(9) J. A. Hersey, *J. Soc. Cosmet. Chem.*, **21**, 259(1970).
(10) J. A. Hersey, *Aust. J. Pharm. Sci.*, **NS1**, 76(1972).
(11) D. Buslik, *Powder Technol.*, **7**, 111(1973).
(12) D. Buslik, *Bull. ASTM No. 165*, **1950**, 66.
(13) K. Stange, *Chem.-Ing.-Tech.*, **26**, 331(1954).
(14) K. R. Poole, R. F. Taylor, and G. P. Wall, *Trans. Inst. Chem. Eng.*, **42**, T305(1964).
(15) K. Stange, *Chem.-Ing.-Tech.*, **35**, 580(1963).
(16) N. Harnby, *Chem. Eng., No. 214*, **1967**, CE270.

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Evidence for Variable Digoxin Absorption as Estimated by Pharmacological Response Intensities

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Abstract □ A dose–effect curve constructed from ventricular rate slowing and oral maintenance doses for digoxin provided evidence for assuming that occupation theory correctly describes drug–receptor site interaction. From the tenets of occupation theory, response intensities were linearly related to biophasic drug levels and provided the input for bi- and triexponential least-squares fits for an intravenously administered 1.2-mg dose of digoxin to patients hospitalized with auricular fibrillation. A biexponential fit best described biophasic drug levels when the biophase was represented by a peripheral compartment. Intravenous biexponential equation parameters were utilized to perform an absorption analysis following oral dosing of 1.2 mg of digoxin to the same patients. From calculations of fractional amounts unabsorbed with time, significant absorption of digoxin was found to be occurring through 24 hr at progressively decreasing but noticeably variable rates; absorption was calculated to be 98.7% complete by 120 hr. Absorption rates were most rapid over the first 5 hr but quite variable thereafter. Oscillations in the intravenous and oral response–time curves, observed between 3 and 12 hr following dosing, likewise produced fluctuations in mathematically derived biophasic drug levels, fractional amounts unabsorbed, and absorption rates for the oral dose, suggesting enterohepatic cycling of digoxin to be more significant than previously thought.

Keyphrases □ Digoxin, variable absorption—estimated by pharmacological response intensities, dose–effect curves, ventricular rate slowing, response–time curves □ Absorption, digoxin—evidence for variability estimated by ventricular rate slowing, dose–effect and response–time curves constructed □ Ventricular rate slowing—used as pharmacological response intensity parameter for studying variable digoxin absorption □ Enterohepatic cycling, digoxin—evidence suggesting new significance

Recent reports (1–3) focused on the variation in bioavailability of digoxin following oral dosing. Blood level curves representing dosing of different brands as well as different lots of the same brand have shown variations in the values[1] of C_{max}, t_{max}, and areas under plasma curves, encouraging authors to conclude that various tablets of digoxin are not uniform. Formulation defects in the drug products administered were cited as probable causes for the differences in bioavailability. From computer simulations of central and peripheral compartment digoxin levels, Sorby and Tozer (4) concluded that bioavailability differences reported for commercial tablets of digoxin could additionally be a consequence of a variable absorption rate. Although variations in tablet formulation and/or method of manufacture are quite often responsible for the observed differences in drug blood level patterns, a variation in absorption rate must also be considered. The purposes of this report are to illustrate the variability in apparent absorption rates of digoxin following oral dosing and to demonstrate the sensitivity of pharmacological response intensities in obtaining this information.

[1] The C_{max} is defined as the maximum concentration of digoxin in plasma corresponding to the time t_{max}.

THEORETICAL

Experimental results often suggest that dose–effect relationships can be mathematically expressed according to drug–receptor (biophasic) occupation theory assuming a single type of receptor:

$$F_I = \frac{I}{I_{max}} = \frac{Q_B}{K_d + Q_B} \qquad \text{(Eq. 1)}$$

where F_I is the fraction of the maximum intensity attainable, Q_B is the quantity of drug in the biophase responsible for eliciting the response, and K_d is a constant which, under the conditions of one-half maximum intensity, equals the quantity of drug in the biophase. Although the relationship is not linear, it is possible to rearrange Eq. 1 to produce Eq. 2 so that dose is directly proportional to the transformed response intensities, f (5):

$$Q_B = K_d f \qquad \text{(Eq. 2)}$$

where:

$$f = \frac{F_I}{1 - F_I} \qquad \text{(Eq. 3)}$$

If pharmacological response is a direct consequence of biophasic drug levels and if drug disposition adheres to first-order kinetics, then it follows that Q_B can be described by Eq. 4:

$$Q_B = K_d f = D\sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(Eq. 4)}$$

