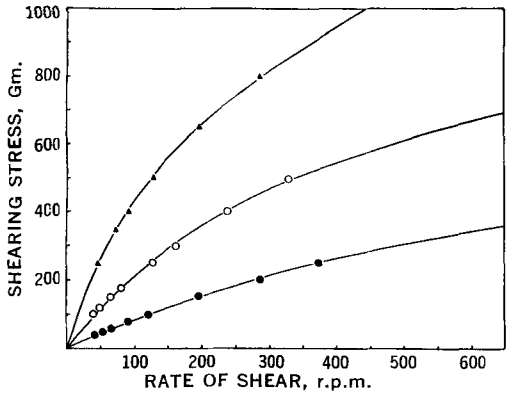

Fig. 4—Computer fits of actual data for CMC flow curves. Computer scale: 1 v. = 100 Gm. and 1 sec. = 50 r.p.m Lines represent computer fit and points represent the actual rheological data. Key: ▲, 2.8%; ○, 2.2%; ●, 1.6%.

TABLE II—VALUES OF *a* AND *c* OBTAINED FROM COMPUTER DATA TO FIT VARIOUS CMC RHEOGRAMS

% w/w CMC	*a*	*c*	$a_{corr.}$	$c_{corr.}$
2.8	0.55	0.55	1.10	1.10×10^{-2}
2.2	0.165	0.225	0.33	4.5×10^{-3}
1.6	0.01	0.09	0.02	1.8×10^{-3}

more than 30 min. instruction even to those completely alien to computer programming.

In summary, this paper has presented a method for the quantitative determination of three parameters which completely characterize the shape of pseudoplastic flow curves. Evidence has been presented regarding the general applicability of the method by making use of actual experimental data. The method is rapid and avoids any extensive calculations. It is hoped that the use of this procedure will lead to a common means of communication among rheologists, working with pseudoplastic systems, who wish to compare experimental data. It is also possible that with the aid of the analog computer some new light may be thrown on the mechanism of pseudoplastic flow. There is a distinct possibility that the same type of treatment presented here may be applied to other rheological systems. Work in these areas is being pursued at the present time.

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Keyphrases

Rheology
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CMC mucilage systems
Analog computer diagram
Simulated system-CMC mucilage comparison flow curves

Apparent pH Dependence of Ethanol Absorption Rate in the Common Guppy

By WILLIAM L. HAYTON and NATHAN A. HALL

The uptake of ethanol from buffered solution by guppies has been studied. There was an apparent increase in the rate of absorption with increasing pH. In contrast, similar experiments made with goldfish failed to show an increase in absorption rate as the pH was increased. The guppy should be used with caution as a model for investigating the effects of varying pH upon drug absorption.

FISH ARE widely used for experimental purposes and for biological assays and have been used for a variety of biological studies including outstanding works in experimental embryology, endocrinology, and nerve physiology (1). The gross anatomy and physiology of fish are comparable to that of mammals, and fish contract many of the same diseases as do mammals. Fish have become popular as experimental tools because they are relatively inexpensive and easily kept in the laboratory.

The use of fish in drug absorption studies has led to the development of a theory by Levy and Gucinski (2) describing the uptake of drugs from a bathing solution by goldfish. The theory describes the uptake of drugs by fish under appropriate conditions as a simple diffusion process dependent upon the concentration of the drug in the external bathing solution.

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The uptake of a weak acid over a pH range encompassing the pKa of the drug has been studied in goldfish and was shown to be consistent with the pH- partition theory. In their studies Levy and Gucinski have shown that the uptake of a nonelectrolyte, ethanol, by goldfish does not depend upon the pH of the external medium.

Levy's theory for goldfish has been applied to the common guppy (*Lebistes reticulatus*) (3). The uptake of ethanol was found to conform to the theory within certain concentration limits. The guppy appeared to show promise as a suitable animal for drug absorption studies. Guppies previously have been used as experimental animals in many types of toxicity studies (4).

