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# Effect of Complex Formation on Drug Absorption X: Effect of Polysorbate 80 on the Permeability of Biologic Membranes

**JUDITH A. ANELLO and GERHARD LEVY***

**Abstract** □ Low concentrations of a nonionic surfactant (polysorbate 80) in the bathing solution increase significantly the absorption and exsorption rate constants of 4-aminoantipyrine in goldfish. This effect is evident in absorption and exsorption studies involving chemical assays as well as in experiments in which absorption rate was determined indirectly on the basis of the time of onset of a pharmacologic effect. The ratio of rate constants with : without surfactant was similar in the three types of experiments. It is concluded that polysorbate 80 enhances drug transfer by a direct effect on the biologic membranes and not by interacting with the drug.

**Keyphrases** □ Complex formation—drug absorption □ Polysorbate 80 effect—membrane permeability □ Absorption, exsorption rates—goldfish □ Overturn time, goldfish—biologic assay □ Colorimetric analysis—spectrophotometry

Studies in this laboratory have shown that polysorbate 80, in concentrations below the critical micelle concentration, significantly increases the absorption of secobarbital and pentobarbital in goldfish (1). This effect was thought to be due either to the formation of a nonmicellar drug-surfactant complex which is absorbed more rapidly than the drug itself, or to a modification of the permeability characteristics of the biologic membranes by the surfactant (1). Subsequent studies, in which the absorption kinetics were determined on fish which had been immersed in surfactant solution, rinsed, and then placed in surfactant-free drug solution, revealed that enhanced absorption could also be obtained by pretreatment of the fish with polysorbate 80 (2). These observations suggested strongly that the surfactant exerts a direct permeability enhancing effect on the biologic membranes. However, the possibility could not be excluded that the enhanced absorption involves an interaction of the drug with surfactant molecules that are adsorbed on the membrane surface. It was believed that studies of drug transfer out of the fish (exsorption), where the surfactant is in the bathing solution, would provide a more definitive indication of the mechanism of the surfactant effect since drug and surfactant are then on opposite sides of the membrane. Also, since previous studies were based on pharmacologic effect data only, it was considered desirable to determine absorption rates by direct chemical assay as well as on the basis of the onset of a defined pharmacological effect. The experimental approach for the absorption and exsorption studies was as previously described by Levy and Miller (3), using 4-aminoantipyrine, a drug that is not measurably metabolized or protein bound in goldfish under the experimental conditions (4).

## EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing 10 g. on the average, were used. All fish in a given experiment were from the same lot.

**Materials**—Polysorbate 80 (lot No. 586 Atlas Powder Company), 4-aminoantipyrine (Eastman Organic Chemicals); Tris(hydroxymethyl) aminomethane (Tris) (Nutritional Biochemicals Corp.); and glycine (Eastman Organic Chemicals).

The drugs were dissolved in 0.05 *M* Tris or 0.05 *M* glycine buffer and the solutions were adjusted to pH 7.0 and 4.0, respectively, with hydrochloric acid.

**Determination of Absorption and Exsorption Rates**—The method has been described previously (3) except for the following modifications. In the absorption experiments, five fish were placed simultaneously in 1 l. of drug solution for designated times, then rinsed quickly in distilled water and stored in individual containers in a freezer until assayed. The fish were never kept frozen for more

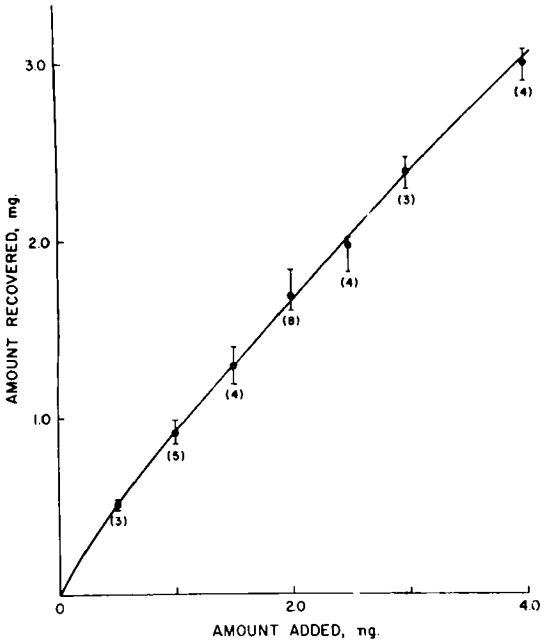


**Figure 1**—*Average recoveries of 4-aminoantipyrine from goldfish homogenate. Shown is a plot of the average amount of drug recovered as a function of the amount added. Vertical bars indicate the range of experimental values, the numbers refer to the number of experiments.*

