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Bioavailability Assessment: Methods to Estimate Total Area (AUC 0–∞) and Total Amount Excreted (A_e^∞) and Importance of Blood and Urine Sampling Scheme with Application to Digoxin

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Five methods are compared to estimate the total area under the digoxin plasma or serum concentration–time curve (AUC 0–∞) after a single dose of drug. To obtain accurate estimates of AUC 0–∞, data required are concentrations at a sufficient number of sampling times to define adequately the concentration–time curve prior to the log-linear phase, and at least three, but preferably four or more equally spaced points in the terminal log-linear phase. One method (designated Method I) requires a digital computer; another (Method III) is the classical method (these two methods do not require equally spaced points in the log-linear phase). Method IIA is the accelerated convergence method of Amidon et al.; *Methods IIB and IIC are modifications of this method, but incorporate usual and orthogonal least squares, respectively, which make them more accurate with real (noisy) data. Methods I and IIC gave very comparable estimates of AUC 0–∞. Results indicate that digoxin administered orally in aqueous solution was completely (100%) absorbed when bioavailability estimates were based on oral and intravenous AUC 0–∞ estimates and the actual doses, whereas formerly only about 80% absorption was reported, based on areas, under plasma concentration curves which were truncated at 96 hr. It is shown that the sampling scheme of blood can produce biased apparent bioavailability estimates when areas under truncated curves are employed, but an appropriate sampling scheme and application of method IIC yield accurate bioavailability estimates. This is important particularly in those bioavailability studies where one is attempting to determine the appropriate label dose for a new "fast-release" digoxin preparation relative to the label dose and bioavailability of currently marketed tablets. It is shown that the magnitudes and variability of apparent elimination rate constants and half-lives of digoxin, estimated from urinary excretion data by the σ^- method, depend on which value of A_e^∞ is used. The formerly reported greater interindividual variability of AUC data compared to A_e data for digoxin is explained in that the AUCs, but not the A_e's, involve the renal clearance, which exhibits considerable inter- and intraindividual variation.*

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KEY WORDS: accelerated convergence method to estimate AUC 0–∞ and A_e^∞; bioavailability; estimation of total areas; estimation of total amounts excreted; blood sampling schemes for digoxin; elimination half-life of digoxin; intra- and interindividual variation of renal clearance of digoxin.

INTRODUCTION

Pharmacokinetic equations appropriate to estimation of absolute or relative bioavailability (used here with the connotation of absorption efficiency only, without the other component of rate of absorption) involve ratios of dose-corrected total areas under plasma or serum concentration–time curves (AUC 0–∞), or total amounts of unchanged drug excreted in the urine in infinite time (A_e^∞) after a single dose of drug (1*a*,2). The word "total" herein refers to AUC 0–∞ or A_e^∞ and not, as often erroneously used in the literature, to indicate AUC 0–T or A_e^T, where T is the investigator's last sampling time; hence AUC 0–T is a *partial* area and A_e^T is a *partial* amount excreted in the urine. In this article, such partial areas and amounts excreted are simply designated by AUC and A_e, respectively.

It is common practice in the digoxin (7–21), as well as the literature for many other drugs, to substitute the particular author's AUC or A_e figures for AUC 0–∞ or A_e^∞ in estimating bioavailability. Such estimates are herein called *apparent bioavailabilities*. For digoxin there have been almost as many blood sampling schemes as investigators. Apparent bioavailabilities depend on the sampling scheme employed, and they may be considerable underestimates of the true bioavailability. This will be very important in establishing the correct dose ratio of new "fast-release" digoxin formulations compared with currently marketed "slow-release" tablets, since an error of the order of 20% could have noticeable effects in the clinical use of digoxin. The shortcomings of reporting such apparent bioavailabilities have been pointed out before by other authors (4,6,16,24). We could find only one article (16) where estimates of A_e^∞ for digoxin had been made, but the method was not given. No article could be found where AUC 0–∞ had been estimated for digoxin after oral administration. Several authors (3,7,9,10,12,14–16,19–21) have collected either 0–6 or 0–10 day digoxin urinary excretion data in those cases when they were employing a radioimmunoassay method for digoxin in plasma or serum which had a sensitivity level of 0.2–0.5 ng/ml and which allowed them to follow digoxin in blood only for about 8 hr. However, as assay has been in the literature (22) since 1972 which allows measurement of digoxin down to 0.08 ng/ml of plasma or serum, and for 96 hr after a single 0.5-mg dose of digoxin (5). This assay has been improved (23) and has a sensitivity limit of 0.05 ng digoxin/ml plasma when a 0.5-ml plasma sample is utilized. The authors have used this improved assay in

several digoxin bioavailability studies where blood was sampled over a 96-hr period.

The articles of Wagner *et al.* (2,5) and Lovering *et al.* (17) suggested that apparent bioavailabilities estimated from partial areas may be close to the true bioavailabilities under certain conditions. In a recent review on digoxin (18) it was stated: "However, if blood sampling is continued for an interval (T) after the dose which is sufficient to allow serum concentrations to become quite small, then AUC 0–T is a good approximation of AUC 0–∞." It was (18) further stated: "In our studies of comparative bioavailability, 4 hr of serum sampling gave results as reliable as 8 or 24 hr of sampling." The same authors (15) also stated: "Extending urine collections beyond 1 day or serum sampling beyond 4 or 8 hr does not necessarily reduce between subject variability or enhance the usefulness of the data." However, Beveridge *et al.* (16) stated: "Therefore, statements on bioavailability [of digoxin] based on areas under plasma curves up to 6 hr may differ from those based on cumulative urinary excretion data, in this case by a factor of about 2 [i.e., a 100% error] and could suggest that bioavailability was much worse than it actually was." This dichotomy of opinion prompted us to examine, in general, the assessment of bioavailability and, in particular, to study digoxin bioavailability. In the process, several new simple methods for estimating AUC 0–∞ and A_e^∞ were devised and applied.

THEORETICAL

Methods for Estimating AUC 0–∞ and A_e^∞

All known methods and the new methods to be presented for estimation of AUC 0–∞ and A_e^∞ depend on accurate estimates of AUC or A_e at various times after administration of a single dose of drug. For AUC the trapezoidal rule (1*b*) is usually employed, and with sufficient sampling times (see rows 6, 7, and 8 of Table III) is accurate for digoxin. An even more accurate method would be that resulting from interpolation by the method of Fried and Zeitz (25), which has been computerized (1*c*), coupled with the trapezoidal rule using both observed and interpolated values. All methods (I, IIA, IIB, IIC, and III) discussed below are applicable *only* to AUC or A_e data in the terminal, log-linear phase. Methods IIA, B, and C below also depend on having three or more blood or urine collections at *equally spaced time intervals* in the terminal log-linear phase of drug elimination; for accurate results, it is later shown that *four* such collections is the minimum necessary. The classical method (Method III) for estimating AUC 0–∞ also depends on an accurate estimate of the apparent elimination rate constant,

which also requires at least three and preferably four or more plasma concentration–time points (i.e., C_p, t pairs).

