follow the patterns demonstrated for catatoxic and syntoxic phenomena in plasma (6, 7).

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Drug Absorption Kinetics in Goldfish

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Abstract □ A model is presented for relating turnover time in goldfish to the concentration of drug in the bathing solution. The model, based on passive transport and the existence of a critical concentration of drug within the fish being required for turnover, was applied to the dose–response data of eight homologous esters of *p*-aminobenzoic acid. Absorption rate constants, relative membrane water partition coefficients, and critical concentrations were evaluated for the methyl through hexyl esters. Heptyl aminobenzoate was only effective in saturated solution. No turnover could be produced by the octyl ester which has a solubility below the critical concentration required for activity.

Keyphrases □ Goldfish—drug absorption kinetics, model and equations relating turnover time and alkyl *p*-aminobenzoate concentration □ Drug absorption kinetics—relationship between turnover time (goldfish) and drug concentration (alkyl *p*-aminobenzoates), model, equations □ *p*-Aminobenzoic acid esters—model and equations relating drug concentration and goldfish turnover time □ Dose–response data—alkyl *p*-aminobenzoate-induced turnover of goldfish

The relationship between the concentration of drug in a bulk solution containing a goldfish and the turnover time of the fish has been studied in several laboratories (1–12) over the past few years. Levy and Gucinski (1) developed a model which relates turnover time to the passive transport rate of the drug across the appropriate membrane of the fish. Their model, while frequently useful, does not account for the commonly observed threshold concentrations of drug, *i.e.*, the minimum concentration producing turnover. Nightingale and Gibaldi (8) recently extended the Levy–Gucinski model to cover situations where a measurable threshold concentration exists. Unfortunately, their derivation appears to predict a dependency on fish volume and on bathing solution volume. This report presents an alternative derivation which overcomes certain difficulties of previous mathematical models.

The equations developed will be used to interpret the dose–response data obtained for several normal alkyl esters of *p*-aminobenzoic acid.

THEORETICAL

In the course of these experiments and in the work of previous investigators, a linear relationship was observed between drug concentration and goldfish turnover time. The linearity and intercept of the reciprocal turnover time–concentration data can be explained by a model based on the following assumptions:

1. Absorption of the drug is passive and is, therefore, a reversible and unsaturable process.
2. Permeability characteristics of the membrane are unchanged during the experiment and unaffected by drug concentration.
3. Drug metabolism or active excretion is negligible during the experiment. There is no development of drug tolerance.

Table I—Comparison of Kinetic Equations for Goldfish Turnover

Model	Relation between $1/t$ and C_B		C_B^e	
Levy–Gucinski (1)	$\frac{1}{t} = \frac{k_1' V_B C_B}{V_F C_F}$	(Eq. 11)	$C_B^e = 0$	(Eq. 12)
Nightingale–Gibaldi (8)	$\frac{1}{t} = \frac{k_1' V_B C_B}{V_F C_F^*} - \frac{k_1' + k_2'}{2}$	(Eq. 13)	$C_B^e = \frac{k_1' + k_2'}{2k_1'} \frac{V_F C_F^*}{V_B}$	(Eq. 14)
This work	$\frac{1}{t} = \frac{k_1}{C_F^*} C_B - \frac{k_2}{2}$	(Eq. 8)	$C_B^e = \frac{k_2}{2k_1} C_F^*$	(Eq. 9)
			$C_B^r = \frac{k_2}{k_1} C_F^*$	(Eq. 10)

4. The end-point is observed immediately after a critical concentration of drug within the fish, C_F^*, is reached. This value is assumed to be independent of the concentration of drug in the bathing solution.

5. The concentration of drug in the bathing solution is unchanged during the experiment.

The first four of these assumptions are essentially equivalent to the assumptions made by Nightingale and Gibaldi (8). The fifth assumption is intuitively agreeable since only a negligible fraction of the total drug present is absorbed by the fish. This latter assumption simplifies both the calculations and the results considerably.

The application of these assumptions to the absorption of drug by goldfish leads to the following differential equation:

$$\frac{dC_F}{dt} = k_1 C_B - k_2 C_F \qquad \text{(Eq. 1)}$$

where C_B and C_F are the concentrations of drug in the bulk or bathing solution and in the fish, respectively; t is time; and k_1 and k_2 are the rate constants for passive absorption and elimination, respectively. The solution to Eq. 1 is:

$$C_F = \frac{k_1}{k_2} C_B + A e^{-k_2 t} \qquad \text{(Eq. 2)}$$

where A is a constant of integration.

