Effect of Complex Formation on Drug Absorption V

Studies on the Mechanism of the Secobarbital Absorption-Enhancing Effect of Polysorbate 80 in Goldfish

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Polysorbate 80 in concentrations of 0.0005 to 0.01 percent increased significantly the absorption rate of secobarbital by goldfish. This absorption-enhancing effect was obtained also if the fish were immersed in polysorbate 80 solution, washed, and then placed in a solution of secobarbital without the surfactant. The ratio of rate constants for absorption of nonionized and ionized secobarbital, respectively, was not affected by polysorbate 80. The results of this study indicate that polysorbate 80 enhances the absorption of secobarbital by increasing the permeability of the biologic membrane to the drug, rather than by forming a more rapidly absorbed nonmicellar polysorbate–secobarbital complex in the bulk phase of the drug solution.

THE EFFECTS of surfactants on drug absorption are of considerable interest and have been investigated extensively (1, 2). Of particular pharmaceutical importance are the possible effects of surfactants at low concentrations, such as might be encountered when dosage forms containing surfactants are dissolved in or diluted with various body fluids following oral or parenteral admistration, or following application to mucous membranes. Unfortunately, appreciable technical difficulties may be encountered in studies of the effect of low concentrations of surfactants on drug absorption (3). The use of goldfish, as described in previous reports from this laboratory (4–6), obviates many of these problems (3). Good correlation has been noted between the results of drug absorption studies with goldfish and rats (5, 7), and similarly between results obtained in rats and in man (8).[1]

A recent study in this laboratory has shown that the absorption rate of secobarbital by goldfish was increased significantly in the presence of 0.01% polysorbate 80, and decreased significantly by high concentrations (1–2%) of the surfactant (3). On the other hand, polysorbate 80 had no effect on the absorption rate of ethanol[2] and certain other alcohols (3). It was noted by appropriate physicochemical studies and from a kinetic analysis of the biologic data that the modification of secobarbital absorption by high concentrations of polysorbate 80 represents the net effect of an enhanced intrinsic absorption rate constant in the presence of the surfactant and a decreased thermodynamic activity of secobarbital resulting from micellar complexation (3). The absorption-enhancing effect of polysorbate 80 was believed to be due either to a direct effect of the surfactant on the permeability of the biologic membrane, or to the formation of a nonmicellar drug–surfactant complex which is more rapidly absorbed than the free drug (3). The available data did not make it possible to distinguish between these possibilities. The purpose of this investigation to be described here was to study the mechanism of the absorption-enhancing effect of polysorbate 80 and to determine the basis for the selectivity of this effect.

EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing 8–10 Gm., were used. All fish used in a given experiment (*i.e.*, listed in any one table) were from the same lot. The composition of drug solutions, methods for determination of absorption rate, and the method for the determination of surface tension were the same as described previously (3) except as noted in the next paragraph.

All absorption studies were carried out on groups of 5 fish immersed simultaneously in 1 L. of drug solution contained in a 2-L. capacity glass beaker placed in a water bath at 20 ± 0.5°. Pretreatment consisted of placing the 5 fish for the indicated time in 1 L. of 0.01% polysorbate 80 in 0.05 *M* THAM buffer (pH 5.9) or in THAM buffer without surfactant. The temperature of these solutions was maintained at 20 ± 0.5° by means of a water bath. After pretreatment, the fish were transferred with a fish net to a pan containing 3 L. distilled water at

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[1] This statement refers to the relative absorption rates of a series of passively absorbed drugs in solution.

[2] Results of recent studies (12) raise the possibility that the mechanism of the pharmacologic effect of ethanol on goldfish differs at low and high concentrations. However, additional experiments conducted in this laboratory have shown that polysorbate 80 has no apparent effect on ethanol absorption even at a relatively low concentration (3.5%) of the latter.

