general, materials that have high elastic contributions (1) (*e.g.*, o/w creams) break down more rapidly than ointments and w/o creams, which are more viscous (1).

The rate constant for breakdown under oscillatory shear is an important parameter when assessing consumer utilization of topical products. In future studies, both nondestructive and destructive oscillatory tests and consumer panels will be used to arrive at a detailed viscoelastic specification for a range of semisolid products.

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Kinetics of Drug Absorption in Goldfish

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Abstract □ A two-compartment reversible model describing the occurrence of pharmacologic effect in goldfish as a function of drug concentration in the bathing solution was examined. The derived expressions were applied to 4-aminoantipyrine-induced overturn. The rate constants of absorption and exsorption for this drug were 4.0×10^{-4} hr.$^{-1}$ and 7.8×10^{-3} hr.$^{-1}$, respectively. The theoretical drug concentration in the fish body necessary for overturn was 19.5 mg. %, which agrees with the experimental results. The mathematical relationships and experimental data demonstrate that a critical concentration is necessary in the fish body before a pharmacologic response will occur. At low drug concentration in the bathing solution, the reciprocal time–drug concentration plots will be hyperbolic rather than linear. The results of this study suggest that 4-aminoantipyrine-induced overturn in the goldfish is absorption rate limited.

Keyphrases □ Goldfish, overturn—4-aminoantipyrine absorption–exsorption kinetics □ Pharmacokinetics, 4-aminoantipyrine absorption–exsorption—goldfish □ 4-Aminoantipyrine, absorption–exsorption kinetics—goldfish

The kinetics of drug absorption in goldfish were recently studied by several workers (1–5). One model, describing the uptake of drugs and toxic chemicals by fish, was developed by Levy and Gucinski (1). As noted by these authors, this theoretical treatment possesses certain limitations due to the simplifying assumptions. The model predicts that a plot of reciprocal time of occurrence of pharmacological effect (time of death or overturn time) *versus* drug concentration in the solution in which the fish are immersed will be linear and pass through the origin. Although the model successfully described the time course of pharmacologic effect of several drugs in the goldfish, some exceptions to the model also were reported. For example, Powers (6) found a number of positive concentration intercept values in plots of reciprocal time of death *versus* concentration of a variety of drug and toxic substances. Powers proposed that there is a concentration of every toxic substance below which no pharmacologic response is observed, regardless of the exposure time. This concentration was designated as a threshold of toxicity. Hall and Hayton (5) subsequently arrived at a similar conclusion by placing goldfish in dilute ethanol solutions for over 24 hr. without observing overturn. In their experiments, it was found that in ethanol-induced overturn and death of goldfish, a plot of reciprocal time of occurrence of the pharmacological end-point *versus* ethanol concentration resulted in a 1% (v/v) intercept. This finding was verified by Gibaldi and Nightingale (2), using the overturn end-point at low ethanol concentrations.

The purpose of the present study was to extend the Levy–Gucinski kinetic model of pharmacologic effect in the goldfish to situations where an apparent threshold concentration does exist.

THEORETICAL

The model of Levy and Gucinski (1) describes the relationship between the drug concentration in the fluids bathing the fish, the absorption rate, and the time of occurrence of pharmacologic effect in the goldfish. The basis of this model is Fick's first law of diffusion:

$$\frac{dA_B}{dt} = \frac{DA}{l}(C_o - C_i) \qquad \text{(Eq. 1)}$$

where A_B is the amount of drug absorbed by the goldfish, (dA_B/dt) is the rate of drug absorption, D is the diffusion coefficient, A is the surface area of biologic membrane, l is the thickness of the biologic membrane, C_o is the drug concentration outside the membrane (the solution bathing the fish), and C_i is the drug concentration on the inside of the membrane.

If one assumes that $C_o \gg C_i$ and integrates Eq. 1 between the limits of zero time and the time necessary to produce a pharmaco-

logic response, *e.g.*, time of death (T_d) or time of overturn (T_o) (2), one obtains:

$$1/T_d = \frac{DAC_o}{l \cdot A_B} = K \cdot C_o \qquad \text{(Eq. 2)}$$

where K is a hybrid constant.

Equation 2 is based upon the following assumptions:

1. Absorption occurs by passive diffusion and, therefore, is not a saturable process.
2. The drug concentration gradient across the absorbing membrane remains essentially constant during the experiment.
3. The permeability characteristics of the membrane do not change with time or drug concentration over the time and concentration range of the experiment.
4. Drug elimination is negligible during the time of the experiment.
5. The pharmacologic effect occurs without significant delay after a given amount of drug has been absorbed and does not involve mechanisms requiring inductive or other delayed effects (1, 2).

