Temperature and Surfactant Dependence of Accumulation of 4-Aminoantipyrine and Ethanol in Fish

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Abstract □ The accumulation of 4-aminoantipyrine and ethanol by goldfish placed in solutions of the drugs was determined from 10 to 35° in the presence and absence of 0.01% polysorbate 80. The volume of distribution of ethanol, 0.68 ml/g of fish, was not affected by surfactant or temperature. The volume of distribution of 4-aminoantipyrine also was not affected by surfactant, but it increased linearly from 0.43 to 0.76 ml/g over 10–35°. The clearance constants for the absorption of both drugs were temperature sensitive. The clearance constant for 4-aminoantipyrine, 11.6 μl/min/g of fish at 20°, conformed to the Arrhenius equation; the activation energy was 12.9 kcal/mole, indicating that 4-aminoantipyrine absorption was probably membrane controlled. Polysorbate 80 increased the clearance constant of 4-aminoantipyrine, and the increase occurred at a measurable rate rather than instantaneously. The clearance constant for ethanol, 46.5 μl/min/g at 20°, was not described by the Arrhenius equation. The activation energy appeared to decrease with increasing temperature, perhaps reflecting a change in the rate-limiting barrier to absorption from the membrane to the aqueous stagnant layer. Alternatively, the ethanol absorption rate may have been limited by blood flow to the absorbing surface. The ethanol absorption rate was not affected by polysorbate 80.

Keyphrases □ 4-Aminoantipyrine—accumulation by goldfish, effect of temperature and surfactant □ Ethanol—accumulation by goldfish, effect of temperature and surfactant □ Absorption—4-aminoantipyrine and ethanol by goldfish, effect of temperature and surfactant □ Distribution, tissue—4-aminoantipyrine and ethanol by goldfish, effect of temperature and surfactant □ Antipyretic–analgesics—4-aminoantipyrine, accumulation by goldfish, effect of temperature and surfactant

Recent reports (1, 2) discussed the effects of temperature on the toxicity of chemical pollutants to fish and showed the complex interrelationships between chemical toxicity and temperature in fish. While the uptake rate of pollutants may increase with temperature, metabolism and excretion rates also may be affected. In addition, the dissolved oxygen concentration, which may affect metabolism, is decreased as temperature increases. Thus, it is not surprising that the toxicity to fish of some pollutants, *e.g.*, endrin and toxaphene, increases with a temperature increase while the toxicity of others, *e.g.*, chlorophenothane (DDT) and methoxychlor, decreases with a temperature increase (1).

The toxicity of a chemical present in the environment of fish is generally a function of the amount of chemical accumulated by the fish. To understand the effects of temperature on the toxicity of pollutants to fish, it is necessary to know the temperature dependence of the processes that determine the extent of accumulation: absorption, distribution, and elimination. Toward this end, the accumulation of 4-aminoantipyrine and ethanol was studied in goldfish at various temperatures. The absorption and distribution of these drugs are rapid compared to their elimination by metabolism or excretion. Thus, their accumulation is related primarily to absorption and distribution, and it was possible to focus on the temperature dependence of these processes. The temperature dependence of the absorption-enhancing effect of a premicellar concentration of polysorbate 80 also was studied.

THEORETICAL

The accumulation rate of substances toxic to fish has been characterized by measuring the time required for fish to turn over or to die (*e.g.*, 3–7). This approach requires no chemical analysis, and it is useful for detection of the relative absorption rates of compounds that elicit the end-point. The time required to produce the end-point depends on both the absorption rate and the dose that causes overturn or death (3). Since the dose required to produce the end-point as well as the absorption rate may be sensitive to temperature, the following alternative approach was used in this study.

The accumulation process was followed by measuring the amount of xenobiotic present in the fish as a function of time. This approach is commonly used; the data are generally modeled as an equilibrium with two first-order rate constants, with units of time^{-1}, for absorption and exsorption of the xenobiotic. For example, an equilibrium model was used recently as the basis for an accelerated test to predict the extent of accumulation of xenobiotics (8). The equilibrium has also been used to model time-for-overturn and time-for-death data (6, 7, 9). For many compounds studied, the accumulation process appears to be represented adequately as a simple equilibrium.

A problem associated with the use of absorption and exsorption rate constants for characterizing accumulation is that the rate constants reflect not only the permeability of barriers to absorption but also the volumes of the fish and bathing solution (7). To overcome this problem, the equilibrium model was modified so that the accumulation process is characterized by two pharmacokinetic constants: a clearance constant, k, that reflects only the permeability of barriers to absorption and exsorption, and a volume of distribution, V, that reflects the capacity for accumulation.

The fish was treated as a single compartment with the xenobiotic equilibrating between it and the external solution. The accumulation rate of the xenobiotic (dX/dt) is:

$$\frac{dX}{dt} = k(C_o - C_i) \qquad \text{(Eq. 1)}$$

where X is the amount of the xenobiotic absorbed in units of milligrams per gram of fish; k is in units of milliliters per minute per gram of fish; and C_o and C_i are the concentrations of the xenobiotic in the external solution and the body water of the fish, respectively, in units of milligrams per milliliter. Experiments with 4-aminoantipyrine showed that the uptake rate was directly related to the size of the fish (Fig. 1). When expressed on a per gram basis, however, uptake was independent of fish size over a range of 1–3 g (Fig. 1); therefore, k and X were normalized to body weight.