Since it is known that $C_F = 0$ at $t = 0$, the constant A must be equal to $-(k_1/k_2)C_B$, and Eq. 2 becomes:

$$e^{-k_2 t} = \left(\frac{\frac{k_1}{k_2} C_B - C_F}{\frac{k_1}{k_2} C_B} \right) \qquad \text{(Eq. 3)}$$

Converting to natural logarithms gives:

$$k_2 t = -\ln \left(\frac{\frac{k_1}{k_2} C_B - C_F}{\frac{k_1}{k_2} C_B} \right) \qquad \text{(Eq. 4)}$$

which can be approximated by:

$$\frac{1}{k_2 t} = \frac{1}{2\left(\frac{X-1}{X+1}\right) + \frac{2}{3}\left(\frac{X-1}{X+1}\right)^3 + \frac{2}{5}\left(\frac{X-1}{X+1}\right)^5 + \dots} \qquad \text{(Eq. 5)}$$

Table II—Solubilities of *n*-Alkyl *p*-Aminobenzoates in Water at 24°

Ester	Solubility, mole/l.
Methyl	8.3×10^{-3}
Ethyl	4.83×10^{-3}
Propyl	2.03×10^{-3}
Butyl	7.35×10^{-4}
Pentyl	1.79×10^{-4}
Hexyl	3.1×10^{-5}
Heptyl	7.0×10^{-6}
Octyl	1.3×10^{-6}

where $X = (k_1 C_B/k_2)/(k_1 C_B/k_2 - C_F)$. For $1 < X < 100$[1], all but the first term in the denominator can be ignored and Eq. 5 becomes:

$$\frac{1}{k_2 t} = \left(\frac{2C_F}{2\frac{k_1}{k_2} C_B - C_F} \right)^{-1} \qquad \text{(Eq. 6)}$$

or[2]:

$$\frac{1}{t} = \frac{k_1 C_B}{C_F} - \frac{k_2}{2} \qquad \text{(Eq. 7)}$$

If, in Eq. 7, t is the turnover time, t^*, then C_F, according to the fifth assumption, must be equal to the threshold concentration of the drug in the fish, C_F^*. Thus, the reciprocal turnover time can be expressed as:

$$\frac{1}{t^*} = \frac{k_1}{C_F^*} C_B - \frac{k_2}{2} \qquad \text{(Eq. 8)}$$

Equation 8 describes a straight line of slope (k_1/C_F^*) and an extrapolated intercept $-(k_2/2)$, which relates reciprocal turnover time to the concentration of drug in the bathing solution. The extrapolated concentration intercept, C_B, of Eq. 8 is determined (by setting $1/t = 0$) to be related to the threshold concentration in the fish, C_F^*, by:

$$C_B^e = \frac{k_2}{2k_1} C_F^* \qquad \text{(Eq. 9)}$$

The existence of a bath concentration that will show no activity over any period of time is well documented in the literature. The value of C_B^e for a particular drug is well defined and consistently observed. Independent studies (4, 5, 7) showed the value of C_B^e for ethanol in goldfish to be between 1 and 2% by volume.

If the series in Eq. 5 is not truncated after the first term, the function deviates from linearity and the real intercept, $(k_1/k_2)\ C_B^r$, approaches C_F^* as $1/t$ approaches zero, *i.e.*:

$$C_B^r = \frac{k_2}{k_1} C_F^* \qquad \text{(Eq. 10)}$$

Figure 1 shows the theoretical relationship between $1/t$ and concentration as described by the exact Eq. 4 and the approximate Eq. 8.

[1] Most of the data to be discussed is within this region.

[2] If one lets $(k_1 C_B)/(k_2 C_F) = p$, then Eq. 4 becomes:

$$\frac{1}{k_2 t} = \frac{1}{\ln\left(\frac{p}{p-1}\right)} \qquad \text{(Eq. 4a)}$$

Equation 4*a* was solved for the following integral values of p between 2 and 100 (p = 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100). The results fit the following least-squares equation with a correlation coefficient of 1.000:

$$\frac{1}{k_2 t} = 1.00\,p - 0.52 \qquad \text{(Eq. 4b)}$$

which is approximately equivalent to:

$$\frac{1}{t} = \frac{k_1 C_B}{C_F} - \frac{k_2}{2} \qquad \text{(Eq. 7)}$$

As can be seen in Fig. 1, the approximate Eq. 7 shows good agreement with the exact solution in p for $2 < p < 100$. However, there is significant deviation from linearity and $1/t \to 0$ as $p \to 1$, *i.e.*, as $k_1/k_2\ C_B \to C_F^*$.