Under these conditions, Eq. 2 predicts that a plot of $1/T_d$ *versus* C_o should be linear with a slope of K and pass through the origin. Levy and Gucinski (1) found this to be true for both ethanol and pentobarbital, using death as the pharmacologic end-point. According to Levy and Gucinski (1), if any of the required conditions used in deriving Eq. 2 are not present, a plot of $1/T_d$ *versus* C_o will not be linear.

It was of interest to modify the Levy–Gucinski model by considering the existence of a threshold concentration. If one assumes that the occurrence of a pharmacologic effect is absorption rate limited, *i.e.*, distribution of drug to the drug receptor, and combination with the receptor to produce the pharmacologic response is very rapid, the following model is applicable:

$$A \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} B \qquad \text{(Eq. 3)}$$

where compartment A is the fluid bathing the fish and compartment B is the fish. The volumes of compartment A (V_A) and compartment B (V_B) are constant. Under these conditions, the following differential equations are appropriate:

$$\frac{dX_A}{dt} = k_{21}X_B - k_{12}X_A \qquad \text{(Eq. 4)}$$

and

$$\frac{dX_B}{dt} = k_{12}X_A - k_{21}X_B \qquad \text{(Eq. 5)}$$

where (dX/dt) is the change in the amount of drug in the respective compartments with respect to time, and the k's are constants with dimensions of reciprocal time.

Solving Eqs. 4 and 5 for X_B, one obtains:

$$X_B = \frac{k_{12}X_A^o}{(k_{12} + k_{21})}\left[1 - e^{-(k_{12}+k_{21})t}\right] \qquad \text{(Eq. 6)}$$

where X_A^o is the initial amount of drug in compartment A (the bathing solution). Changing the amounts of drug (X) to concentration terms ($C = X/V$) and rearranging, one obtains:

$$e^{-(k_{12}+k_{21})t} = \frac{KC_A^o - C_B}{KC_A^o} \qquad \text{(Eq. 7)}$$

where C is the concentration of drug in the respective compartment, V is the volume of the respective compartment, and $K = k_{12}V_A/(k_{12} + k_{21})V_B$.

Assuming that the concentration of drug in the fish (C_B) reaches the level necessary to produce a pharmacologic response (C_B^*) at time t^*, then:

$$e^{-(k_{12}+k_{21})t^*} = \frac{KC_A^o - C_B^*}{KC_A^o} \qquad \text{(Eq. 8)}$$

Equation 8 may be rewritten as follows:

$$1/t^* = \frac{k_{12} + k_{21}}{\ln\left(\dfrac{KC_A^o}{KC_A^o - C_B^*}\right)} \qquad \text{(Eq. 9)}$$

The denominator on the right side of Eq. 9 may be approximated by the following logarithmic series:

$$\ln Z = 2\left[\frac{Z-1}{Z+1} + \frac{1}{3}\left(\frac{Z-1}{Z+1}\right)^3 + \frac{1}{5}\left(\frac{Z-1}{Z+1}\right)^5 + \cdots\right] \qquad \text{(Eq. 10)}$$

where Z is greater than zero and equals: $KC_A^o/(KC_A^o - C_B^*)$.

If all terms of Eq. 10 except the first are assumed to be negligible, then:

$$1/t^* \cong \frac{k_{12} + k_{21}}{2\left(\dfrac{Z-1}{Z+1}\right)} \qquad \text{(Eq. 11)}$$

and upon substitution for Z,

$$1/t^* \cong \frac{K(k_{12} + k_{21})C_A^o}{C_B^*} - \frac{(k_{12} + k_{21})}{2} \qquad \text{(Eq. 12)}$$

Hence a plot of $1/t^*$ *versus* C_A^o should have a linear portion with a slope of $(k_{12} + k_{21}) \cdot K/C_B^*$ and an intercept of $-(k_{12} + k_{21})/2$.

