Drug Absorption and Exsorption Kinetics in Goldfish

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The utility of goldfish as test animals for studies of biologic membrane permeation has led to a detailed analysis of the kinetics of drug transfer, in both directions, across goldfish body membranes. Methodology was developed to study permeation solely across gill membranes as well as across the total body surface. 4-Aminoantipyrine was used in the investigation because this drug is neither metabolized nor protein-bound in goldfish. The kinetics of absorption and exsorption was determined under various conditions (across the gills only, across the total body surface, from and to external media of different pH, exsorption after drug injection and after drug absorption). Drug elimination after immersion of fish in 4-aminoantipyrine solution and subsequent transfer into buffer solution was describable by simple first-order kinetics, while data describing the exit of drug from the fish after intraperitoneal injection could be resolved usually into two separate first-order components (leakage from puncture at site of injection and exsorption across body membranes). Drug absorption was found to be first-order with respect to 4-aminoantipyrine. However, under the conditions of most of the experiments (which permitted the maintenance of an essentially constant concentration gradient across the absorbing membranes) drug absorption proceeded by apparent zero-order kinetics. Changes in pH of the external medium had no effect on exsorption kinetics, but had a marked effect on absorption rates. These observations indicate that changes in pH from 4.0 to 7.0 have no significant effect on the permeability characteristics of the biologic membranes as such, but modify drug absorption only by changing the extent of ionization of a weak acid or base.

FIFTY years ago, Pittenger and Vanderkleed described a method for the standardization of digitalis fluid extract by means of a bioassay using goldfish (1). Since that time, goldfish have been used to study the response of the central nervous system to a variety of ataraxics, psychomimetics, stimulants, and depressants (2); to determine drug toxicity (3, 4); to evaluate topical anesthetics (5); to assess the neurotoxicity of streptomycin (6); to test gall bladder evacuants (7); to study the mechanisms of action of the sulfonyl ureas (8) and general anesthetics (9); and to investigate biochemical correlates of behavior (10). These are only a few examples to illustrate the extensive use of goldfish in pharmaceutic and pharmacologic investigations. The utility of fish for the study of drugs is enhanced further by the fact that they are subject to many diseases similar to those of man (11). Fish may develop tuberculosis and mycosis, trypanosomiasis and schistosomiasis, tumors and cancers. They are subject to vitamin deficiency diseases such as polyneuritis and anemia, degenerative diseases such as cirrhosis of the liver, and metabolic diseases such as cataracts, gall stones, and diabetes.

Recent investigations have illustrated the usefulness of goldfish for studies of the permeability of biologic membranes to certain drugs, and for determinations of the effect of environmental factors, such as pH and complex formation, on drug absorption (12, 13). These investigations also have resulted in the development of a method for the determination of drug absorption rates without chemical assay (14). There remained, however, a number of unresolved questions with respect to the transfer of drugs and other foreign substances across fish membranes: What are the relative rates of absorption and exsorption[1] of a given substance under similar conditions? Does drug absorption and exsorption occur across the gill membranes only (16) or across all body membranes (17)? What is the effect of route of administration on drug elimination kinetics? What is the effect of pH on the permeability characteristics of the membranes as such? Since fish apparently rely on exsorption by passive diffusion rather than on biotransformation for the elimination of drugs (16), it is important (from a pharmacologic, economic, and public health point of view) to obtain answers to the questions posed above. The authors have endeavored to provide these answers in the present report.

EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing from 8–12 Gm., were used. All the fish used in any one of the experiments were of the same lot to minimize variation in results due to differences in characteristics of the fish.

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[1] Exsorption refers to the transfer of drug from the tissues to the outside environment (15).

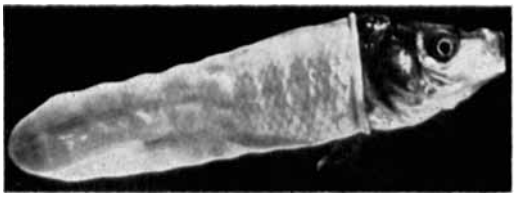

Fig. 1.—Goldfish with body enclosed in rubber bag for determination of rate constants for drug absorption and exsorption across the gill membranes.

