Effect of Polysorbate Hydrolysis Products on Biologic Membrane Permeability

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Abstract □ Low concentrations (0.01 percent or less) of polysorbate 20 and polysorbate 80 increase the rate of absorption of secobarbital in goldfish. Equimolar concentrations of the polyol moiety [polyoxyethylene (20) sorbitan] have no effect, but the respective fatty acids (lauric and oleic) have a significant enhancing effect on secobarbital absorption. Polysorbate 20 is more effective than polysorbate 80; the opposite is true for their respective fatty acid components. The relationship between absorption-enhancing effect and surface tension (or concentration) differs with each of the surfactants.

Keyphrases □ Membrane permeability—polysorbate hydrolysis products □ Secobarbital absorption—goldfish □ Polysorbate fatty acid components—secobarbital absorption □ Surface tension effect—secobarbital absorption

Studies in this laboratory have shown that low concentrations of polysorbate 80 [polyoxyethylene (20) sorbitan monooleate] increase the rate of absorption of certain lipoid soluble compounds by goldfish (1–3). This surfactant also increases the permeability of rat ascites hepatoma cells to nitrogen mustard *N*-oxide (4). The data of Matsumoto (5) and Yamada *et al.* (6) suggest that polysorbate 80 may increase the absorption of free sulfisoxazole, sulfanilamide, and methyl salicylate from the rat small intestine since the observed absorption rate constants in the presence of the surfactant, when corrected for the degree of micellar complexation of the respective drugs, are consistently somewhat higher than the absorption rate constants in the absence of the surfactant. The polysorbates are readily hydrolyzed by pancreatic lipase in the gastrointestinal tract to fatty acid and polyol (polyoxyethylene sorbitan) but there is little or no splitting of the ether bond between the polyoxyethylene group and the sorbitan moiety (7–10). It is of interest therefore to determine the effect of the physiologic hydrolysis products of polysorbate, *i.e.*, the fatty acid and the polyol, on membrane permeability. The study to be described here deals with the effect of polysorbates 20 and 80, their polyol moiety, and their respective fatty acids, on the rate of absorption of secobarbital by goldfish.

Table I—Effect of Polysorbate 80 and its Hydrolysis Products on Secobarbital Absorption by Goldfish

Composition of Solution: Sodium Secobarbital, %	pH[a]	Additives[b]	Time of Death min. (*SD*)[c]	Time of Death Ratio[d]	Surface Tension, dynes/cm.[e]
0.020	5.9	—	55.8 (4.3)	1.000	63.6
0.020	5.9	Polysorbate 80	23.9 (2.1)	0.428	43.0
0.020	5.9	Polyol[f]	54.2 (5.2)	0.971	60.5
0.020	5.9	Oleic acid	Not soluble		
0.130	8.9	—	46.0 (6.4)	1.000	63.6
0.130	8.9	Polysorbate 80	25.0 (3.7)	0.543	41.5
0.130	8.9	Polyol	50.6 (11.0)	1.100	62.7
0.130	8.9	Oleic acid	10.0 (2.0)	0.217	33.2
—	5.9	Polysorbate 80	—[g]		
—	8.9	Polysorbate 80	—		
—	5.9	Polyol	—		
—	8.9	Polyol	—		
—	5.9	—	—		
—	8.9	—	—		
—	5.9	Oleic acid	Not soluble		
—	8.9	Oleic acid	234 (±23)		35.7

[a] 0.05 *M* Tris, at 20 ± 0.5°. [b] $7.05 \times 10^{-5} M$, equivalent to 0.01% polysorbate 80. [c] Mean of five fish. [d] Relative to solution of same pH without additive. [e] Mean of four determinations. [f] Polyoxyethylene (20) sorbitan. [g] No deaths in 24 hr.

EXPERIMENTAL

The absorption rate of secobarbital by goldfish was determined by the time of death method (2) and surface tensions were measured with the Cenco du Nouy tensiometer (1) as described previously.