TABLE I—EFFECT OF POLYSORBATE 80 ON SECOBARBITAL ABSORPTION BY GOLDFISH[a]

Group	Polysorbate 80 Concn., % w/v	Time of Death,[b] min.	Significantly Different ($p < 0.01$) from Group	Surface Tension,[c] dyne/cm. Before	After
A	None	27.9 (9.4)[d]	*B*, *C*, *D*	63.6	64.0
B	0.0005	17.8 (4.2)	*A*, *D*	51.0	52.7
C	0.001	16.0 (3.6)	*A*	48.1	49.1
D	0.01	14.2 (1.3)	*A*, *B*	43.0	42.6

[a] Fish were immersed in 0.020% sodium secobarbital at pH 5.9 and 20 ± 0.5°. [b] Mean of 10 fish. [c] Mean of 4 determinations. [d] Standard deviation in parentheses.

TABLE II—EFFECT OF PRETREATMENT WITH POLYSORBATE 80 ON SECOBARBITAL ABSORPTION BY GOLDFISH AND ON SURFACE TENSION OF SECOBARBITAL SOLUTIONS

Pretreatment Conditions		Time of Death in Secobarbital Soln.,[a] min.	Surface Tension of Secobarbital Soln. at End of Expt.,[b] dyne/cm.
Soln.	Immersion Time, min.		
0.05 *M* Tris, pH 5.9	30	27.0 (5.8)[c]	63.7
0.01% polysorbate 80 in 0.05 *M* Tris, pH 5.9	30	21.4[d] (4.1)	63.7

[a] Mean of 20 fish immersed in 0.020% sodium secobarbital at pH 5.9 and 20 ± 0.5°. [b] Mean of 4 determinations. [c] Standard deviation in parentheses. [d] Statistically significantly different ($p < 0.01$) from group not pretreated with polysorbate 80.

TABLE III—EFFECT OF DURATION OF PRETREATMENT WITH POLYSORBATE 80 ON SECOBARBITAL ABSORPTION BY GOLDFISH

Pretreatment Conditions		Time of Death in Secobarbital Soln.,[a] min.	Significance of Difference
Soln.	Immersion Time, min.		
0.05 *M* Tris, pH 5.9	1	19.1 (4.1)[b]	$p < .05$
0.01% polysorbate 80 in 0.05 *M* Tris, pH 5.9	1	16.6 (3.1)	N.S.
0.05 *M* Tris, pH 5.9	60	20.4 (3.2)	N.S
0.01% polysorbate 80 in 0.05 *M* Tris, pH 5.9	60	16.8 (3.3)	$p < .01$

[a] Mean of 20 fish in 0.020% sodium secobarbital at pH 5.9 and 20 ± 0.5°. [b] Standard deviation in parentheses.

room temperature where they remained for 4 min. The latter procedure was repeated twice for a total of 3 washes of 4 min. each. The fish were then placed in secobarbital solution as described above.

RESULTS AND DISCUSSION

The principal investigational approach used in this study was to determine the absorption rate of secobarbital in fish previously exposed to polysorbate 80 and in fish exposed only to buffer before being immersed in secobarbital solution. An initial concern was the possibility that sufficient surfactant may be transferred mechanically together with the polysorbate 80 treated fish to the secobarbital solution so that the latter would not really be free of surfactant. It was desirable therefore to determine the concentration range in which polysorbate 80, in the presence of secobarbital, will enhance significantly the rate of absorption of the latter. The data listed in Table I show that polysorbate 80 concentrations as low as 0.0005% have a pronounced absorption-enhancing effect. Polysorbate 80 as such, in the concentrations employed in this study, has no obvious deleterious effect on goldfish (3). It was noted that surface tension measurements provide a sensitive semiquantitative indication of surfactant concentration in the range employed in this study. There was only a small and possibly insignificant increase in the surface tension of the solutions at the end of the experiment; this suggests that only a small amount of the surfactant could have been adsorbed on the fish membranes.