Determination of Absorption Rate.—Goldfish were placed in large glass jars containing 150 ml. of drug solution per fish. The solutions were maintained at a temperature of 20 ± 0.5° by means of a constant-temperature refrigerated water bath. At the end of the absorption period, the fish were removed rapidly from the solution by draining the jars over a colander. The colander was then immersed momentarily in distilled water in order to remove the drug solution from the body surface of the fish. The fish were killed, placed in individual glass containers, and stored in a freezer until assayed.

To determine absorption across the gills only, single fish with body enclosed in a rubber bag were placed in 250-ml. capacity glass beakers containing 150 ml. of drug solution. These beakers were placed in a water bath at 20 ± 0.5°. Removal, washing, and storage of fish was done in the manner described above.

The drug solutions consisted of 250 mg.% 4-aminoantipyrine (Eastman) in Tham buffer (0.05 *M*, pH 7.0) or in glycine buffer (0.05 *M*, pH 4.0).

Determination of Exsorption Rate.—Exsorption rate constants were determined after administering the drug (*a*) by injection or (*b*) by immersing the fish in drug solution. In procedure (*a*), 1 mg. 4-aminoantipyrine per Gm. body weight was injected intraperitoneally as a 2% aqueous solution, using a tuberculin syringe with a 27-gauge, 1/4-in., regular point hypodermic needle. In procedure (*b*), the fish were placed for 30 min. in 860 mg.% 4-aminoantipyrine in Tham buffer (0.05 *M*, pH 7.0) and rinsed subsequently in distilled water. The latter procedure resulted in the absorption of about 1 mg. drug/Gm. fish.

After injection or absorption of drug, single fish were placed in 600-ml. capacity glass beakers containing 500 ml. of buffer solution (either pH 7.0 Tham or pH 4.0 glycine) at 20 ± 0.5°. Fifteen-milliliter aliquots of solution were removed every 30 min. for 6 hr. Fifteen milliliters of buffer solution was added after each sampling to maintain a constant volume of solution in the beakers. The aliquots were filtered through Millipore membranes (type HA) and stored for subsequent assay. At the end of the experiment, fish used in procedure (*b*) were rinsed with distilled water, killed, placed in glass jars, and stored in a freezer for subsequent assay.

Treatment of Injection Site.—In some exsorption experiments after intraperitoneal injection of drug, the injection site was treated with a protein precipitant in one of the following ways. (*a*) A commercial styptic pencil (90% aluminum potassium sulfate) was moistened and applied to the injection site immediately after withdrawal of the hypodermic needle, or (*b*) a 10% solution of tannic acid in ethanol was applied by means of a cotton-tipped applicator. In each case, the injection site was blotted lightly with filter paper after application of the protein precipitant.

Enclosure of Fish Body in Rubber Bag.—To study absorption or exsorption across the gills, goldfish were placed in rubber bags (surgical latex finger cots, small size) so that only the head was exposed. The opening of the finger cot was expanded and held by means of a ring clamp, and 4 ml. of distilled water was introduced. The fish was then inserted tail first into the bag, and eased carefully in position such that the finger cot covered the entire body except the head and gills (Fig. 1). The rim of the finger cot was immediately caudal to the pectoral fins.

Determination of 4-Aminoantipyrine.—The analytical method was a modification of the colorimetric method of Brun (18) for the determination of *p*-aminohippuric acid. Aqueous solutions (*i.e.*, samples of aqueous medium from the exsorption studies) were diluted, if necessary, to yield a 4-aminoantipyrine concentration of 0.1 to 0.5 mg.%. One milliliter of *p*-dimethylaminobenzaldehyde reagent solution was added to 10 ml. of drug solution. A blank was prepared by adding 1 ml. of reagent solution to 10 ml. of solvent. Absorbance was measured at 430 mμ with a Hitachi–Perkin-Elmer spectrophotometer, model 139.