Materials—Sodium secobarbital, USP,[1] tris (hydroxymethyl) aminomethane (Tham),[2] polysorbate 20 and polysorbate 80,[3] total polyol of polysorbate 80,[4] sodium oleate purified,[5] lauric acid 99%.[6] The molecular weight of the surfactants was calculated by adding the molecular weight of the respective fatty acids, minus 18, to the weight average molecular weight of 1,155 for the polyol moiety (11).

RESULTS AND DISCUSSION

The results of studies with polysorbate 80 and its hydrolysis products are listed in Table I. The surfactant, at a concentration of 0.01%, decreased significantly ($p < 0.01$) the time of death of the fish in secobarbital solutions of pH 5.9 (where essentially all of the barbiturate is in nonionized form) and pH 8.9 (where the barbiturate is 90% ionized). The polyol moiety had no measurable effect at either pH. Oleic acid had a pronounced effect, significantly greater ($p < 0.01$) than that of an equimolar concentration of polysorbate 80, on the time of death at pH 8.9. At that pH the fatty acid is likely to be completely ionized, since its pKa is probably around 5 (12). Fish observed for more than 24 hr. (far longer than the duration of any experiment) appeared completely normal in pH 5.9 and 8.9 buffer, and in solutions of polysorbate 80 and polyol. Fish in pH 8.9 oleate solution survived for an average of 234 min. It appears unlikely that the effect of oleate on the time of death of fish exposed to secobarbital was due to a direct toxic action of the fatty acid.

The results of studies with polysorbate 20 and its fatty acid hydrolysis product (lauric acid) are listed in Table II. The polyol moiety of polysorbate 20 is the same as that of polysorbate 80, and its lack of effect is already established (Table I). Polysorbate 20, at a molar concentration equivalent to 0.01% polysorbate 80 and at one-tenth that concentration, decreased significantly ($p < 0.01$) the time of death of goldfish in secobarbital solutions of pH 6.9 and 8.9, *i.e.*, at ±1 pH unit from the pKa of 7.9 for secobarbital (13). An equimolar concentration of lauric acid also had a significant effect ($p < 0.01$ at pH 6.9 and < 0.05 at pH 8.9 *versus* the control), but this effect was not as pronounced as that of polysorbate 20 ($p < 0.01$ comparing lauric acid to polysorbate 20). While the dissociation constants for

[1] Ruger Chemical Co.
[2] Nutritional Biochemicals Corp.
[3] Tween 20 (lot no. 745) and Tween 80 (lot no. 586), Atlas Chemical Industries, Wilmington, Del.
[4] Obtained through the courtesy of Dr. Joseph F. Treon (TC No. 4880, Atlas Chemical Industries).
[5] Lot no. 792829, Fisher Scientific Co.
[6] Lot no. 5574, Nutritional Biochemicals Corp.

Table II—Effect of Polysorbate 20 and its Fatty Acid Hydrolysis Product (Lauric Acid) on Secobarbital Absorption by Goldfish

Composition of Solution					
Secobarbital, %	pH[a]	Additives[b]	Time of Death min. (SD)[c]	Time of Death Ratio[d]	Surface Tension, dynes/cm.[e]
0.020	6.9	—	41.6 (4.6)	1.000	63.8
0.020	6.9	Polysorbate 20	12.7 (0.9)*	0.305	50.6
0.020	6.9	Polysorbate 20	8.7 (1.1)	0.209	38.5
0.020	6.9	Lauric acid	26.1 (3.9)	0.627	57.1
0.130	8.9	—	49.3 (8.7)	1.000	64.2
0.130	8.9	Polysorbate 20	15.4 (1.2)*	0.312	52.0
0.130	8.9	Polysorbate 20	11.3 (1.9)	0.229	38.9
0.130	8.9	Lauric acid	33.4 (8.7)	0.677	58.7
—	6.9	Lauric acid	—[f]		62.6
—	8.9	Lauric acid	—		66.6
—	5.9	Polysorbate 20	—		
—	8.9	Polysorbate 20	—		