These results differ from those of Levy and Gucinski (1) and Nightingale and Gibaldi (8) in several respects. The final equations and the concentration intercepts of the three derivations (in the present notation) are summarized in Table I. The major difference between the equation developed here and the other equations found in the table is that the latter appear to be dependent upon the volume of the fish, V_F, and the volume of the bathing solution, V_B. The independence of goldfish turnover time on fish size was demonstrated by DiSanto and Wagner (7) using ethanol and in these laboratories for various esters of *p*-aminobenzoic acid[3]. As long as the volume of the bathing solution is greater than about 50 times the volume of the fish, the turnover time is independent of solution volume. Both Eqs. 11 and 13 predict that reciprocal turnover time will increase linearly with increasing solution volume at a constant concentration. However, if k_1' is converted to units based on concentration rather than amounts by $k_1' = k_1(C_F/C_B)$, the slope of the Nightingale–Gibaldi Eq. 13 becomes identical to the slope of the present Eq. 8. The differences in the intercepts of Eqs. 8 and 13 are simply a reflection of the mathematical approximations involved in the two treatments.

If the value of C_F^* refers to the aqueous portion of the fish, it is possible to make one further simplifying assumption; namely, that $k_1 = k_2$. In other words, as is implicit in the first assumption, there is no directional specificity in the membrane barriers. With this simplification the extrapolated concentration intercept, C_B^e, of a reciprocal turnover times *versus* concentration plot is equal to one-half the critical concentration within the fish required to produce turnover:

$$C_B^e = {}^1/_2 C_F^* \qquad \text{(Eq. 15)}$$

Equation 15 is consistent with the fact that C_B^e is equal to ${}^1/_2 C_B^r$ (Fig. 1) and the hypothesis (Eq. 10) that the threshold concentration in the bulk, C_B^r, must be equal to the critical concentration within the fish.

If C_F^* is the total fish concentration of drug, this discussion is still qualitatively applicable. Since over 80% of the goldfish is aqueous, $k_1 \approx k_2$ and, thus, $2C_B^e \approx C_B^r$. The equilibrium constants of several simple semipolar compounds between gudgeons and water were determined (13) to be 0.94 for methanol, 0.86 for ethanol, 0.79 for isopropanol, 0.88 for acetone, and 0.84 for methyl acetate. Likewise, the data of Nightingale (9) indicate a goldfish–water partition coefficient of 0.92 for 4-aminoantipyrine. From these values, it can be seen that the approximation of k_2/k_1 by unity will be off by less than 20% for these compounds. Further verification of the equivalency of the two rate constants is found in reported results (3, 9, 11, 14) that have shown experimentally that $C_B^r \approx C_F^*$ or $k_1 \approx k_2$.

Thus, Eq. 8 can be rewritten as:

$$\frac{1}{t} = \frac{k_1}{C_F^*}C_B - \frac{k_1}{2} = \left(\frac{C_B}{C_F^*} - \frac{1}{2}\right)k_1 \qquad \text{(Eq. 16)}$$

EXPERIMENTAL

Materials—Methyl *p*-aminobenzoate, ethyl *p*-aminobenzoate, and butyl *p*-aminobenzoate were used as received. The remaining esters were synthesized from the appropriate alcohol[4] and *p*-nitrobenzoyl chloride[5] as described previously (15, 16).

Aqueous Solubility—The aqueous solubility of these esters was determined at 24 ± 1° by UV spectroscopy of stock solutions which were equilibrated for at least 3 days.

Bathing Solutions—All bathing solutions were prepared from the stock saturated solutions by dilution.

Measurement of Turnover Time—Goldfish, *Carassius auratus*, between 5.08 and 7.62 cm. (2 and 3 in.) in length, were purchased locally. The shorter turnover time was determined by placing a single fish in 200 ml. of bathing solution. The longer times were determined by placing several fish in a larger volume. In all cases, the end-point was taken as the time it took the fish to become unable to bring itself to an upright position after being turned onto its side by a glass rod, *i.e.*, lose its righting reflex.

