Drug Absorption III: Effect of Membrane Storage on the Kinetics of Drug Absorption

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Abstract □ Monoexponential and biexponential disappearance of drug was observed both from the simulated gut phase of a three-phase *in vitro* model for drug absorption and from the lumen of an *in situ* rat gut. Monoexponential disappearance occurred when accumulation of drug in the membrane phase was low or absent, whereas biexponential disappearance occurred when membrane accumulation was appreciable. In all cases, overall transfer of drug to blood was essentially irreversible. Kinetic analysis of biexponential lumen phase data, in terms of a two-compartment open model, was accomplished by applying the technique of "feathering." An analog computer was programmed with the calculated rate constants and the computer generated curves were compared with experimental absorption data from the three phases of the *in vitro* model. Good agreement between the computer curves and the experimental data confirmed the validity of the computational technique and the accuracy of the individual rate constants. An analog computer was also used to simulate the fraction of the dose of drug in the membrane for the *in situ* experiments since the membrane was not available for analysis.

Keyphrases □ Drug absorption—membrane storage effect □ Membrane storage effect—drug absorption kinetics □ Intestinal drug transfer—*in vitro*, *in situ* preparations □ Model, three-phase—drug absorption kinetics □ Analog computer generated curves, experimental data curves—comparison

This paper reports on two kinetic cases of drug transfer commonly observed in an *in vitro* three-phase model for drug absorption (1), and in an *in situ* rat intestinal preparation (2). The objectives of this investigation were (*a*) to correlate kinetic models observed in the *in vitro* system with the kinetics of drug disappearance from the lumen in the *in situ* system; (*b*) to evaluate the role of drug accumulation in a "membrane compartment" on the observed kinetics; and (*c*) to compare various computational methods for estimating the rate constants involved.

Many investigators have attempted to correlate o/w partitioning of drugs in various solvent systems with their gastrointestinal absorption rates. Although rank ordering of a homologous series of drugs in terms of their pH-partition behavior and apparent gastrointestinal absorption rates may be possible, a direct correlation between these two factors is often difficult to demonstrate (3). Possible reasons for the lack of correlation are: (*a*) there is no solvent system that mimics all the physicochemical properties of the absorbing membrane; (*b*) o/w partition coefficients are equilibrium parameters whereas gastrointestinal drug absorption is a dynamic process; and (*c*) no experimental procedure was available which would allow determination of reproducible realistic drug absorption rates in a living system under closely controlled conditions.

Previous reports from these laboratories (1) have dealt with the use of an *in vitro* model for the drug absorption process which employs an artificial lipid-like barrier (organic solvent) separating two aqueous buffered phases representing gut lumen fluid and blood. This technique allows a study of the o/w partition behavior of drugs in a dynamic system which mimics the *in vivo* absorption process and in which all phases can be analyzed so that each rate constant can be evaluated individually. In this model several kinetic mechanisms involving reversible and irreversible transfer among the phases were observed (4). A frequently observed mechanism was one involving reversible transfer of drug from "gut lumen" (Phase A) to "absorbing membrane" (Phase B) followed by irreversible transfer from "absorbing membrane" to "blood" (Phase C); that is, $A \rightleftharpoons B \rightarrow C$. This mechanism fits the expectations of the pH-partition hypothesis because it suggests partitioning of drugs into the membrane. The *in vivo* gut to blood transfer of many drugs may be described by the $A \rightleftharpoons B \rightarrow C$ mechanism because factors such as the volume, pH, and protein-binding capacity of the blood as well as the large tissue distribution and rapid metabolism and excretion of these drugs often tend to make the rate constant for the $C \rightarrow B$ process very small. Consequently, the transport from gut to blood may take on some characteristics of a unidirectional process.

The development of a method for determining drug absorption rates from segments of the gastrointestinal tracts of rats, *in situ*, which yields rates which are closely reproducible from animal to animal and are comparable to those calculated from blood concentration data following oral drug administration to humans and intact animals has also been reported (2). By following the concentration of drug in the lumen of the intestine at closely spaced intervals and analyzing the data using methods for estimating appropriate rate constants in multicompartmental systems when only one phase is available for analysis (5, 6), it is possible to quantitate the kinetic model which describes the absorption of drugs showing biexponential disappearance from the gut lumen.

