

Hence $\hat{k}_a$ is $0.409\ \text{hr}^{-1}$ and the real value was 0.41, and

$$\begin{aligned}\hat{C}_0 = A_\infty/V_1 &= 4.6158/\{1 - e^{-(k_a)(\Delta t)}\} = 4.6158/\{1 - e^{-(0.409)(0.50)}\} \\ &= 24.96\end{aligned} \tag{36}$$

whereas the real value is 25.

Hence if the estimates are rounded off the estimates are equal to the real values.

Usual Methods of Estimating k_a and C_0

Usually the estimate of A_∞/V_1 is taken from the terminal values of A_{t_n}/V_1, i.e., the asymptote of the A_{t_n}/V_1 vs. t_n plot. If we take the average value of the three last A_{t_n}/V_1 values, namely 24.7491, as the A_∞/V_1 value and then do linear least-squares regression on $\ln(24.7491 - A_{t_n}/V_1), t_n$ values, we obtain

$$\ln(24.7491 - A_{t_n}/V_1) = 3.2337 - 0.428t\ (r = 0.9999) \tag{37}$$

whence

$$\{24.7491 - A_{t_n}/V_1\} = 25.375\, e^{-0.428t} \tag{38}$$

Hence using this method we have 0.428 as the estimate of k_a and two estimates of A_∞/V_1, namely 24.7491 and 25.375, and we really don't know which one is the "correct" one.

Note that the Guggenheim method of estimating k_a and C_0 or A_∞/V_1 is the more accurate in this case and is less ambiguous.

The product of $(k_{el})_{app}$ and the total area under the "plasma concentration" curve is also an estimate of A_∞/V_1. With the example given, the result

is shown below:

$$\int_0^\infty C_1(t)\,dt = A_1/\alpha + A_2/\beta + A_3/k_a$$
$$= -23.4105/0.68 + 11.9066/0.08 + 11.5039/0.41 \quad (39)$$
$$= 142.5$$

$$A_\infty/V_1 = (k_{el})_{app}\int_0^\infty C_1(t)\,dt = (0.1755)(142.5) = 25. \quad (40)$$

Effect of Bias in Parameters Estimated from Intravenous Data

Simulation Example 2. For the intravenous data, the parameter values $k_{12} = 1.162$, $k_{21} = 0.515$, $k_{el} = 0.038$, and $C_0 = 100$ (corresponding to $V_1 = 41.0$) were used to substitute into equation 41, which is appropriate for Model II. Such substitution gave equation 42:

$$C_1(t) = C_0[(k_{21} - \beta)e^{-\beta t} - (k_{21} - \alpha)e^{-\alpha t}]/(\alpha - \beta) \quad (41)$$

$$C_1(t) = 29.758\,e^{-0.0115t} + 70.2419\,e^{-1.7035t} \quad (42)$$

Intravenous C_1, t values were generated with equation 42 and 20 sets of concentrations "with noise" were generated by adding 5% random error with normal deviates. Each of these 20 sets of concentration data was fitted to equation 41 using the program NONLIN and an IBM digital computer. The initial estimates used to initiate the iteration in each case were values of k_{12}, k_{21}, k_{el}, and V_1 which were *twice* the known values. The averages of the 20 estimated parameters of each type were $k_{12} = 1.853$, $k_{21} = 0.797$, $k_{el} = 0.068$, and $V_1 = 32.6$ (corresponding to $C_0 = 125.8$). The real values of α and β were $\alpha = 1.7035$ and $\beta = 0.0155$. The values of α and β obtained from the averaged parameters were $\alpha = 2.6979$ and $\beta = 0.0201$. The averages of the parameters, obtained by this method, are quite biased and represent an extreme case. We could have used only one of 20 sets of biased estimates but reasoned that it was fairer to use the averages of the 20. The equation for the estimated concentrations (C_1), obtained by substituting the averaged parameters into equation 41, is given by equation 43:

$$\hat{C}_1(t) = 36.489\,e^{-0.0201t} + 89.2805\,e^{-2.6979t} \quad (43)$$

A table of the $\hat{C}_1$ and real C_1 values clearly shows the bias in that when $t < 12$ then $\hat{C}_1 > C_1$, and when $t \geq 24$, $\hat{C} < C_1$. However, because of the rapid falloff and then flattening of the curve, it is almost impossible to show the nature of the systemic deviations or trends on cartesian coordinate graph