The following assumptions were made:

1. Under fixed conditions, uptake obeys the principle of superposition. This assumption appears valid since the uptake of ethanol (Fig. 2) and 4-aminoantipyrine (data not shown) was directly proportional to C_o.
2. There is no absorption from the gut of the fish. This assumption seems to be reasonable since freshwater fish generally do not swallow water (10).
3. Except for exsorption, the elimination of ethanol and 4-aminoantipyrine is negligible during the experiment. While one report suggests that goldfish may metabolize ethanol (11), the metabolism rate apparently is small compared to the absorption rate of ethanol in this study and the effect of metabolism on the shape of the accumulation curves would be negligible. 4-Aminoantipyrine is apparently not metabolized in goldfish (12).

The volume of the solution bathing the fish was sufficiently large that C_o could be considered constant; it decreased less than 0.5% during any

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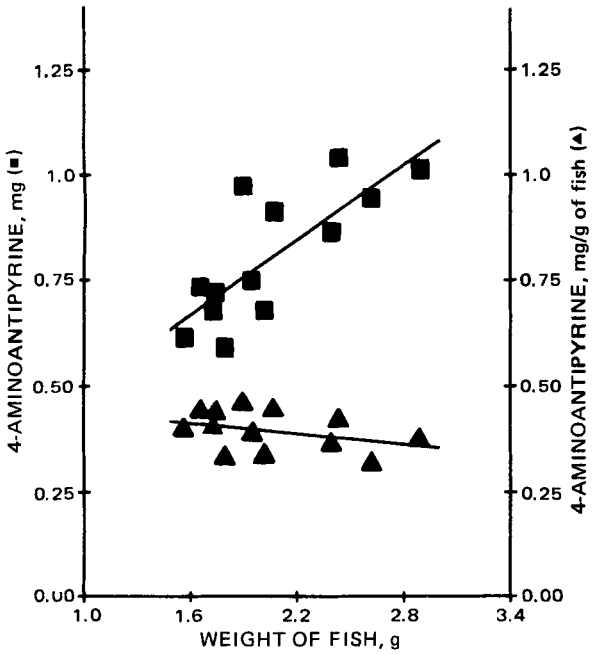

Figure 1—*4-Aminoantipyrine (■) and 4-aminoantipyrine per gram of fish (▲) as a function of the weight of the fish. Fish were in the solution for 30 min at 35°. Each point represents one fish; the lines were fit by the least-squares method.*

absorption experiment. A volume of distribution, V, was defined such that $C_i = X/V$, where V has units of milliliters per gram of fish. Equation 1 may be integrated to:

$$X = VC_o[1 - e^{-(kt/V)}] \qquad \text{(Eq. 2)}$$

Equation 2 predicts that X approaches an equilibrium value of VC_o, and the half-life for equilibration is 0.693 V/k. The volume of distribution is somewhat different from that used in standard compartmental pharmacokinetic models (13) in that the "reference tissue" is the external solution rather than blood or plasma.

The interpretation of k depends on the rate-limiting step or steps operative for a particular xenobiotic. When the uptake rate is controlled by an aqueous stagnant layer, the clearance constant is:

$$k = (D_a)(A/h) \qquad \text{(Eq. 3)}$$

where D_a is the diffusion coefficient of the xenobiotic in the aqueous layer in units of square centimeters per minute, A is the effective area of the absorbing surface in square centimeters per gram of fish, and h is the effective thickness of the aqueous stagnant layer in centimeters. When the uptake rate is limited by diffusion across a membrane, the clearance constant is:

$$k = (D_m)(A)(K_m/\delta) \qquad \text{(Eq. 4)}$$

where D_m is the diffusion coefficient of the xenobiotic in the membrane, K_m is the membrane–water partition coefficient of the xenobiotic, and δ is the thickness of the membrane in centimeters. For compounds with high membrane permeability, the uptake rate may be limited by blood flow and:

$$k = (Q)(K_b) \qquad \text{(Eq. 5)}$$

where Q is the blood flow rate to the absorbing surface in milliliters per minute per gram of fish and K_b is the effective blood–external solution partition coefficient of the xenobiotic. In the general case, the absorption rate may be limited by all three barriers and the clearance constant for absorption would be:

$$k = \left[\frac{h}{D_a A} + \frac{\delta}{D_m A K_m} + \frac{1}{QK_b}\right]^{-1} \qquad \text{(Eq. 6)}$$

Any one of the three terms on the right can be omitted to give an expression relevant to an absorption barrier composed of two rate-limiting steps.