For the determination of 4-aminoantipyrine in goldfish tissue, single fish were homogenized with 20 ml. of distilled water in a tissue homogenizer. The homogenate was transferred quantitatively to a 100-ml. graduated cylinder, 1 or 2 drops of *n*-octanol was added to remove the foam resulting from homogenization, and water was added to bring the total volume to five times that of the fish. The homogenate was centrifuged at 2400 × *g* for 20 min., 5 ml. of the supernatant phase was pipeted into a 15-ml. centrifuge tube, and 1 ml. of 15% trichloroacetic

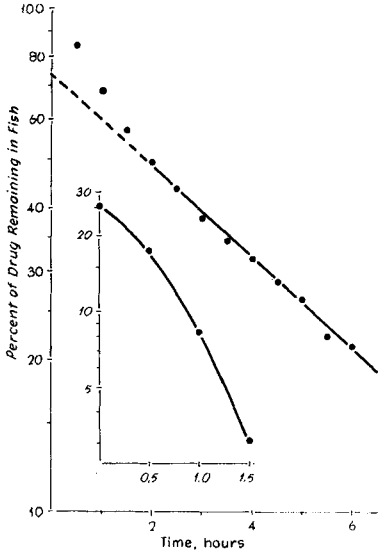

Fig. 2.—Exsorption of 4-aminoantipyrine from a single goldfish after intraperitoneal injection of 1 mg./Gm. body weight. Inset is a plot of the difference between the early experimental points and values obtained by extrapolation of the linear segment of the curve.

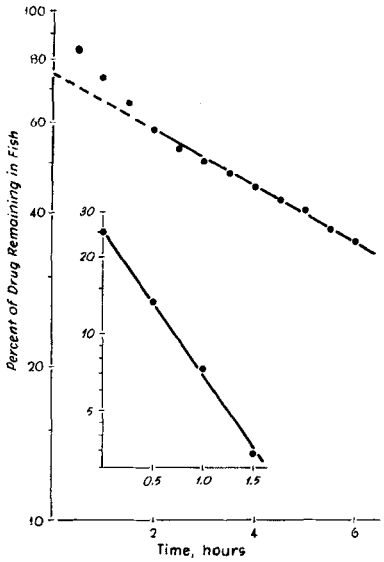

Fig. 3.—Exsorption of 4-aminoantipyrine from a single goldfish after intraperitoneal injection of 1 mg./Gm. body weight followed by application of tannic acid solution to injection site. Inset as in Fig. 2.

acid solution[2] and 4 ml. of distilled water were added. The solution was shaken and centrifuged at 2400 × *g* for 10 min. Five milliliters of the clear supernatant phase was diluted to 20 ml. with distilled water, and 2 ml. of *p*-dimethylaminobenzaldehyde reagent solution was added. Absorbance of the solution[3] was determined at 430 mμ with a Hitachi–Perkin-Elmer spectrophotometer, model 139. Tissue blanks were prepared as described above, using goldfish not exposed to the drug. Blank values averaged 0.02 mg. 4-aminoantipyrine equivalent per Gm. fish tissue and were used to correct the analytical results.

Determination of Water Content of Fish Tissue.—Fish were killed, blotted with filter paper, cut in small pieces, placed on a watch glass, and dried for 24 hr. at 50° in an electric oven. The watch glass with tissue was weighed on an analytical balance before and after drying. Subsequent heating at 110° for 2.5 hr. resulted in no change in weight (< 0.5%).

Determination of pH of Fish Tissue.—Fish were killed and homogenized immediately in an ice bath. The homogenate was brought to room temperature, and the miniature electrodes of a Leeds and Northup pH meter, model 7664, were placed in the homogenate. The entire procedure was carried out as rapidly as possible.

RESULTS AND DISCUSSION

The drug chosen for these studies, 4-aminoantipyrine, is neither metabolized nor protein-bound in goldfish (16). Unlike marine fish, goldfish do not swallow water in detectable amounts over a 24-hr. period (19). There is evidence (*Reference 20* and data collected in the present study) that renal excretion does not contribute significantly to drug elimination in goldfish. For these reasons, studies of absorption and exsorption kinetics involving passive diffusion processes are relatively uncomplicated when the goldfish is used as the experimental animal.