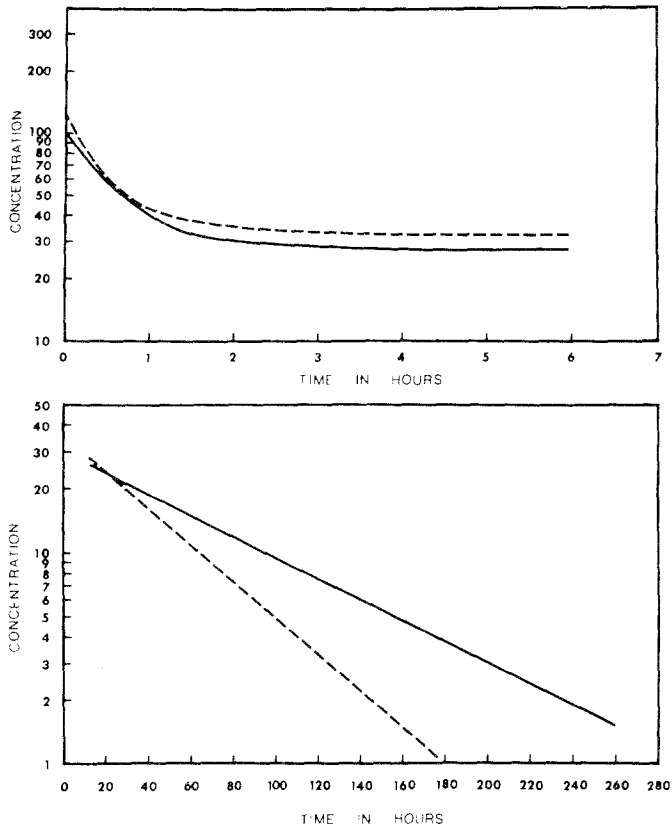


Fig. 1. Simulated intravenous blood level curve (——) generated with equation 42 and biased estimations (– – –) generated with equation 43. Top: Expanded scale of first 6 hr only. Bottom: Terminal part of curves.

paper. Figure 1 consists of two semilogarithmic plots which show the nature of the trends. At the top of Fig. 1 is an expanded scale plot showing the data for the first 6 hr. At the bottom of Fig. 1 some of the data beyond 10 hr are plotted. A weighted residual plot would also show such systematic deviations.

Oral C_1, t data were generated by using the same real values of the disposition parameters as used to generate the original intravenous data. Three sets were generated using $C_0 = 100$ and $k_a = 0.5$, $C_0 = 75$ and $k_a = 1.25$, and $C_0 = 75$ and $k_a = 2$. The parameter values were substituted into equation 31 and the value of $k_{21} = 0.515$ was used for E_2. The Loo–Riegelman method was then applied to the C_1, t data of each of the three sets using the biased parameters $k_{12} = 1.853$, $k_{21} = 0.797$, and $k_{el} = 0.068$. In each set, concentrations were used at times $t = 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4,$

Table II. Estimates of A_∞/V_1 and k_a Obtained by Using Biased Disposition Parameters in Applying the Loo–Riegelman Method in Simulation Example 2

	Set 1		Set 2		Set 3	
	A_∞/V_1	k_a	A_∞/V_1	k_a	A_∞/V_1	k_a
Real value	100	0.50	75	1.25	75	2.00
Guggenheim method	123	0.48	108	0.91	96.4	1.64
Sigma-minus method	119	0.53	99.5	1.48	101.0	2.26
$\left(\text{real}\ \frac{A_\infty}{V_1}\right)_{\text{oral}} \times \frac{(\text{biased } C_0)_{\text{i.v.}}}{(\text{real } C_0)_{\text{i.v.}}}$	125.8	—	94.4	—	94.4	

1.6, 1.8, 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312. These are more sampling times than one would have available in a human study, but we wished not to introduce another source of error discussed later.