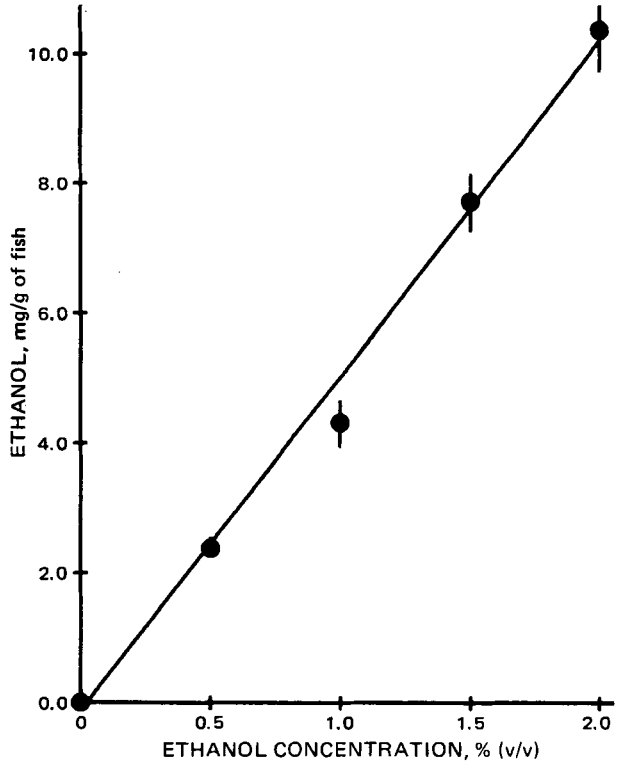

Figure 2—*Ethanol absorbed by fish as a function of ethanol concentration. Fish were in each ethanol solution for 30 min at 25°. Each point represents the mean of five fish; bars represent ±1 SD. The line was fit by the least-squares method.*

EXPERIMENTAL

Materials—4-Aminoantipyrine[1], ethanol[2], tromethamine[3], and polysorbate 80[4] were used as received. ^{14}C-Ethanol[5] was dissolved in distilled water and stored at 4°; all other reagents and solvents were reagent grade.

Absorption—Goldfish (*Crassius auratus*), 1–3 g, were housed in a rectangular glass tank containing 56 liters of 0.075% NaCl solution that was aerated and filtered continuously. The tank was suspended in a water bath that could be maintained at constant temperature[6] over 10–35°. Five lots of fish were used. All fish used in each experiment were from the same lot to overcome the problem of lot-to-lot variability.

Preliminary experiments showed that the absorption rate rapidly stabilized after a change in temperature (Fig. 3). Fish were acclimated to the temperature being studied for a minimum of 24 hr. They were placed individually in 100 ml of 4-aminoantipyrine (1.5 mg/ml) or 75 ml of ethanol (1.0%) solution buffered at pH 7.0 with 0.05 M tromethamine. When present, polysorbate 80 was at a concentration of 0.01%. Ethanol solutions contained 7 nCi of ^{14}C-ethanol/ml; all solutions were prepared on the day of use. After immersion in drug solution for varying periods, the fish were rinsed with distilled water, blotted with tissue paper, and weighed. Fish were immediately either assayed for drug or frozen for assay later.

Analytical Methods—Fish exposed to 4-aminoantipyrine were homogenized with 15 ml each of chloroform and 0.05 M, pH 7.0 tromethamine solution. The homogenate was centrifuged, and 5.0 ml of the chloroform layer was removed and shaken with 10 ml of 0.01 N HCl solution for 20 min. To 2 ml of the aqueous phase were added 7.0 ml of water and 2 ml of p-dimethylaminobenzaldehyde reagent (14). The absorbance was measured[7] at 430 nm and converted to the amount of 4-aminoantipyrine per gram of fish.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] U.S. Industrial Chemical Co., New York, N.Y.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Atlas Chemical Industries, Wilmington, Del.
[5] New England Nuclear, Boston, Mass.
[6] Model TE9 Tamson circulating bath and model PBC-4 bath cooler, Neslab Instruments, Portsmouth, N.H.
[7] Beckman DU, Beckman Instruments, Fullerton, Calif.; with Gilford model 2000 multiple sample recorder, Gilford Instrument Laboratories, Oberlin, Ohio.

Table I—Volume of Distribution of 4-Aminoantipyrine and Ethanol with and without 0.01% Polysorbate 80 at Various Temperatures

Temperature	Volume of Distribution[a], ml/g of Fish			
	4-Aminoantipyrine		Ethanol	
	With	Without	With	Without
10°	0.448	0.427	0.655	0.680
20°	0.581	0.554	0.675	0.681
30°	0.709	0.724	0.673	0.688
35°	—[b]	0.765	0.705	0.722

[a] Estimated from nonlinear least-squares fit of Eq. 2 to data shown in Figs. 4–7. [b] Not determined.

To measure the extent of adsorption of 4-aminoantipyrine to the fish surface, fish were immersed in the drug solution for 30 sec and analyzed for 4-aminoantipyrine; the amount detected was negligible. There was no detectable difference between the absorbances of blanks prepared without fish and with fish that had not been exposed to 4-aminoantipyrine. Blanks prepared without fish were used routinely as a reference.

To determine the amount of ethanol absorbed, each fish was homogenized with 5 ml of distilled water and the homogenate was centrifuged. One-half milliliter of the supernate was transferred to a scintillation vial, mixed with 10 ml of scintillation fluid[8], and counted[9] until a minimum of 10,000 counts was collected. ^{14}C-Toluene was used as an internal standard to correct for quenching; the efficiency of the counting system was approximately 75%. The recovery of ethanol added to fish homogenate was 100%.