Method I

Method I depends on nonlinear least-squares fitting to equation 1 of log linear AUC, t or A_e, t data using a suitable program and a digital computer:

$$y = P(1) - P(3)\, e^{-P(2)t} \tag{1}$$

For AUC data, y represents AUC at time t, $P(1)$ represents AUC 0–∞, $P(2)$ represents λ_1, and $P(3)$ represents B_1/λ_1, such that the plasma concentration, C_p, in the log-linear phase is described by equation 2. The B_1 in equation 2 (and later equation 13) is a complex function of model parameters:

$$C_p = B_1\, e^{-\lambda_1 t} \tag{2}$$

For A_e data, y represents A_e at time t, $P(1)$ represents A_e^{∞}, $P(2)$ represents λ_1, and $P(3)$ is equivalent to $\mathrm{Cl}_R B_1$, where Cl_R is the renal clearance.

To apply the method, we used the program NONLIN (26) and the Amdahl 470V/6 digital computer. $P(1)$, $P(2)$, and $P(3)$ are the parameters estimated in the fittings. The method has two advantages: (a) the points may be equally or nonequally spaced and (b) one obtains the standard deviations of the estimated AUC 0–∞ or A_e^{∞} as well as measures of fit of predicted AUC or A_e to observed AUC or A_e values.

Method IIA

Method IIA is the accelerated convergence method of Amidon *et al.* (27). For a series of points, (t_i, Y_i), approaching an asymptote, Y_∞, and obeying first-order kinetics from time t', the general equation 3 applies, where λ_1 is the first-order rate constant:

$$Y_i = Y_\infty[1 - e^{-\lambda_1(t_i - t')}] \tag{3}$$

For three equally spaced points, at intervals, Δt, particular cases of equation 3 may be written as

$$Y_1 = Y_\infty[1 - e^{-\lambda_1 \Delta t}] \tag{4}$$

$$Y_2 = Y_\infty[1 - e^{-2\lambda_1 \Delta t}] \tag{5}$$

$$Y_3 = Y_\infty[1 - e^{-3\lambda_1 \Delta t}] \tag{6}$$

Amidon *et al.* (27) used different symbolism such that Y_1 = their X_n, Y_2 = their X_{n+1}, Y_3 = their X_{n+2}, and Y_∞ = their X_n'. They also plotted the differences on the ordinate but ended up deriving the expression corres-

ponding to these differences being plotted on the abscissa. Hence we prefer to plot the differences on the abscissa in order to apply Methods IIB and IIC discussed later.

Consider a rectalinear plot of $Y = Y_i$ (ordinate) vs. $X = Y_{i+1} - Y_i$ (abscissa) with the equation of the straight line being $Y = a + bX$; two points on the line are $(Y_2 - Y_1)$, Y_2 and $(Y_3 - Y_2)$, Y_3; the line extrapolates back to give an ordinate intercept, a, equal to Y_∞; the slope of the line, b, is given by equation 7, and the slope is *negative* since $Y_1 < Y_2 < Y_3$ and $(Y_2 - Y_1) > (Y_3 - Y_2)$.

$$b = \frac{(Y_2 - Y_3)}{(Y_2 - Y_1) - (Y_3 - Y_2)} = \frac{Y_3 - Y_2}{Y_3 - 2Y_2 + Y_1} \tag{7}$$

When $Y = Y_3$, the equation of the line is given by

$$Y_3 = Y_\infty + \left[\frac{Y_3 - Y_2}{Y_3 - 2Y_2 + Y_1}\right](Y_3 - Y_2) = Y_\infty + \frac{(Y_3 - Y_2)^2}{Y_3 - 2Y_2 + Y_1} \tag{8}$$

Rearrangement of equation 8 gives equation 9, which is equivalent to the equation given by Amidon *et al.* (27):

$$Y_\infty = Y_3 - \frac{(Y_3 - Y_2)^2}{Y_3 - 2Y_2 + Y_1} \tag{9}$$

The validity of equation 9 with respect to first-order kinetics is readily checked by substituting for Y_1, Y_2, and Y_3 in equation 9 from equations 4 through 6 and showing that the right-hand side is equal to the left-hand side.

As a simulation for AUC data after bolus intravenous administration of digoxin, let the values of the parameters of equation 1 be $P(1) = 44.67$, $P(2) = 0.0122$, and $P(3) = 37.44$. Substitution of these values and $t = 24$, 48, and 72 hr into equation 1 yielded the values below.

t	Y_i
24	$16.73 = Y_1$
48	$23.82 = Y_2$
72	$29.12 = Y_3$

Substitution of the above values into equation 9 and simplification gave $Y_\infty = 44.81$. The actual value of $P(1) = Y_\infty = \text{AUC}\ 0\text{–}\infty = 44.67$, while the estimated value by this method is 44.81. Hence with these error-free data the method gave an answer with an error of +0.45%.

Method IIB

Method IIB is a modification of Method IIA, but three or more points may be utilized (see Fig. 3 as an example). Equation 10 is a generalization of

equation 8:

$$Y_i = Y_\infty - (\text{slope})(Y_{i+1} - Y_i) \tag{10}$$

Equation 10 indicates that a plot of Y_i vs. $(Y_{i+1} - Y_i)$ will have an ordinate intercept equal to Y_∞ and a negative slope, when the method of least squares is applied to the data and first-order kinetics is obeyed. To illustrate the method, the data used to illustrate Method IIA were extended by adding a Y_4 value for 96 hr and these data are shown below:

t	$Y = Y_i$	$X = Y_{i+1} - Y_i$
24	$16.73 = Y_1$	
		7.09
48	$23.82 = Y_2$	
		5.30
72	$29.12 = Y_3$	
		3.94
96	$33.06 = Y_4$	

Using ordinary least-squares linear regression and the pairs of values $X = 7.09$ and $Y = 16.73$, $X = 5.30$, and $Y = 23.82$, and $X = 3.94$ and $Y = 29.12$, equation 11 was obtained with a correlation coefficient of 1.000. The intercept, 44.64, is

$$Y = 44.64 - 3.935X \tag{11}$$

the estimate of AUC 0–∞ and is within −0.07% of the actual value of 44.67. Methods IIA and IIB give the same answer when there are only three Y_i values and the line is based on only two points.

Method IIC

Method IIC is the same as Method IIB, with the exception that orthogonal least squares (28) is used in place of ordinary least squares. In applying both methods, trapezoidal areas should *not* be rounded off before calculating the parameters of the least-squares line, but final intercept values should be rounded off to the same number of places as the original blood level or urinary excretion data. The equations used to obtain the slope and intercept of the orthogonal least-squares line are shown in the Appendix. With the same data as used to illustrate Method IIB, Method IIC gave the same intercept, 44.64, with an error of −0.07%. However, with other data sets orthogonal least squares and ordinary least squares do not give the same answer. Since both Y_1 and $Y_{i+1} - Y_i$ contain errors, orthogonal least squares is preferred from a statistical point of view. Hence Method IIC is preferred to Method IIB.