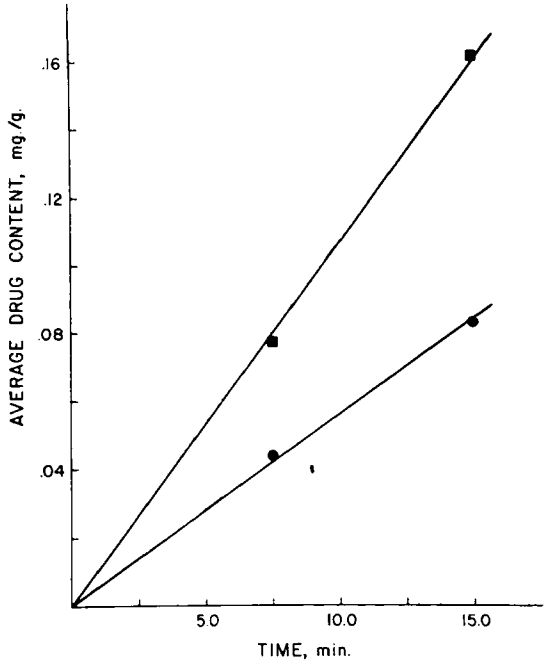


**Figure 2**—*Effect of polysorbate 80 on the absorption of 4-aminoantipyrine by goldfish. Fish were immersed in 250 mg. % 4-aminoantipyrine with (squares) or without (circles) 0.01% polysorbate 80 in 0.05 M Tris buffer pH 7.0 at 20° ± 0.5°. Each point represents the average of five fish.*

than 1 week (usually only a few days) to avoid degradation of drug which becomes significant with longer storage times.

For the exsorption study, the fish were immersed first in 850 mg. % 4-aminoantipyrine solution (pH 7.0 Tris buffer at 20°) for 30 min. This resulted in the absorption of about 1 mg. of 4-aminoantipyrine/g. of fish. The fish were then rinsed momentarily with distilled water and placed in buffer solution or in buffer with surfactant. Drug concentrations in the buffer solutions were determined at designated intervals, and drug remaining in the fish was determined at the end of the experiment.

**Determination of 4-Aminoantipyrine in Tissue and Aqueous Solutions**—The method used for the determination of 4-aminoantipyrine in solution and in fish tissue was a modification (3) of the colorimetric method of Brun (5). Tissues were homogenized in a Lourdes homogenizer. Blank values were obtained from tissues of fish which had been immersed in buffer solution or in buffer solution with 0.01% polysorbate 80 for 15 min., rinsed for a few seconds with distilled water, and frozen until assayed. There was no difference in the blank values of fish tissues from animals exposed to buffer solutions with surfactant or pure buffer solution, respectively. Therefore, the average blank value of 0.013 mg. 4-aminoantipyrine equivalent/g. fish tissue was used to correct all the analytical data.

Drug recovery determinations were carried out by immersing fish in buffer or in buffer with surfactant for 15 min., rinsing, injecting intraperitoneally known amounts of drug, and then homogenizing the fish and assaying as described above. Polysorbate 80 pre-

**Table I**—Rate Constants for Absorption of 4-Aminoantipyrine in Goldfish

| pH | Concn. of Polysorbate, % w/v | Rate Constant ($\pm SD$) min.$^{-1}$ $\times 10^3$ | Ratio with: without Surfactant |
|---|---|---|---|
| 4.0 | 0.01% | 2.2 (±1.0)[a] | 1.8 |
| 4.0 | None | 1.2 (±0.4)[a] | |
| 7.0 | 0.01% | 4.2 (±0.7)[b] | 1.8 |
| 7.0 | None | 2.3 (±0.2)[b] | |

[a] pH 4.0 values are based on the 10- and 20-min. data, total of 10 fish. [b] pH 7.0 values are based on the 7.5- and 15.0-min. data, total of 10 fish.

treatment did not affect drug recovery and therefore all data were averaged. Recoveries were found to be dependent on the type and intensity of homogenization and on the total amounts of drug present. The percent recovery decreased with increasing amounts of drug (Fig. 1). All analytical results were corrected accordingly.