The Guggenheim method was applied to the equally spaced $\Delta A_T/V_1, t_1$ values (here t_1 is time at the beginning of the interval) in the 0- to 2-hr range, and the sigma-minus method was also applied to estimate A_∞/V_1 and k_a from each set of data. Results are shown in Table II. It may be seen that k_a is reasonably well estimated in each case but that A_∞/V_1 is always appreciably higher than the real value. This is due to the bias in the fitting of the intravenous data. The ratio of volumes is 41/32.6 = 1.258, hence the ratio

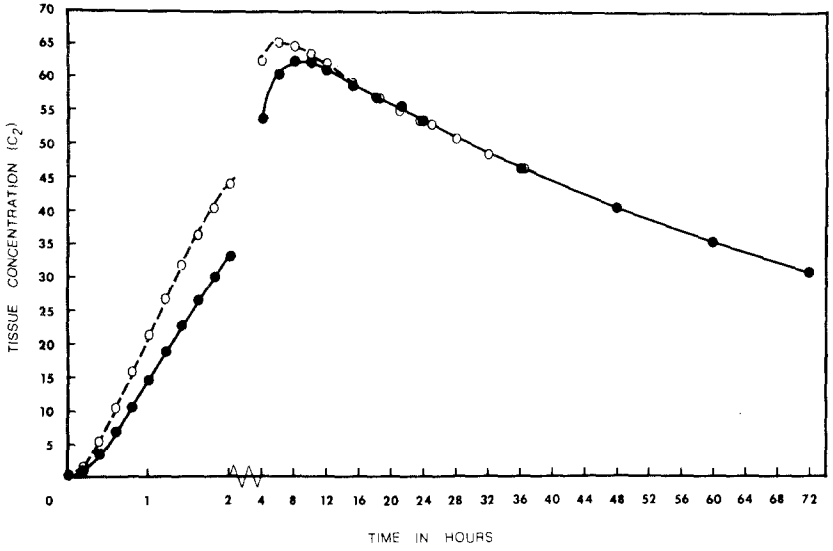


Fig. 2. Solid line gives actual C_2 values for simulation example 2 (oral). Dotted line gives estimated C_2 values calculated with the biased parameter values.

of biased C_0/true C_0 = 125.8/100 = 1.258. Equation 44 approximately holds in each case:

$$(\hat{A_\infty}/V_1)_{\text{oral}} \simeq (\text{real } A_\infty/V_1)_{\text{oral}} \cdot (\text{biased } C_0)_{\text{i.v.}}/(\text{real } C_0)_{\text{i.v.}} \qquad (44)$$

That is, the poor estimate of A_∞/V_1 obtained by application of the Loo–Riegelman method to the oral data is accounted for by the bias in the estimate of C_0 in the fitting of the intravenous data. This can be seen by comparing the values of the right-hand side of equation 44 with the A_∞/V_1 estimates in Table II. It is somewhat remarkable that k_a is estimated as well as it is under conditions of such biased estimates. This is accentuated by Fig. 2, which is a plot of the real C_2 values and the values of $\hat{C}_2$ obtained by the Loo–Riegelman method using the biased estimates of k_{12}, k_{21}, and k_{el} for set 1 where $k_a = 0.5$. It is obvious that the $\hat{C}_2$ values during the absorption phase are considerably higher in this case than the real C_2 values.

Effects of Time Interval Between Plasma Concentrations

Many simulations have been performed, but results of only one will be given.

Simulation Example 3. The original parameters of example 2, namely $k_{12} = 1.162$, $k_{21} = 0.515$, $k_{el} = 0.038$, $k_a = 0.5$, and $C_0 = 100$, were used. These values, substituted into equation 31, gave the equation for C_1 shown in the footnote to Table III. Substitution into the appropriate equation for Model II also give the equation for C_2 shown in the same footnote. Using equation 2, $\hat{C}_2$ values were calculated from the C_1, t values listed in columns 1 and 2 of Table III; in this case, the real values of k_{12}, k_{21}, and k_{el} (above) were used and not the biased estimates. Hence this simulation is a test of the criticality of the values of ΔC_1 and Δt and of the distribution of the C_1 values with respect to time. Four different sets of C_1, t values were used and the generated $\hat{C}_2$ values are shown in Table III in the last four columns. All calculations were carried out to the number of significant places shown in Table III. Although the trapezoidal areas are not listed, the error introduced by use of the trapezoidal rule in this simulation was of minor importance in determining the results. Since Δt appears in the exponent of "e" in two terms of equation 1, and both ΔC_1 and Δt appear in the third term, the value of Δt and its change with time are the major source of poor estimates of C_2. When a very large number of C_1, t values are used, as in set 1, $\hat{C}_2$ is essentially the same as the real C_2 over the whole time range. However, in sets 2 and 3, when Δt suddenly jumps from 2 hr (in the 2- to 12-hr range) to 12 hr (between 12 and 24 hr) then the $\hat{C}_2$ estimated at 24 hr is appreciably lower than the real C_2 value. At least with this set of data, when fewer "blood samples" are taken in the absorption phase (sets 3 and 4 compared with set