RESULTS AND DISCUSSION

Equation 2 adequately described the accumulation of both drugs at various temperatures and in the presence and absence of 0.01% polysorbate 80 (Figs. 4–7). Equation 2 was fit to the data by a nonlinear least-squares method to obtain estimates of V and k.

The volume of distribution characterizes the capacity of the fish to accumulate drug. The volume of distribution of 4-aminoantipyrine increased with temperature while that of ethanol was relatively temperature independent (Fig. 8). This result suggests that the distribution of 4-aminoantipyrine involves temperature-sensitive equilibria, possibly tissue or plasma protein binding, or partitioning into a nonaqueous phase. The lack of temperature dependence of V for ethanol indicates that its distribution is limited primarily to body water. Polysorbate 80 did not affect the V values of either drug (Table I).

For both drugs, the clearance constants for absorption were quite sensitive to temperature (Table II). Two of the potential barriers to absorption involve diffusion (Eqs. 3 and 4); therefore, the clearance constants were plotted according to the Arrhenius equation. The plots were linear for 4-aminoantipyrine but convex for ethanol (Fig. 9). The activation energy for the absorption of 4-aminoantipyrine in the absence of surfactant was determined from the slope of the Arrhenius plot to be 12.9 kcal/mole. Since this activation energy is considerably greater than the activation energy for diffusion of a substance in water, approximately

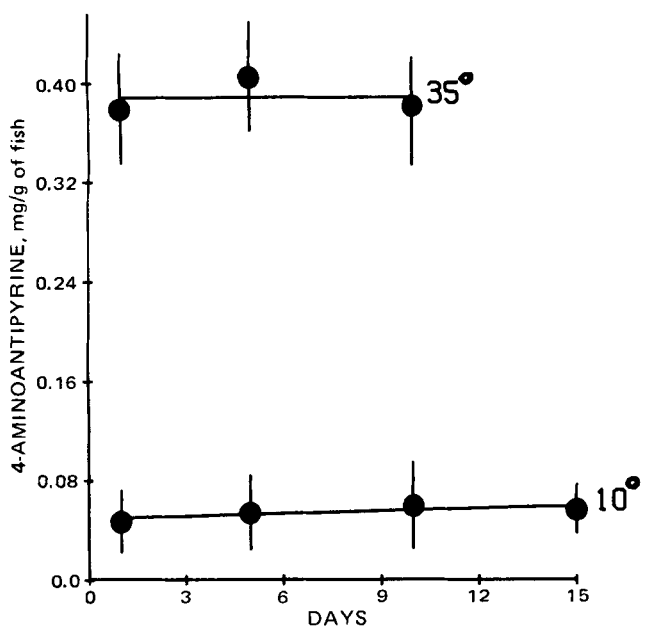

Figure 3—*4-Aminoantipyrine absorbed by fish at various times after adjusting the temperature of the fish tank from room temperature to either 10 or 35°. Fish were exposed to the drug solution for 30 min. Each point represents the mean of five fish; bars represent ±1 SD. The lines were fit by least squares.*

[8] PCS solubilizer, Amersham/Searle, Arlington Heights, Ill.
[9] Packard Tri-Carb 3320, Packard Instrument Co., Downers Grove, Ill.

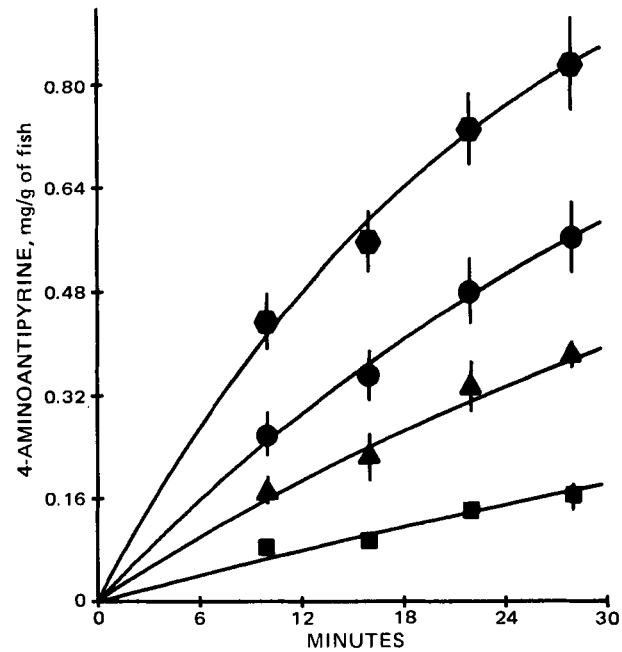

Figure 4—*4-Aminoantipyrine absorbed by fish as a function of time at 10° (■), 20° (▲), 30° (●), and 35° (●). Each point represents the mean of five fish; bars represent ±1 SD. Lines were fit by nonlinear least squares according to Eq. 2. The experiments were performed in the sequence: 10, 20, 30, and 35°.*