Initially, drug exsorption kinetics was determined after intraperitoneal injection of 4-aminoantipyrine. During these experiments, the concentration of drug in the external medium was negligible (relative to drug concentration in fish tissues) at all times, due to the large volume of the medium. Exsorption data, when plotted as per cent of dose remaining in fish (logarithmic scale) *versus* time (linear scale), could be resolved usually into two separate first-order processes. One of these processes was suspected to be leakage of drug solution from the site of injection. Attempts to prevent this leakage by application of protein precipitants (tannic acid solution or styptic pencil) to the site of injection were unsuccessful. Figures 2, 3, and 4 are representative plots of 4-aminoantipyrine exsorption data for untreated fish, fish treated with tannic acid solution, and fish treated with styptic pencil, respectively. The insets in these figures represent plots of the difference between early experimental points and values obtained by extrapolation of the linear segment of the respective curves. Plots obtained by this method were usually first order (Figs. 3 and 4) but occasionally approached zero-order characteristics (Fig. 2). The contribution of leakage to the total elimination of drug can be determined on the basis of the intercept value obtained when the linear segment of the exsorption curve is extrapolated to zero time. These values were essentially equal for untreated and treated fish (Table I), which shows that application of protein precipitant to the site of injection neither prevented nor reduced leakage under the conditions of the experiment. The half-life for exsorption was essentially the same for untreated and styptic pencil-treated fish, but longer for tannic acid-treated fish (Table I). The latter effect may be due in part to spreading of tannic acid

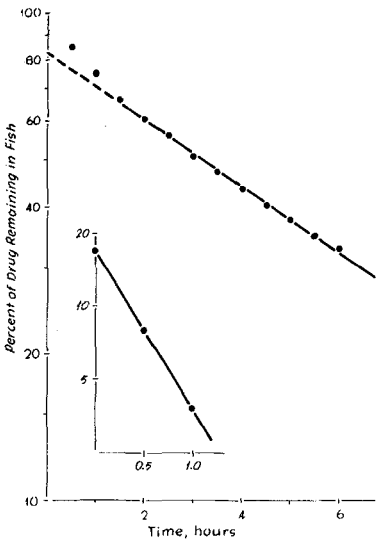

Fig. 4.—Exsorption of 4-aminoantipyrine from a single goldfish after intraperitoneal injection of 1 mg./Gm. body weight followed by application of styptic pencil to injection site. Inset as in Fig. 2.

[2] Higher concentrations of trichloroacetic acid interfere with the assay and should not be used.

[3] Absorbance of the solutions decreases about 10% in 6 hr. Spectrophotometric measurements should therefore be performed within 1 hr. after addition of reagent solution.

TABLE I.—EXSORPTION OF 4-AMINOANTIPYRINE AFTER INTRAPERITONEAL INJECTION IN GOLDFISH

Exptl. Conditions	Animals, No.	Av. Half-Life for Exsorption, min.	Av. Intercept Value at Zero Time, % of Drug in Fish
Controls	5	209	75.8
Local application of styptic pencil	5	238	75.6
Local application of tannic acid	5	331	77.1
Body enclosed in rubber bag	6	549	91.2

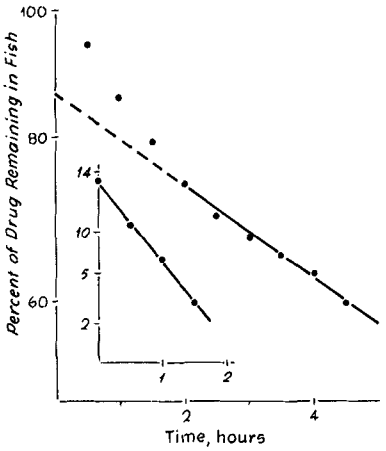

Fig. 5.—Exsorption of 4-aminoantipyrine from the gills of a single goldfish (with body enclosed in rubber bag) after intraperitoneal injection of 1 mg./Gm. body weight. The ordinate represents the per cent of drug remaining in the fish tissues and in the solution enclosed by the rubber bag. Insert as in Fig. 2.