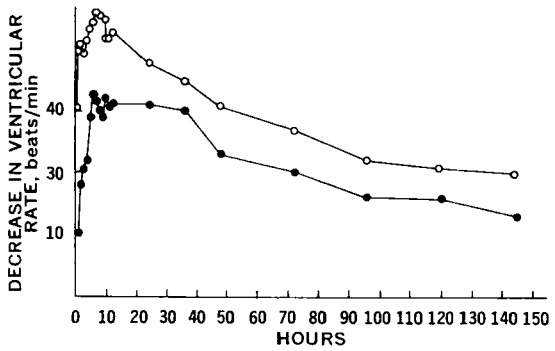


Figure 1—*Time course of ventricular rate slowing for intravenous* (○) *and oral* (●) *doses of 1.2 mg of digoxin administered to patients hospitalized with auricular fibrillation. Each data point represents a mean of 17 patients. Original data were taken from Ref. 7. Fluctuations occurring at approximately* t_{max} *are suggestive of enterohepatic cycling.*

or:

$$\frac{Q_B}{K_d} = f = \frac{D}{K_d}\sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(Eq. 5)}$$

where A_i and m_i are equation parameters evaluated from the data, and D denotes the dose absorbed. Under the conditions of Eq. 5, a plot of f *versus* time gives a graphic representation for the disposition of proportionate biophasic drug levels with time; however, because of the inclusion of K_d in Eqs. 4 and 5, one too many parameters is present so that all of the individual model parameters (*i.e.*, k_{ij}'s or transfer constants between compartments i and j) may not be calculated.

By assuming Eq. 2 to hold at all times following dosing, it is possible to eliminate K_d by dividing it by the maximum transformed response intensity, f_{max}, occurring at t_{max} and corresponding to $(Q_B)_{max}$ to yield:

$$\frac{Q_B}{(Q_B)_{max}} = \frac{f}{f_{max}} \qquad \text{(Eq. 6)}$$

Under the conditions of Eq. 6, experimental f values recorded at each time and divided by the value[2] of f_{max} can be plotted against time to represent the time course of fractional biophasic drug levels.

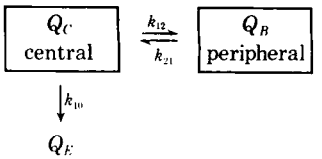
If K_d can be obtained, then Q_B may be plotted *versus* time according to Eq. 4 to yield the appropriate model parameters. Experimentally, this would require: (*a*) identification of the specific anatomical site associated with the biophase, which for drugs acting on a cellular level may not be known; and (*b*) development of a sensitive assay for drug and/or metabolite, whichever is responsible for eliciting a response. This would obviously not be practically feasible for many drugs. As will be subsequently shown, it is not always necessary to calculate values for the model parameters or, for that matter, for all equation parameters to estimate apparent absorption rates; only the equation parameters m_i need be available.

Provided the biophase is kinetically associated within a peripheral compartment and drug first enters the central compartment, it then becomes possible to calculate A_t, the cumulative amount of drug absorbed up to time t, using Eq. 7:

$$A_t = \frac{1}{k_{12}}\frac{dQ_B}{dt} + \left(\frac{k_{21} + k_{12} + k_{10}}{k_{12}}\right)Q_B + \frac{k_{21}k_{10}}{k_{12}}\int_0^t Q_B\,dt \qquad \text{(Eq. 7)}$$

Equation 7 is derived (*Appendix*) for the simplest case, in which the biophase is contained within a peripheral compartment and is communicating directly with the central compartment from which drug is eliminated, *i.e.*, the familiar two-compartment model (Scheme I).

Equation 7, divided by A_∞, can be expressed totally in terms of



Scheme I

equation parameters, obviating the necessity for calculating model parameters when absorption rate analyses are to be performed (6):

$$\frac{A_t}{A_\infty} = \frac{\dfrac{dQ_B}{dt} + (m_1 + m_2)Q_B + m_1m_2\displaystyle\int_0^t Q_B\,dt}{m_1m_2\displaystyle\int_0^\infty Q_B\,dt} \qquad \text{(Eq. 8)}$$

The m_i's are related to the model parameters by:

$$m_1m_2 = k_{10} + k_{12} + k_{21} \qquad \text{(Eq. 9)}$$

$$m_1m_2 = k_{10}k_{21} \qquad \text{(Eq. 10)}$$

Equation 8 is valid regardless of the compartment to which elimination is assigned. By substituting $K_d f$ from Eq. 2 for Q_B in Eq. 8, canceling the unknown K_d from the numerator and the denominator, and subtracting the resulting value from unity, Eq. 11 results. Equation 11 describes the fractional amount remaining to be absorbed as a function of time, calculated wholly from pharmacological response intensities:

$$1 - \frac{A_t}{A_\infty} = \frac{\dfrac{df}{dt} + (m_1 + m_2)f + m_1m_2\displaystyle\int_0^t f\,dt}{m_1m_2\displaystyle\int_0^\infty f\,dt} \qquad \text{(Eq. 11)}$$