EXPERIMENTAL

In Vitro Three-Phase Model—*Apparatus and Reagents*—All chemicals were reagent grade unless otherwise specified. A Leeds & Northrup 7401 pH meter, a Beckman model DB spectrophotometer, a Hitachi-Perkin-Elmer spectrophotometer, and an Electronic Associates, Inc. TR-48 analog computer were utilized.

The glass tubes, rocking apparatus, and aqueous buffers used in these studies were described in previous communications (1, 4).

Procedure—The procedure for studying drug transfer kinetics in the *in vitro* three-phase model has been described in previous communications (1, 4).

In Situ Rat Gut—*Apparatus and Reagents*—All chemicals were reagent grade unless otherwise specified. Chlorpromazine hydrochloride,[1] prochlorperazine edisylate,[1] trimeprazine tartrate,[1] trifluoperazine hydrochloride,[1] and haloperidol were used.[2] All solutions were prepared with distilled, deionized, boiled water. A Beckman

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[2] McNeil Laboratories, Fort Washington, Pa.

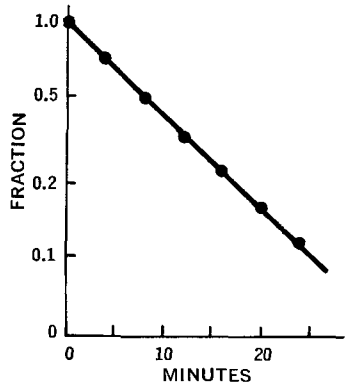

Figure 1—*Semilogarithmic plot of fraction of dose of salicylic acid remaining in rat intestinal lumen,* in situ, versus *time. Dose = 22 mg., half-life = 8 min., lumen solution pH = 6.0.*

Zeromatic II pH meter, a Haake type FBE constant-temperature water bath, and a Cary model 15 spectrophotometer were utilized.

The perfusion and drug solutions used in these studies were described in a previous communication (2).

Test Animals—Male Sprague Dawley albino rats weighing 220–260 g. were fasted 15–20 hr. prior to use.

Procedure—The procedure for studying drug disappearance from the *in situ* rat gut lumen and the analytical methods employed for these drugs have been described in previous communications (2, 7).

RESULTS AND DISCUSSION

Figure 1 illustrates the disappearance of salicylic acid (SA) from the *in situ* rat gut lumen when the pH of the lumen solution is 6.0 (2). The figure shows that the disappearance follows first-order kinetics (monoexponential) for the entire experiment (three half-lives). In another experiment, only trace amounts of salicylic acid could be demonstrated in the lumen solution when 75 mg. of drug was administered intravenously and drug-free buffer was placed in the gut lumen. These results suggest that the kinetics of oral salicylic acid absorption under these conditions may be described in terms of the following model:

$$SA_{lumen} \underset{(\leftarrow)}{\rightarrow} SA_{blood} \qquad \text{(Eq. 1)}$$

that is, overall drug absorption follows apparent irreversible first-order kinetics.

The disappearance of prochlorperazine (PCZ) from the *in situ* rat gut lumen (pH 6.0) is shown in Fig. 2. In this case, the disappearance follows a biexponential profile in contrast to the monoexponential profile observed with salicylic acid. These results suggest that the kinetics of prochlorperazine absorption may be described in terms of the following model:

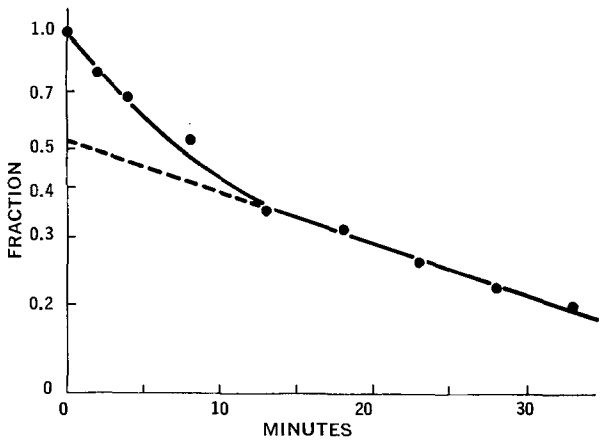

Figure 2—*Semilogarithmic plot of fraction of dose of prochlorperazine hydrochloride in rat intestinal lumen,* in situ, versus *time. Dose = 10 mg., half-life (of straight line) = 23 min., lumen solution pH = 6.0.*