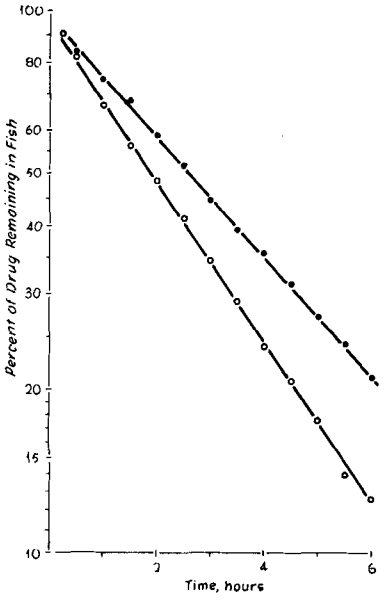

Fig. 6.—Exsorption of 4-aminoantipyrine from two goldfish after absorption of about 1 mg./Gm. body weight by immersion in 4-aminoantipyrine solution.

over the skin and a resulting modification of skin permeability.

Exsorption kinetics after intraperitoneal injection of drug was studied also under conditions where the body of the fish was enclosed in a rubber bag. While this did not prevent leakage of drug solution from the site of injection, it caused retention of this solution in the rubber bag so that only drug exsorbed across gill membranes could appear in the external medium. The data obtained in these studies (Fig. 5 and Table I) show that exsorption does occur across the gills. Since the half-life for exsorption across the gills was about double the half-life for unrestricted exsorption (*i.e.*, in fish with body not enclosed in rubber bag), it can be concluded also that exsorption is not restricted to the gills only. The initial rapid exsorption (illustrated in Fig. 5), under conditions where leakage does not contribute to the drug concentration changes with time in the external solution, may be explained in the following way. Initially, drug is exsorbed across the gills into the external medium and across the skin into the small volume of water present in the rubber bag. When the concentrations of 4-aminoantipyrine in fish tissue and in the solution enclosed by the rubber bag are equal, exsorption occurs solely across the gills. Thus, the initial rapid exsorption evident in Fig. 5 represents essentially an initial distributive phase.

To circumvent the apparent leakage problem, subsequent exsorption experiments were carried out after administering the drug by immersion of the fish in 4-aminoantipyrine solutions under conditions (concentration, time of exposure) which resulted in the absorption of about 1 mg. drug/Gm. body weight. The exact amount of drug present in the fish at the beginning of an exsorption experiment was determined from the amount of drug in the external medium (including corrections for aliquots removed for assay) and the amount of drug in the fish at the end of the experiment. Figure 6 depicts representative data from two such experiments which show that exsorption occurred by a single exponential process.[4] These observations constitute strong support for the assumption that the initial rapid exsorption phase evident in Figs. 2–4 was due to leakage of drug solution from the site of injection.

Table II contains data obtained in exsorption experiments in which 4-aminoantipyrine was administered by immersion of the fish in drug solution, and where the body of each fish was enclosed subsequently in a rubber bag. The average rate constant for exsorption across the gills only was 1.6×10^{-3} reciprocal minutes. This value is based on data obtained from fish *3*, *4*, and *5* (Table II); the duration of exsorption in the other two fish was too short for adequate fitting of curves to the data. The information listed in Table II permits assessment of the role of the kidneys in drug elimination. At no time did the concentration of drug in the small volume of aqueous medium enclosed in the rubber bag exceed the drug concentration in the tissue fluids of the fish. In the 4.0- and 4.5-hr. experiments, drug concentration in the bag was appreciably lower than that in tissue fluids; in the 6-hr. experiments, it occasionally approached equilibrium. If renal

[4] The lines intercept the ordinate slightly below the 100% mark, apparently due to the rapid initial expulsion of a small volume of drug solution retained in the pharyngeal and gill chambers.

TABLE II.—CONCENTRATION OF 4-AMINOANTIPYRINE IN GOLDFISH TISSUE, TISSUE WATER, IMMERSION MEDIUM, AND IN RUBBER BAG ENCLOSING BODY, AT TERMINATION OF EXSORPTION EXPERIMENT

Fish	Duration of Expt., hr.	Wt. of Fish, Gm.	Initial Drug Content, mg.[a]	Drug in Rubber Bag Amt., mg.	Drug in Rubber Bag Concn., mg./ml.	Drug Concn. in Medium, mg./ml.	Drug Concn. in Fish mg./Gm. Tissue	Drug Concn. in Fish mg./ml. Body Water[b]
1	4.0	9.6	10.9	0.887	0.222	0.0064	0.642	0.823
2	4.5	10.0	14.1	1.59	0.436	0.0089	0.706	0.905
3	5.0	9.9	8.15	0.997	0.324	0.0068	0.304	0.390
4	6.0	7.8	6.94	1.07	0.261	0.0062	0.262	0.336
5	6.0	8.9	7.29	0.931	0.369	0.0053	0.347	0.445

[a] Sum of drug in fish tissue, rubber bag, and immersion medium (pH 7.0) at end of experiment. [b] Based on 78% water content of fish.