The corresponding equation for a three-exponential model is given by Eq. 12:

$$1 - \frac{A_t}{A_\infty} = \frac{\dfrac{d^2f}{dt^2} + (m_1 + m_2 + m_3)\dfrac{df}{dt} + (m_1m_2 + m_2m_3 + m_1m_3)f + m_1m_2m_3\displaystyle\int_0^t f\,dt}{m_1m_2m_3\displaystyle\int_0^\infty f\,dt} \qquad \text{(Eq. 12)}$$

METHODS

Gold *et al.* (7) reported on the decrease in ventricular heart rate in hospitalized patients with auricular fibrillation following oral and intravenous dosing of 1.2 mg of digoxin. Subsequent data pertaining to digoxin and retreated in this article were taken from that report. Computerized fits of the dose–effect curve and biophasic drug levels as a function of time were obtained[3]. Beginning with an initial set of parameter values, the program obtains a least-squares fit to experimental data using stepwise Gauss–Newton iterations (8). To obtain initial estimates, the response intensities (Eq. 5) were plotted as a function of time on semilogarithmic paper. By using back-extrapolation procedures (9), the curve was resolved into the appropriate number of exponential components to yield values for the A_i's and m_i's. A Scatchard-type plot representing a linear transformation of Eq. 1 enabled its initial estimates to be obtained for use in the nonlinear curve-fitting program. Other necessary calculations were also performed by computer[3].

RESULTS

Figure 1 shows the time course of ventricular rate slowing for the oral and intravenous doses. Each point represents a mean of 17 subjects; zero time values were determined following a control period of several days in which patients were put to bed in an at-

[2] The f_{max} is a function of dose and the time course of drug at the active site, whereas I_{max} is independent of time and represents the maximum theoretical response obtainable according to Eq. 1.

[3] Using the BMDX.85 FORTRAN IV computer program and a CDC 6400 digital computer, Computer Science Center, University of Washington, Seattle, Wash.

Table I—Mean Ventricular Rates (Beats per Minute) and Standard Deviations for Two Time Determinations following Dosing of 1.2 mg of Digoxin to 17 Human Subjects[a]

Time of Determination	Mean	Standard Deviation	Coefficient of Variation
	Oral[b]		
Zero	107.7	26.0	24.1
Peak	74.6	11.2	15.0
	Intravenous[c]		
Zero	107.4	30.8	28.7
Peak	62.9	10.6	16.8

[a] Data calculated from Fig. 2 of Ref. 7. [b] $n = 18$. [c] $n = 16$.

tempt to establish a stable baseline, a prerequisite in quantifying pharmacological response intensities. Table I lists the standard deviations calculated for zero and peak time response values corresponding to the 1.2-mg oral and intravenous doses. For digoxin, a perceptible graded response, a slowing of ventricular rate, was measured through 168 hr. It is necessary that drug and not metabolite be responsible for eliciting a response and that the rate-determining step is the time required for drug to reach the biophase. The closest evidence for the latter comes from a report (10) in which the temporal change in left ventricle ejection time and Q-S_2 intervals were closely associated with computer-generated amounts of drug in the tissue compartment of a two-compartment open model. Additional reports have shown a relationship between myocardial digoxin concentration and hemodynamic effects (11) and the selective affinity that myocardial tissue exhibits for digoxin as compared to serum and other organs (12). Moreover, the biochemical activity of digoxin, namely, its role in the inhibition of adenosine triphosphatase to promote myocardial contraction, suggests an intracellular biophase which would be expected to behave kinetically distinguishable from the rapidly perfused heart muscle.

Assuming that occupation theory (Eq. 1) correctly describes response as a function of Q_B, values of K_d and I_{max} must be determined to obtain estimates for Q_B as a function of time following dosing (Eq. 4). If Eq. 1 contains a dose term in place of Q_B, the value of K_d under the conditions of one-half maximum intensity does not equal Q_B but equals Q_B times an unknown proportionality factor and, therefore, cannot be used in Eq. 4. The I_{max} value, however, remains the same regardless of whether Q_B or dose is plotted *versus* response intensities because the abscissa values, but not the ordinate values, are multiplied by the proportionality factor. The proportionality factor equals the summation terms on the right-hand side of Eq. 4 relating Q_B to dose where t equals a fixed time. Its value need not be known since the use of K_d is obviated by the utilization of Eqs. 6 and 11.