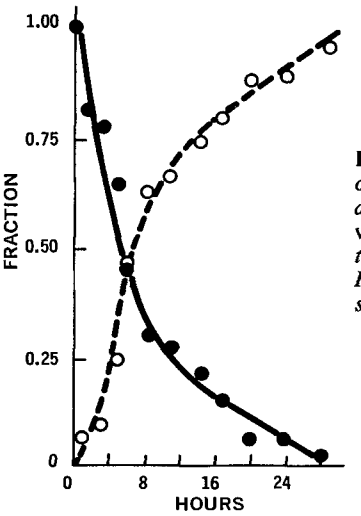

Figure 3—*Plot of fraction of salicylic acid in Phases A and C of a three-phase* in vitro *model for drug absorption. pH: Phase A, 2.0; Phase C, 7.4. Phase B consisted of cyclohexane.*

$$PCZ_{lumen} \underset{(\leftarrow)}{\rightleftharpoons} PCZ_{membrane} \rightarrow PCZ_{blood} \qquad \text{(Eq. 2)}$$

that is, rapid distribution of prochlorperazine occurs between the gut lumen and a compartment representing the membrane followed by slower, essentially irreversible, transfer of the drug from the membrane compartment into the blood. As with salicylic acid, when prochlorperazine was administered intravenously, only a negligible amount of drug could be detected in the gut lumen, confirming that at least one step in the transfer process is essentially irreversible. It can be assumed that the initial transfer was reversible since the disappearance of drug from the gut lumen was not simple first-order (monoexponential).

Current concepts of passive drug absorption suggest that drugs pass through the absorbing membrane after first dissolving or partitioning into it. In the *in vitro* model, drug partitioning into the "Membrane" phase is the only way drug can transfer from Phase A (gut lumen) to Phase C (blood); and the membrane could be considered a discrete compartment in any absorption model. Based on this premise, Eq. 2 is the explicit equation describing the absorption of many passively absorbed drugs. However, if accumulation of drug in the membrane compartment is negligible, Eq. 1 may adequately fit the data. Since both salicylic acid and prochlorperazine are essentially irreversibly transferred from lumen to blood, it would appear that these drugs differ greatly in the degree to which they accumulate in the membrane. This phenomenon can be easily illustrated by means of data obtained in the three-phase *in vitro* model for drug absorption which have previously been described (1, 4). Figure 3 shows plots of the fractions of salicylic acid in Phases A and C of the model system when the simulated gut Phase A is buffered at pH 2.0 and the simulated membrane Phase B is cyclohexane. Figure 4 shows a semilogarithmic plot of the Phase A data, and the plot suggests that the disappearance of salicylic

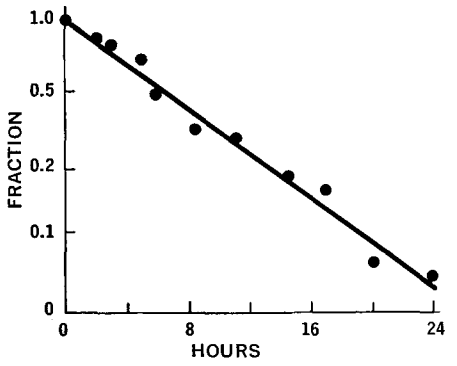

Figure 4—*Semilogarithmic plot of Phase A data from Fig. 3 showing first-order nature of drug transfer.*