The effect of pH on the rate of absorption of nonelectrolytes has not been examined in detail. A variable pH should have no profound effect on the physical characteristics of a nonelectrolyte, but absorbing membranes might be sensitive to these changes. Membranes generally are believed to possess pores, allowing for the diffusion of small molecules through the membrane without entering the lipid phase. Kavanau (5) has proposed a mechanism whereby the diameter of the pores changes between open and closed configurations. In his theory the transformation is mediated by proton activity. It is possible then that varying pH could significantly alter the rate of absorption of a neutral molecule by causing a structural change in the absorbing membrane. The study reported here was undertaken to find what effect pH variability might have on the rate of absorption of a neutral molecule in guppies and goldfish.

EXPERIMENTAL

To investigate the effect of varying pH upon the absorption of ethanol through intact membranes both the guppy and the goldfish were used. The time for a pharmacological effect to occur has been shown previously to be a function of the rate of absorption (2, 3). A change in the time required for the effect to occur at constant ethanol concentration was considered to reflect a change in the absorption rate.

Two different pharmacological effects were used as end points—overturn (loss of equilibrium) and death (cessation of gill and mouth movement). The times required to attain the end point from the immersion were termed "overturn time" and "survival time," respectively. The overturn end point was used with the guppies because cessation of gill and mouth movements was difficult to detect due to their size. The death end point was used on goldfish because they did not give a "sharp" overturn end point but appeared to lose their equilibrium by degrees. Both end points used on the same lot of fish gave parallel log-log plots of time for effect *versus* concentration, indicating that both end points were the same function of concentration.

Materials—The ethanol solutions were prepared from absolute ethanol of reagent quality (U. S. Industrial Chemical Co.). The solutions were prepared by accurately measuring the ethanol into a volumetric flask. A quantity of buffer and sodium chloride necessary to give 0.05 *M* buffer concentration and an ionic strength of 0.05 was added with distilled water; the pH was adjusted to the desired value with dilute NaOH or HCl, and the solution was made to volume. The ionic strength adjustment was based upon calculation of the fraction of the buffer in its ionic form at each pH. All solutions contained 0.05 *M* buffer adjusted to 0.05 in ionic strength.

Three buffers were used in preparing the solution: 2-(*N*-morpholino)-ethane-sulfonic acid.H_2O(MES, Calbiochem), *N,N* - bis(2 - hydroxyethyl)glycine (bicine, Calbiochem), and tris-(hydroxymethyl)-aminomethane (tris or THAM, Fisher Scientific Co.). The buffers supplied by Calbiochem are relatively new in biological studies. The pKa of MES is 6.09, of bicine 8.26, and of tris 8.14 at 25° (6). The buffers were found by preliminary experiments to be innocuous to the fish.

Fish—Common guppies (*Lesbistes reticulatus*) were obtained from a local supplier of tropical fish. They were maintained at 25° and sized as described previously (3). Only male fish were used.

Goldfish (*Carassius auratus*), approximately 4 cm. in length, were purchased in a single lot from a local pet shop.

Method—A 4% ethanol solution was used with the guppies and a 6% ethanol solution with the goldfish. Nine solutions were used in the guppy study and 10 in the goldfish study. The solutions in each study were identical except for pH and buffer. The solutions were hypotonic to fish blood so the osmotic pressure vector across the absorbing membranes was in the same direction as in natural environmental conditions, although not quite so large (7). Prior to use, the solutions were placed in a water bath at 25° and allowed to attain temperature equilibrium.

The fish were exposed to the drug solutions by removing them from the tank with a plastic net and dropping them into the ethanol solution. Thirty milliliters of solution in a 100-ml. beaker was used for the guppies, and 100 ml. of solution in a 250-ml. beaker was used for the goldfish. A stopwatch was started when the fish made contact with the solution. The 100- or 250-ml. beaker was then placed inside a 600-ml. beaker that was suspended in a constant-temperature bath at 25°. The temperature of the bathing solution was thus maintained at 24–25°. Each fish was observed until the end point was reached. The stopwatch was stopped, the fish removed from the solution, and the time recorded. A fresh solution was used for each fish, and the fish were used only once. Twelve guppies and six goldfish were used for each solution.

To minimize bias in determining the end point, the solutions were coded by letter, the code being unknown to the person determining the end point. The solutions were administered in a random order established from a table of random numbers. These procedures were designed to eliminate the effects of the order in which the fish were removed from the tank and the bias of the observer in end point determination and fish selection.