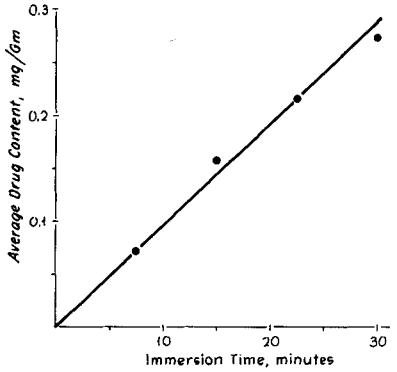

Fig. 7.—Absorption of 4-aminoantipyrine by goldfish immersed in 250 mg.% 4-aminoantipyrine solution of pH 7.0. Each point represents the average of five fish.

excretion had been significant compared with exsorption across the skin, the drug concentration in the solution contained in the rubber bag would have rapidly equaled and even exceeded the concentration

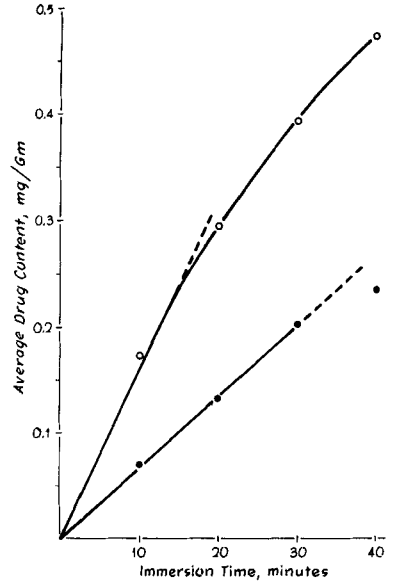

Fig. 8.—Absorption of 4-aminoantipyrine by goldfish immersed in 250 mg.% 4-aminoantipyrine solution of pH 4.0 (●) and pH 7.0 (○), respectively. Each point represents the average of seven fish.

of drug in tissue fluids. In goldfish, the kidneys are concerned mainly with the elimination of water against an osmotic gradient; they are constructed in a manner which facilitates reabsorption of solutes (21). For example, injection of bicarbonate in goldfish does not increase the pH of urine—bicarbonate is apparently eliminated principally by exsorption (20).

Absorption of 4-aminoantipyrine was studied under conditions where the drug concentration in the external medium was very much higher throughout the experiment than the concentration in fish tissues. Also, the large volume of external solution relative to the volume of fish caused the drug concentration in the external medium to remain essentially constant. This resulted in drug absorption by apparent zero-order kinetics (Fig. 7) where the first-order absorption rate constant (K_A) could be calculated from the relationship K_A = absorption rate/drug concentration.

Average rate constants for absorption and exsorption were 3.9×10^{-3} reciprocal minutes and 4.9×10^{-3} reciprocal minutes, respectively. The similarity of these values as well as anatomical considerations (*i.e.*, the vascularity of the fish body) suggest that the absorption and exsorption processes are subject to the same rate-limiting barriers. However, this conclusion is justified only if the degree of ionization of the weak base 4-aminoantipyrine in fish tissue fluids and in the external medium is the same. Since the pH of goldfish tissue fluids was found to be approximately 7.0, the pH of the external medium had been adjusted to the same value. The pKa of 4-aminoantipyrine is 4.1 (22), and the drug is therefore essentially nonionized at pH 7.0.

To determine the effect of pH on the absorption of 4-aminoantipyrine, goldfish were exposed to solutions of the drug in pH 4.0 buffer and pH 7.0 buffer, respectively. Results of these experiments, shown in Fig. 8, indicate that pH has a marked effect on the absorption of 4-aminoantipyrine. The drug is essentially nonionized at pH 7.0 but only about 44% nonionized at pH 4.0. The experimental data are compatible with the assumption that 4-aminoantipyrine is absorbed primarily or solely in nonionized form. The deviation from linearity of some of the data shown in Fig. 8 is due to drug concentration build-up in the fish tissues. Concentrations above 0.25 mg./Gm. (10% of drug concentration in external medium) result in an appreciable change in the drug concentration gradient across the absorbing membranes.