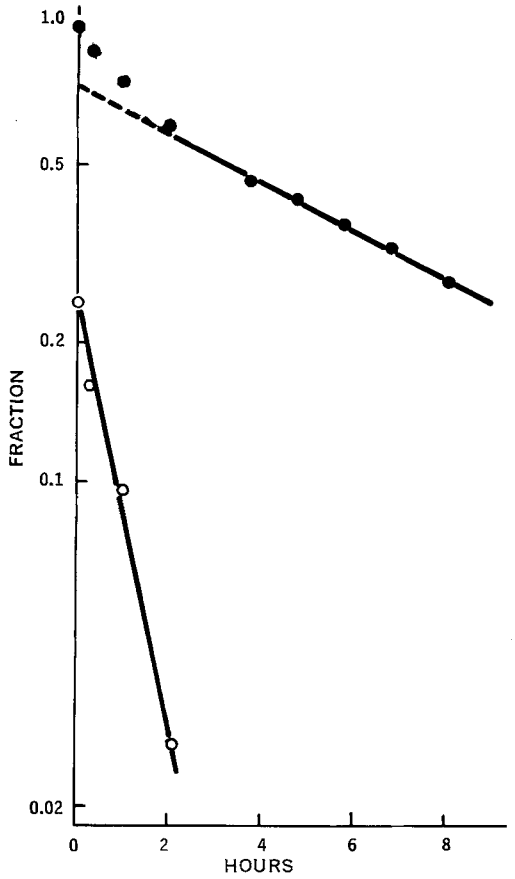

Figure 5—*Semilogarithmic plot of fraction of salicylic acid in Phase A of a three-phase* in vitro *model for drug absorption. The pH of Phase A = 2.0. The pH of Phase C = 7.4. Phase B consisted of 0.1% n-octanol in cyclohexane. Values of the intercepts and slopes were determined graphically: $X_1 = 0.27$; $X_2 = 0.73$; a = 1.08; and b = 0.12. The following values for the rate constants in the model (Eq. 5) were calculated using Eqs. 6–8: $k_{12} = 0.37$; $k_{21} = 0.48$; and $k_{23} = 0.35\ hr.^{-1}$.*

acid from Phase A may be described in terms of an irreversible first-order transfer of drug between Phases A and C:

$$F_A \xrightarrow{b} F_C \qquad \text{(Eq. 3)}$$

where F_A and F_C are the fractions of total drug in the respective phases, and b is the apparent first-order rate constant describing the overall transfer process. A semilogarithmic plot of $(F_C^{\infty} - F_C)$ *versus* time has the same slope as the plot in Fig. 4. Consequently, Eq. 3 can be used to describe this system even though the equation ignores the existence of Phase B. This simplification is justifiable because F_B is extremely low, therefore essentially constant, throughout the experiment. (The concentrations of salicylic acid in cyclohexane were so low that they could not be measured experimentally.) Thus, F_B may be considered to be at steady state; and, under these circumstances, b is a complex function of the rate constants controlling transfer of drug to and from Phase B (4). Because F_B cannot be measured experimentally, and because the overall transfer is monoexponential, the individual rate constants

Table I—Kinetic Data for the Disappearance of Drug from Rat Intestinal Lumen, *In Situ* (Lumen pH = 6.0)

	X_1[a]	X_2[a]	a[a]	b[a]	k_{12}[b]	k_{21}[b]	k_{23}[b]
Prochlorperazine	0.47	0.53	0.20	0.030	0.11	0.065	0.055
Chlorpromazine	0.40	0.60	0.70	0.036	0.30	0.35	0.084
Trifluoperazine	0.41	0.59	0.28	0.026	0.13	0.12	0.056
Trimeperazine	0.18	0.82	0.36	0.031	0.091	0.18	0.12
Haloperidol	0.16	0.84	0.35	0.030	0.082	0.17	0.13

[a] Intercepts (X_1 and X_2 as fraction of dose) and Slopes (a and b in $hr.^{-1}$) were determined as illustrated in Fig. 5 from semilogarithmic plots of fraction of dose in the lumen *versus* time. [b] Rate constants (in $hr.^{-1}$) were calculated by means of Eqs. 6–8.