Exposure of goldfish to 0.01% polysorbate 80 for 30 min., followed by three washings in water, resulted in a significantly enhanced absorption of secobarbital when the fish were placed in secobarbital solutions which did not contain polysorbate 80 (Table II). The magnitude of this effect was independent of the duration of exposure of the fish to surfactant; fish placed in polysorbate 80 solution for 1 min. subsequently absorbed secobarbital as rapidly as did fish maintained in polysorbate 80 solution for 60 min. before being placed in secobarbital solution (Table III). There was no detectable mechanical carry-over of polysorbate 80 to the secobarbital solution; the effectiveness of the three washings is evidenced by the fact that the secobarbital solutions used for surfactant-treated and control fish, respectively, had the same surface tension at the end of the experiment (Table II). Results obtained in control fish were not affected by duration of immersion in buffer solution (1 *versus* 60 min.) preceding their placement in secobarbital solution. Preliminary experiments had shown also that the response to secobarbital of fish

TABLE IV—EFFECT OF POLYSORBATE 80 ON ABSORPTION OF NONIONIZED AND IONIZED FORMS OF SECOBARBITAL BY GOLDFISH

Compn. of Soln.					
Polysorbate 80, %	Sod. Secobarbital, %	pH[a]	Time of Death,[b] min.	Ratio of Time of Death With:Without Polysorbate	k_n/k_i[c]
None	0.020	6.9	41.4 (8.5)[d]	0.52	16.6
0.01	0.020	6.9	21.6 (3.8)		
None	0.125	8.9	40.7 (13.3)	0.52	16.5
0.01	0.125	8.9	21.2 (3.4)		

[a] 0.05 *M* Tris buffer at 20 ± 0.5°. [b] Mean of 10 fish. [c] Ratio of absorption rate constants for nonionized and ionized secobarbital. [d] Standard deviation in parentheses.

subjected to the several transfers required for pretreatment and subsequent washings did not differ from the response of fish placed directly in secobarbital solution. The times of death of control fish and pretreated fish in the experiments listed in Table II were somewhat longer than those in the experiments listed in Table III, but the relative magnitude of the surfactant effect was similar in each set of experiments. The absolute difference in death times of the control fish in the two sets of experiments is due to the use of different lots of fish. This lot to lot difference has been found previously and necessitates the use of appropriate controls in each set of experiments.

The experimental results listed in Tables II and III show that polysorbate 80 can increase the absorption rate of secobarbital in goldfish even if the two substances are not present together in the bulk phase of the solution. This leads to the conclusion that the absorption-enhancing effect of polysorbate 80 is due to a direct effect of the surfactant on the permeability characteristics of the fish membranes. The experimental results do not support an assumption that the enhanced absorption of secobarbital is due to the formation of a more rapidly absorbed nonmicellar drug–surfactant complex (9). In addition, no physicochemical evidence for such a complex in aqueous solutions containing up to 0.01% polysorbate 80 has been found in this laboratory (3).

The experimental data suggest that a small amount of polysorbate 80 is adsorbed rapidly on the absorbing membranes of fish, and that this in turn causes a more rapid absorption of secobarbital and certain other drugs. The fact that exposure of the fish to polysorbate 80 for 1 min. elicits the same effect as exposure for 60 min. shows not only that the surfactant acts rapidly, but also that the effect is relatively time independent. This is in agreement with the result of the previously reported kinetic analysis of the effect of polysorbate 80 on secobarbital absorption (3); the surfactant effect could be expressed in terms of a rate constant for the enhanced absorption and thus appeared to be essentially time independent. This would not be expected to occur if the surfactant acts by slowly removing lipoid constituents from the membrane or by causing the leaching out of some other membrane component. The rapid effect of polysorbate 80 is more consistent with a mechanism involving adsorption of surfactant molecules on the biologic membrane, thereby making the latter more permeable to certain drugs. Such a mechanism *could* involve interaction of adsorbed surfactant with the drug, thereby "augmenting the amount of drug on the surface of the biologic membranes" (9). Thus, a distinction between a membrane effect and a complexing effect as the basis for the absorption-enhancing mechanism of polysorbate 80 is a matter of semantics; the present study distinguishes between a membrane effect (including a possible complex formation between *membrane-bound* surfactant and drug) and an effect due to the formation of nonmicellar drug–surfactant complex in the *bulk phase* of the solution. It is apparent from the tabulated data that polysorbate 80 exerts a more pronounced effect on secobarbital absorption in the presence of the drug than when used as a pretreatment. This is probably due to partial desorption of surfactant from the fish membranes during the washings.