Table III. How "Tissue Concentration" (C_2) Estimated by the Loo–Riegelman Method Depends Markedly on the Values of ΔC_1 and Δt (Simulation Example 3)

Time (hr)	Actual values[a]		$\hat{C}_2$ estimated by Loo–Riegelman method			
	C_1	C_2	Set 1	Set 2	Set 3	Set 4
0	0	0	0	0	0	0
0.2	8.4772	1.0050	0.9851	0.9851		
0.4	14.5103	3.4940	3.4617	3.4617	3.3722	
0.6	18.8027	6.8653	6.8260	6.8260		6.5546
0.8	21.8551	10.7075	10.6649	10.6649	10.5464	
1.0	24.0237	14.7430	14.6994	14.6994		
1.2	25.5620	18.7878	18.7446	18.7446	18.6247	18.4459
1.4	26.6506	22.7231	22.6815	22.6815		
1.6	27.4180	26.4756	26.4360	26.4360	26.3261	
1.8	27.9558	30.0031	29.9659	29.9659		29.7084
2.	28.3292	33.2846	33.2500	33.2500	33.1537	
4.	28.8842	53.7895	53.6152	53.6152	53.5808	53.5108
6.	28.3629	60.6871	60.4402	60.4402	60.4279	
8.	27.7577	62.3045	62.0230	62.0230	62.0186	61.0682
10.	27.1408	61.9908	61.6965	61.6965	61.6949	
12.	26.5289	60.9871	60.6907	60.6907	60.6901	59.5752
15.	25.6317	59.1009	58.4714			
18.	24.7630	57.1373	56.2262			
21.	23.9233	55.2088	54.4844			
24.	23.1121	53.3386	52.6725	36.0372	36.0372	36.0349
27.	21.8109	51.5302	50.0094			
30.	21.0815	49.7828	48.1112			
33.	20.3673	48.0946	46.4378			
36.	20.1329	46.4637	45.6494	31.3437		31.3437
39.	19.4502	44.8880	44.2838			
42.	18.7906	43.3658	42.8209			
45.	18.1534	41.8952	41.3771			
48.	17.5377	40.4745	39.9756	27.3032	−23.5814	30.5258
51.	16.9430	39.1019	38.6204			
54.	16.3684	37.7759	37.3107			
57.	15.8134	36.4945	36.0456			
60.	15.2771	35.2572	34.8232	23.7842		23.7909
63.	14.7590	34.0616	33.6422			
66.	14.2585	32.9065	32.5013			
69.	13.7750	31.7906	31.3992			
72.	13.3079	30.7125	30.3345	20.7185	−19.4101	20.7185
75.	12.8566	29.6710	29.3058			
78.	12.4206	28.6648	28.3120			
81.	11.9994	27.6928	27.3519			
84.	11.5925	26.7537	26.4243	18.0477		18.0477
87.	11.1993	25.8464	25.5281			
90.	10.8196	24.9900	24.6625			
93.	10.4527	24.1231	23.8262			
96.	10.0982	23.3051	23.0182	15.1212	−14.7295	15.7212

[a]Used equations for two-compartment open model with first-order absorption with $k_{12} = 1.162$, $k_{21} = 0.515$, $k_{e1} = 0.038$, and $k_a = 0.5\ \text{hr}^{-1}$, $C_0 = 100$. These parameters gave equations

$$C_1 = 30.458\, e^{-0.0115t} - 29.1825\, e^{-1.7035t} - 1.2757\, e^{-0.5t}$$

$$C_2 = 70.2929\, e^{-0.0115t} + 28.5318\, e^{-1.0735t} - 98.8247\, e^{-0.5t}$$

2) a similar dramatic effect on $\hat{C}_2$ is not seen. Of course, there is a lower limit, and one must take sufficient samples to get a good distribution of points at least up to the peak of the C_1, t curve (which in this example is near 4 hr). Notice that although set 3 consists of 15 samples and set 4 consists of only 14 samples the C_2 values are better in set 4 than in set 3. The large Δt of 24 hr (from 24 to 48 hr) in set 3 caused $\hat{C}_2$ to become a large negative value at 48 hr.

