Effect of Complex Formation on Drug Absorption III

Concentration- and Drug-Dependent Effect of a Nonionic Surfactant

By GERHARD LEVY, KAREN E. MILLER, and RICHARD H. REUNING*

The effect of various concentrations of the nonionic surfactant polysorbate 80 on the absorption of a number of alcohols and barbiturates by goldfish has been studied. The absorption rate of the barbiturates was increased significantly in the presence of low concentrations of polysorbate 80, and decreased by higher concentrations of the surfactant. The absorption rate of the alcohols studied was not affected significantly by the surfactant. The retardation of barbiturate absorption at higher polysorbate 80 concentrations, which occurred also during mechanical agitation of the solution (when diffusion of drug to the absorbing membranes is definitely not absorption rate limiting), is interpreted as being indicative of the absence of a dissociating effect of the biologic membranes on the drug–micelle complex. The drug–micelle complexes differ in this respect from the nonmicellar dye complexes studied previously, apparently due to the greater exposure of substances in simple 1:1 complexes. Equilibrium dialysis and surface tension determinations have been carried out in an attempt to elucidate the mechanisms of the effects of polysorbate 80 on drug absorption. It is shown by kinetic analysis that the modification of barbiturate absorption by polysorbate 80 represents the net effect of enhanced absorption and decreased thermodynamic activity of the drug due to micellar complexation.

NUMEROUS studies of the effect of surfactants on drug absorption have shown that these agents can either increase, decrease, or exert no apparent effect on the transfer of drugs across biologic membranes (1). Some of the complexities and biopharmaceutical aspects of this problem have been reviewed recently (2). It is now appreciated that the type and magnitude of effect can be a function of the concentration (3) and chemical nature of the surfactant and that a given surfactant also may have certain specific pharmacologic properties of its own (2). It has been suggested by one of the authors that the observed effect of a surfactant on absorption may represent, in a certain concentration range, the net result of both enhancement and retardation of absorption (2).

Most of the studies of surfactant effects on drug absorption have been carried out on microbial systems. The results thus obtained may have limited applicability to multicellular organisms, since the latter are able to maintain homeostasis much more effectively. Moreover, the presence of enzymes and other vital cell constituents in the cell membrane makes unicellular organisms particularly sensitive to direct effects of surfactants. Absorption studies using small animals, isolated intestines, or man present other difficulties—dilution effects and interaction with mucus and/or other components of intestinal fluids and tissues make it practically impossible to maintain a constant known concentration of surfactant and drug. The results of such experiments, therefore, can be interpreted only qualitatively or, at best, semiquantitatively. These difficulties are not encountered when using fish for absorption studies (4–6). The major advantage of the fish system is due to the large volume of drug solution which may be used; this permits the maintenance of an essentially constant concentration gradient of drug and surfactant across the biologic membrane, despite continuous absorption of the drug and possible binding of some of the surfactant to mucus and/or membrane constitutents. Previous studies have shown that the drug absorption characteristics of fish membranes are similar to those of rats (5), while the latter yield results similar to those obtained in man (7).[1]

The investigation described here is part of a continuing study of the effect of complex formation of drug absorption (8, 9). The purpose of the presently described investigation was to determine the effect of various concentrations of a representative nonionic surfactant, polysorbate 80, on the absorption of certain noninteracting and interacting drugs by goldfish. It was desired also to elucidate the mechanism of the observed effects, and to examine their relationship to certain physico-chemical characteristics of the drug–surfactant system.

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[1] This statement refers to absorption by passive diffusion; there are appreciable differences between species in active transport characteristics.

EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing from 15 to 25 Gm., were used. All fish utilized in a given set of experiments were from the same lot.

Drug Solutions.—All solutions were prepared in bulk on the day of the experiment from reagent, U.S.P., or N.F. grade chemicals. The drugs were dissolved in 0.05 *M* tris(hydroxymethyl)aminomethane (Tham) buffer, adjusted to pH 5.9 at 20° with hydrochloric acid.

Determination of Absorption Rate.—Single goldfish were placed in 250-ml. capacity beakers containing 175 ml. of drug solution at 20 ± 1°. The time of death, evidenced by cessation of gill and mouth movements, was noted. Absorption rate constants were calculated as previously described (5).

All determinations of time of death were carried out by the same individual. Prior to the experiment, all beakers containing the various drug solutions were labelled with code numbers by an individual not otherwise associated with the study. The codes were broken only after completion of the experiment.

Effect of Stirring on Time of Death.—Single goldfish were placed in 250-ml. capacity beakers containing 100 ml. drug solution at 28 ± 1°. Half of the solutions were stirred with a magnetic stirrer at about 500 r.p.m. The dimensions of the stirring bar were: diameter 0.25 cm., length 1.7 cm. The solutions could not be coded in this experiment since foaming on stirring made the surfactant-containing solutions readily recognizable.

Determination of Micellar Complexation.—The possible binding of ethanol by polysorbate 80 was investigated by equilibrium dialysis, using a method similar to that described previously (8). Ten milliliters of 2% w/v polysorbate 80 in 0.05 *M* Tham, pH 5.9, was placed in nylon dialysis bags.[2] Each bag was suspended in a 125-ml. conical flask containing 110 ml. of 2% v/v ethanol in 0.05 *M* Tham, pH 5.9. The solutions were equilibrated for 14 to 20 days at room temperature. The concentration of ethanol inside and outside the dialysis bag was then determined by the method of Hoult and Pawan (10).