**Determination of Overturn Time**—Five fish at a time were placed in 1 l. of drug solution in 0.05 *M* tris buffer of pH 7.0 at 20 ± 0.5°. Overturn time was the time elapsed until a fish placed on its side with a stirring rod could not right itself immediately (6).

## RESULTS AND DISCUSSION

The effect of 0.01% polysorbate 80 on the absorption of 4-aminoantipyrine by goldfish is shown in Fig. 2 and Table I. Absorption proceeded at a constant rate since the fish acted as an infinite sink and drug concentration in the solution remained essentially constant during the experiment, The absorption rate constants were determined by dividing absorption rate by the concentration of the drug in the solution. Polysorbate 80 increased substantially the absorption rate constant of 4-aminoantipyrine. The absorption rate constant at pH 7.0 was twice as large as at pH 4.0. This shows that the drug is absorbed solely or predominantly in the nonionized form [the pKa of 4-aminoantipyrine is 4.1 (7)]. The degree of absorption enhancement by polysorbate 80 was the same at pH 4.0 and 7.0, so that there was no measurable absorption of ionized 4-aminoantipyrine even in the presence of the surfactant. Had there been pronounced absorption of ionized drug in the presence of surfactant, the degree of absorption enhancement

**Table II**—Effect of polysorbate 80 on Exsorption of 4-Aminoantipyrine in Goldfish

| Concn. of Polysorbate, % w/v[a] | No. of Animals | Rate Constant ($\pm SD$) min.$^{-1}$ $\times 10^3$ |
|---|---|---|
| None | 6 | 3.3 (±1.0) |
| 0.005 | 2 | 5.2 (5.3, 5.1)[b] |
| 0.01 | 7 | 5.6 (±1.1) |
| 0.1 | 2 | 5.7 (6.1, 5.3)[b] |

[a] In 0.05 *M* Tris, pH 7.0, at 20° ± 0.5°. [b] Individual values given.

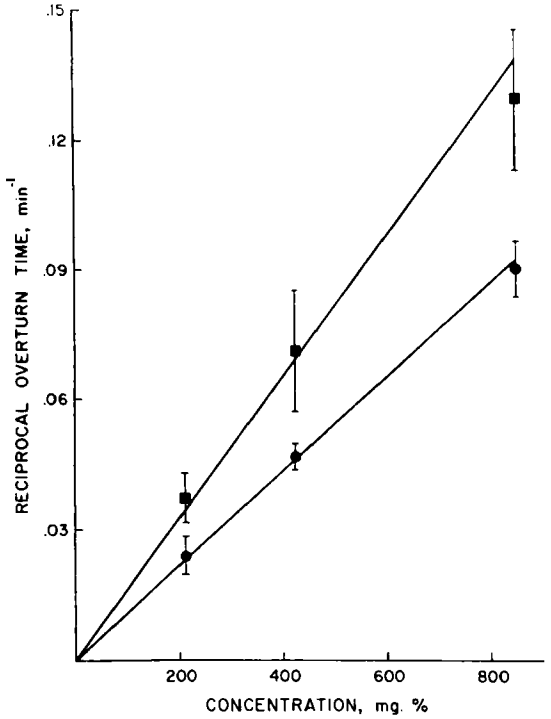


**Figure 3**—*Effect of polysorbate 80 on reciprocal overturn time in goldfish. Squares, 4-aminoantipyrine with 0.01% polysorbate 80; circles, without polysorbate 80. All solutions at pH 7.0. Each point is the average of five fish; vertical bars represent ±1 SD.*

by polysorbate 80 would have been greater at pH 4 (when 4-aminoantipyrine is half ionized) than at pH 7.0 (when the drug is essentially nonionized). The absorption rate constants determined in the control experiments are in good agreement with previously obtained values (3).

The effect of the polysorbate 80 on 4-aminoantipyrine absorption was also studied by a pharmacologic method, the theoretical basis of which has been presented previously (8, 9). Since time of death of the fish could not be determined with sufficient accuracy, overturn time (6, 10) was used as the pharmacologic end point. The results of this study, which are summarized in Fig. 3, also show that 0.01% polysorbate 80 significantly increased the absorption rate constant of 4-aminoantipyrine.