[3] S. H. Yalkowsky, G. L. Flynn, and T. G. Slunick, unpublished data.
[4] Eastman or Aldrich.
[5] Matheson.

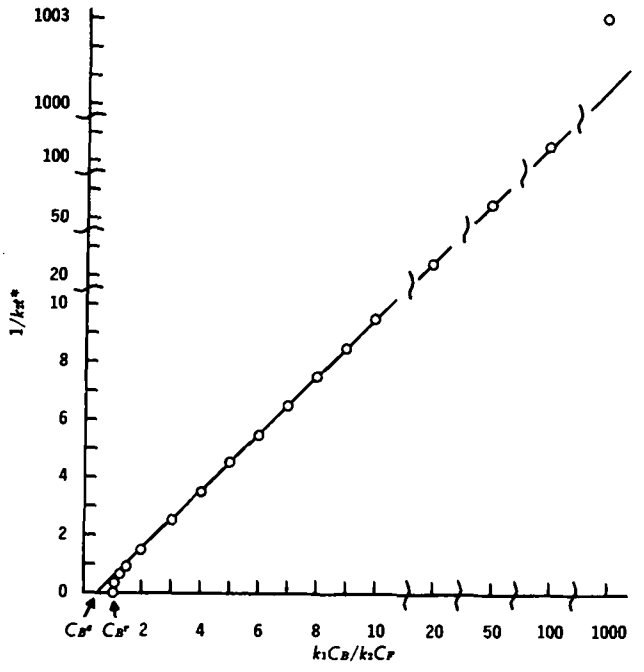

Figure 1—*Comparison of exact and approximate solutions to Eq. 4. Key:* O, *exact solution; and* ——, *approximate solution given by Eq. 7.*

RESULTS AND DISCUSSION

Solubility—The 24° solubilities of the eight esters studied are listed in Table II. Past butyl, the solubility decreases by factor of 5.0 for each additional methylene group; the solubilities of shorter homologs decrease more slowly with chain length. The solubilities of the *n*-alkyl *p*-aminobenzoates were already interpreted in terms of other physical properties of these compounds (16).

Turnover Time—Mean turnover times obtained as a function of concentration for the various *n*-alkyl *p*-aminobenzoates are given in Table III. With the exception of the four values discussed below, all of the values are the average of 10 individual experimental points. The asterisks represent conditions under which at least three out of 10 fish did not turn over within 48 hr. These data are shown graphically in Figs. 2 and 3. The graphs are presented in terms of fractional saturation, C/S, rather than the more conventional concentration, C, for two reasons: (*a*) all of the data in Table III, which range from 1×10^{-6} to 8×10^{-3} M, can be plotted simultaneously, and (*b*) a direct comparison of the esters can be made on a so-called "equal thermodynamic scale" (17).

In Fig. 2, reciprocal turnover time is plotted against fractional saturation. The minimum turnover time observed is about 0.4 min., and this value was obtained for saturated solutions of the lower esters. Furthermore, these points fall well below the least-squares line through the other data. The authors believe that these deviations from predicted behavior are related to the time required for the establishment of a quasi-steady-state concentration gradient across the membrane barriers. This lag time for turnover is then the minimum attainable turnover time.

The bulk of the data in Fig. 2 falls on lines having positive concentration intercepts. The equations for the least-squares lines (all points weighted according to the reciprocal of their variance) for each ester are given in Table IV. Dividing the slope of the solubilities of the esters enables concentration to be expressed in moles per liter instead of fractional saturation. It is necessary to weight the data as already described to obtain more accurate values for the intercepts. The scatter in the reciprocal turnover time produced by the higher concentrations of drug precluded accurate determination of the intercepts by nonweighted regression analysis of all of the data. This same difficulty was noted by Nightingale and Gibaldi (8) for 4-aminoantipyrine-induced turnover. They overcame this difficulty by ignoring the data for the higher drug concentrations in determining the intercept.

The log–log curves of Fig. 3 are also revealing. All of the curves approach an infinite slope as $C_B \to C_B^r$; but for $C_B \gg C_B^r$, the

slopes all approach unity. If there were no intercept in the $1/t$ *versus* C data, the log-log plots would have a slope of -1.0 throughout the range studied.