The Levy–Gucinski model can be readily shown to be an approximation of the proposed absorption-rate-limited model. This can be seen from Eq. 5, assuming that X_A is large and constant and X_B is negligible:

$$\frac{dX_B}{dt} = k_{12}X_A^o \qquad \text{(Eq. 13)}$$

Upon integration:

$$X_B^* = k_{12}X_A^o \cdot t^* \qquad \text{(Eq. 14)}$$

where X_B^* is the amount of drug in the fish after exposure to the bathing solution containing X_A^o drug for a period of time (t^*) necessary to cause the pharmacologic response. Equation 14 may be rewritten as:

$$X_B^* = k_aC_A^ot^* \qquad \text{(Eq. 15)}$$

where $k_a = k_{12}V_A$. Upon rearrangement:

$$1/t^* = K \cdot C_A^o \qquad \text{(Eq. 16)}$$

where $K = k_a/X_B^*$.

Equation 16 is similar to Eq. 2 derived by Levy and Gucinski (1) using a different approach. While the hybrid rate constant, K, has a different meaning in each model, they are kinetically indistinguishable.

Since Eq. 12 is not exact, it will only approximate the kinetics of drug transfer in the proposed model. To gain insight into the exact relationship between reciprocal overturn time ($1/t^*$) and drug concentration in the fish bathing solution (C_A^o), it is necessary to solve Eq. 9 using reasonable estimates of the critical parameters. When this is done, as can be seen from Fig. 1, hyperbolic curvature occurs at low drug concentrations. Since this type of curvature has not been previously reported, the purposes of the *Experimental* portion of this report were to characterize the $1/t^*$ *versus* C_A^o plot at low drug concentrations and to calculate the constants k_{12}, k_{21}, and C_B^* for 4-aminoantipyrine from Eq. 9.

EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing about 8–10 g., served as the test animal. All fish used in the experiment were from the same lot. The test drug, 4-aminoantipyrine[1], was dissolved in distilled water. The final solutions were adjusted to pH 6.1 with either 1 *N* HCl or NaOH. The fish were immersed in 250 ml. of drug solution at 26–28°, and the overturn time was determined as previously described (2, 7).

RESULTS AND DISCUSSION

The results of the present study indicate that 4-aminoantipyrine-induced overturn apparently follows the Levy and Gucinski model

[1] Eastman.

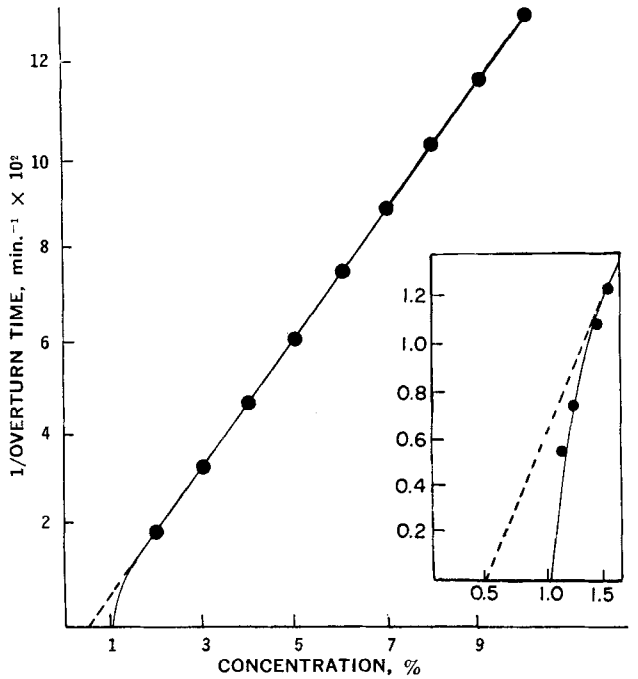

Figure 1—*Reciprocal times of overturn of goldfish as a function of concentration. The data were simulated from the proposed drug absorption-rate-limited model, using a Control Data Corp. 6400 digital computer (9). The parameters were:* $k_{12} = 2.8 \times 10^{-4}$ *min.*$^{-1}$, $k_{21} = 1.4 \times 10^{-3}$ *min.*$^{-1}$, $X_B^* = 0.05$ *mg.*, $V_B = 5$ *ml., and* $V_A = 250$ *ml. Inset represents data at low concentrations.*

at high drug concentrations. This is clearly shown in Fig. 2, where a plot of reciprocal overturn time *versus* concentration is linear over a concentration range from 40 to 800 mg. %. Analysis of the data using an IBM 360 digital computer resulted in a regression equation of:

$$1/T_o = 0.00042X - 5.13 \times 10^{-6} \qquad \text{(Eq. 17)}$$

where X is concentration in mg. %, and T_o is the overturn time in minutes. The correlation coefficient of the line was 0.999. The mean data obtained at each concentration of 4-aminoantipyrine, along with standard deviations, are summarized in Table I. These findings (concentration range: 40–800 mg. %) agree with the data of Anello and Levy (4) in that the curve is linear and apparently passes through the origin. Examination of Table I reveals, however, that the mean overturn times become disproportionately large at

