Absorption of Ethanol by the Common Guppy (*Lebistes reticulatus*)

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The uptake of ethanol by guppies (*Lebistes reticulatus*) from solutions of varying concentrations has been observed at 25°. Results of the observations were compared with those obtained with goldfish (*Carassius auratus*) at 20° and 25°. Both time of death and time of overturn were used as criteria of pharmacological effect. Plots of the reciprocal time of effect against ethanol concentration failed to intersect the origin in all cases, except for goldfish at 20° where time of death was observed. The experimental demonstration of a threshold concentration for each fish implies that the actual plot should be a sigmoid curve. The results are discussed in relation to the theory of Levy and Gucinski. Guppies appear to be suitable for drug absorption studies.

A THEORY describing the uptake of drugs and toxic chemicals by fish from their aqueous environment has been developed by Levy and Gucinski (1). They have tested the theory with goldfish and have shown it to be useful in the study of factors influencing biological membrane permeation by passive diffusion of drugs (2–4). This investigation represents an attempt to apply the theoretical concepts of Levy to another experimental fish, the common guppy.

The guppy has been used extensively in water pollution studies (5), is easily kept under laboratory conditions, and is sensitive to many toxic substances. Since the usual adult is small (200–500 mg.), it was thought that this fish might be suited for the study of drugs available in limited quantities or for drugs which give poorly predictable responses with other fish.

EXPERIMENTAL

Fish—Common guppies (*Lebistes reticulatus*) were obtained from a local supplier of tropical fish in lots of 100 fish. They were kept in an aerated aquarium filled with tap water and fitted with a submersion filter and a thermostatically controlled heater set at 25°. Regulation of the relative size for use in experiments was accomplished by allowing the fish to escape from polystyrene cups punctured by holes of known size. Only those fish able to pass through 5-mm. holes but unable to pass through 3-mm. holes were used.

Goldfish (*Carassius auratus*) were purchased at a local pet shop.

Method—A single adult guppy was transferred by net from the aquarium to a 100-ml. beaker containing 30 ml. of the drug solution, and a stopwatch was started. Beaker sizes and solution volumes for goldfish were those of Levy and Gucinski (1). The fish was observed for drug effect and the time recorded. Prior to use all drug solutions were equilibrated in a constant-temperature water bath at 25°, and the beaker containing the drug solution was placed inside a 600-ml. beaker suspended in a 25° constant-temperature water bath. The temperature of the test medium was thus maintained at 24 to 25°. A similar procedure was used for studies at 20° with a refrigerated constant-temperature bath.

At each drug concentration several fish were run. None of the solutions were re-used, thus each fish was exposed to fresh solution. Results were expressed as the average time for a group of fish to show an effect, and the standard deviations of means were routinely calculated. Ethanol solutions were prepared by pipeting the calculated quantity of absolute ethanol into a volumetric flask and bringing to volume with distilled water.

Absorption of Ethanol

To gain experience in the use of fish and to test the reproducibility of the previously reported method, a series of experiments with graded concentrations of ethanol were conducted with goldfish at two different temperatures, 25° and 20°. Results were plotted as the reciprocal time of death *versus* percentage by volume of ethanol. Table I lists the regression equations obtained for the plotted lines and those of Levy and Gucinski for comparison. Six fish were used to determine each point at 25° and four at 20°.

The variation among lots of fish was expected and has been previously reported. The higher temperature was observed to increase the slope of the plotted line and to cause a larger negative intercept than previously reported.

A series of experiments was then conducted in a similar manner with guppies. Since these fish have a natural tropical habitat and preliminary experiments showed them sensitive to low temperatures, absorption at only 25° was determined. Six fish were run for each point and at least 5 points were plotted for each run. The regression equations for the plotted experimental data are listed in Table II. The data indicated a greater sensitivity or rate of absorption for guppies than for goldfish, and a lot to lot variation was evident. All plots showed negative intercepts.

Criteria of Drug Effect—Considerable difficulty was encountered in determining the end point of death with guppies because of their size. Although a magnifying lens was helpful in observing the cessation of mouth and gill movements, optical difficulties were complicated by the beakers housing the fish. Investigators in river pollution studies have utilized the loss of balance equilibrium or "overturn" as an indication of toxic effect (6), especially where the death end point is uncertain. Accordingly, observations were made using the overturn time as the criterion of drug effect. Figure 1 is a representative plot of the experimental data for goldfish and guppies with both overturn and death end points at 25°, and Table III lists regression equations for the plotted lines with overturn as the end point.