Method III

Method II is the classical method (1*a*) for estimating AUC 0–∞ using

$$\text{AUC } 0\text{–}\infty = \text{trapezoidal area } 0\text{–}T + \hat{C}_T/\lambda_1 \tag{12}$$

In equation 12, T is the last sampling time in the log-linear phase and $\hat{C}_T$ is the estimated concentration at that time obtained with equation 14. The value of λ_1 is obtained by least-squares regression based on equation 13.

$$\ln C_p = \ln B_1 - \lambda_1 t \tag{13}$$

$$\hat{C}_T = e^{(\ln B_1 - \lambda_1 T)} \tag{14}$$

As indicated formerly, one should have a minimum of three and preferably four or more C_p,t or A_e,t pairs in the log-linear phase and adequate sampling in the early time period with blood data to apply equation 12.

Other Methods

When the Guggenheim method (1*d*,29) was applied to the data which were used to illustrate Methods IIB and IIC, the AUC 0–∞ estimate was 37.50, with a large error of −16.1%. When the "rate method" (1*e*) was applied to the same data, the AUC 0–∞ estimate was 37.64, with a large error of −15.3%. Since these two methods gave such large errors even with "error-free" data, they were not considered further for application to digoxin AUC,t data. The Results and Discussion section indicates that the same conclusion was reached with respect to digoxin urinary excretion data.

Variability of AUC and A_e Data

There have been several reports (9,15,18) that interindividual AUC data are more variable than interindividual A_e data, and the conclusion was reached that A_e data provide a more reliable estimate of apparent bioavailability than AUC data. There is a simple pharmacokinetic explanation for such differences in variability. AUC data are influenced by an additional variable, namely renal clearance, Cl_R, which does not influence A_e data. If the system obeys linear pharmacokinetics, then equations 15–17 apply:

$$A_e = \text{Cl}_R \cdot \text{AUC} \tag{15}$$

$$A_e^{\infty} = fFF^*D \tag{16}$$

$$\text{AUC } 0\text{–}\infty = fFF^*D/\text{Cl}_R \tag{17}$$

In equations 16 and 17, f is the fraction of the drug which reaches the circulation which is excreted in the urine, F is the bioavailability factor concerned with incomplete absorption ($0 \leq F \leq 1$), F^* is the bioavailability factor concerned with the "first-pass effect" ($0 \leq F^* \leq 1$), and D is the dose administered orally. For the intravenous route, $F = 1$ and the same equations apply. F^* has meaning only when both oral and intravenous data are considered together.

Koup *et al.* (14) reported renal clearances of digoxin in eight subjects following both bolus intravenous injections and intravenous infusions. Interindividual coefficients of variation of the Cl_R values were 40.8% and 37.8% for bolus and infusion, respectively. Intrasubject variability of Cl_R is reflected by the coefficient of variation calculated from the differences in the pairs of Cl_R values for the eight subjects; this coefficient of variation was 43.8%. This suggests that intraindividual variation and interindividual variation of the Cl_R of digoxin are very similar. Similar calculations made by the authors from Cl_R data collected in two recent digoxin bioavailability studies, one involving 12 and the other involving 15 subjects, also gave little differences between inter- and intraindividual variation of Cl_R. Thus one can readily see with such an additional variable (Cl_R) being "contained in" AUC data, but not in A_e data, why AUC data are more variable than A_e data. Equations for both AUC and A_e will be analogous to equations 16 and 17, but will also contain exponential terms; those equations for data in the log-linear phase will contain a term with $e^{-\lambda_1 t}$ (like equation 1); those for data in the postabsorptive, distribution phase will contain two terms, one with $e^{-\lambda_1 t}$ and one with $e^{-\lambda_2 t}$. Therefore, on a theoretical basis one would expect AUC to a given time to be more variable than AUC 0–∞, and A_e to a given time to be more variable than A_e^{∞}.

Estimation of AUC 0–∞ from Data of Wagner *et al.* (5)

Wagner *et al.* (5) reported digoxin plasma concentration–time data for two subjects administered labeled doses of 0.5 mg of digoxin by 1-hr constant-rate intravenous infusion, as a solution orally, as two B & W tablets, and as two Fougera tablets (both 0.25 mg/tablet) orally. The blood sampling scheme employed is shown in the sixth row of Table III. For each subject and each treatment, AUC 0–∞ was estimated by Methods I, IIA, IIB, IIC, and III. The needed AUC,t data were obtained by application of the trapezoidal rule (1b) to each set of C_p,t data. Bioavailability estimates, based on ratios of dose-corrected AUC 0–∞'s, were compared with apparent bioavailabilities, calculated from dose-corrected AUCs at various times after administration, using the 1-hr intravenous infusion data as the "standard" in both cases.

Importance of Blood Sampling Scheme

Digoxin plasma concentration–time data were simulated for a "fast-release" (A) and a "slow-release" (B) digoxin formulation. Equations used are shown in the Appendix. The advantage of such a procedure is that the exact answers are known against which "experimental answers" may be compared. Eight different blood sampling schemes were compared: six of these were taken from the literature; a seventh scheme was used by the authors in a recent unpublished study; the eighth scheme led to the points shown in Fig. 2. The equations used to generate these data were chosen so that the simulated plasma concentrations from 3 hr to infinity were identical to the third decimal place for A and B. This provided a *minimum* (not a maximum) test to show the effect of the blood sampling scheme on apparent bioavailabilities. It also provided a means to show that the reasoning of Lovering *et al.* (17) is faulty, when applied to digoxin.

Estimation of A_e^∞ from Data of Juhl *et al.* (20)

The individual subject/treatment sets of A_e,t data of Juhl *et al.* (20) were employed to obtain estimates of A_e^∞ by Methods I, IIA, IIB, and IIC. Bioavailability estimates, based on ratios of the A_e^∞'s, were compared with apparent bioavailabilities, based on ratios of A_e's to various times. Apparent elimination rate constants, λ_1, were calculated, using the method of least squares, the different A_e^∞ estimates, and equation 18, in which $\ln I$ is the intercept and its value depends on the particular model which applies.

$$\ln (A_e^\infty - A_e) = \ln I - \lambda_1 t \tag{18}$$

RESULTS AND DISCUSSION

Estimation of AUC 0–∞ from Data of Wagner *et al.* (5)

The averages of duplicate assay values reported by Wagner *et al.* (5) were used as the C_p,t data. The AUCs were obtained by trapezoidal rule. AUCs in the log-linear phase, corresponding to 24, 48, 72, and 96 hr for oral treatments and 25, 49, 73, and 97 hr for the 1 hr constant-rate intravenous infusion, were employed to estimate AUC 0–∞ values, which are shown in Table I. Plotting of data according to equation 10 (see Fig. 3 for example with urinary excretion data) indicated that elimination was not apparent first order at 12 hr, but was at 24 hr, when plasma data were evaluated. Data for the Fougera tablet in subject 2 were anomalous in that the data did not obey apparent first-order kinetics (see footnotes to Table I). For the other seven sets of data, the AUC 0–∞'s estimated by Method IIC agreed extremely well

Table I. Summary of AUC 0–∞ for Digoxin Estimated from the Plasma Concentration–Time Data of Wagner *et al.* (5)