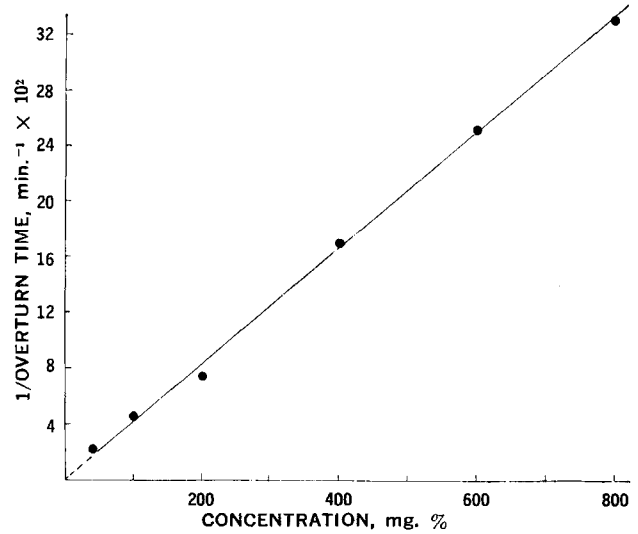

Figure 2—*4-Aminoantipyrine-induced overturn data at high concentrations. Regression equation* $1/T_0 = 0.00042X - 5.13 \times 10^{-6}$, *where X is concentration in milligrams percent. Data are from Table I.*

Table I—Overturn Times of Goldfish in Various Concentrations of 4-Aminoantipyrine

Concentration, mg. %	Number of Animals	Mean Overturn Time, min.[a]	*SD*
800	8	2.99	0.083
600	8	3.95	0.104
400	8	5.86	0.130
200	8	13.30	0.131
100	8	22.00	1.260
50	16	45.06	1.372
40	8	58.14	0.793
30	12	86.02	2.052
25	8	106.70	6.139
20	8	194.95	3.738
15	8	∞[b]	
10	8	∞[b]	

[a] All values different by t-test, $p < 0.01$. [b] > 24 hr.

low drug concentrations. In fact, concentrations of 4-aminoantipyrine of 15 mg. % and lower do not cause overturn. The data at low drug concentrations are shown in Fig. 3. It is apparent that the curve does not pass through the origin but has a finite intercept on the positive X-axis, with a definite deviation from linearity as predicted by the proposed model.

An attempt was made to utilize the experimental data to calculate the forward and reverse rate constants, k_{12} and k_{21}, using Eq. 9. By setting V_A, the volume of the bathing solution, as 250 ml. and V_B, the volume of the fish, as 10 ml., it was possible to write a program for the digital computer capable of calculating k_{12}, k_{21}, and C_B^* (8). This program calculates the sum of squared deviations of the experimental data from those calculated according to Eq. 9. This sum was obtained by setting initial conditions for k_{12}, k_{21}, and C_B^* and incrementing these parameters in a stepwise fashion so that every possible combination of the three constants was tested. Each set of parameters was tested by calculating a $1/t^*$ value for each pair of data points, taking the absolute value of the difference between the calculated $1/t^*$ and experimental $1/t^*$, squaring, and summing. This value was the sum for the function. Each sum was compared to the previously calculated sum, and the larger value was rejected. The parameters giving the smallest sum were taken as the best set of constants for the experimental data. Only one such set was ever found utilizing this program, although it is theoretically possible to have more than one "best" set.

The constants were incremented as follows: k_{12} from 0.0 to 0.01 in increments of 0.0001; k_{21} from 0.0 to 0.1 in increments of 0.001; and C_B^* from 10.0 to 20.0 in increments of 0.1. All other possibilities were previously rejected using this program with larger increments. Computation time can be quite lengthy due to the large number of calculations involved. For example, using 20 data points, 1×10^8 calculations are needed, resulting in computer usage of approximately 60 min. This time can be appreciably reduced to about 10 min. by using a smaller number of increments.

After the critical parameters were obtained, another digital computer program was written to solve Eq. 9 for $1/t^*$, given concentrations of drug from 5 to 800 mg. %.