A_{t_n}/V_1 values for each set were estimated by applying equation 1, the trapezoidal rule for the areas, and the $\hat{C}_2$ values listed in Table III. Then the $A_{t_n}/V_1, t_n$ values were analyzed by two different methods to estimate the A_∞/V_1 and k_a values. The Guggenheim method was applied to the equally spaced values in the 0- to 2-hr range. Since absorption had essentially ceased at 10 hr all the A_{t_n}/V_1 values from 12 to 96 hr were averaged to obtain an estimate of the asymptote of each set: the early A_{t_n}/V_1 values were then subtracted from these asymptotes and the natural logarithms of the differences were treated by least squares to obtain the estimate of k_a by the usual sigma-minus method. Results are shown in Table IV. In each case, the Guggenheim method very accurately estimated both A_∞/V_1 and k_a. However, only for set 1 did the sigma-minus method give good estimates of either k_a or A_∞/V_1. The sigma-minus plots are shown in Fig. 3. The unwary (with real data) might interpret data set 2 as being a case of biexponential absorption! The reason for these results is that the poor estimates of C_2 beyond 12 hr in sets 2–4 caused great fluctuation in the terminal A_{t_n}/V_1 values and all the values were lower than the real values.

DISCUSSION

The derivations have shown that under certain conditions the Loo–Riegelman method will provide the correct A_∞/V_1 value and the correct

Table IV. How Both the Values of ΔC_1 and Δt (Data Set) and the Method of Plotting A_T/V_1 Data Affect the Estimates of A_∞/V_1 and k_a Obtained (Simulation Example 3)

Data set	Parameter		Estimate obtained from A_T/V_1 data by	
	Symbol	Real value	Guggenheim method	Sigma-minus plot
1	A_∞/V_1	100.0	100.1	99.6
	k_a	0.50	0.4988	0.5164
2	A_∞/V_1	100.0	100.1	90.2
	k_a	0.50	0.4988	0.5887
3	A_∞/V_1	100.0	100.5	Curved plot
	k_a	0.50	0.4941	
4	A_∞/V_1	100.0	101.1	98.1
	k_a	0.50	0.4863	0.6138

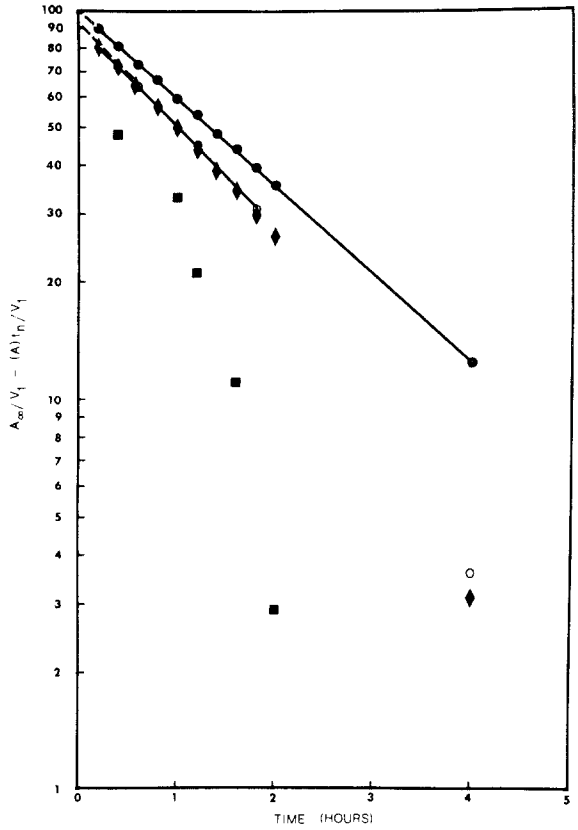


Fig. 3. Sigma-minus plots for simulation example 3. ●, Set 1; ◆, set 2; ■, set 3; ○, set 4.

kinetics of absorption (or the correct A_T/V_1, T plot if the kinetics of absorption are nonuniform) independently of whether there is metabolism in compartment 2 or not. In those cases where metabolism does occur in compartment 2, the disposition parameters estimated from the intravenous data are only apparent and not the real values. The relationships among the parameters of Models I, II, and III are shown. This explains the results reported by Kaplan (5) and Breckenridge and Orme (6). The treatment herein is simpler than that of Suzuki and Saitoh (4).