From the equations in Table IV, the values of the kinetic parameters, k_2, $(k_1/C_B{}^s)$, and $(k_2/k_1)C_B{}^s$, can be obtained for each ester, and they are listed in Table V. It is possible to determine a range for $C_B{}^0$ from the raw material data in Table III. These ranges are also in agreement with the data in Table V.

The low solubility of the heptyl ester is responsible for the lack of conclusive estimates of its kinetic parameters. The inability of the octyl ester to produce turnover, even from saturated solutions, suggests that its solubility is below the threshold concentration. Likewise, the nonyl ester was found to be totally inactive.

The data indicate quite clearly that k_2 values decrease and $k_1/C_B{}^s$ values increase with increasing chain length of the *p*-aminobenzoate ester moiety. However, these changes are small compared to the dramatic decreases in $k_2C_B{}^s/k_1$ that accompany assension of the homologous series. Unfortunately, the proposed model does not enable the definitive mathematical separation of k_1 and $C_B{}^s$. If it is assumed that $k_1 = k_2$ as described, it is evident that $C_B{}^s$ is highly dependent upon chain length. On the other hand, if it is assumed that the intrinsic activities of all of the esters are identical, it is

Table III—Average Turnover Times Produced by Various Concentrations of *n*-Alkyl *p*-Aminobenzoates

Ester	Fractional Saturation	Concentration, mole/l.	Number of Fish	Mean Turnover Time, min.	Standard Error of Mean	Mean Reciprocal Turnover Time
Methyl	Saturated	8.3×10^{-3}	5	0.320	0.051	3.15
	2/3	5.52×10^{-3}	10	0.410	0.030	2.44
	1/2	4.15×10^{-3}	10	0.640	0.098	1.52
	1/3	2.76×10^{-3}	10	0.933	0.088	1.07
	1/4	2.07×10^{-3}	10	1.230	0.076	0.813
	1/6	1.40×10^{-3}	10	2.270	0.183	0.44
	1/10	8.3×10^{-4}	10	4.390	0.189	0.23
	1/20	4.15×10^{-4}	10	45.0	15.7	0.022
	1/40	2.07×10^{-4}	10	—[a]	—	—
	1/100	8.3×10^{-5}	10	—[a]	—	—
Ethyl	Saturated	4.83×10^{-3}	5	0.340	0.062	2.95
	2/3	3.16×10^{-3}	10	0.270	0.015	3.70
	1/2	2.37×10^{-3}	10	0.460	0.040	2.17
	1/3	1.58×10^{-3}	10	0.550	0.045	1.82
	1/4	1.18×10^{-3}	10	0.990	0.095	1.01
	1/6	7.8×10^{-4}	10	1.210	0.159	0.83
	1/10	4.83×10^{-4}	10	2.800	0.226	0.36
	1/20	2.37×10^{-4}	10	6.50	0.64	0.15
	1/40	1.18×10^{-4}	10	47	9.3	0.021
	1/100	4.83×10^{-5}	10	—[a]	—	—
Propyl	Saturated	2.03×10^{-3}	6	0.333	0.042	3.0
	2/3	1.3×10^{-3}	10	0.450	0.037	2.22
	1/2	9.8×10^{-4}	10	0.510	0.038	1.96
	1/3	6.5×10^{-4}	10	0.680	0.039	1.47
	1/4	4.9×10^{-4}	10	0.900	0.097	1.11
	1/6	3.3×10^{-4}	10	1.150	0.097	0.87
	1/10	2.03×10^{-4}	10	3.150	0.442	0.32
	1/20	9.8×10^{-5}	10	8.250	0.720	0.12
	1/40	4.9×10^{-5}	10	30.50	3.06	0.03
	1/100	2.03×10^{-5}	10	—[a]	—	—
Butyl	Saturated	7.35×10^{-4}	5	0.340	0.025	2.94
	2/3	4.7×10^{-4}	10	0.640	0.072	1.56
	1/2	3.6×10^{-4}	10	0.730	0.070	1.37
	1/3	2.4×10^{-4}	10	1.110	0.094	0.90
	1/4	1.8×10^{-4}	10	1.510	0.157	0.66
	1/6	1.2×10^{-4}	10	2.600	0.256	0.39
	1/10	7.35×10^{-5}	10	4.650	0.289	0.22
	1/20	3.6×10^{-5}	10	9.950	0.664	0.10
	1/40	1.8×10^{-5}	10	70.60	18.44	0.014
	1/100	7.35×10^{-6}	10	—[a]	—	—
Pentyl	Saturated	1.79×10^{-4}	10	1.370	0.104	0.73
	3/4	1.34×10^{-4}	10	1.830	0.146	0.55
	2/3	1.29×10^{-4}	10	2.380	0.133	0.42
	1/2	9.0×10^{-5}	10	3.310	0.293	0.30
	1/3	5.95×10^{-5}	10	4.500	0.632	0.22
	1/4	4.5×10^{-5}	10	6.380	0.495	0.16
	1/6	2.98×10^{-5}	10	8.850	0.311	0.11
	1/10	1.79×10^{-5}	10	15.50	1.06	0.065
	1/20	9.0×10^{-5}	10	43.33	3.67	0.023
	1/40	4.5×10^{-6}	6	63.60	5.05	0.016
	1/100	1.79×10^{-6}	5	—[a]	—	—
Hexyl	Saturated	3.1×10^{-5}	10	6.70	0.26	0.15
	3/4	2.4×10^{-5}	10	9.20	0.41	0.11
	2/3	2.0×10^{-5}	10	13.00	0.45	0.078
	1/2	1.5×10^{-5}	10	14.30	1.68	0.070
	1/3	1.0×10^{-5}	10	25.00	2.17	0.040
	1/4	7.5×10^{-6}	10	31.20	5.88	0.032
	1/6	5.0×10^{-6}	10	—[a]	—	—
Heptyl	Saturated	7.2×10^{-6}	6	30.00	3.72	0.033
	1/2	3.6×10^{-6}	6	—[a]	—	—
Octyl	Saturated	1.3×10^{-6}	2	—[a]	—	—