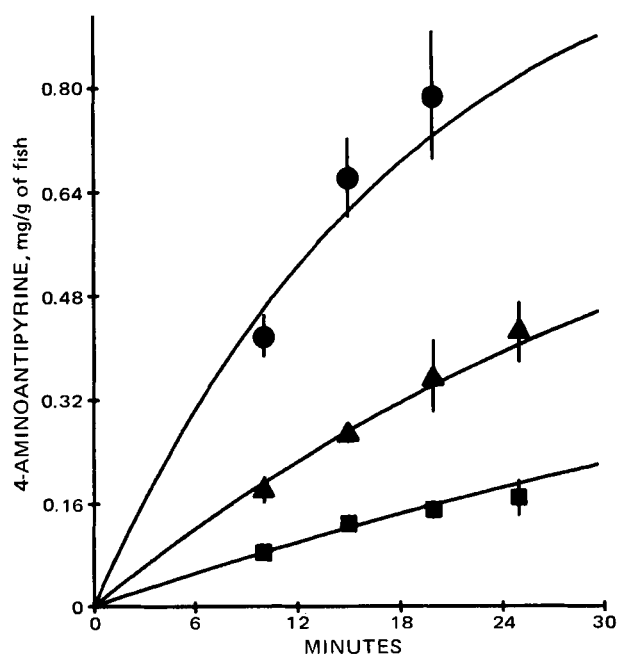

Figure 5—*Same as Fig. 4 except that 0.01% polysorbate 80 was present in the 4-aminoantipyrine solution. The experiments were performed in the sequence: 30, 20, and 10°.*

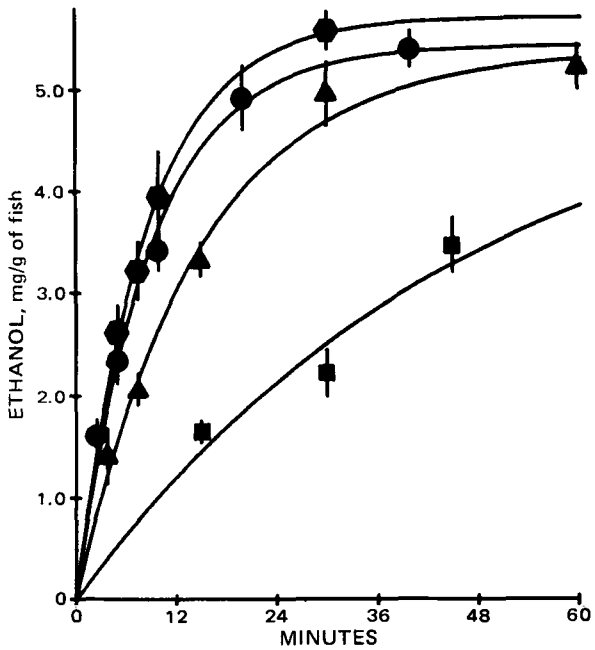

Figure 6—*Ethanol absorbed by fish as a function of time at 10° (■), 20° (▲), 30° (●), and 35° (●). Each point represents the mean of five fish; bars represent ±1 SD. Lines were fit by nonlinear least squares according to Eq. 2. The experiments were performed in the sequence: 30, 35, 10, and 20°.*

5 kcal/mole, the rate-limiting step in 4-aminoantipyrine absorption appears to be diffusion across a biological membrane rather than an aqueous stagnant layer. Similar activation energies were reported for the passive transport of small molecules across lipoid membranes. For example, the activation energies for transport of malonamide and urea across egg–lecithin liposomes were 13.0 and 9.3 kcal/mole, respectively (15), and activation energies for urea and antipyrine transport through rabbit gallbladder epithelium were 10.1 and 10.5 kcal/mole, respectively (16).

The rate constant (k/V) for equilibration of 4-aminoantipyrine was also plotted according to the Arrhenius equation. This rate constant is equivalent to the absorption rate constant when the equilibrium is characterized by absorption and exsorption rate constants (see *Theoretical*). The activation energy was 9.3 kcal/mole, considerably less than the value obtained when the clearance constant alone was used. The discrepancy occurs because the absorption rate constant is a function of both membrane permeability and the volume of distribution. Both parameters are temperature sensitive for 4-aminoantipyrine, and the activation energy determined from the absorption rate constant reflects this fact. The clearance constant is a better characterization of the permeability of barriers to absorption than the absorption rate constant because changes in the latter may be due to a change in either absorption or distribution of drug. The clearance constant is less ambiguous because it reflects only the permeability of barriers to absorption.