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TABLE I—REGRESSION EQUATIONS FOR RECIPROCAL TIME OF DEATH ($1/T_L$) *Versus* ETHANOL CONCENTRATION (C) WITH GOLDFISH

Source	Eq.	Temp.	No. of Points Determining Line
Levy and Gucinski (1)	$1/T_L = 0.62\ C - 0.01$	20°	6
Levy and Gucinski (1)	$1/T_L = 0.64\ C + 0.36$	20°	4
Levy and Gucinski (1)	$1/T_L = 0.63\ C - 0.098$	20°	3
This study	$1/T_L = 0.48\ C + 0.036$	20°	4
This study	$1/T_L = 0.89\ C - 0.93$[a]	25°	5

[a] Plotted in Fig. 1.

TABLE II—REGRESSION EQUATIONS FOR RECIPROCAL TIME OF DEATH *Versus* ETHANOL CONCENTRATION WITH GUPPIES

Fish Lot No.	Eq.	Ethanol Concn. Range, % by Vol.
I G	$1/T_L = 1.81\ C - 1.91$	2–16
IV G	$1/T_L = 2.26\ C - 3.50$	2–16
VII G	$1/T_L = 2.04\ C - 4.12$[a]	3–7

[a] Plotted in Fig. 1.

TABLE III—REGRESSION EQUATIONS FOR RECIPROCAL OVERTURN TIME ($1/T_{OT}$) *Versus* ETHANOL CONCENTRATION (C)

Fish Lot No.	Eq.	Temp.
I F (Goldfish)	$1/T_{OT} = 7.65\ C - 17.24$[a]	25°
I F (Goldfish)	$1/T_{OT} = 4.82\ C - 12.68$	20°
II G (Guppies)	$1/T_{OT} = 5.74\ C - 13.45$	25°
IV G (Guppies)	$1/T_{OT} = 5.87\ C - 14.70$	25°
VII G (Guppies)	$1/T_{OT} = 5.70\ C - 15.07$[a]	25°

[a] Plotted in Fig. 1.

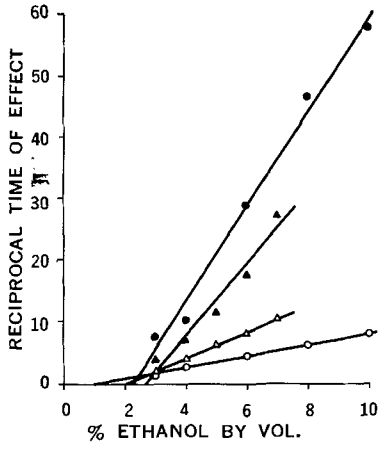

Fig. 1—A plot of reciprocal time of effect as a function of ethanol concentration at 25° for goldfish and guppies. Key: ○, goldfish, death end point; ●, goldfish, overturn end point; △, guppies, death end point; ▲, guppies, overturn end point.

DISCUSSION

The theory developed by Levy and Gucinski (1) to describe the goldfish as an experimental animal in drug absorption states $1/T_L = (K/L)\ C$, in which T_L is time of death, L the lethal dose, K the apparent absorption rate constant, and C the concentration of the drug in the medium, offers a number of attractions including the avoidance of involved chemical assays and tedious lethal dose determinations. It seems, however, that the theoretical model may possess certain limitations in application due to the simplifying assumptions upon which it is based, as pointed out by Levy and Gucinski.

Levy states that a number of assumptions are necessary for the model to be applicable. These include: (*a*) absorptiono ccurs only by passive diffusion; (*b*) the concentration gradient is essentially constant during the experiment; (*c*) the membrane permeability characteristics are unchanging under the experimental conditions; (*d*) drug elimination, including biotransformation, is negligible during the experiment; and (*e*) the pharmacologic end point occurs without significant delay after the lethal dose of the drug has been absorbed. He recommends using the experimental data themselves to indicate if the requirements are met. A plot of the reciprocal death time *versus* concentration should be linear and intersect the origin (2).

Initial experiments with goldfish showed that our experimental techniques would yield results conforming to the theory for ethanol with death as the observed pharmacological effect. (See Table I.) The lower slope of our plot as compared to those of Levy and Gucinski at 20° was ascribed to variations among lots of goldfish. The increase in temperature to 25° increased the slope of the plot and resulted in a negative intercept; the increase in slope would be expected from both more rapid diffusion and greater lethality of the ethanol for the fish at the higher temperature.

When guppies were used as the experimental fish, there was observed a three to fourfold greater slope than with goldfish, but most notable was the consistent negative intercept of the plotted line. Initially, this intercept was thought to be due to the lesser precision of experimental points where death occurred under 10 min.; therefore, a run was designed to obtain a plot where all points were derived from data in which death times were greater than 10 min. (Table II, lot VII G). The negative intercept appeared to be consistent.