The dose-effect curve presented as Fig. 2 represents a steady-state response to a particular daily oral maintenance dose. Its use in this report serves to establish that occupation theory describes the interaction between drug and receptor and to obtain an estimate of I_{max} for calculation of f. The pharmacological response intensity, I, is represented by a decrease in ventricular rate measured in reference to an average controlled baseline of 108 beats/min. Each point is an average of response intensities obtained from 30 ambulatory patients with auricular fibrillation, to whom a digitalizing dose was first administered to produce a ventricular heart rate of approximately 70 beats/min followed by 4 weeks of daily maintenance dosing. It is assumed that due to the large number of subjects studied, the fraction absorbed for each dose level is constant so that the I_{max} value is not significantly altered. Weekly monitoring of ventricular rate provided the steady-state response for the particular dose level. When the dose was adjusted to another maintenance level, a week elapsed before measurements were resumed so that a steady-state response representative of the new dosing level would be attained. Under the conditions of steady state, the daily oral maintenance dose would be expected to be directly related to Q_B; consequently, a plot of maintenance dose *versus* steady-state response intensities would give the same I_{max} value as a plot of Q_B *versus* the corresponding response intensity following the administration of single doses, intravenous or oral. As long as dose, either intravenous or oral, remains proportional to Q_B, the abscissa values of a dose-effect curve can be multiplied by any constant factor without influencing I_{max}.

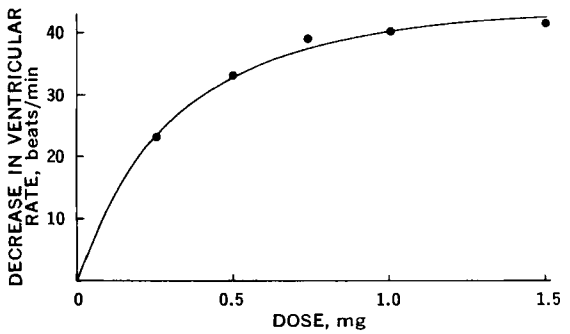


Figure 2—*Relationship of steady-state ventricular rate slowing to oral maintenance dosing of digoxin. Each patient was digitalized prior to a 4-week period of daily maintenance dosing. Each circle (●) denotes an average of four determinations taken weekly from 30 ambulatory patients with auricular fibrillation. Data were taken from Ref. 7.*

The hyperbolic shape of Fig. 2 is characteristic of what one might expect of a drug adhering to the tenets of occupation theory. Evidence for assuming occupation theory to describe drug-receptor interaction at biophasic levels correctly comes from the good least-squares nonlinear fit of the experimental dose-effect data, yielding an I_{max} value of 51.3 beats/min. From this value, the fraction of the maximum response attainable for the 1.2-mg intravenous and oral doses was calculated to be 0.906 and 0.633, respectively. Although these values are somewhat higher than anticipated for a drug with a low margin of safety such as digoxin, nausea and vomiting were experienced by 37.5% of those taking the 1.2-mg oral maintenance dose.

Values of f were calculated according to Eq. 3 following a single intravenous injection to 17 patients (7). Reciprocal weighting was used to provide a biexponential and a triexponential least-squares fit of the data to Eq. 5 as a function of time, yielding the following expressions with accompanying mean sum of squares (SS):

$$f = 2.842e^{-0.0144t} - 3.134e^{-1.200t} \quad \text{(Eq. 13)}$$

$$SS = 1.25 \times 10^{-2}$$

$$f = 1.708e^{-0.0086t} + 2.138e^{-0.073t} - 3.478e^{-0.500t} \quad \text{(Eq. 14)}$$

$$SS = 1.03 \times 10^{-2}$$

The regression line corresponding to the triexponential fit only slightly improved the fit as compared to the biexponential as judged by the similar SS values. A closer fit would normally be expected on the basis of more degrees of freedom associated with the triexponential case. Systematic deviation was not excessively apparent with either fit. According to Scheme I, the initial conditions of the system dictate that at time zero no drug has distributed to the biophasic compartment; therefore, a sum of the A_i's should equal zero. Initially, this constraint was added to the two-exponential case but a poor fit resulted. The added constraint to the three-exponential case produced equation parameters that gave identical absorption rate results as those obtained from the unconstrained biexponential fit.

Although the curve-fitting method followed by most investigators is to use the simplest model consistent with the experimental data, an attempt was made to constrain each case to a known criterion to choose between bi- and triexponential fits more confidently. It is reasoned that the cumulative amount of drug absorbed at all times following a rapid intravenous dose of drug should approximate the dose. In terms of Eq. 11 for the biexponential case and of Eq. 12 for the triexponential case, the fractional amount of drug remaining to be absorbed at all times should correspondingly approximate zero. The mean ($n = 24$) and the standard deviation of fractional amounts unabsorbed for the biexponential case, 0.030 ± 0.164, as compared to the widely varying values calculated for the triexponential case, 0.676 ± 28.8, indicated the biexponential case to approximate physiological con-