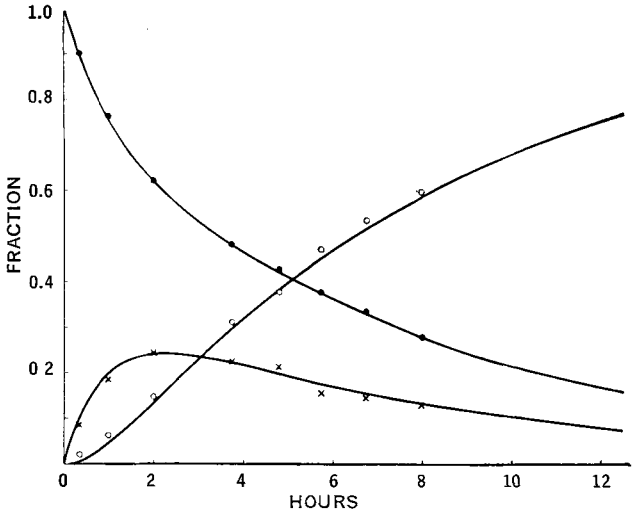

Figure 6—*Plots of the fractions of salicylic acid in Phases A (•), B (×), and C (○) for the experiment shown in Fig. 5. The points were obtained experimentally, and the lines were drawn by an analog computer programmed with Eq. 5 and the following values of the rate constants:* $k_{12} = 0.37$; $k_{21} = 0.50$; *and* $k_{23} = 0.40\ hr.^{-1}$.

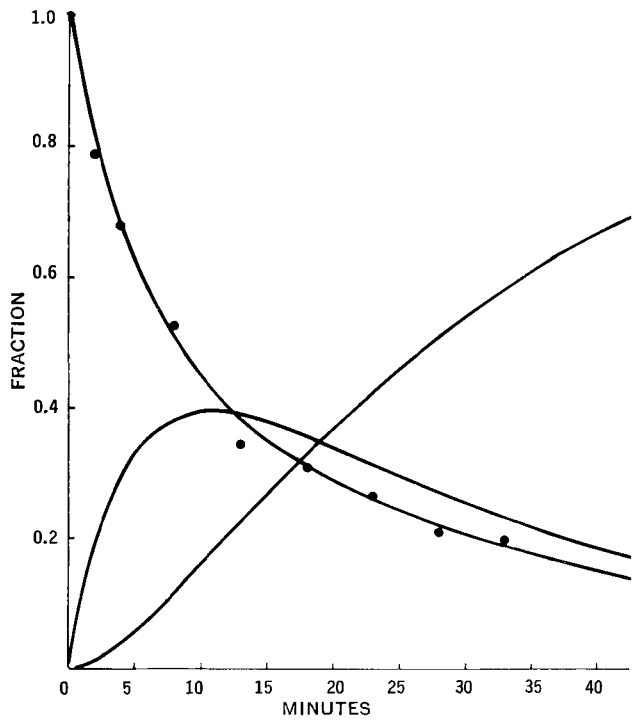

Figure 7—*Plots of the fractions of prochlorperazine hydrochloride in the lumen, the membrane compartment, and the blood compartment of the* in situ *rat intestinal preparation* versus *time. The points were obtained experimentally from the lumen solution (pH = 6.0). Initially, these data were plotted on semilogarithmic paper and values of the intercepts and slopes for the biexponential equation were determined as shown in Fig. 2. Values for the rate constants (Eq. 4) were then calculated using Eqs. 6–8; and these values* ($k_{12} = 0.11$, $k_{21} = 0.065$, $k_{23} = 0.055\ hr.^{-1}$) *were used to program an analog computer which generated the membrane compartment and blood compartment curves.*

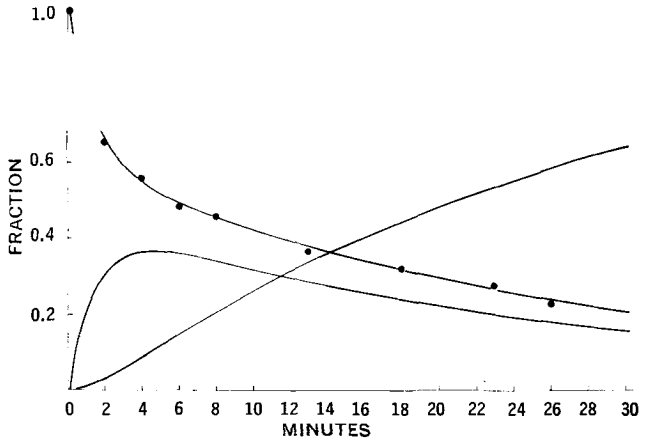

Figure 8—*Plots for chlorpromazine similar to those shown in Fig. 7;* ($k_{12} = 0.30, k_{21} = 0.35, k_{23} = 0.084\ hr.^{-1}$).