[a] 0.05 *M* Tris, at 20 ± 0.5°. [b] 7.05 × 10^{-5} *M*, equivalent to 0.01% polysorbate 80, except items marked by asterisk, which are 7.05 × 10^{-6} *M*. [c] Mean of five fish. [d] Relative to solution of same pH without additive. [e] Mean of at least three determinations. [f] No deaths up to 550 min.

the normal fatty acids have apparently been determined only up to a chain length of nine carbons due to the poor water solubility of the acids of longer chain length (12), it is likely that lauric acid is almost completely ionized at pH 6.9 and 8.9. Solutions containing surfactant or lauric acid, but not secobarbital, did not cause death of the fish in 550 min., the duration of this experiment.

The utility of the time of death method for determining drug absorption rates in goldfish has been demonstrated previously (14, 15). The time of death due to immersion of the fish in drug solution is inversely proportional to the absorption rate of the drug and the results obtained by this method agree well with results obtained by chemical assay (3). Of interest is the relationship between the surface tension of the various solutions and their effect on secobarbital absorption. This is shown in Fig. 1, where times of death are expressed as a ratio relative to the times of death observed with solutions containing only secobarbital and buffer. This method of data presentation corrects for differences in the sensitivity of different lots of animals to secobarbital, and for differences in pH and barbiturate concentration. It is evident that the effect of the two surfactants and their respective fatty acids on secobarbital absorption is not simply reflected by the surface tension of the solutions, although there is a rank-order relationship between time of death ratio (TDR) and surface tension with any one surfactant and its fatty acid component. This relationship is not affected by pH in the pH range 5.9 to 8.9. The shape of the TDR *versus* surface tension curve is different for each of the surfactants (Fig. 1). Oleate is much more effective than laurate. Esterification with the polyol reduces the surface tension-lowering and absorption-enhancing effect of oleate, but has the opposite effect on laurate. Polysorbate 20 has a much more pronounced effect on secobarbital absorption than does polysorbate 80, regardless of whether the comparison is based on the effect of equimolar concentrations or on solutions of equal surface tension.[7] These observations raise interesting questions about the effect of fatty acids, their salts, and their esters on the permeability of biologic membranes. Studies are now in progress in this laboratory to determine the relationship between these effects and the physicochemical properties of fatty acids and their derivatives.

[7] The experiments with the two surfactants were repeated with different lots of polysorbates, and yielded essentially the same results. This indicates that the quantitative differences in the effects of the two surfactants are unlikely to have been due to the accidental presence of a contaminant in one of the materials.

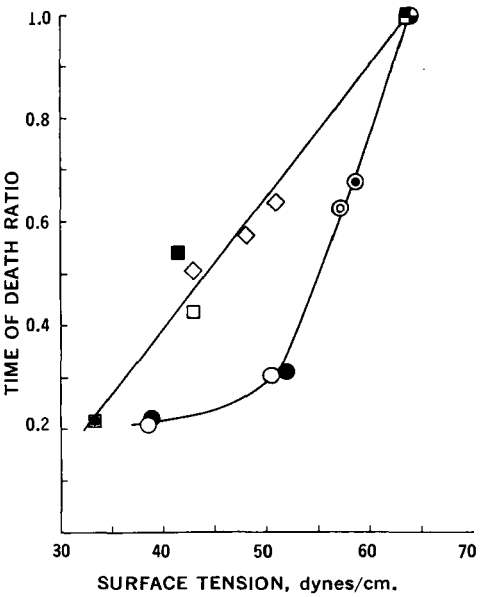

Figure 1—*Relationship between time of death ratio (with : without surfactant or fatty acid) of goldfish and surface tension of secobarbital solutions containing polysorbate or its fatty acid hydrolysis product. Key: Circles, polysorbate 20 series; squares, polysorbate 80 series. Open symbols, pH 5.9 or 6.9; closed symbols, pH 8.9. Symbols with border represent the fatty acid of the series. Diamonds, data from a previous study (2).*

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