The results of these calculations are shown in Fig. 3, where the solid line is calculated and the points are experimental. The overturn data used to generate the critical parameters and the theoretical curve ranged from 15 to 100 mg. %. The data at high drug concentrations were not used because they were found to bias the results; their inclusion introduced a predominance of linear data which effectively linearized the curve. Experimental variation tends to influence the slope of the line appreciably at high drug concentrations and to bias, through an averaging process, the curvature at low drug concentrations. The use of low drug concentration data did not appreciably affect the slope of the curve. The slope of the linear portion of the curve was found to be 5.1×10^{-2} ml./min. mg. and compares favorably to the least-squares regression slope of 4.2×10^{-2} ml./min. mg. obtained at high drug concentrations (Fig. 2).

Anello and Levy (4) found that goldfish, when exposed to a 250-mg. % 4-aminoantipyrine solution, overturned in approximately 37 min. (as estimated from their Fig. 3). Using this overturn time, it was possible to calculate the corresponding amount of drug pres-

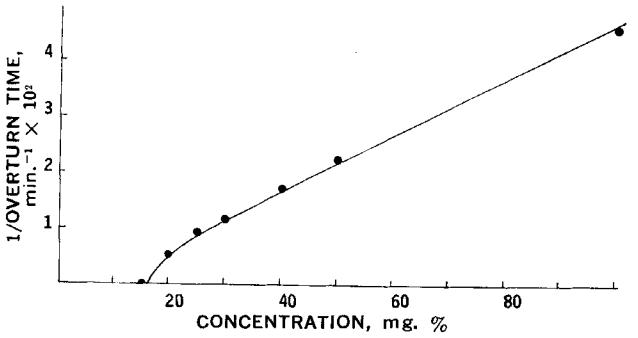

Figure 3—*4-Aminoantipyrine-induced overturn data at low concentrations. The parameters are:* $k_{12} = 4.0 \times 10^{-4}$ *min.*$^{-1}$, $k_{21} = 7.8 \times 10^{-3}$ *min.*$^{-1}$, $C_B^* = 19.5$ *mg.%*, $V_A = 250.0$ *ml., and* $V_B = 10.0$ *ml.*

ent in the goldfish at overturn from their zero-order absorption-rate plot. The amount of drug in the fish at overturn was estimated to be 2.03 mg./10-g. fish. Assuming that a 10-g. fish has a volume of 10 ml., the drug concentration at overturn is 20.3 mg. %, which is in excellent agreement with the theoretical computer-generated C_B^* value of 19.5 mg. % found for the same drug in the present study.

Figure 3 shows the curvature at low 4-aminoantipyrine levels. The shape of the curve is a hyperbola whose general equation, written in terms of reciprocal time and drug concentration, is:

$$1/t^* = \frac{b}{a}\sqrt{(C_A^o)^2 - a^2} \qquad \text{(Eq. 18)}$$

where a and b are constants and correspond to the distance from the origin to the point of intersection of the curve with the X-axis (point P) and the distance from point P to the extrapolated straight-line portion of the graph (asymptote), respectively. Using the "Step-Function" program (8), a and b were calculated from Eq. 18; the equation was then solved for reciprocal overturn time at various 4-aminoantipyrine concentrations. The generated data were plotted and found to yield a curve superimposable to that shown in Fig. 3, which indicates that Eq. 9 is a hyperbolic function.

Based on the data obtained with 4-aminoantipyrine, one can visualize the process of drug absorption in the goldfish. Drug is passively absorbed from the bathing solution. The rate of drug absorption is a function of the rate constant k_{12} and the concentration of drug in the bathing solution (C_A^o). Initially and at high drug concentration, $C_A^o \cdot k_{12} \gg C_B \cdot k_{21}$, and diffusion out of the fish is negligible. Under these conditions the Levy–Gucinski model is applicable, and a plot of $1/t^*$ *versus* C_A^o is linear and should intersect the origin. As C_B becomes larger or as C_A^o becomes smaller, the product $C_B \cdot k_{21}$ approaches $C_A^o \cdot k_{12}$ in magnitude, and deviations from linearity in a $1/t^*$ *versus* C_A^o plot are observed. The curve is hyperbolic and may have a finite positive X-axis intercept. This will occur if X_B^* has a relatively high value or if $k_{21} \gg k_{12}$. When $C_B^* \cdot k_{21} > C_A^* \cdot k_{12}$, the critical concentration necessary for pharmacologic effect in the goldfish will not be reached. The relationship between X_B^* and overturn times has not been experimentally determined; however, these relationships are under study at present.

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