cannot be determined easily. It might be concluded that when simple first-order disappearance of drug from the lumen is observed in the *in situ* preparation it can be presumed that membrane accumulation of drug is not great. Inherent in this conclusion is the assumption that transfer of drug from gut lumen to membrane is reversible. If drug transfer from gut to membrane were irreversible, disappearance from the gut lumen would always be monoexponential.

Figure 5 shows a semilogarithmic plot of fraction of salicylic acid in Phase A of the three-phase model when Phase A is buffered at pH 2.0 and Phase B is 0.1% *n*-octanol in cyclohexane. In this case, disappearance of salicylic acid from Phase A follows a biexponential profile, suggesting that there is appreciable accumulation of salicylic acid in Phase B. The presence of significant amounts of drug in Phase B was confirmed experimentally (see Fig. 6). The kinetic model may be depicted as follows:

$$F_A \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} F_B \xrightarrow{k_{23}} F_C \qquad \text{(Eq. 4)}$$

Mathematically, F_A is given by the following biexponential equation:

$$F_A = X_1 e^{-at} + X_2 e^{-bt} \qquad \text{(Eq. 5)}$$

Table II—Data Illustrating the Accuracy of the Feathering Technique for Determining Rate Constants from Curves Generated by an Analog Computer

Value	k_{12}	k_{21}	k_{23}
Analog[a]	0.25	1.00	0.20
Feathering[b]	0.25	0.98	0.19
Analog	0.25	1.00	0.080
Feathering	0.23	0.87	0.065
Analog	0.25	0.35	0.050
Feathering	0.25	0.35	0.048

[a] Values used to program an analog computer which generated curves. [b] Values obtained by feathering the computer generated curves.

The values of X_1, X_2, a, and b can be determined graphically from the semilogarithmic plot as shown in Fig. 5; and k_{12}, k_{21}, and k_{23} can be calculated from these values using the following equations (5, 6):

$$k_{12} = \frac{X_1 a + X_2 b}{X_1 + X_2} \qquad \text{(Eq. 6)}$$

$$k_{23} = \frac{ab}{k_{12}} \qquad \text{(Eq. 7)}$$

$$k_{21} = a + b - k_{12} - k_{23} \qquad \text{(Eq. 8)}$$

The results of these calculations are summarized in the legend of Fig. 5.

A distinct advantage of the three-phase model over the *in situ* gut preparation is that all phases are easily accessible for analysis. In cases such as the one under discussion, it is possible to confirm values estimated for the individual rate constants by using them to predict data for Phases B and C and then comparing the predicted data with experimental data for these phases. Such a comparison is shown in Fig. 6 for the salicylic acid data. In this figure, the lines were drawn by an analog computer programmed with Eq. 4, and the points are actual experimental data for the three phases. The values of the computer constants used to generate the lines are shown in the legend of Fig. 6. These values are slightly different from those calculated using Eqs. 6–8 (see Fig. 5) but the agreement is close and the discrepancies can be attributed to experimental error and to error in feathering the semilogarithmic plot (see Fig. 5).