Subject	Method	AUC 0–∞ [(ng/ml) × hr]			
		1 hr i.v. infusion	Solution orally	B & W tablet orally	Fougera tablet orally
1	I	43.2[a]	40.1[a]	20.1[a]	9.03[a]
		(0.82)[b]	(3.42)	(0.08)	(0.20)
	IIA	41.5[c]	51.2[c]	20.3[c]	9.50[c]
	IIB	43.1[a]	39.2[a]	20.1[a]	9.00[a]
	IIC	43.2[a]	39.3[a]	20.1[a]	9.02[a]
	III	44.8[a]	37.6[d]	20.0[a]	8.53[a]
2	I	44.7[a]	42.0[a]	21.7[a]	15.2[a]
		(4.97)	(0.02)	(1.06)	(8.56)
	IIA	37.3[c]	42.1[c]	24.9[c]	—[e]
	IIB	42.8[a]	42.0[a]	21.5[a]	—[e]
	IIC	44.3[a]	42.0[a]	21.7[a]	—[e]
	III	47.5[a]	49.1[d]	20.2[a]	23.4[a]

[a] Four partial areas to 25, 49, 73, and 97 hr were used for the intravenous data and those to 24, 48, 72, and 96 hr were used for the oral data.
[b] Numbers in parentheses are standard deviations of the estimated areas.
[c] Three partial areas to 25, 49, and 73 hr were used for the intravenous data and those to 24, 48, and 72 hr were used for the oral data.
[d] Three partial areas to 48, 72, and 96 hr were used.
[e] Methods gave ridiculous answers, indicating that data were not obeying first-order kinetics; this was also indicated by the very large standard deviation of 8.56 for an estimated area of 15.2 by Method I and the discrepancy of the answers obtained by Methods I and III.

with the AUC 0–∞'s estimated by Method I, the mean absolute deviation being 0.17 (ng/ml) × hr (0.5% of mean by Method I), and in four out of the seven sets the estimates were identical. For Methods I and IIB the mean absolute deviation was 0.45 (ng/ml) × hr (0.7% of mean by Method I), for Methods I and III it was 2.3 (ng/ml) × hr (7.3% of mean by Method I), and for Methods I and IIA it was 3.45 (ng/ml) × hr (11% of mean by Method I).

Figure 1 illustrates the variation in apparent digoxin bioavailability as a function of time. The "true" bioavailabilities, based on dose-corrected AUC 0–∞ ratios, are shown above the infinity signs at the far right of the two graphs. These "true" bioavailabilities, based on AUC 0–∞'s obtained by Method I, are very similar to those estimated by Method IIC. This new interpretation of the data of Wagner *et al.* (5) indicates that digoxin, administered as an aqueous solution orally in those studies, was completely absorbed. This agrees with similar estimates made from 10-day urinary excretion data (3) and disagrees with data summarized by Greenblatt *et al.* (18) and with the original estimates of Wagner *et al.* (5), based on apparent bioavailabilities. The graphs in Fig. 1 illustrate how impossible it is to compare apparent bioavailabilities of different investigators who sample

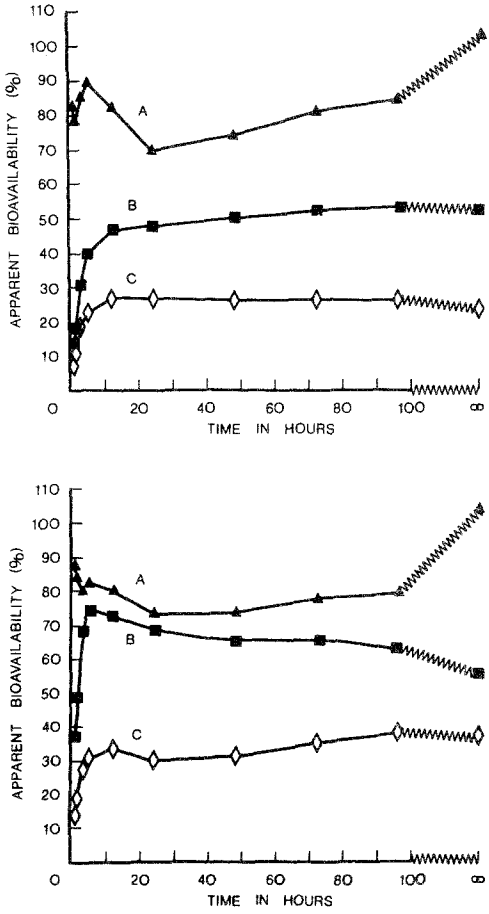

Fig. 1. Apparent bioavailabilities as a function of time for two subjects based on the digoxin plasma concentrations of Wagner *et al.* (5). Apparent bioavailabilities were calculated from the dose-corrected ratio of areas under the plasma concentration–time curves. The "true" bioavailabilities are given by the points above the infinity sign and are based on total areas estimated by Method I. Key: A, oral solution relative to 1-hr intravenous infusion; B, B & W (Lanoxin) tablet relative to 1-hr intravenous infusion; C, Fougera tablet relative to 1-hr intravenous infusion. Top, subject 1; botton, subject 2.

Table II. Comparison of Results Based on Partial Areas with Those Based on Total Areas Obtained by Method I

	Apparent bioavailability of digoxin in the Fougera tablet relative to that in the B & W tablet				
Area utilized	Subject 1		Subject 2		Average
0–5 hr	57.7	Decrease	41.3	Increase	49.5
0–96 hr	48.5	with	60.7	with	54.6
0–∞	44.7	time	69.4	time	57.1

blood for different times, such as 3, 4, 5, 6, 8, 12, 24, 48 and 96 hr (7–21). It also should be noted that in three out of four examples the "true" bioavailability estimates for the tablets were *lower* than the values estimated from AUC 0–96 hr data. Also, throughout the 12–96 hr period, the trend lines rise for subject 1 (curves A and B) and subject 2 (curves A and C), but the trend line falls in the same period for subject 2 (curve B). It is also obvious from the figure that a 6-hr sampling scheme, as recommended by the Food and Drug Administration for digoxin (31), cannot provide "true" bioavailability estimates for digoxin.

Table II compares results based on partial areas with those obtained by total areas, when the Fougera tablet is compared to the B & W tablet as a "standard." For subject 1 there is a decrease with increase in time, and for subject 2 there is an increase with increase in time. Klink *et al.* (13) also reported data on the apparent bioavailabilities of digoxin from the B & W tablet and a Towne-Paulsen tablet, relative to digoxin elixir. For the B & W tablet the values were 71%, 83%, 96%, and 106%, and for the Towne-Paulsen tablet they were 65%, 74%, 85%, and 101%, based on ratios of AUC values to 5, 12, 24, and 48 hr, respectively. These results make questionable the conclusions of Greenblatt *et al.* (15,18) and Lovering *et al.* (17) that areas under truncated plasma or serum concentration–time curves are satisfactory to estimate digoxin bioavailability.