[a] Inactive.

Table IV—Dose–Response Equations

Ester	Equation	Number of Data Points Used	Correlation Coefficient
Methyl	$1/t = (-0.164 \pm 0.0045) + (3.95 \pm 0.055) + 8.3 \times 10^{-3} \times C$	6	1.000
Ethyl	$1/t = (-0.105 \pm 0.0124) + (5.37 \pm 0.278) + 4.83 \times 10^{-3} \times C$	8	0.989
Propyl	$1/t = (-0.0742 \pm 0.0147) + (4.41 \pm 0.305) + 2.03 \times 10^{-3} \times C$	8	0.979
	$(-0.0895 \pm 0.0131) + (4.91 \pm 0.336) + 2.03 \times 10^{-3} \times C$	6	0.989
Butyl	$1/t = (0.0398 \pm 0.0124) + (2.76 \pm 0.168) + 7.35 \times 10^{-4} \times C$	9	0.980
	$(-0.0449 \pm 0.0072) + (2.87 \pm 0.130) + 7.35 \times 10^{-4} \times C$	6	0.998
Pentyl	$1/t = (-0.00302 \pm 0.00082) + (0.676 \pm 0.0241) + 1.79 \times 10^{-4} \times C$	10	0.993
Hexyl	$1/t = (-0.000570 \pm 0.000846) + (0.135 \pm 0.0103) + 3.1 \times 10^{-5} \times C$	7	0.981

obvious from the data that k_1 cannot equal k_2. These two assumptions are consistent with one another if equal concentrations of the esters have equal activities only in some lipid or membrane portion of the fish; *i.e.*, at turnover, C_M^* is the same for each ester. Since the membrane water partition coefficient of each ester is given by:

$$PC = \frac{C_M}{C_W} \approx \frac{C_M}{C_F} \approx \frac{C_M^*}{C_F^*} \qquad \text{(Eq. 17)}$$

and if C_M^* is assumed independent of chain length, the relative partition coefficients of the esters are approximately proportional to the reciprocal of their critical fish concentrations, C_F^*. Interpretation of the changes in k_1, k_2, C_F^*, and PC that accompany changes in alkyl chain length will be published later.

As a further test of its validity, the turnover times produced by various concentrations of 4-aminoantipyrine (Table I of *Reference 8*) were analyzed according to the model. To get meaningful estimates of the intercepts, only the data for the lower concentrations of drug were considered.

The resulting least-squares line was then used to calculate the kinetic parameters. These values (Table VI) were in good agreement with values determined independently by Nightingale (9) and Anello and Levy (11).