The exsorption kinetics of 4-aminoantipyrine were determined by placing the fish in drug solution long enough to absorb about 1 mg. of 4-aminoantipyrine/g. of body weight, rinsing the fish, and placing it in drug free solution which acted as an infinite sink. Figure 4 shows the exsorption of 4-aminoantipyrine into pH 7.0 buffer with and without 0.01% polysorbate 80. The exsorption rate constant was considerably larger when the bathing solution contained the surfactant. The surfactant effect was relatively independent of concentration in the range studied; polysorbate 80 concentrations from 0.005 to 0.1% yielded essentially the same rate constants (Table II). The exsorption rate constants for 4-aminoantipyrine are similar in value to the absorption rate constants, under the same conditions (either with or without surfactant). The small difference between the two constants, apparent here as well as in a previous study (3), may possibly reflect the contribution of an excretory pathway to the apparent exsorption rate constant. These results show that the drug transfer rate-limiting barrier is the external membranes (skin and gills) of the fish and that the surfactant has a direct effect on the permeability characteristics of these membranes. Polysorbate 80 itself does not cross biologic membranes (11) and thus cannot interact with drug on the body side of the membranes when fish are placed in surfactant solution.

The relative concentration independence of the polysorbate 80 effect on membrane permeability (Table II) is consistent with previous observations on the effect of polysorbate 80 (0.0005 to 0.01%) on the absorption of secobarbital (2). The absorption *inhibiting* effect of high (above CMC) concentrations of

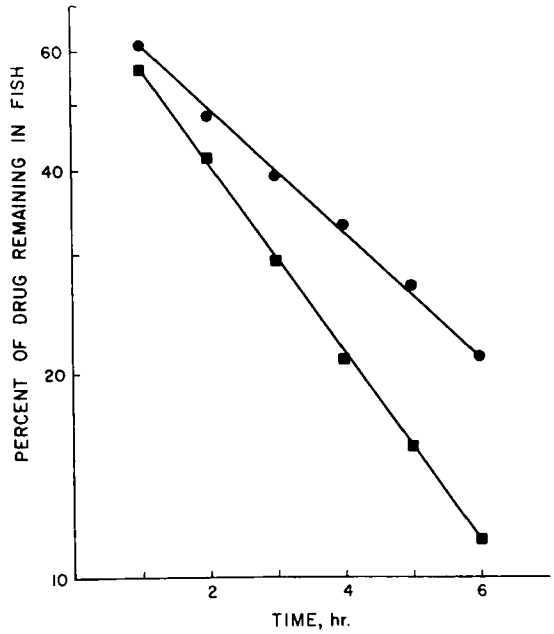


**Figure 4**—*Effect of polysorbate 80 on exsorption of 4-aminoantipyrine from goldfish after absorption of approximately 1 mg./g. body weight by immersion in 4-aminoantipyrine solution. Circles represent exsorption into 0.05 M Tris buffer pH 7.0 (average of six fish); squares indicate exsorption into buffer containing 0.01% polysorbate 80 (average of seven fish). All experiments at 20° ± 0.5°.*

surfactant (1), which is due to micellar complexation of drug, is of course not operative in the case of drug exsorption. The relative concentration independence of the surfactant effect on membrane permeability may be due to a high affinity of the surfactant to membrane constituents so that maximum absorption of surfactant can occur even from solutions containing very low concentrations of polysorbate 80. Since the surfactant effect is at least partly reversible [drug absorption enhancement is more pronounced in fish immersed in surfactant solution than in fish pretreated with the surfactant (2)], the increased permeability of the membranes is