RESULTS

Table I lists the results of the studies with guppies. At each pH the means from all buffers are averaged to give a collected mean. A *t* test of differences among the means in the guppy experiments showed that at the 95% confidence level there are significant differences between the collected

TABLE I—OVERTURN TIMES IN MINUTES FOR GUPPIES IN 4% ETHANOL[a]

	pH				
	5.0	6.0	7.0	8.0	9.0
		MES			
	17.25(3.93)	15.30(2.22)	12.20(2.59)	...	...
		Tris			
	...	14.88(2.59)	16.01(1.68)	14.81(1.80)	...
		Bicine			
	...	...	14.65(2.76)	12.65(1.74)	11.34(1.76)
Collected mean	17.25	15.09	14.28	13.73	11.34

[a] Each reported time represents the mean of 12 fish. Standard deviation in parentheses.

TABLE II—SURVIVAL TIMES IN MINUTES FOR GOLDFISH IN 6% ETHANOL[a]

	pH				
	5.0	6.0	7.0	8.0	9.0
		MES			
	14.30(1.25)	12.30(1.17)	17.05(1.51)	...	...
		Tris			
	...	15.28(0.98)	13.91(1.98)	14.23(0.79)	14.91(1.54)
		Bicine			
	...	...	15.61(2.72)	13.56(0.40)	14.08(0.96)
Collected mean	14.30	13.79	15.52	13.89	14.49

[a] Each reported time represents the mean of six fish. Standard deviation in parentheses.

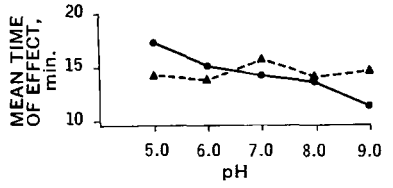

Fig. 1—Variation of mean time of the effect of ethanol in buffered solutions at different pH levels. Points represent collected means for all solutions buffered with MES, tris, and bicine. Key: ▲, survival times (min.) for goldfish in 6% ethanol; ●, overturn times (min.) for guppies in 4% ethanol.

means at pH 5 and 6, pH 6 and 8, pH 7 and 9, pH 8 and 9, as well as between pH values farther apart than these. With the individual buffers there is a trend toward shorter overturn times with increasing pH, except for the tris-buffered series. The differences among the means for the tris series are not statistically significant.

Table II lists the results of the studies with goldfish. There are no statistically significant differences at the 95% confidence level among any of the collected means, and there appears to be no trend paralleling the changes in pH with individual buffers.

Figure 1 depicts the comparison between goldfish and guppies of the effect of ethanol at various pH levels.

DISCUSSION

Several factors may have an appreciable effect on the absorption rate of a molecule. The more important factors are concentration, temperature, pH, osmotic pressure, ionic strength, degree of ionization of the molecule, size of the molecule, and its lipid solubility. If a buffer is present, as in this study, the degree of ionization of the buffer may be important.

In these experiments, the concentration of the ethanol (4% for guppies and 6% for goldfish) and the buffer (0.05 *M*) was held constant. The osmotic pressure of all the solutions was nearly equal although the exact osmotic pressure exerted by the buffers is not known. The ionic strength of the solutions was constant at 0.05. Because ethanol is nonionized in aqueous solutions, the ionization of the molecule was not a variable. The size of the molecule and its lipid solubility also remained constant in all of the solutions.

The degree of ionization of the buffer could possibly have an effect upon the absorbing membranes of the fish due to ion-membrane interactions. In their buffer ranges MES and bicine are anionic as their molecules have fully dissociated carboxyl groups and partially ionized amino groups depending on the pH of the solution. As the pH is increased in the buffering range of the molecule the amino group becomes less ionized, resulting in an increased net negative charge on the molecule from the fully ionized carboxyl group. Tris, however, is not zwitterionic but is a substituted amine, and as the pH is increased, the number of positively charged buffer ions decreases in the buffering range of the molecule. It is unlikely that charges upon the buffer molecules would influence the absorption rate of ethanol in this study as all solutions were of equal ionic strength, and there is no qualitative difference among the charges on the buffer ions and the sodium and chloride ions present. To maintain the pH of the solutions it is necessary that buffers be present.