Importance of Blood Sampling Scheme

Figure 2 shows simulated digoxin plasma concentrations for a "fast-release" (A) and a "slow-release" (B) digoxin formulation. Table III summarizes apparent bioavailabilities as a function of time, estimated from AUCs obtained by trapezoidal rule from the data shown in Fig. 2. Care must be taken to read Table III correctly. The "true" bioavailability is 97.4%. Values listed in rows 1 to 8 under "Apparent bioavailability" are those which would be estimated with the given sampling scheme and the areas under the truncated curves up to the last sampling time. Thus with the 3-hr

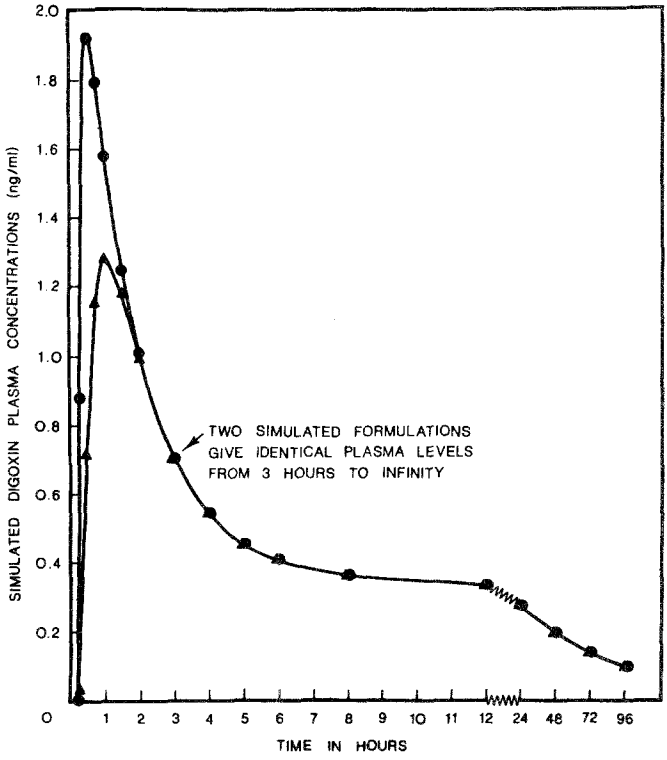

Fig. 2. Simulated digoxin plasma concentrations for "fast-release" and "slow-release" digoxin formulations.

sampling scheme in row 1 one underestimates the "true" bioavailability by 100 (97.4–84.3)/97.4 = 13.4%. With the 6-hr sampling scheme in row 2 one underestimates the "true" bioavailability by 14.7%. For this simulation the 96-hr sampling schemes of rows 6–8 give AUC 0–96 hr estimates between 99.3% and 99.8% of the "true" bioavailability, while Method IIC (last column of Table III) gives estimates from 100.1% to 100.4% of the "true" bioavailability. Values listed in the last row of Table III are those obtained with equations 23 and 25 of the Appendix. Discrepancies between the numbers when one reads vertically in the table—for example, comparing 84.3 in row 1 with 76.9 in row 9—are caused by the sampling scheme's not truly defining the actual curves. This is a *minimum* (not a maximum) test and does show that sampling schemes only up to 8 hr do introduce appreciable error in bioavailability estimates. As stated in the Introduction, Beveridge *et al.* (16) claimed that with real data areas under digoxin plasma concentrations up to 6 hr can lead to a 100% underestimate of bioavailability.

Table III. Importance of the Blood Sampling Scheme in Estimating Bioavailability Using Simulated Data for "Fast-Release" (A) and "Slow-Release" (B) Digoxin Formulations (Fig. 2)

		Apparent bioavailability (%) $= \frac{\text{AUC for B}}{\text{AUC for A}} \times 100$						Bioavailability $= \frac{\text{AUC 0–}\infty\text{ for B}}{\text{AUC 0–}\infty\text{ for A}} \times 100$
Reference[a]	Blood sampling scheme (hr)	3	6	8	12	24	96	∞
(6)	0, 0.5, 1.0, 1.5, 2, 3	84.3						—[d]
(8)	0, 0.5, 1, 3, 6		83.1					—[d]
(19)	0, 0.5, 1, 1.5, 2, 3, 4, 6		88.1					—[d]
(10)	0, 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8			86.1				—[d]
(21)	0, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 4, 7, 24					94.2		—[d]
(5)	0, 0.25, 0.5, 0.75, 1, 1.5, 3, 5, 12, 24, 48, 72, 96						97.2	97.8[e]
b	0, 0.25, 0.5, 0.75, 1, 1.5, 3, 5, 7, 9, 12, 24, 48, 72, 96						97.7	97.5[e]
c	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96						96.8	97.5[e]
	True values from equations	76.9	84.1	86.2	88.9	92.7	96.6	97.4[f]

[a] Reference for the sampling scheme shown, which was used in an actual digoxin bioavailability study.
[b] Sampling scheme used in recent (unpublished) study of K. S. Albert, J. W. Ayres, J. G. Wagner, *et al.*
[c] Sampling scheme for points shown in Fig. 2.
[d] Bioavailability estimate cannot be made by Methods I through III since data are not in log-linear phase.
[e] Values obtained from AUC 0–∞'s estimated by Method IIC from AUCs to 24, 48, 72, and 96 hr.
[f] Actual bioavailability for the simulation.

Lovering *et al.* (17) stated: "The AUC ratios at $t = 2T$ are, in most cases, within a few percentage points of the AUC ratio at infinite time and experimentally indistinguishable from it." Here T is the time absorption was allowed to proceed in their simulations. In the present simulations the "fast-release" formulation had released 99% of the "drug" in 0.66 hr, hence we can consider $2T$ equal to 1.32 hr; the "slow-release" formulation had released 99% of the "drug" in 1.735 hr, hence we can consider $2T$ equal to 3.5 hr. The actual apparent bioavailabilities were 76.9% and 80.4% at 3 and 4 hr, respectively, which are 20.5% and 17.4% from the "true" bioavailability of 97.4%. Hence the conclusions of Lovering *et al.* (17) with respect to the validity of use of truncated blood level curves should not be applied to digoxin.

Estimation of A_e^∞ Data of Juhl *et al.* (20)

Juhl *et al.* (20) measured urinary excretion of digoxin in ten subjects over a 10-day period after oral administration of 0.5 mg digoxin (as B & W elixir, 0.05 mg/ml) alone, then again when the subjects had been administered sulfasalazine for 6 days. Individual subject data were kindly supplied to us by Dr. Juhl. Table IV lists the mean amounts of digoxin excreted to the various times, with their standard errors, both in micrograms and expressed as a percent of the means. In the last three columns of Table IV are given the apparent bioavailabilities in percent under the heading "Ratio of means × 100," as well as the standard error (SE) of the ratio, and these expressed as a percentage of the mean ratio. The formula used for the SE of the mean ratios is shown in footnote *b* to Table IV. Since, usually, bioavailability estimates are made from a ratio of means, the variances of values from which both the numerator and denominator were obtained influence the variance of the ratio. The formula for the SR of the ratio given takes this into consideration; this formula does not appear to have been employed in bioavailability studies formerly, but should be employed in the future.