The contribution of gill and skin membranes, respectively, to drug absorption is shown by the data

TABLE III.—EFFECT OF pH AND BODY SURFACE AREA ON THE ABSORPTION OF 4-AMINOANTIPYRINE BY GOLDFISH

Immersion Time,[a] min.	pH	Body Enclosed in Rubber Bag	Amt. of Drug Absorbed,[b] mg./Gm. Av.	S.D.
30	4.0	Yes	0.119	0.016
30	7.0	Yes	0.216	0.038
30	7.0	No	0.434	0.077
10	7.0	No	0.159	0.046

[a] Fish were immersed in 250 mg.% 4-aminoantipyrine at 20°. [b] Seven fish each.

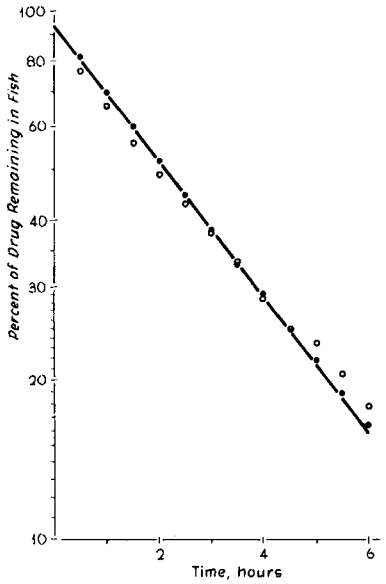

Fig. 9.—Exsorption of 4-aminoantipyrine from goldfish into aqueous media of pH 4.0 (○) and pH 7.0 (●), respectively, after absorption of about 1 mg./Gm. body weight by immersion in 4-aminoantipyrine solution. Each point represents the average of nine fish.

listed in Table III. The gills contribute about 50% to the total absorption. This is similar to the relative contribution of gill and skin membranes to drug exsorption, as shown in Table I. As expected, the effect of pH on drug absorption across the gills is the same as the effect on absorption across the entire body surface.

Most studies of the effect of pH on drug absorption have not been designed in a manner which makes it possible to distinguish between the effect of pH change (*a*) on the extent of ionization of the drug and (*b*) on the permeability of the biologic membrane to nonionized and/or ionized drug molecules. Studies of drug exsorption from fish tissue into solutions of different pH permit this distinction; the homeostatic mechanism of goldfish causes the pH of tissue fluids and therefore the degree of ionization of drug in these fluids to be unaffected by changes in the pH of the external medium. Thus, it is possible to determine the effect of pH changes on the permeability characteristics of the membranes as such.

As shown in Fig. 9, there was no significant difference in the exsorption rate of 4-aminoantipyrine from goldfish into solutions of pH 7.0 and pH 4.0, respectively. The two curves in Fig. 9 are based on nine fish each. (It was possible to present average curves in these instances because the individual values were very similar.) Results of this experiment indicate that pH variations from 4.0 to 7.0 have no significant effect on the permeability characteristics of the fish membranes. Similar conclusions were arrived at by Levy and Gucinski (12) from the results of studies with different experimental design and in the pH range of 6.9–8.9.

The results of the investigation reported here are summarized in Table IV. Rate constants for absorption and/or exsorption under different experimental conditions are listed in a manner which permits comparison of data obtained in any one experiment or in different experiments. There is some variation between lots of fish which accounts probably for the range of rate constants for absorption across the whole body at pH 7.0. However, it is apparent that (*a*) absorption and exsorption occur at similar rates (when pH is the same), (*b*) absorption and exsorption of 4-aminoantipyrine involve passive diffusion across both gill and body membranes, (*c*) changes in pH from 4.0 to 7.0 have no significant effect on the permeability characteristics of the membranes as such, and (*d*) 4-aminoantipyrine is absorbed mainly or solely in nonionized form. It is not quite clear if the relatively low exsorption rate constants obtained in experiments in which the drug was administered by intraperitoneal injection (experiment *D*) are due to lot-to-lot variations of the fish or if diffusion of drug from the injection site through the tissues (rather than diffusion through gill and skin membranes) became the exsorption rate-limiting process under these experimental conditions.