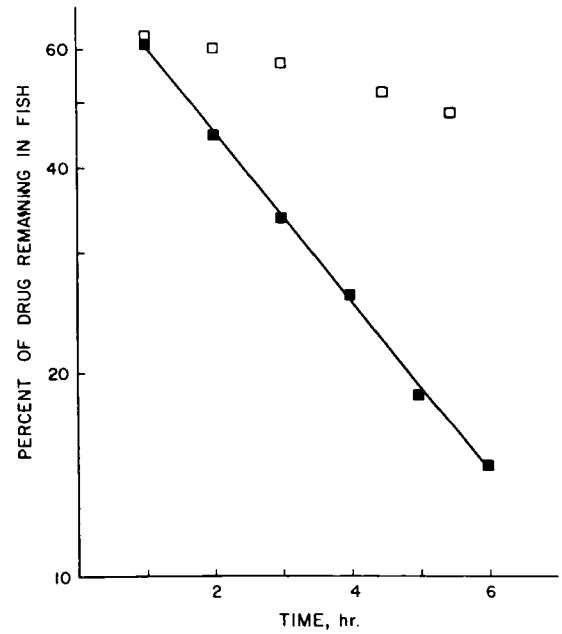


**Figure 5**—*Effect of death on exsorption of 4-aminoantipyrine from goldfish into 0.05% polysorbate 80. Closed squares represent a single live fish; open squares represent a fish which died approximately 30 min. after the beginning of the experiment.*

**Table III**—Effect of polysorbate 80 on the Permeability of Goldfish Membranes to 4-Aminoantipyrine, as Shown by Absorption, Exsorption, and Pharmacologic Effect Measurements

| Type of Experiment[a] | Rate Constant with 0.01% Polysorbate min.$^{-1}$ ($\pm SD$) $\times 10^3$ | Rate Constant without Polysorbate min.$^{-1}$ ($\pm SD$) $\times 10^3$ | Ratio with : without Surfactant |
|---|---|---|---|
| Absorption | 4.2 ($\pm$0.7)[b] | 2.3 ($\pm$0.2)[b] | 1.8 |
| Exsorption | 5.6 ($\pm$1.1)[c] | 3.3 ($\pm$1.0)[c] | 1.7 |
| Overturn | —[d] | —[d] | 1.5 |

[a] All experiments done at pH 7.0 at 20° ± 0.5°. [b] Mean of rate constants calculated from absorption data for each fish at each time. [c] Mean of individual rate constants. [d] Mean CT values, as g. min./l. ($\pm SD$): 61.2 ($\pm$10.2) with polysorbate; 91.5 ($\pm$10.2) without polysorbate.

probably not due (solely) to the leaching or extraction of membrane components.

The accidental death of one fish shortly after the start of an exsorption experiment, apparently due to an overdose of drug, provided a means of assessing the role of blood circulation on exsorption. The exsorption of 4-aminoantipyrine from the dead fish was much slower than from a living fish (Fig. 5). This is interpreted as being indicative of a change in the rate-limiting step in the exsorption process. Apparently, diffusion of drug through body tissues rather than through the external membranes becomes exsorption rate limiting when there is no blood circulation.

The results of these studies show that polysorbate 80 increases directly the permeability of biologic membranes of the goldfish to nonionized 4-aminoantipyrine. As shown in Table III, this effect is evident in absorption and exsorption studies involving chemical assays as well as in experiments in which absorption rate was determined indirectly on the basis of the time of onset of a pharmacologic effect. The ratio of rate constants with : without surfactant was similar in the three types of experiments.

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# New Local Anesthetics II: Lidocaine Analogs Embodying Pyrazolidine as the Basic Moiety


**MILTON J. KORNET and POO AN THIO**



**Abstract** □ The synthesis of a number of novel pyrazolidinylacylanilides and their precursors is reported. In the course of molecular modification, the anilinocarbonyl or anilinocarbonylmethyl group has been attached to the 1-, 3-, and 4-positions of the pyrazolidine ring. These anilides exhibited varying degrees of local anesthetic activity.



**Keyphrases** □ Lidocaine analogs—local anesthetic □ Pyrazolidinylacylanilides and precursors—synthesis □ Anesthetic activity—lidocaine analogs □ IR spectrophotometry—identity □ NMR spectrometry—identity □ Vapor phase chromatography—separation, analysis


The local anesthetic activity of a series of esters containing the pyrazolidine ring has been reported by these laboratories (1). Subsequently, further testing of the compounds on the isolated nerve indicated that in general they were more potent than lidocaine.[1] These promising results prompted the authors to prepare a number of novel pyrazolidinylacylanilides and evaluate their local anesthetic activity, especially since the amide linkage possesses the special advantage of hydrolytic stability as compared with an ester bond.

Local anesthetics have been the subject of many reviews (2, 3) and it has been shown that the distance between the amide carbonyl and the basic amino group influences the activity to a very large extent. Maximum activity is generally observed when the two groups are separated by either one or two carbon atoms (4). With this in mind, efforts were directed to the synthesis of the several analogs in this series which would result

[1] Unpublished results from the Astra Research Laboratories.