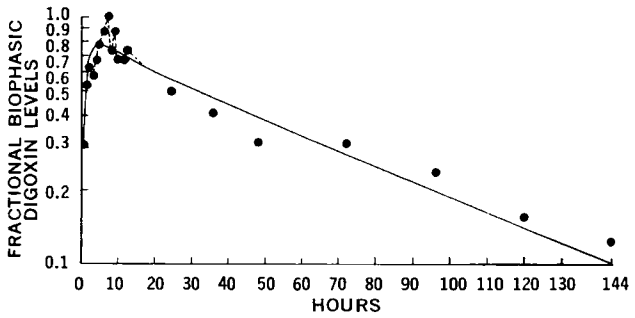


Figure 3—*Semilogarithmic plot of fractional amount of drug in biophase* versus *time after intravenous dosing of 1.2 mg of digoxin to 17 patients hospitalized with auricular fibrillation. Each point (●) represents an average calculated from ventricular rate slowing. The smooth line represents a least sum of squares, biexponential, computerized fit to the data taken from Ref. 7. Fluctuations about the theoretical line occurring at approximately* t_{max} *are suggestive of enterohepatic cycling.*

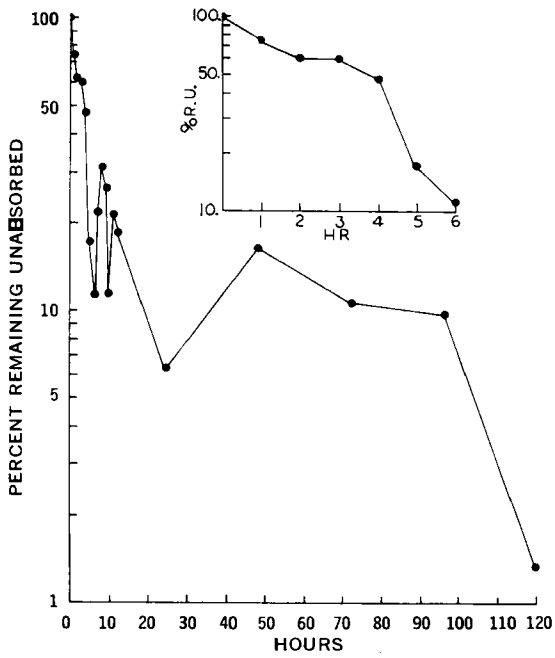


Figure 4—*Semilogarithmic plot of percent digoxin remaining unabsorbed following oral dosing of 1.2 mg to 17 patients hospitalized with auricular fibrillation. Each point (●) represents an average calculated for a two-compartment model utilizing ventricular rate slowing, taken from Ref. 7, as input data. The insert represents the same data but with an expanded time scale and shown over the first 6 hr only.*

ditions more closely. The unconstrained biexponential fit to the experimental data, denoted by Eq. 13, is shown in Fig. 3. The *f* values were normalized according to Eq. 6 and, as such, represent the fractional amount of digoxin present in the biophase with time.

By repeated administration of oral and intravenous doses, Gold *et al.* (7) found that an oral dose[4] 1.5 times the intravenous dose produced approximately the same maximum response intensity; however, Gold *et al.* (7) did not mention whether aqueous solutions, alcoholic solutions, or tablets were administered. From these oral dosing studies, it was concluded that the extent of absorption for an oral dose approximated 50–60%. From the same data and assuming first-pass effects to be negligible, 57.9% of the oral dose reached the biophase and elicited a response as calculated from the ratio of the total areas under the average *f versus* time curves for the oral and intravenous doses. Doherty *et al.* (13), on the other hand, reported values of 85% for the absolute (including recycling) absorption of digoxin based upon studies of tritiated digoxin serum levels as well as urine and stool excretion of patients with surgically induced biliary fistula. The drug was administered orally as an alcoholic solution. Wagner *et al.* (14) also found that the average absolute absorption was 80% of the dose calculated from areas under plasma–time curves when digoxin was administered orally in 5% dextrose solution, but when administered as commercially available tablets only 56.7 and 30.7% of the dose was absorbed. The extent of bioavailability, therefore, appears to be very dependent upon the choice of oral dosage form used as well as upon formulation factors varying within a dosage form.

The fractional amount of digoxin remaining to be absorbed following oral dosing was calculated according to Eq. 11 and plotted logarithmically *versus* time. Inspection of Fig. 4 reveals that absorption is 89% complete over the first 6 hr[5]. A log linear relationship would indicate simple first-order absorption; however, the insert shown in Fig. 4 indicates that this is not the case. From 6 through 24 hr, the absorption pattern is quite variable, fluctuating between 67.1% at 8 hr and 93.7% at 24 hr. Analysis of the absorption rate calculated from the cumulative absorption data illustrates the relatively rapid rate of absorption occurring over the first 5 hr (Fig. 5). The absorption rate becomes negative (net drug leaving body) at 6 hr and, with the exception of the 10-hr rate, continues negative until 12 hr. A positive absorption rate, although small, continues throughout the entire time course of 168 hr.