TABLE IV.—RATE CONSTANTS FOR ABSORPTION AND EXSORPTION OF 4-AMINOANTIPYRINE IN GOLDFISH

Exptl. Conditions[a]	pH of Medium	Rate Constant (± S.D.), min.$^{-1}$ × 10^3
Experiment A		
Absorption, whole body	7.0	3.9 (±0.8)
Exsorption, whole body	7.0	4.9 (±0.8)
Exsorption, whole body	4.0	4.6 (±0.6)
Experiment B		
Absorption, whole body	7.0	6.4 (±1.2)
Absorption, whole body	4.0	2.7 (±0.7)
Experiment C		
Absorption, whole body	7.0	5.9[b] (±1.5)
Absorption, gills only	7.0	2.9 (±0.5)
Absorption, gills only	4.0	1.6 (±0.2)
Experiment D		
Exsorption, whole body, after i.p. injection	7.0	3.3 (±1.4)
Exsorption, gills only, after i.p. injection	7.0	1.3 (±0.4)
Experiment E		
Exsorption, gills only	7.0	1.6 (±0.2)

[a] Unless indicated otherwise, exsorption experiments were carried out after immersing fish in drug solution under conditions (time, concentration) which resulted in the absorption of about 1 mg. 4-aminoantipyrine/Gm. fish. [b] Based on average of 10- and 30-min. data (see Table III).

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Dielectric Solubility Profiles of Acetanilide and Several Derivatives in Dioxane–Water Mixtures

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The solubilities of acetanilide, *p*-methyl acetanilide, and *p*-ethoxyacetanilide (phenacetin) were determined in dioxane–water mixtures of known dielectric constants. The solubility curves that were obtained showed a multiplicity of peak solubility values as a function of the dielectric constant. These peak solubilities or dielectric requirements for the subject compounds were found to vary to some extent and may reflect solute polarity with respect to acetanilide as the parent compound.

IN A RECENT communication (1), the existence of multiple solubility peaks for the xanthines as a function of the dielectric constants of dioxane–water mixtures was presented. It was felt that this multipeak array might be evidenced for other pharmaceutical solutes as well. To this end, several substances in the antipyretic class were chosen—acetanilide and the *p*-methyl and *p*-ethoxy derivatives of acetanilide. It was felt that these compounds might allow for a qualitative picture in terms of solute polarity and possible dielectric requirement (DR) shifts. These compounds may be representative of substances for which at some future time quantitation of solubility as a function of dielectric constant is needed.

Enhanced solubility of pharmaceutical substances in solvent mixtures may have usefulness in the control of solubility, especially in pharmaceutical vehicles. Enhanced or maximum solubility for various substances has been reported previously as a function of either solvent composition (2–5), dielectric constants (6–8), or solubility parameters (9, 10). Increased solubility as a function of the dielectric constant of syrup vehicles has also been reported (11). An interesting paper by Gorman and Hall (12), summarizing some aspects of solubility with solubility parameters and dielectric constants, has also been presented.

Thus, the present studies were conducted with a view toward establishing a multiple solubility peak array for drugs in the antipyretic class and to illustrate the possible generalization of multiple solubility peaks for diverse compounds.

EXPERIMENTAL

Materials.—Acetanilide (Eastman-Kodak No. 3 White Label), *p*-methyl acetanilide (Eastman-Kodak No. 425 White Label), phenacetin (Mallinckrodt No. 2244) were the solutes used in this study. Distilled water (pH 6.8–7.2) and *p*-dioxane (Fisher certified D-111) were the solvents used.

Equipment.—A Beckman DK-2 was used for the spectrophotometric analysis. An Ainsworth type 12 balance was used for the gravimetric analysis. A Sargent water bath with attendant thermonitor unit for equilibration of samples at 25° and temperature control.

Methods.—Solvents of dioxane–water mixtures in 2.5% v/v increments were added to 22-ml. screw-capped Teflon-lined vials and excess solute added. Sample vials were tied down to a rotating

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