Also listed in Table IV are the bioavailabilities, calculated from the ratios of mean A_e^∞ values, obtained by Methods I, IIA, IIB, and IIC, as well as the mean A_e^∞ values with their standard errors. Method IIA, employing only 3-, 4-, and 5-day excretion data, gave rather poor estimates of A_e^∞, with a consequent low bioavailability estimate of 75.5% and a high value of 12.3% for 100 (SE of ratio)/ratio. As with the former AUC,t data, Methods I and IIC, using 3–10 day excretion data, gave the same bioavailability estimate of 82.1%, with essentially the same standard errors, namely 5.64 and 5.57. Method IIC, using only 3–6 day data, gave a similar estimate of 82.3% for bioavailability, with a standard error of 5.04. In applying Methods I through IIC to these data we excluded day 1 and 2 excretion,

Table IV. Average Amounts of Digoxin Excreted in the Urine of Ten Subjects and Apparent Bioavailabilities of Digoxin When Administered with Sulfasalazine Relative to Digoxin Alone with Their Standard Errors, Estimated from the Data on Juhl *et al.* (20)

		Amount of digoxin excreted (μg)								
		Digoxin alone			Digoxin + sulfasalazine			Apparent bioavailability (%)		
Time (days)	Method	Mean	SE[a]	$\frac{SE}{Mean} \times 100$	Mean	SE	$\frac{SE}{Mean} \times 100$	Ratio of means × 100	SE of ratio[b]	$\frac{SE}{ratio} \times 100$
0.08		37.7	3.09	8.19	27.9	3.01	10.8	74.0	10.8	14.6
0.17		53.3	3.89	7.30	41.2	4.40	10.7	77.3	8.81	11.4
0.33		67.7	4.21	6.21	59.3	6.39	10.8	87.6	8.85	10.1
0.5		80.3	5.31	6.62	68.5	6.83	9.97	85.3	8.20	9.61
1.0		116	8.35	7.20	95.5	9.46	9.90	82.2	8.89	10.8
2.0		167	11.4	6.80	137	12.3	8.96	82.0	6.94	8.46
3.0		205	13.7	6.66	167	14.1	8.45	81.5	6.50	7.98
4.0		229	15.0	6.54	187	15.3	8.20	81.7	6.48	7.93
5.0		245	15.8	6.44	201	15.5	7.69	82.0	6.18	7.54
6.0		257	16.3	6.36	210	15.7	7.45	81.7	6.05	7.40
7.0		265	16.6	6.28	216	15.7	7.29	81.5	5.87	7.20
8.0		271	16.9	6.23	221	15.8	7.17	81.6	5.83	7.14
9.0		275	17.1	6.23	225	15.8	7.03	81.8	5.85	7.15
10.0		278	17.3	6.22	228	16.0	7.02	82.0	5.84	7.12
∞	I(3–10)[c]	285	17.5	6.14	234	15.4	6.58	82.1	5.64	6.87
∞	IIA(3–5)	303	23.4	7.72	229	18.7	8.17	75.5	9.32	12.3
∞	IIB(3–6)	281	18.2	6.48	224	16.3	7.28	79.7	6.37	7.99
∞	IIB(3–10)	284	17.5	6.16	232	15.5	6.68	81.6	5.74	7.03
∞	IIC(3–6)	282	17.6	6.24	232	13.8	5.95	82.3	5.04	6.13
∞	IIC(3–10)	285	17.4	6.61	234	15.3	6.54	82.1	5.57	6.79

[a] SE is the standard error of the mean calculated in the usual manner.

[b] SE of ratio $= \{(\Sigma Y_1^2 - 2R \Sigma Y_1 Y_2 + R^2 \Sigma Y_2^2)/[N(N-1)(\bar{Y}_2)^2]\}^{1/2}$, where Y_1 and Y_2 are the individual values from which the two means were calculated, R is the ratio of means, and $\bar{Y}_2$ is the mean in the denominator of the ratio (30).

[c] Numbers in parentheses indicate the data used in days.

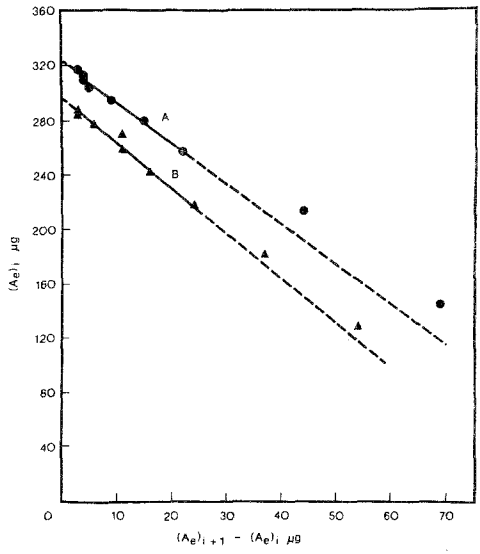

Fig. 3. Plots of $(A_e)_i$ vs. $(A_e)_{i+1}-(A_e)_i$ prepared from the data of Juhl *et al.* (20) for subjects 1 and 2 given digoxin alone. The orthogonal least-squares lines, based on 3–10 day excretion of digoxin, have been extrapolated to show that the points based on 1- and 2-day excretion lie above the extrapolated line, indicating that urinary excretion is not apparent first order until day 3. Key: A, subject 1, digoxin alone, intercept = $A_e^{\infty} = 322\,\mu g$; B, subject 2, digoxin alone, intercept = $A_e^{\infty} = 297\,\mu g$.

since there was evidence with many data sets that the A_e,t data were not truly log linear until day 3. This is reflected in the larger standard errors for both mean amounts excreted and apparent bioavailabilities for days 1 and 2 compared to days 3 through 10 (Table IV).

In applying Methods IIB and IIC one should first plot $(A_e)_i$ vs. $(A_e)_{i+1}-(A_e)_i$ or $(AUC)_i$ vs. $(AUC)_{i+1}-(AUC)_i$, as shown in Fig. 3. In our modification, data are plotted as shown, while Amidon *et al.* (27) plotted in the reverse manner, i.e., $(A_e)_{i+1}-(A_e)_i$ vs. $(A_e)_i$. Plots prepared from the data of Juhl *et al.* (20) for subjects 1 and 2 given digoxin alone are shown in Fig. 3. The orthogonal least-squares lines (Method IIC), based on 3–10 day excretion, are drawn through the points, and then these lines are extrapolated. One can see that the points derived from 1- and 2-day excretion, at far right of the figure, lie above the extrapolated lines. Because of the increasing separation of the points on such a plot, the inclusion of the points based on

day 1 and 2 excretion in calculation of the orthogonal least-squares line would unduly influence the estimated value of the intercept, A_e^∞; inclusion of such points leads to estimates of A_e^∞ which are lower than the value of A_e at the last sampling time. Deviation of such points from the extrapolated line, based on *later time* points, strongly suggests that day 1 and 2 excretion data are not in the log-linear phase. This *may* be caused by the nonspecificity of the digoxin radioimmunoassay (22), more rapid excretion of some cross-reacting metabolites than digoxin, and thus a changing metabolite/unchanged drug ratio in urine with time, as formerly pointed out by Stoll and Wagner (32). Hence we chose to estimate A_e only from the 3–6 and 3–10 day urinary excretion data. Similarly, with the data of Wagner *et al.* (5) such plots indicated that the plasma concentration and AUC,t data from 24 to 96 hr were in the log-linear phase but that the 12-hr data were not. The reason for the discrepancy between plasma concentration and urinary excretion data for digoxin with respect to the time when the log-linear phase commences is unknown at this time.