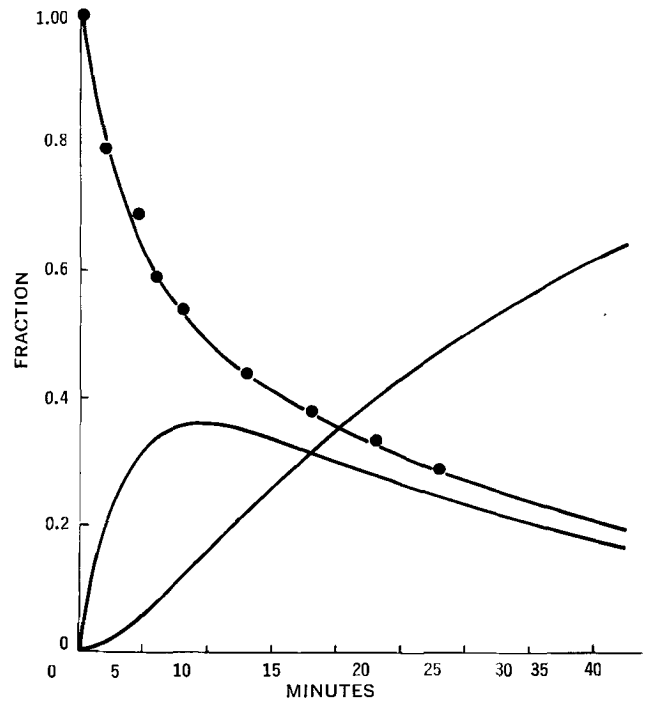

Figure 9—*Plots for trifluoperazine similar to those shown in Fig. 7;* ($k_{12} = 0.13, k_{21} = 0.12, k_{23} = 0.056\ hr.^{-1}$).

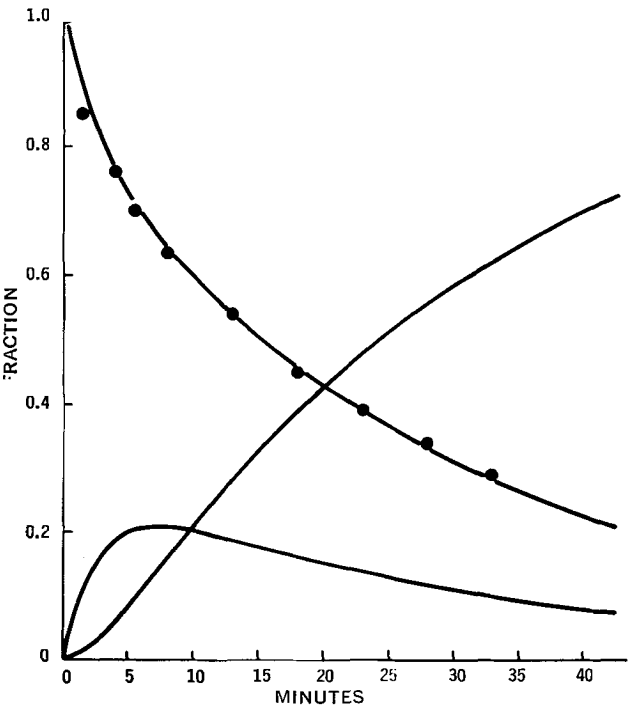

Figure 10—*Plots for trimeperazine similar to those shown in Fig. 7;* ($k_{12} = 0.091, k_{21} = 0.18, k_{23} = 0.12\ hr.^{-1}$).

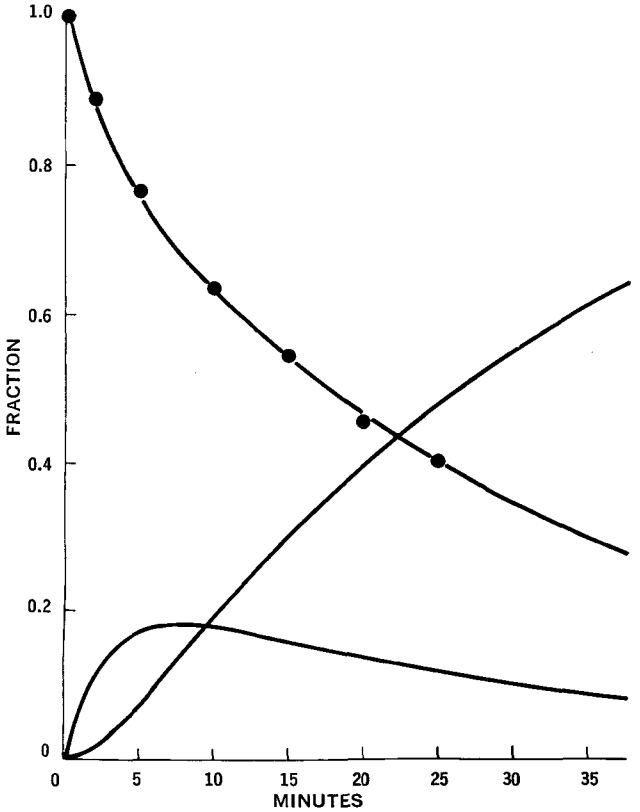

Figure 11—*Plots for haloperidol similar to those shown in Fig. 7;* ($k_{12} = 0.082, k_{21} = 0.17, k_{23} = 0.13\ hr.^{-1}$).