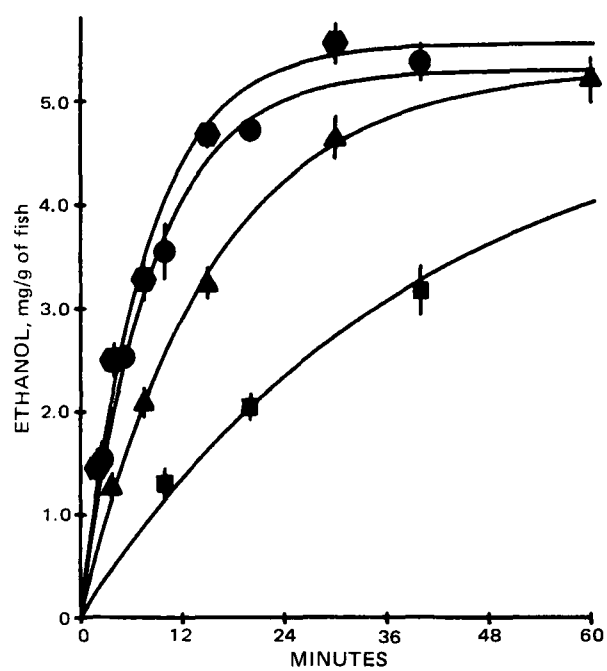

Figure 7—*Same as Fig. 6 except that 0.01% polysorbate 80 was present in the ethanol solution. The experiments were performed in the sequence: 10, 20, 30, and 35°.*

Table II—Clearance Constants of 4-Aminoantipyrine and Ethanol with and without 0.01% Polysorbate 80 at Various Temperatures

Temperature	Clearance Constant[a], μl/min/g of Fish			
	4-Aminoantipyrine		Ethanol	
	With	Without	With	Without
10°	6.0	4.8	16.4	14.3
20°	14.5	11.6	44.3	46.5
30°	39.7	20.0	80.6	76.9
35°	—[b]	33.5	92.5	85.4

[a] Estimated from nonlinear least-squares fit of Eq. 2 to data shown in Figs. 4–7. [b] Not determined.

In the presence of 0.01% polysorbate 80, the clearance constant of 4-aminoantipyrine increased significantly over the 10–35° range (Fig. 9). The absorption-enhancing effect of premicellar concentrations of surfactants is well known (17). It is due to an increase in the permeability of the absorbing membrane rather than to a nonmicellar surfactant–drug complex. The mechanism of the surfactant effect is not well understood. It has been postulated that the surfactant molecules enter the lipid portion of membranes and either increase the fluidity of the hydrocarbon lipid chains (18) or cause dissociation of structural lipoproteins (19, 20).

While the presence of polysorbate 80 appeared to increase the energy of activation for the absorption of 4-aminoantipyrine (Fig. 9), this result may be an artifact. The increased slope of the Arrhenius plot may reflect a temperature dependence of the effect of the surfactant on the membrane. Thus, at higher temperatures, the membrane would be more susceptible to the effects of the surfactant than at lower temperatures and the slope of the Arrhenius plot would appear to be increased.

Alternatively, if the structure of the membrane in the presence of polysorbate 80 is unchanged over the 10–35° range, the increased slope of the Arrhenius plot and Eq. 4 suggest that the surfactant reduces the diffusion coefficient of 4-aminoantipyrine in the membrane and that the reduced diffusion coefficient is more than compensated for by: (*a*) an increase in the area available for absorption, (*b*) an increase in the

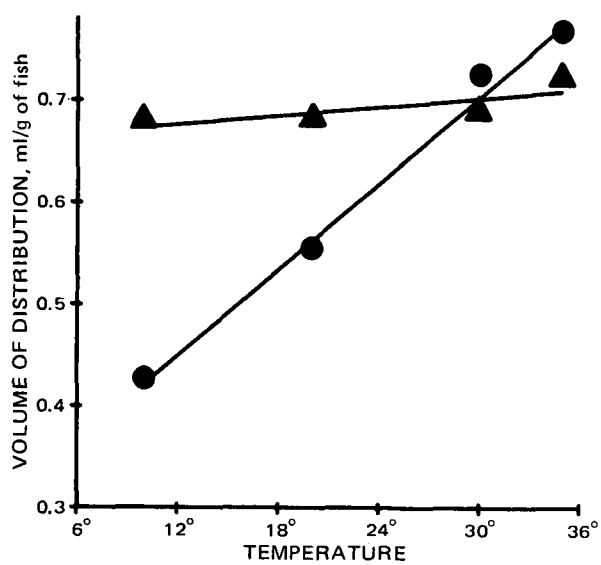

Figure 8—*Volume of distribution as a function of temperature for 4-aminoantipyrine (●) and ethanol (▲). The volumes of distribution were obtained from fits of Eq. 2 to the plots shown in Figs. 4 and 6.*

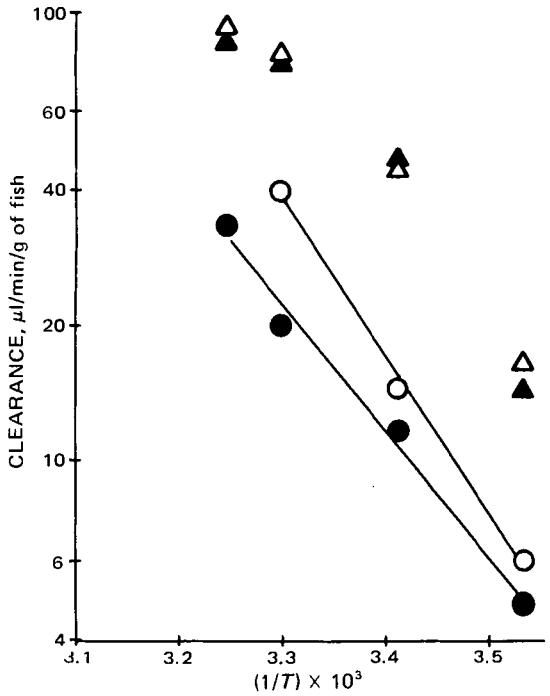

Figure 9—*Arrhenius plots for the absorption clearance constants of 4-aminoantipyrine (circles) and ethanol (triangles) in the absence (closed) and presence (open) of 0.01% polysorbate 80.*

membrane-water partition coefficient of the drug, and/or (c) a decrease in the thickness of the membrane.