Table V—Kinetic Parameters for *n*-Alkyl *p*-Aminobenzoate-Induced Turnover of Goldfish

Ester	k_2, min.$^{-1}$	k_1/C_B^*, l./min./mole	$(k_2/k_1)C_B^*(=C_F^*)$, mole/l.
Methyl	0.328	475	6.9×10^{-4}
Ethyl	0.210	1100	1.88×10^{-4}
Propyl	0.148	2160	6.9×10^{-5}
Butyl	0.080	3750	2.13×10^{-5}
Pentyl	0.0060	3780	1.59×10^{-6}
Hexyl	0.0011	4310	2.6×10^{-7}

CONCLUSIONS

The equations derived in this report not only describe the dose–response data for alkyl benzoate-induced turnover of goldfish but also provide a convenient means of estimating the following parameters: (*a*) elimination rate constant, and (*b*) the critical or threshold concentration of each drug required to produce turnover.

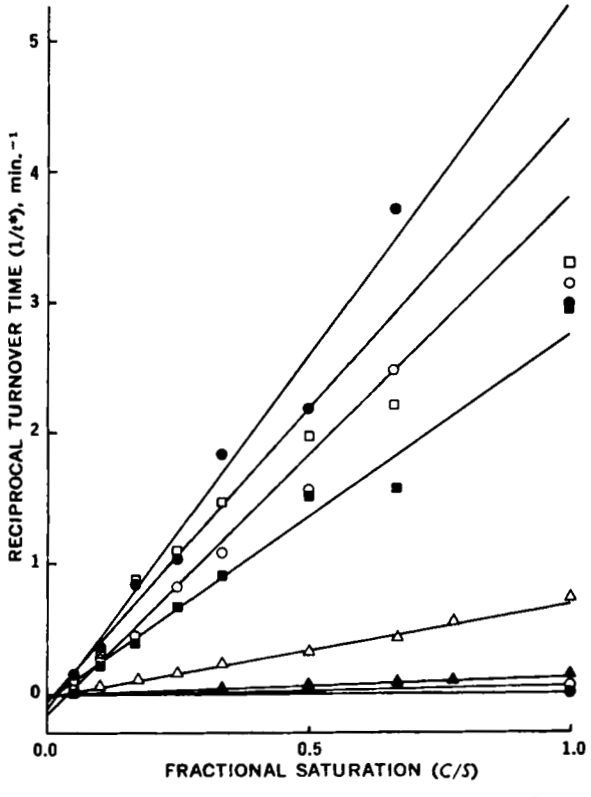

Figure 2—*Reciprocal turnover time as a function of the the fractional saturation of* p*-aminobenzoate ester in the bathing solution. Key:* ○, *methyl;* ●, *ethyl;* □, *propyl;* ■, *butyl;* △, *pentyl;* ▲, *hexyl;* ◇, *heptyl; and* ◆, *octyl. Lines are the weighted least-squares lines given in Table III.*

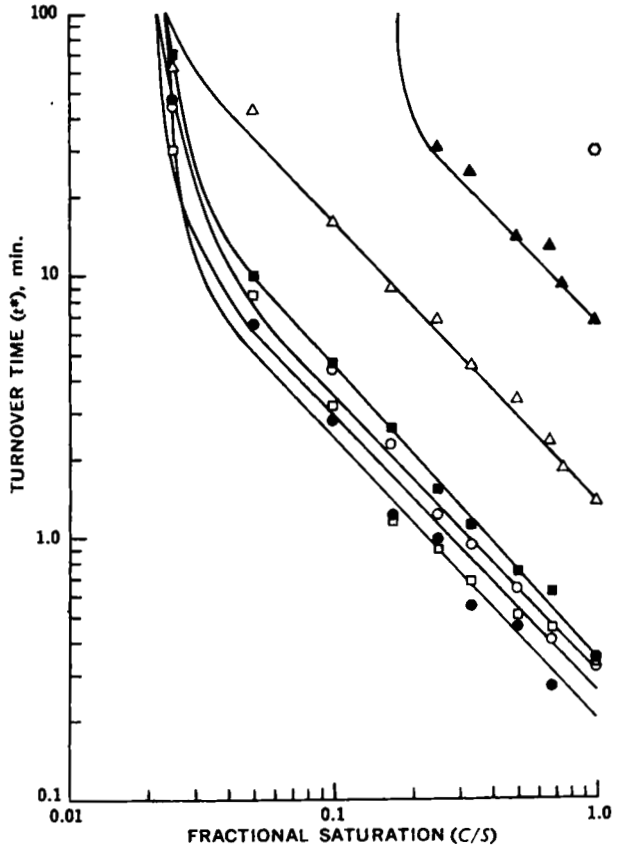

Figure 3—*Log–log relationship between bathing solution concentration (expressed as fractional saturation) and turnover time. The symbols have the same meaning as those in Fig. 2.*