When the absorption of highly lipid soluble drugs such as the phenothiazine or butyrophenone tranquilizers was studied in the *in situ* rat gut preparation, biexponential loss of drug from the lumen solution was always observed. Such experiments were carried out with prochlorperazine, chlorpromazine, trifluoperazine, trimeperazine, and haloperidol. In each case the data were plotted on semilogarithmic paper, and values for X_1, X_2, a, and b (Eq. 5) were determined by feathering as illustrated in Fig. 5. These values along with values for the three rate constants k_{12}, k_{21}, and k_{23} (calculated by means of Eqs. 6–8) are shown in Table I. The three rate constants were used as input to an analog computer programmed with Eq. 4, and curves for each of the three compartments were generated by the computer. The results for the four phenothiazines and haloperidol are shown in Figs. 7–11. The lines are computer generated curves and the points are experimental data from the gut lumen. These figures show that, as might be expected, there is good agreement between the theoretical curve and the experimental data for the gut lumen compartment. Also with these highly lipid soluble drugs, the three-compartment model predicts that the amount of drug in the membrane compartment reaches a maximum of between 20 and 40% of the dose. Thus, there is appreciable membrane storage of these drugs during gastrointestinal absorption.

One might question whether analog computer simulations such as those described truly reflect drug behavior in the inaccessible phases of the model. The accuracy of these simulations depends in turn upon the accuracy of the rate constants calculated by means of Eqs. 6–8. The results of an experiment based on ideal data generated by an analog computer suggest that the accuracy of the rate constants depends directly upon the accuracy of the data obtained in the gut lumen. An analog computer programmed with the three-compartment model and with known values of k_{12}, k_{21}, and k_{23} (see Table II) was used to generate an ideal plot of the fraction of the dose in the "gut lumen" compartment *versus* time. Points were picked off the curve and plotted on semilogarithmic paper. The curve was feathered to obtain X_1, X_2, a, and b and values for k_{12}, k_{21}, and k_{23} were calculated using Eqs. 6–8. The results in Table II show excellent agreement between the calculated and known values of the rate constants suggesting that rate constants calculated from experimental data in this manner and analog simulations based on these rate constants are as accurate, but no more accurate, than the experimental data itself. The *in situ* rat gut preparation yields such precise and reproducible data that the analog simulations in Figs. 7–11 might be expected to truly represent distribution of these drugs among the gut lumen, absorbing membrane, and blood compartments of the experimental animals.

SUMMARY

The disappearance of salicylic acid and certain other drugs (2) from the *in situ* rat gut lumen was found to be monoexponential, whereas certain highly lipid soluble drugs exhibited biexponential disappearance in the same preparation. In each case, the overall transfer of drug from gut lumen to blood was essentially irreversible. Experiments with an *in vitro* three-phase model for drug absorption showed that monoexponential disappearance from the lumen can be expected when accumulation of the drugs in the membrane is negligible. Conversely, biexponential disappearance from the lumen can be expected when accumulation of the drugs in the membrane is appreciable.

By feathering the lumen data which exhibited biexponential drug loss and applying standard mathematical techniques, specific rate constants were determined for drug transfer into and out of the membrane. The validity of these derived rate constants was confirmed by means of an analog computer for data obtained with the three-phase *in vitro* model in which the amounts of drug in all three phases were determined experimentally. The validity of the computational method was confirmed using ideal data generated by an analog computer programmed with known rate constants.

Resolution of the absorption process into its individual components should allow closer and more meaningful study of the factors influencing it. For example, fasting of animals has been reported to slow drug disappearance from the gut lumen (7); and studies are presently being conducted to determine which rate constants are affected by this fasting. Experiments are also being carried out to determine the effect of pH on the individual rate constants, and simultaneous sampling of serum and lumen contents are also being conducted to further elucidate the overall gut to blood transfer process.

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