DISCUSSION

Significant absorption of digoxin appears to be occurring through 24 hr at progressively decreasing, but noticeably variable, rates until 120 hr when only 1.3 of the percent unabsorbed remains to be absorbed. This is evident from Figs. 4 and 5 and also from Fig. 1 in which a decrease in ventricular rate for the intravenous and oral curves returns to normal in a parallel fashion only after 24 hr following dosing. Although a peak response for the oral data is observed at 11 hr in Fig. 1, a general plateau effect is evident through 36 hr. The same trend was reported previously (14); following a slow intravenous infusion of digoxin, plasma levels maintained a plateau from about 2 to 7 hr.

One possible explanation is that absorption is occurring continuously along the various segments of the small intestine, duodenum, jejunum, and ileum but at decreasing rates as drug encounters less effective surface area per unit length while traversing the small intestine. Enterohepatic cycling could also account for the long duration associated with digoxin absorption and, along with variable gallbladder emptying time, could additionally explain the variable absorption rate observed between 5 and 12 hr for the oral dose. Oscillations occurring immediately before and after t_{max} in the intravenous and oral response–time curves of Fig. 1 and the corresponding Q_B–time curve of Fig. 3 for the intravenous data were observed between 1 and 12 hr. Fluctuations in data might have been detected over larger segments of the curves if more frequent determinations had been made beyond 12 hr. However, if the fluctuations in data were a result of the error associated with the determination of f/f_{max}, then the variation of experimental points in Fig. 1 and of the derived Q_B values for the intravenous data in Fig. 3 would be expected to be similar along the entire curve instead of occurring at approximately peak times. Moreover, the variations between subjects (expressed in Table I as coefficients of variation for peak and zero times) would indicate scattering about the theoretical curve to be less at t_{max} than at other times, gradually increasing as f/f_{max} returns to predosing values. Although no direct proof is available that significant intermittent recycling is occurring, the medical literature suggests the possibility. Similar trends can be observed in the plasma curves of digoxin reported (3) for patients taking 0.5 mg of digoxin 0.5 hr after food daily. Plasma curves for fasting subjects did not exhibit these fluctuations. It is known that the release of gallbladder contents occurs when food present in the stomach and intestine stimulates the release of GI hormones,

[4] Prepared from material supplied by Burroughs Wellcome & Co., Inc., as the pure form which served as the USP reference standard of digoxin.

[5] The 89% complete refers to the percentage of drug absorbed that will ultimately be absorbed.

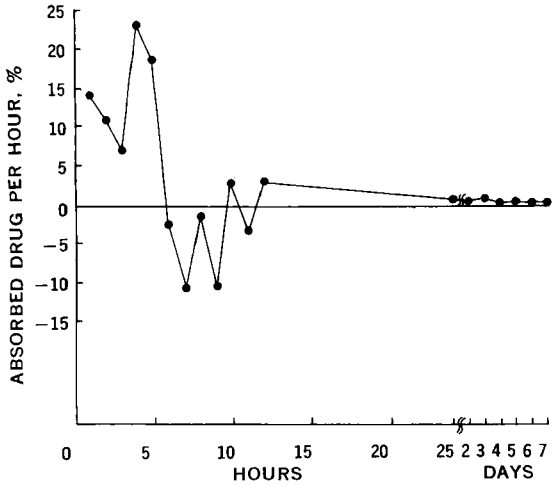
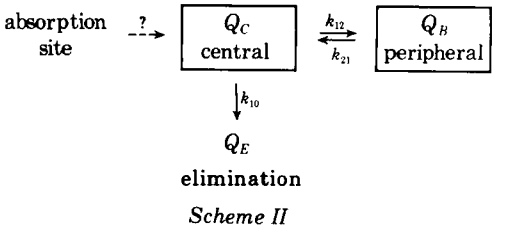


Figure 5—*Absorption rate of 1.2-mg oral dose of digoxin* versus *time calculated according to a two-compartment model. Each data point* (●) *calculated from ventricular rate slowing, taken from Ref. 7, represents a mean of 17 patients hospitalized with auricular fibrillation.*

which causes the coordinated contraction of the gallbladder and dilation of the sphincter of Oddi. Bile containing digoxin is subsequently released into the duodenum. Bile, although continuously secreted by the liver, does not continuously enter the duodenum but is stored along the common bile duct and in the gallbladder prior to relaxation of the sphincter of Oddi. The total evacuation period of the gallbladder varies from 15 min to several hours (15) but can be considered intermittent over the long disposition time of digoxin (16, 17).