The parameter which this study concerns is pH and its effect upon the absorbing membranes of guppies. From the data collected in this study it appears that the pH of the solution has a significant effect upon the rate of absorption of ethanol by guppies. The absorbing membranes of the fish are

apparently altered, causing an increase in the rate of absorption as the hydrogen-ion activity becomes less. The nature of this alteration could be a change in the membrane pore size or some other structural change in the membrane.

The extremes of the pH range used in this study are not harmful to either of the fish which may live for extended periods at either pH 5.0 or 9.0. Further, it is doubtful that the changes in pH have interfered with the integrity of the membrane. The possibility of a synergistic toxicity of the hydrogen ion–ethanol combination, independent of absorption rate, could not be ruled out. Preliminary experiments on guppies with strychnine, however, showed a similar increase in absorption rate at high pH levels. The observed absorption rate increase was considerably greater than that accounted for by calculation of the amount of strychnine present as the free base at elevated pH levels.

Results from this study indicate that the absorbing membranes of the guppies are pH sensitive. The use of this fish as an experimental model in studying drug absorption probably should be limited to those studies in which the pH of the solutions used is constant. Absorption studies that are run with pH as a variable factor probably will be influenced by the changing absorption rates due to varying pH, and any change in absorption rate in such a study may not be meaningful. Goldfish, on the other hand, appear to have absorbing membranes which are not appreciably altered by pH changes *per se*.

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Keyphrases

Absorption of ethanol
pH dependence of absorption rate in guppies
pH independence of absorption rate in goldfish
Membrane sensitivity to pH

Mixed Glycol Acetal Dimethylcarbamates *via* Transacetalation of Certain Di- and Trihydric Alcohols

By CLAUDE PIANTADOSI, FRED T. SEMENIUK, and ROBERT K. RAUCH

Mixed acetals of a new type were synthesized by an acetal exchange reaction between the mono dimethylcarbamate of ethylene, propylene, and trimethylene glycols and the diethyl acetals of butyraldehyde, heptaldehyde, and benzaldehyde. The glycerol carbonate (followed by dimethylaminolysis) also underwent transacetalation and dimethylcarbamate esters of the dioxolane-type were synthesized.

A NUMBER of acyclic acetals (1), cyclic acetals (2), and their derivatives (3–9) have been reported in the literature. Their potentialities as hypnotics, anticonvulsants, and skeletal muscle relaxants (3–9) have been noted. In view of the fact that mixed acetals are similar in structure to the pharmacologically active glycerol ethers, it was of interest to synthesize some of the mixed acetal carbamates in order to determine the feasibility of the transacetalation reaction for preparing such compounds. Exchange reactions between alcohols in acetal linkage and alcohols have long been known. They have been reported to be applicable for primary alcohols (10, 11), for secondary and tertiary alcohols and phenols (11), for ethylene and trimethylene glycol (12), and for glycerol (13–15). The term "transacetalation" has been coined and may be applied generally to cover all such acetal exchange reactions (13). The experimental procedures presented in this paper describe the use of this reaction with the glycol monocarbamates of ethylene, propylene, trimethylene, and glycerol with the diethyl acetals of benzaldehyde, butyraldehyde, and heptaldehyde. Attempts to prepare the unsubstituted carbamate derivatives by this method were not successful.

The diethyl acetals of butyraldehyde, heptaldehyde, and benzaldehyde were prepared according to the method of Claisen (16). The method of Najer *et al.* (17) was used for the preparation of propylene glycol carbonate and the method of Carothers *et al.* (18) for trimethylene glycol carbonate. An adaptation of the method of Delaby *et al.* (19) for the synthesis of ethylene glycol monocarbamate was used in preparing the carbamates. The synthesis of heptaldehyde and benzaldehyde mixed glycerol carbonate acetals and dioxolane dimethylcarbamates were carried out as previously described by Piantadosi *et al.* (13). An examination of the infrared spectrum of the compounds synthesized revealed the presence of characteristic ester absorption due to C=O stretching in the region of 1620–1829

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