The apparent bioavailability estimate of 82.2% from 1-day urinary excretion is essentially the same as the bioavailability estimate of 82.1% based on A_e^∞ values obtained by Methods I (3–10 days) and IIC (3–10 days), but the standard error of 8.89% for 1-day excretion is higher than the values of 5.64% and 5.57% obtained using Methods I and IIC to obtain A_e^∞. The greater variability of apparent bioavailability based on 1-day excretion than bioavailability based on estimates of A_e^∞ with these data supports arguments made in the Theoretical section.

Table V lists the individual subject/treatment values of A_e and apparent elimination rate constants, λ_1, obtained by Methods I and IIC and equation 18. The standard deviations of these two estimated parameters and the average absolute percent deviation and range of percent deviations of $\hat{A}_e$ from observed A_e obtained by Method I are also shown in the table. These standard deviations of the estimated parameters are very small relative to the estimates, and the percent deviations are very small compared to most other nonlinear least-squares fitting with which the senior author is familiar. This strongly supports obeyance of first-order kinetics in the 3–10 day period. The mean absolute deviation of A_e^∞'s estimated by Methods I and IIC is only 1 μg, and in five out of the 20 sets of data both methods gave the same estimate of A_e^∞. Differences in the λ_1 values obtained by Methods I and IIC just reflect how sensitive such values are to change in asymptote (A_e^∞ value) used when applying equation 18. Frequently authors use the observed amount excreted to the end of their observation period (here 10 days) as the asymptote, with consequent considerable error in estimated apparent elimination rate constant or elimination half-life. This was discussed formerly by Wagner (33).

Table V. Estimated Amounts of Digoxin Excreted in the Urine in Infinite Time (A_e^∞) Obtained by Methods I and IIC Using 3–10 Day Urinary Excretion Data of Juhl *et al.* (20) and Apparent Elimination Rate Constants (λ_1)

	Digoxin alone						Digoxin + sulfasalazine					
	Method I		Deviations (%)		Method IIC		Method I		Deviations (%)		Method IIC	
Subject	A_e^∞ (μg)	λ_1 (days^{-1})	Average[a]	Range	A_e^∞ (μg)	λ_1 (days^{-1})	A_e^∞ (μg)	λ_1 (days^{-1})	Average	Range	A_e^∞ (μg)	λ_1 (days^{-1})
1	323	0.409	0.20	−0.38 to	322	0.459	256	0.323	0.56	−0.88 to	253	0.453
	(1.19)[b]	(0.0224)		+0.36			(3.19)	(0.0445)		+1.4		
2	295	0.367	0.08	−0.11 to	297	0.327	246	0.333	0.14	−0.19 to	246	0.333
	(0.469)	(0.0061)		+0.13			(0.73)	(0.0098)		+0.37		
3	253	0.323	0.19	−0.34 to	253	0.323	205	0.299	0.30	−0.33 to	204	0.308
	(1.09)	(0.0105)		+0.34			(1.44)	(0.013)		+0.61		
4	232	0.356	0.36	−0.55 to	231	0.382	274	0.362	0.69	−1.24 to	273	0.492
	(1.55)	(0.0225)		+0.97			(3.77)	(0.057)		+1.17		
5	342	0.329	0.34	−0.58 to	339	0.381	179	0.301	0.92	−2.98 to	181	0.272
	(2.58)	(0.0228)		+1.02			(3.98)	(0.0990)		+1.38		
6	306	0.333	0.23	−0.58 to	305	0.348	283	0.409	0.29	−0.38 to	284	0.406
	(1.62)	(0.0149)		+0.44			(1.44)	(0.0220)		+0.39		
7	310	0.403	0.23	−0.35 to	311	0.407	300	0.344	0.11	−0.22 to	300	0.347
	(1.27)	(0.0181)		+0.48			(0.79)	(0.010)		+0.15		
8	216	0.327	0.39	−0.80 to	217	0.309	158	0.227	0.12	−0.17 to	158	0.227
	(1.73)	(0.0217)		+0.58			(0.77)	(0.0060)		+0.35		
9	366	0.343	0.11	−0.34 to	367	0.333	255	0.357	0.15	−0.22 to	254	0.375
	(0.87)	(0.0071)		+0.21			(0.69)	(0.0092)		+0.31		
10	205	0.318	0.16	−0.51 to	204	0.335	186	0.439	0.37	−0.40 to	186	0.444
	(0.92)	(0.0140)		+0.32			(0.93)	(0.0227)		+0.55		
Mean	285	0.351			285	0.360	234	0.339			234	0.366

[a] Average absolute deviation (signs ignored) of estimated AUC from observed AUC.
[b] Numbers in parentheses are standard deviations of the estimated parameters.

Table VI. Mean Apparent Elimination Rate Constants of Digoxin ($\bar{\lambda}_1$) and Corresponding Half-Lives Obtained from the Urinary Excretion Data of Juhl *et al.* (20) for a Panel of Ten Subjects

		Digoxin alone			Digoxin + sulfasalazine		
Method[a]	Data used (days)	$\bar{\lambda}_1$ (days^{-1}) ($t_{1/2}$ hr)	95% C.I. of $\bar{\lambda}_1$ ($t_{1/2}$ hr)	C.V.[b] (%)	$\bar{\lambda}_1$ (days^{-1}) ($t_{1/2}$ hr)	95% C.I. of $\bar{\lambda}_1$ ($t_{1/2}$ hr)	C.V. (%)
I	3–10	0.351 (47.4)	0.328–0.375 (44.4–50.7)	9.34	0.339 (49.1)	0.297–0.381 (43.7–56.0)	17.5
IIA	3–5	0.400 (41.6)	0.268–0.532 (31.3–62.1)	46.0	0.407[c] (40.9)	0.258–0.556 (29.9–64.5)	51.2
IIB	3–6	0.392 (42.4)	0.341–0.443 (37.6–48.8)	18.0	0.435[c] (38.2)	0.358–0.512 (32.5–46.5)	24.8
IIB	3–10	0.368 (45.2)	0.341–0.395 (42.2–48.8)	10.1	0.384 (43.3)	0.327–0.441 (37.7–50.9)	20.8
IIC	3–6	0.380 (43.8)	0.330–0.430 (38.7–50.4)	18.5	0.427[c] (39.0)	0.351–0.503 (33.1–47.9)	24.8
IIC	3–10	0.360 (42.3)	0.327–0.393 (42.3–50.9)	12.9	0.366 (45.9)	0.306–0.426 (39.0–54.4)	23.1

[a] Indicated method was used to obtain the amount excreted at infinite time (A_e^∞), and then the apparent elimination rate constant (λ_1) was obtained by linear least-squares regression using equation 18.
[b] C.V. (%) = (standard deviation/mean) × 100.
[c] Based on only nine subjects; subject 5 was excluded since a ridiculous answer for A_e^∞ was obtained by the indicated method.

The Guggenheim method (1*d*,29) and the "rate method" (1*e*) gave very poor estimates for A_e^∞ of 150 and 151, respectively, from the data of subject 1 (digoxin plus sulfasalazine), compared to values of 256 and 253 obtained by Methods I and IIC, respectively. As a result, these methods were not evaluated further with respect to digoxin urinary excretion data.