According to Doherty *et al.* (13), a relatively small fraction of digoxin, 6.5%, is involved in enterohepatic cycling. This value was reported for human subjects with surgically induced biliary fistula. The bile was found to contain about 9% of the administered digoxin, which approximated the usual total stool excretion of parenteral digoxin. The serum half-life of these patients did not differ from those without biliary fistula, indicating to these authors that this factor is of minor importance to the drug's pharmacokinetic behavior. Half-life determinations, however, are calculated from widely spaced plasma concentrations which are approximately 4% or less of peak concentration levels. In view of the deviations observed at peak times for response, biophasic digoxin levels, and plasma levels of digoxin, it is conceivable that a small fraction of peak circulating concentrations of digoxin could be stored in the gallbladder in high enough concentrations such that its release and subsequent absorption would be in large enough quantities to account for the observed trends. Additionally, if the actual volume of the biophase is small in comparison to the circulating volume and if affinity of digoxin for biophasic receptors was great, the small fluctuations in plasma concentrations, particularly at peak times, could extrapolate to large changes in biophasic drug levels. Fluctuations in the normalized f values of Fig. 3 immediately before and after t_{max}, at 3 and 7 hr, deviated by 23.4 and 35.5%, respectively, from the computer-generated line of best fit for the intravenous dose. However, a compensating factor exists to nullify partially these large differences in terms of pharmacological response. Because of the hyperbolic shape of the dose–effect curve, large changes in biophasic drug levels translate into disproportionately smaller changes in response as higher biophasic drug levels are reached. This is exemplified by the pharmacological data; maximum response for the intravenous dose was estimated from the dose–effect curve to be 90.6% of the theoretical maximum. Within this range on the dose–effect curve, large deviations calculated for fractional biophasic drug levels represent a theoretical difference in response of 5.6 beats/min; experimentally, a difference of 4.5 beats/min was observed. It is likely that dose–effect curves representing various electromechanical events occurring in the heart and collectively referred to as systolic time intervals (18) would show greater sensitivity to digitalis intoxication than dose–effect curves constructed from ventricular rate slowing. This is indicated from a



Scheme II

study (19) in which systolic time intervals as well as heart rate were measured in newborn infants before and again at 4 and 8 hr after oral administration of digoxin. Varying degrees of statistically significant differences were found for all measurements after dosing as compared to predosing values except for heart rate; however, a relatively small pediatric dose (30 μg/kg) was used. In addition, the maximal inotropic effect, as measured by preejection changes, occurred with smaller doses of digoxin. If toxicity was apparent along lower segments of the dose–effect curve, smaller changes in fractional biophasic drug levels would translate into larger changes in response.

From the observed trends in the response data, it is apparent that the significance of enterohepatic cycling as it relates to the kinetics of pharmacolological effect warrants further study.

APPENDIX

The derivation of A_t for an open two-compartment system (Scheme II) as a function of time is based on a material balance which accounts for drug absorbed at all times. For this particular system, material balance dictates that A_t at any time is the sum of the quantities of drug present in the biophase, Q_B (a component of the peripheral compartment), central compartment, Q_C, and that which has been eliminated, Q_E:

$$A_t = Q_B + Q_C + Q_E \qquad \text{(Eq. A1)}$$

Equation A1 can be written in differential form:

$$dA_t = dQ_B + dQ_C + dQ_E \qquad \text{(Eq. A2)}$$

By referring to Scheme II, the following relationships can be derived:

$$\frac{dQ_B}{dt} = k_{12}Q_C - k_{21}Q_B \qquad \text{(Eq. A3)}$$

$$\frac{dQ_E}{dt} = k_{10}Q_C \qquad \text{(Eq. A4)}$$

Equation A5 is obtained by solving Eq. A3 for Q_C, differentiating with respect to time, and expressing in terms of dQ_C:

$$dQ_C = \left(\frac{1}{k_{12}}\frac{d^2Q_C}{dt^2} + \frac{k_{21}}{k_{12}}\frac{dQ_B}{dt}\right)dt \qquad \text{(Eq. A5)}$$

Solving Eq. A3 for Q_C, substituting into Eq. A4, and simplifying yield:

$$dQ_E = \left(\frac{k_{10}}{k_{12}}\frac{dQ_B}{dt} + \frac{k_{10}k_{21}}{k_{12}}Q_B\right)dt \qquad \text{(Eq. A6)}$$

Substituting Eqs. A5 and A6 for the appropriate terms in Eq. A2 and simplifying give the expression:

$$dA_t = \frac{1}{k_{12}}\left(\frac{d^2Q_B}{dt^2} + (k_{12} + k_{21} + k_{10})\frac{dQ_B}{dt} + k_{21}k_{10}Q_B\right)dt \qquad \text{(Eq. A7)}$$

Upon integration between limits 0 and t, Eq. A7 becomes:

$$A_t = \frac{1}{k_{12}}\left(\frac{dQ_B}{dt} + (k_{12} + k_{21} + k_{10})Q_B + k_{21}k_{10}\int_0^t Q_B\,dt\right) \qquad \text{(Eq. A8)}$$