The failure of polysorbate 80 to enhance the absorption of low molecular weight alcohols and its effectiveness in increasing the absorption of two lipoid-soluble barbiturates of considerably higher molecular weight suggested that the surfactant might selectively increase absorption across the lipoid portion of biologic membranes without affecting the rate of drug permeation through membrane pores (3). These considerations (and a provisional assumption that secobarbital anion is absorbed solely or mainly by the pore route) led to an experiment to assess the relative effect of polysorbate 80 on the absorption rate of the nonionized and ionized forms of secobarbital, respectively. This involved determination of secobarbital absorption at pH 6.9 and 8.9 with and without surfactant (Table IV). Secobarbital has a pKa of 7.9 at 20° (10) and is about 90% nonionized at the low pH and 90% ionized at the higher pH. A more pronounced surfactant effect on the absorption of the nonionized form of secobarbital would be reflected by a greater overall effect at low pH than at higher pH where ionized secobarbital contributes appreciably to the total absorption. In fact, the surfactant affected the absorption of both species to an equal degree (Table IV).[3] This is evident not only from the respective ratios of times of death with and without surfactant, but also from the ratios of the rate constants for the absorption of nonionized and ionized secobarbital in the absence and presence of polysorbate 80. The ratios of these rate constants were calculated from two simultaneous equations of the form:

[3] Note also that the *relative* effect of 0.01% polysorbate 80 in the experiments listed in Table IV is exactly the same (50% decrease in time of death) as in the experiments listed in Table I.

$$L = k_n C_n T_L + k_i C_i T_L \qquad \text{(Eq. 1)}$$

where L is the lethal dose of secobarbital, k_n and k_i are the rate constants for the absorption of nonionized and ionized secobarbital, respectively, C_n is the concentration of nonionized secobarbital, C_i is the concentration of ionized secobarbital, and T_L is the time of death (4, 5). The experimental data obtained at pH 6.9 and 8.9 were used for each pair of equations, one for drug absorption in the absence of polysorbate 80, the other for absorption in the presence of the surfactant. The $k_n:k_i$ ratio was 16.5 in each case, showing that the absorption of ionized and nonionized secobarbital was enhanced equally by polysorbate 80. The experiments listed in Table IV were designed to yield a relatively constant T_L irrespective of pH by using an appropriately higher over-all concentration of secobarbital at pH 8.9 as determined from preliminary experiments. The $k_n:k_i$ ratio of 16.5 obtained in this manner is in relatively good agreement with the ratio of about 10 determined several years ago in this laboratory by an entirely different experimental approach where the over-all concentration of secobarbital was kept constant and T_L was variable (4).

The results of this study provide a better understanding of the nature of the absorption-enhancing effect of polysorbate 80 although the exact mechanism, and the basis for its specificity, must still remain speculative. One possibility is that surfactant molecules adsorbed to the surface of the biologic membrane interact with barbiturates [but not with the low molecular weight alcohols studied previously (3)] and thereby enhance to an equal degree the penetration of nonionized barbiturates across the lipoid portion of the membrane and the diffusion of barbiturate anions through pores in the membrane. This mechanism is difficult to accept on a rigorous basis, particularly since there is no evidence for nonmicellar complexation of secobarbital with polysorbate 80 at concentrations up to 0.01% of the latter. In addition, significant micellar complexation of secobarbital by polysorbate 80 occurs only with the nonionic form of secobarbital (11). It seems much more likely that secobarbital anion (mol. wt. >200) is absorbed solely or primarily across the lipoid portion of biologic membranes rather than through pores in the membrane, *i.e.*, by the same route as nonionized secobarbital. The similarity in the absorption-enhancing effect of polysorbate 80 on the ionized and nonionized forms of secobarbital, and the lack of effect on the absorption of low molecular weight alcohols, can then be explained by assuming that the surfactant affects drug absorption across the lipoid barrier portion of the biologic membrane but does not affect diffusion of drug through membrane pores.

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Keyphrases

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