Table VI lists the mean apparent elimination rate constants, λ_1, the 95% confidence intervals of λ_1, and the corresponding apparent elimination half-lives, as well as the coefficients of variation of λ_1, obtained by the estimation of A_e^∞ by Methods I through IIC and then applying equation 18. Such a comparison is even more sensitive than a comparison of A_e^∞ values (Tables IV and V). Data in Table VI show that the most elegant method, Method I, gave the smallest 95% C.I. for λ_1 and the smallest coefficients of variation. The poorest method, Method IIA, gave the largest 95% C.I.'s of λ_1 and the largest coefficients of variation. Method IIB (3–10 days) gave 95% C.I.'s and coefficients of variation just slightly different than those of Method I, with Method IIC (3–10 days) a close second. With just 3–6 day excretion, Methods IIB and IIC gave larger confidence intervals and coefficients of variation, but the estimates themselves are quite good when compared to those obtained by Method I (3–10 days). Table VI clearly shows what is often attributed to intersubject variation is just the result of the method used to estimate apparent elimination rate constants and half-lives. From the λ_1 values estimated by Method I, intersubject variation in apparent elimination half-life of digoxin is indicated by a mean normalized difference of 16.0%, with a range of 1.7–36%, when equation 19 is employed.

$$\text{Normalized difference (\%)} = \frac{|(t_{1/2})_d - (t_{1/2})_{d+s}|}{[(t_{1/2})_d + (t_{1/2})_{d+s}]/2} \times 100 \qquad (19)$$

In equation 19, $(t_{1/2})_d$ is the half-life obtained with digoxin alone and $(t_{1/2})_{d+s}$ is the half-life obtained when digoxin was administered after sulfasalazine. Of course, in this case, intersubject variation may be confounded with a possible effect of sulfasalazine on the half-life of digoxin, but there was no real evidence of such an effect.

A reevaluation (34) of the digoxin intravenous data of Koup *et al.* (14) gave a mean elimination half-life of 42.1 hr, with a range of 33.0–53.3 hr. The half-lives shown in Table VI, obtained from a different panel of subjects and from urinary excretion data obtained after oral administration, agree very well with those data.

CONCLUSIONS

1. Method IIC, requiring only a desk calculator, and Method I, requiring a digital computer, give extremely similar estimates of both

AUC 0–∞ and A_e^{∞}, provided that there are at least four values of AUC or A_e in the terminal log-linear phase. Method IIC is preferred over Method IIB, both on a statistical basis and on the basis of results obtained with real AUC data. Methods IIB and IIC, based on the method proposed by Amidon *et al.* (27), but incorporating a least-squares extrapolation, are more accurate with real (noisy) data than Method IIA.

2. The plots of $(A_e)_i$ vs. $(A_e)_{i+1} - (A_e)_i$ (see Fig. 3) or similar plots of $(AUC)_i$ vs. $(AUC)_{i+1} - (AUC)_i$ are often a more sensitive indicator of when the log-linear phase begins than classical semilogarithmic plots.

3. Because of 1 and 2 above it may be wise for investigators seriously to consider writing protocols which provide for the taking of four equally spaced blood and urine samples in the log-linear phase after administration of single doses of not only digoxin but also any drug where the purpose of the study is to gather bioavailability or pharmacokinetic information. There must, of course, also be enough samples drawn prior to the commencement of the log-linear phase to describe adequately the plasma concentration–time curve and/or the amount excreted–time curve.

4. A 0–6 hr blood sampling scheme for digoxin will yield a significant underestimate of relative bioavailability when "slow-release" and "fast-release" digoxin preparations are compared after oral administration.

5. Digoxin administered orally as an aqueous solution is most probably more bioavailable than formerly reported. The senior author formerly reported (5) about 80% absorption from the aqueous solution (relative to 1-hr intravenous infusion) based on comparison of dose-corrected AUC 0–96 hr's. Reevaluation of these data in this report indicates 100% absorption. Hence in estimating absolute bioavailabilities of oral dosage form of digoxin (i.e., intravenous route as the standard) the appropriate answer appears to be obtained only when one estimates AUC 0–∞'s after both routes.

6. The formerly reported greater interindividual variability of AUC data compared with A_e data for digoxin is explained in that the AUCs but not the A_e's involve the renal clearance.

7. Preliminary data suggest that the intraindividual variability of the renal clearance of digoxin is very similar in magnitude to the interindividual variability.

8. When bioavailabilities are estimated from the ratio of mean dose-corrected AUCs or A_e's, the standard error calculated by the formula in footnote *b* of Table IV may be used as a measure of the error of the bioavailability estimate.

9. The magnitudes and variability of apparent elimination rate constants and half-lives of digoxin (and presumably other drugs), estimated from urinary excretion data by the σ^- method, depend on the value used for

the asymptotic amount excreted. Method IIC provides a simple procedure to estimate the appropriate A_e^{∞} for application of the σ^- method.

10. As far as the relative bioavailability estimate is concerned, following oral administration of digoxin there appears to be no particular advantage in collecting urine beyond 24 hr. However, the standard error of the bioavailability estimate does decrease somewhat as the urinary collection period is extended up to 10 days.

APPENDIX

Orthogonal Least Squares (28)

The slope and intercept of the orthogonal least-squares lines when Method IIC was applied in this study were obtained with

$$\text{Slope} = [(s_y^2 - s_x^2) + \sqrt{(s_y^2 - s_x^2)^2 + 4(s_{xy})^2}]/2s_{xy} \tag{20}$$

$$\text{Intercept} = \bar{y} = (\text{slope})\,\bar{x} \tag{21}$$

where

$$s_x^2 = \Sigma x^2 - (\Sigma x)^2/N \qquad s_y^2 = \Sigma y^2 - (\Sigma y)^2/N \qquad s_{xy} = \Sigma xy - \Sigma x\,\Sigma y/N$$

$$\bar{x} = \Sigma x/N \qquad \bar{y} = \Sigma y/N.$$

Simulation to Show Importance of Blood Sampling Scheme

Plasma concentrations, $\hat{C}_p$, for the "fast-release" formulation, A, were given by

$$\hat{C}_p = 0.4\,e^{-0.0146(t-0.2)} + 2.0\,e^{-0.65(t-0.2)} + 1\,e^{-10(t-0.2)} - 3.4\,e^{-10(t-0.2)} \tag{22}$$

The corresponding AUCs were given by

$$\text{AUC} = 30.2342 - [27.3973\,e^{-0.0146(t-0.2)} + 3.0769\,e^{-0.65(t-0.2)} + 0.1\,e^{-10(t-0.2)} - 0.34\,e^{-10(t-0.2)}] \tag{23}$$

Plasma concentrations for the "slow-release" formulation, B, were given by

$$\hat{C}_p = 0.4\,e^{-0.0146(t-0.2)} + 2.0\,e^{-0.65(t-0.2)} + 1\,e^{-10(t-0.2)} - 3.4\,e^{-3(t-0.2)} \tag{24}$$

The corresponding AUCs were given by

$$\text{AUC} = 29.4409 - [27.3973\,e^{-0.0146(t-0.2)} + 3.0769\,e^{-0.65(t-0.2)} + 0.1\,e^{-10(t-0.2)} - 1.1333\,e^{-3(t-0.2)}] \tag{25}$$

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