The initial absorption rate of 4-aminoantipyrine was lower than would be predicted if polysorbate 80 produced an instantaneous increase in membrane permeability. For example, the amount of 4-aminoantipyrine absorbed at 10 min at 30° was appreciably below the least-squares fitted line (Fig. 5). To determine whether the absorption-enhancing effect of the surfactant was instantaneous, the amount of 4-aminoantipyrine absorbed was measured after a 5-min exposure of the fish to 1.5 mg of 4-aminoantipyrine/ml under the following conditions: A, control fish were exposed for 5 min to 1.5 mg of 4-aminoantipyrine/ml; B, fish were exposed for 5 min to 1.5 mg of 4-aminoantipyrine/ml containing 0.01% polysorbate 80; C, fish were immersed in 0.01% polysorbate 80 solution for 10 min, removed, rinsed, and placed for 5 min in 1.5 mg of 4-aminoantipyrine/ml of solution containing 0.01% polysorbate 80; and D, fish were pretreated with surfactant as in Condition C and then placed in 1.5 mg of 4-aminoantipyrine/ml of solution without surfactant.

As shown in Table III, the absorption rate of 4-aminoantipyrine under Condition B tended to be greater than the control as observed previously (Figs. 4 and 5). However, pretreatment of the fish with 0.01% polysorbate 80 for 10 min (Condition C) further increased the absorption rate of 4-aminoantipyrine. Thus, the action of the surfactant apparently is not instantaneous. When fish are placed in drug solutions containing surfactant, membrane permeability apparently is at or near the control value, and it increases with time until it reaches a maximum value. This observation agrees with the results of *in vitro* studies of the kinetics of the surface pressure of an air–aqueous solution interface after injection of cetrimonium bromide beneath the interface into the stirred subphase (21). The surface pressure exponentially approached an equilibrium value with a half-life of approximately 5 min, consistent with the concept of a potential energy barrier to surfactant adsorption at the interface.

The absorption rate of 4-aminoantipyrine under Condition D was also significantly greater ($p < 0.05$) than the control absorption rate. The permeability-enhancing effect of the surfactant persists after the surfactant has been removed from the drug solution. The surfactant-mediated alteration in the permeability of the absorbing membrane apparently is relatively slow. A similar observation was made with regard to the effect of 1% poloxalene on pentobarbital absorption (22).

The relatively small surfactant-induced increase in the k of 4-aminoantipyrine at low temperature (Fig. 9) may be explained in part by the kinetics of the surfactant effect. At low temperatures, a longer time may be required for the surfactant to increase permeability than is required

Table III—4-Aminoantipyrine Absorption in Fish Pretreated with Polysorbate 80

Condition[a]	4-Aminoantipyrine[b], mg/g of Fish
A	0.138 ± 0.013[c,d]
B	0.162 ± 0.020[e]
C	0.251 ± 0.030[c,e]
D	0.209 ± 0.025[d]

[a] A = control, B = 0.01% polysorbate 80 present in the drug solution, C = 10-min pretreatment in 0.01% polysorbate 80 and 0.01% polysorbate 80 present in the drug solution, and D = 10-min pretreatment in 0.01% polysorbate 80 and no surfactant in drug solution. [b] Mean of five fish ±1 *SD*. [c,d,e] Values having same superscript are significantly different ($p < 0.05$).

for equilibration of 4-aminoantipyrine between the fish and the external solution.

The convex curvature of the Arrhenius plot for ethanol clearance constants (Fig. 9) indicates that the activation energy for ethanol absorption decreases with a temperature increase. Such curvature has been attributed to a phase change in the lipids of absorbing membranes. For example, the kinetics of absorption of chlortetracycline by *Staphylococcus aureus* are temperature dependent, and there is a pronounced change in the slope of the Arrhenius plot at 27° (23). This transition temperature is sensitive to the fatty acid composition of the *S. aureus* cells and to the temperature at which the cells are cultured. Since no break in the Arrhenius plot of the 4-aminoantipyrine clearance constants was evident, the curvature of the Arrhenius plot for the ethanol clearance constants apparently was not due to a phase change in the membrane.

An alternative explanation for the curved Arrhenius plot is that the absorption rate of ethanol is controlled both by diffusion to the membrane across a stagnant aqueous layer and by diffusion across the membrane. Since the activation energy for diffusion to the membrane would be lower than for diffusion across the membrane, the former process would be less sensitive to a change in temperature than the latter. Thus, at high temperature the rate-limiting barrier could be an aqueous stagnant layer, with the permeability of the underlying membrane being sufficiently large that the membrane would not act as a barrier. A decrease in temperature would cause a greater decrease in the permeability of the membrane than the aqueous stagnant layer. At low temperature, the rate-limiting barrier would be the membrane, with the permeability of the aqueous layer being large enough not to control the absorption rate. A similar mechanism was proposed to explain a convex Arrhenius plot observed for the permeation of 1-butanol across the toad urinary bladder (24). The plot was linear if the permeability coefficients were corrected for unstirred layer effects.