Equation A8 is Eq. 7 in the *Theoretical* section. In the limit when

$t = \infty$, Eq. A8 reduces to Eq. A9:

$$A_\infty = \frac{k_{21}k_{10}}{k_{12}}\int_0^{t_\infty} Q_B\,dt \qquad \text{(Eq. A9)}$$

By dividing Eq. A8 by Eq. A9 and canceling like terms, an expression results which permits the evaluation of the fractional amount of drug ultimately absorbed, A_t/A_∞, at any time:

$$\frac{A_t}{A_\infty} = \frac{\frac{dQ_B}{dt} + (k_{12} + k_{21} + k_{10})Q_B + k_{21}k_{10}\int_0^t Q_B\,dt}{k_{21}k_{10}\int_0^t Q_B\,dt} \qquad \text{(Eq. A10)}$$

The validity of Eq. A10 is upheld providing drug first enters the central systemic compartment before being distributed to the biophasic compartment. It is also required that the differential equation written for Q_B does not contain a term representing the absorption process. The rate constants in Eq. A10 can be written in terms of the m_i's shown in Eqs. 9 and 10 such that:

$$\frac{A_t}{A_\infty} = \frac{\frac{dQ_B}{dt} + (m_1 + m_2)Q_B + m_1m_2\int_0^t Q_B\,dt}{m_1m_2\int_0^{t_\infty} Q_B\,dt} \qquad \text{(Eq. A11)}$$

Equation A11 is the same as Eq. 8. In a similar manner, absorption equations can be derived for two-compartment open models in which either $k_{10} = 0$ or $k_{10} \neq k_{20} \neq 0$. The relationships between the m_i's and k_{ij}'s are not altered, and each equation becomes identical in form to Eq. A11; only the numerical values of the m_i's vary. Therefore, Eq. A11 corresponds to a generalized two-compartment model in which the existence of a specified elimination rate constant need not be established. A general equation for A_t representing a three-compartment model can be similarly derived in which elimination also need not be assigned as a particular compartment; only the m_i's are required (20, 21).

REFERENCES

(1) T. R. D. Shaw, M. R. Howard, and J. Hamer, *Lancet*, **2**, 303(1972).

(2) J. Lindenbaum, W. H. Mellow, M. O. Blackstone, and U. P. Butler, *N. Engl. J. Med.*, **285**, 1344(1971).

(3) R. J. White, D. A. Chamberlain, M. Howard, and T. W. Smith, *Brit. Med. J.*, **1**, 380(1971).

(4) D. L. Sorby and T. N. Tozer, *Drug Intel.*, **7**, 78(1973).

(5) G. Serge, "International Conference on Radioactive Isotopes in Pharmacology," Wiley, London, England, 1969, pp. 383–389.

(6) R. D. Schoenwald, Ph.D. thesis, Purdue University, Lafayette, Ind., 1971.

(7) H. Gold, M. Cattell, T. Greiner, L. W. Hanlon, N. T. Kwit, W. Modell, E. Cotlove, J. Benton, and H. L. Otto, *J. Pharmacol. Exp. Ther.*, **109**, 45(1953).

(8) R. I. Jennrich and P. F. Sampson, *Technometrics*, **10**, 63(1968).

(9) D. C. Riggs, "The Mathematical Approach to Physiological Problems," Williams & Wilkins, Baltimore, Md., 1963, p. 146.

(10) R. H. Reuning, R. A. Sams, and R. E. Notari, *J. Clin. Pharmacol. New Drugs*, **13**, 127(1973).

(11) R. N. Deutscher, D. C. Harrison, and R. H. Goldman, *Amer. J. Cardiol.*, **29**, 47(1972).

(12) J. E. Doherty, W. H. Perkins, and W. J. Flanigan, *Ann. Intern. Med.*, **66**, 116(1967).

(13) J. E. Doherty, W. J. Flanigan, M. L. Murphey, R. T. Bullock, G. L. Dalrymple, O. W. Beard, and W. H. Perkins, *Circulation*, **42**, 867(1970).

(14) J. G. Wagner, M. Christensen, E. Sakmer, D. Blair, J. D. Yates, P. W. Willis, III, A. J. Sedman, and R. G. Stoll, *J. Amer. Med. Ass.*, **224**, 199(1973).

(15) F. H. Netter, "Ciba Collection of Medical Illustrations," vol. 3, Ciba Publications, New York, N.Y., 1969, p. 52.

(16) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1970, p. 687.

(17) A. C. Guyton, "Textbook of Medical Physiology," 3rd ed., Saunders, Philadelphia, Pa., 1971, p. 749.

(18) A. M. Weissler, W. S. Harris, and C. D. Schoenfeld, *Circulation*, **37**, 149(1968).

(19) A. M. Levy, D. M. Leaman, and J. S. Hanson, *ibid.*, **46**, 816(1972).

(20) V. F. Smolen, *J. Pharm. Sci.*, **60**, 878(1971).

(21) *Ibid.*, **60**, 354(1971).

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