Table VI—Values of Kinetic Parameters for 4-Aminoantipyrine-Induced Overturn of Goldfish

Parameter	From Eq. 8 and Dose–Response Data	Literature Values	Reference
k_1	6.54×10^{-3} min.$^{-1}$	2.3×10^{-3} min.$^{-1}$	11
k_2	6.54×10^{-3} min.$^{-1}$	3.3×10^{-3} min.$^{-1}$	11
C_F*	6.67 mg.%	6.8 mg.%	9

The proposed model for the action of homologous narcotic drugs on goldfish was tested using *n*-alkyl *p*-aminobenzoates. The experimental data were interpretable by the derived equations. Apparent discrepancies between the model and high concentration data were accounted for by recognizing that there is a finite lag time for turnover.

Literature data for 4-aminoantipyrine-induced narcosis of goldfish were also interpretable by the model.

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Correlation of Viscoelastic Functions for Pharmaceutical Semisolids: Comparison of Creep and Oscillatory Tests for Oil-in-Water Creams Stabilized by Mixed Emulsifiers

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Abstract □ Liquid paraffin-in-water emulsions stabilized by mixed emulsifiers of a surfactant (cetrimide or cetomacrogol) and a long-chain alcohol were used as model systems to represent pharmaceutical semisolids. They were examined in their linear viscoelastic regions using creep and oscillatory (Weissenberg rheogoniometer) techniques. Storage and loss compliances (J' and J'') over the frequency range 2.5×10^{-8} to 1.5×10^{-1} Hz. were calculated from creep data using numerical formulas. The transformed compliances were used to derive the other oscillatory functions, the storage and loss moduli (G' and G''), the storage and loss viscosities (η' and η''), and the loss tangent (tan δ) at the same frequencies. The agreement between transformed functions and those obtained directly from oscillatory measurements at frequencies ranging from 7.91×10^{-4} to 1.5×10^{-1} Hz. was generally good. Discrepancies at the extremities of this range were a result of the practical difficulties involved when creep measurements are made at very short times (representing high frequencies) and evaporation of water from the parallel plates of the rheogoniometer during low frequency dynamic experiments. Neither small strain technique alone covered the entire frequency or time range necessary for an exhaustive study of semisolids. Creep data were unavoidably inaccurate at high frequencies, and the limitations of the rheogoniometer gear box prevented oscillatory measurements from being made at extremely low frequencies. The unification of data from oscillatory and creep measurements so as to provide a dynamic description over an extended frequency range (10^{-8}–25 Hz.) is discussed. The variations of each function with frequency are considered with reference to linear viscoelastic theory; the limiting values and the shapes of the plots at low frequencies agreed well with the theoretical values for viscoelastic liquids. It was concluded that such interconversion techniques are applicable and valuable for interpreting the rheological behavior of a pharmaceutical system.

Keyphrases □ Creams, oil-in-water, stabilized by mixed emulsifiers—viscoelastic properties, correlation between creep and oscillatory tests □ Creep measurements—correlation with oscillatory functions, oil-in-water creams stabilized by mixed emulsifiers □ Oscillatory functions—correlation with creep measurements, oil-in-water creams stabilized by mixed emulsifiers □ Viscoelastic properties, oil-in-water creams stabilized by mixed emulsifiers—correlation between creep and oscillatory tests

The rheological properties of semisolid preparations affect all stages of manufacture, and they should be considered in quality control. The release of medicaments from semisolid vehicles is sometimes related to these properties, so a knowledge of the rheological effects of changes in formulation may also be important for bioavailability studies.

In the past, a major difficulty encountered in the rheological evaluation of semisolid ointments and creams was how to obtain a true measure of consistency. The approach described frequently in the literature is to use a continuous shear technique and then to attempt to correlate derived parameters, *e.g.*, spur points or yield values, with the qualitative terms "consistency