The plots of the experimental data for both goldfish and guppies when overturn time was used as the criterion of drug action (Table III) showed a greater slope and greater negative intercept than when death was used. The increased slope was expected, for all fish lost their sense of balance before expiring. The greater slope for goldfish at 25° (lot I F) than for guppies (lots II G, IV G, VII G) was somewhat surprising since guppies seemed more

sensitive to ethanol lethality than goldfish (Tables I, II, and Fig. 1). Goldfish, however, are considerably deeper (measuring from ventral to dorsal surface) than are guppies; thus, their sense of balance is evidently more sensitive to slight CNS depression.

The repeated negative intercepts for plots of all the guppy experiments, for the goldfish experiment at 25°, and for the goldfish experiments where overturn time was observed seem to indicate that the theory fails to describe the experimental observations. Although plots of the data are apparently linear, they do not intersect the origin. Consideration of the assumptions upon which the theory is based may indicate that the failure of guppies to produce plots in agreement with the theory might be due to unknown characteristics of the fish. However, it does not appear reasonable that with guppies factors such as biotransformation, delay of pharmacological effect, and changing membrane permeability would be greatly different from goldfish when the plots exhibit similar linearity. Furthermore, it is difficult to ascribe deviation of the goldfish data for overturn times to factors much different from those functioning where time of death is observed.

One consistency is apparent. Whenever the slope of the plot increases, the negative intercept increases. This observation demonstrates that the kinetic model does not apply under conditions where one or more of the simplifying assumptions is not fulfilled. In 1917 Powers (7) concluded, after extensive experiments with goldfish and various toxicants, that the survival time is inversely related to the concentration only over a limited concentration range. In both low and high concentrations considerable deviation from linearity is evident and the true curve has a sigmoid form. Extrapolation of the linear portion of the curve to zero on either axis will tend to obscure the real behavior of the fish. A plot which intersects the origin cannot show a threshold concentration—that concentration which causes no observable effect. Most toxic substances studied in fish exhibit a threshold concentration (8).

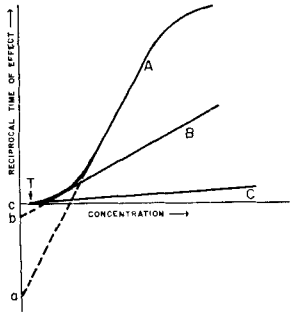

Fig. 2—A hypothetical plot of reciprocal time of effect versus *concentration of drug. Curves* A, B, *and* C *have a common threshold concentration* T. *Extrapolation of the linear portion of each curve intersect the ordinate at* a, b, *and* c.

Both goldfish and guppies have been kept in this laboratory for over 24 hr. in appreciable ethanol concentrations without loss of balance or death. For guppies the threshold at 25° lies between 0.25 and 0.50% and for goldfish between 1.0 and 2.0%. Therefore, the true plot of reciprocal time of effect *versus* concentration should intersect the concentration axis at some point other than the origin; the curves should assume a sigmoid form. If the hypothetical situations depicted in Fig. 2 are considered, the three illustrated cases parallel the results obtained in this study. Extrapolation of the linear part of curve A will give a large negative intercept, a. Curve B will give a smaller negative intercept, b, and curve C will give a negligible intercept, c. The regression equation obtained for goldfish with the time-of-death end point (Table I, 20°) corresponds to hypothetical curve C; equations for goldfish and guppies with the time-of-death end point at 25° (Table I, 25°, Table II) are similar to hypothetical curve B; and equations for goldfish and guppies with the overturn time end point (Table III) resemble curve A.

Since threshold concentrations can be demonstrated experimentally, a deviation from linearity for all plots is implied. The theory in its original form was not designed for low concentrations of ethanol, and the observed deviations from linearity therefore are to be expected. Similar behavior might be expected for other chemicals. The experimental system upon which the theory was based was stated to be useful "due to the relatively large volumes of aqueous medium and high drug concentrations" (1), however, a later publication stated that plots of the data "should be linear and intersect the origin" as a test of the validity of the mathematical model for a particular set of experimental conditions (2). From the foregoing considerations it seems unwise to assign theoretical or physiological significance to the intercept values.

This investigation points up some of the limitations of the original theory which can be encountered, some of which would be expected from the simplifying assumptions. Levy and co-workers have utilized the kinetic model with agents other than ethanol and have found goldfish absorption to correlate well with other biological data (1–3). Italian investigators, working with three different drugs, have also verified the usefulness of the model by making direct comparisons between goldfish absorption and oral absorption in rats (9).

The theory of Levy and Gucinski (1) appears to be applicable to guppies as well as goldfish, provided that the selected drug concentrations are well above the threshold levels.

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