If the membrane controlled the absorption rate of ethanol at low temperature, the absorption rate should have been increased by polysorbate 80 at low temperature; it was not (Fig. 9). This lack of effect may have resulted from a relatively slow onset of surfactant-induced permeability. Alternatively, it may reflect the existence of two separate routes, membrane lipid and pores, for 4-aminoantipyrine and ethanol, the permeability of the latter being insensitive to surfactant (25). However, the existence of a pore route is open to question (24, 26).

The convex curvature noted in the Arrhenius plot for ethanol also may be explained by postulating that the absorption rate of ethanol is controlled by the blood flow rate to the absorbing membranes. There is no theoretical basis for the blood flow rate to follow the Arrhenius equation. The relationship between k for ethanol and temperature would mirror the relationship between blood flow and temperature. In addition, postulation of blood flow as the rate-limiting step is consistent with the lack of effect of polysorbate 80 on the ethanol absorption rate. While the surfactant may increase the permeability of the absorbing membranes to ethanol, the permeability in the absence of surfactant is sufficiently large so that it would not control the absorption rate. A further increase in permeability when surfactant was present would not alter the capacity of the blood to remove absorbed ethanol.

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Radioimmunoassay for Psychotropic Drugs I: Synthesis and Properties of Haptens for Chlorpromazine

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Abstract □ For the development of radioimmunoassay procedures for chlorpromazine and its active metabolites, three chlorpromazine haptens, 7-(or 8-)(3-carboxypropionyl)chlorpromazine, *N*-(3-carboxypropionyl)desmethylchlorpromazine, and *N*-(2-carboxyethyl)desmethylchlorpromazine, were synthesized and characterized by GLC–mass spectrometry, PMR spectrometry, and IR spectrophotometry. Each hapten was coupled to bovine serum albumin, and the number of hapten residues per mole of bovine serum albumin was calculated by UV spectrophotometric methods. Antibodies to each hapten–protein conjugate were obtained in rabbits, and titers of the antiserums were checked by evaluating their binding characteristics to tritiated chlorpromazine.

Keyphrases □ Chlorpromazine—three haptens synthesized and characterized, antibodies developed in rabbits, antiserum titers determined □ Haptens, chlorpromazine—three synthesized and characterized, antibodies developed in rabbits, antiserum titers determined □ Antibodies—to three chlorpromazine haptens developed in rabbits □ Tranquilizers—chlorpromazine, three haptens synthesized and characterized, antibodies developed in rabbits, antiserum titers determined

Chlorpromazine is widely used in the treatment of certain psychiatric disorders. It is extensively metabolized in humans, and several of its metabolites are pharmacologically active. For thorough study of the metabolism, pharmacokinetics, and clinical monitoring of this drug, sensitive specific procedures are required for the determination of chlorpromazine levels in biological fluids.

BACKGROUND

Various current techniques with adequate sensitivity to determine plasma chlorpromazine concentrations are GLC with electron-capture detection (1), labeled derivative formation (2), fluorescent labeling with dansyl chloride (3), TLC of a quaternary ammonium derivative (with 9-bromomethylacridine) followed by UV photolysis and subsequent spectrofluorometric determination (4), and GLC with mass spectrometric detection (5). But these methods are tedious, require extraction of the drug from biological fluids and/or its derivatization, and are not easily amenable for routine clinical monitoring.

Radioimmunoassay also may be applicable for determining plasma chlorpromazine concentrations. This technique is simple, requires no extraction, and is readily applied to routine analysis. A reported radioimmunoassay procedure for chlorpromazine (6), in which the protein (bovine serum albumin) was presumably coupled through the side chain of the drug, is not sufficiently sensitive (*cf.*, 7) and does not differentiate between chlorpromazine and its didemethylated metabolite at any level or among chlorpromazine, 7-hydroxychlorpromazine, and chlorpromazine sulfoxide at the 20-ng level.

Another published procedure (7), in which the bovine serum albumin was coupled to the ring system of chlorpromazine through a carboxyphenylazo bridge, was claimed to distinguish to some extent between chlorpromazine and several of its ring-modified and side-chain-modified metabolites, but it has not been widely evaluated or applied.

The present approach toward a sensitive, specific radioimmunoassay for chlorpromazine and other tricyclic antidepressants was to synthesize three chlorpromazine–protein conjugates in which the drug was coupled through a modified side chain as well as through a ring-modified chlorpromazine hapten. These conjugates will subsequently be evaluated to determine which had the specificity and sensitivity to measure the concentration of unchanged chlorpromazine and the total concentration of chlorpromazine and its active metabolites such as monodesmethyl- and didesmethylchlorpromazine.

This paper describes the synthesis and characterization of three new drug–protein conjugates, the production of antibodies to these conjugates in rabbits, and the titers of the antiserums.

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