In situations where the disposition parameters are biased, such as when they are obtained by the "feathering" or "back-projection" technique or when the computer converges on a local minimum in the least-squares surface, use of such biased parameters in application of the Loo–Riegelman

method to oral data results in reasonable estimate of the true absorption rate, k_a (if absorption is first order), but in a biased value of $\hat{A_\infty}/V_1$ which is approximately given by equation 44. This strongly suggests that in the fitting of the intravenous concentration data one should use as good initial estimates of the parameters k_{12}, k_{21}, k_{el}, and C_0 for Model II as possible, and fit the data to equation 41 repeatedly on the computer until one has the best curve-fit possible—that is, there should be no systemic deviations of the model-predicted $\hat{C}_1$ from the observed C_1 values.

The reason there is bias when one "feathers" or "strips" the intravenous data is that to obtain the estimate of β one assumes $e^{-\alpha t} = 0$ at some point and then for terminal concentration data assumes $\beta = -\Delta \ln C/\Delta t$. This biased estimate of β is higher than the true value, hence the residuals are biased, and a biased estimate of α is also obtained. The amount of bias is very dependent on the particular set of data which is "stripped." Besides the usual definition of β, one may define it as

$$[d \ln C/dt]_{\substack{t \to \infty \\ C \to 0}} = -\beta$$

When the digital computer obtains a fit without systematic deviations, it is really using this latter interpretation of β rather than the former.

The Loo–Riegelman method assumes that the plasma level curve is linear between adjacent points. Because of this assumption, the values of C_2 estimated by the method depend on the time intervals between samples. Hence, optionally, in applying the method to oral concentration data one should have a large number of concentration–time points which are closely spaced. But this is impractical since one can take only so many blood samples following any given treatment in a human subject. To circumvent the problem, one can fit a function or functions to the data points such that the "line" goes through each observed concentration and is "smooth" between observed concentrations. An ideal approach, which the author has studied, is use of the spline and Akima methods reported by Fried and Zeitz (9). With a very "steep-slope" plasma concentration curve (sum of four exponential terms), Sedman (personal communication) has shown that the method gives interpolated concentrations between adjacent "observed" points which differ only 0–2% from the real values. Hence in applying the Loo–Riegelman method to oral concentration data one should generate a large number of such interpolated points and use these and the observed concentrations to estimate the C_2 values. We now have a computer program to interpolate such values and apply equations 1 and 2 to the interpolated and observed values.[2]

[2]P. Larson and A. Sedman, University of Michigan, Ann Arbor, Mich.

In most simulations, the A_∞/V_1 value, and sometimes the k_a value, is better estimated by means of the Guggenheim method from the early A_T/V_1 values than by the classical sigma-minus method. The Guggenheim method requires equally spaced A_T/V_1 values and hence equally spaced blood samples during the absorption phase. When using the curve-fitting method above, one can generate interpolated concentrations at equally spaced time values, and hence the blood samples need not actually be taken at equally spaced intervals to apply the Guggenheim method. However, if the operator wishes to insure that he used all *observed* concentrations in the absorption phase, then to apply the Guggenheim method the study should be designed for taking blood samples at equally spaced intervals at least up to the time of the expected maximum plasma concentration.

If absorption is not first order, then both the Guggenheim plot of $\log(\Delta A_{t_n}/V_1)$ vs. t_1 and the sigma-minus plot will be curved if the data give a sufficient span of time compared with the time for half of the drug to be absorbed. In these cases, the user usually likes to present the A_{t_n}/V_1 vs. t plot. If only the observed concentration data are used, then the problem presented by simulation example 3 (Table III) may occur and the A_∞/V_1 value may be difficult to determine. However, if one uses a large number of interpolated values, then an estimate close to the true value of A_∞/V_1 would be obtained: this is supported by set 1 in Table III. The relative A_∞/V_1 values from different treatments are important in bioavailability studies and it is desirable to obtain the best possible estimates of this parameter.

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