The binding of secobarbital by polysorbate 80 was determined also by equilibrium dialysis. A number of different drug and surfactant concentrations were employed. The solvent system was 0.05 *M* Tham at pH 5.9. Due to the very poor permeability of the nylon membrane to secobarbital, cellulose dialyzer tubing[3] was used. In agreement with observations by others (11), this material was found to be impermeable to polysorbate 80. Equal concentrations of secobarbital were placed initially inside and outside the dialysis bag, and the solutions were equilibrated for 2 to 4 days at 20 ± 1°. The concentration of secobarbital on each side of the dialysis bag then was determined spectrophotometrically at 255 mμ, using 0.5 *M* sodium hydroxide solution as the diluent and blank. Polysorbate 80 interference in the secobarbital assay was additive; analytical results from polysorbate 80-containing solutions were corrected appropriately. The binding data yielded Freundlich-type isotherms (8) which permitted determination of the degree of binding at various total secobarbital concentrations.

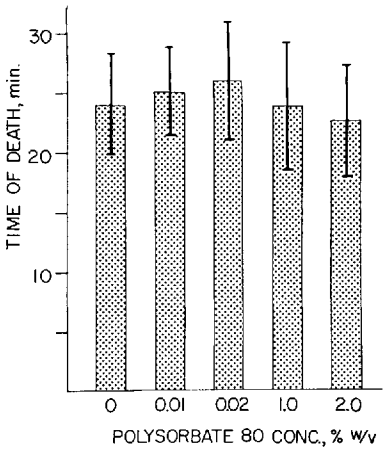


Fig. 1.—Effect of polysorbate 80 on the time of death of goldfish immersed in 5% ethanol solution (pH 5.9, 20°). Mean of 10 fish each. Vertical bars indicate ±1 standard deviation.

Surface Tension Determinations.—Surface tensions were determined with a Du Nouy tensiometer[4] at 20 ± 1°, using standard procedures for cleaning of the ring and for correction of the instrumental readings (12). To reduce surface aging effects, the solutions were swirled and agitated moderately immediately before each reading. The reported values are therefore dynamic rather than equilibrium values.

RESULTS AND DISCUSSION

It was found in preliminary experiments that immersion of goldfish in 0.01 to 2.0% polysorbate 80 solutions for 24 hr. had no apparent deleterious effect on the fish. This is consistent with the very low acute systemic toxicity of polysorbate 80 in other animals; for example, Nissim (13) reported that subcutaneous injection of up to 8 Gm. of polysorbate 80 per Kg. body weight did not produce any untoward effects in mice.

Polysorbate 80 had no significant effect on the rate of absorption of ethanol by goldfish, as judged by the time of death of the fish after immersion in 5% ethanol solution containing up to 2.0% of the surfactant (Fig. 1). Equilibrium dialysis showed that there was no binding of ethanol by polysorbate 80.

The surfactant had a pronounced, concentration-dependent effect on the absorption rate of secobarbital (Fig. 2). The barbiturate was absorbed more rapidly in the presence of low concentrations of polysorbate 80; higher concentrations of the surfactant decreased the rate of absorption significantly. Equilibrium dialysis indicated that there was considerable binding of secobarbital by polysorbate 80 when the concentration of the surfactant was in the range which caused retardation of secobarbital absorption. Since a previous study

[2] Tomac Nylon bags, American Hospital Supply Corp., Evanston, Ill.
[3] Fisher Scientific Co Model 70545, Central Scientific Co. Chicago Ill.

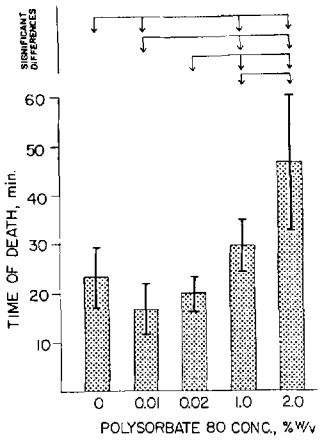


Fig. 2.—Effect of polysorbate 80 on the time of death of goldfish immersed in 0.020% sodium secobarbital solution (pH 5.9, 20°). Mean of 10 fish each. Vertical bars indicate ±1 standard deviation. Arrows connect values which differ significantly ($p < 0.05$) from one another.

(9) has shown that biologic membranes can have a dissociating effect on certain types of complexes, an experiment was designed to determine if the absorption-retarding effect of polysorbate 80 was due to the decreased thermodynamic activity of secobarbital as a consequence of its partial micellar complexation, or if the effect is due to a decreased rate of diffusion of the drug in micelles to the biologic membranes. For this purpose, the effect of polysorbate 80 on secobarbital absorption from intensively stirred solutions was compared to the effect obtained in unstirred solutions. There was no difference in the respective ratios of the times of death in solutions with polysorbate 80 to those without polysorbate 80 (Table I). Since the absorption-retarding effect of the surfactant was present also during rapid stirring, where diffusion of drug molecules and molecular aggregates is definitely not absorption rate limiting,[5] it may be concluded that fish membranes do not have a dissociating effect on secobarbital–polysorbate 80 micellar complexes. The difference in the effect of biologic membranes on micellar complexes and certain simple 1:1 complexes (9) is due probably to the greater exposure of drugs in the latter. This permits better contact and facilitates the interaction between the drug and the biologic membrane.

Differences in the times of death listed in Table I and those shown in Fig. 1 reflect the effect of temperature on the absorption rate and the lethal dose of secobarbital, and on the binding of the drug by polysorbate 80. Technical difficulties made it impossible to carry out the stirring experiments at 20°. Stirring itself apparently enhanced drug absorption; this was due probably to the more rapid flow of drug solution through the mouth and thereby across the gills. The gills are responsible for about 50% of the total drug absorption (6).

In view of the apparent lack of dissociating effect of the biologic membranes on the micellar complex

[5] It is believed that this is true even in unstirred solutions, due to the constant movement of the fish. The described experiment was carried out to establish this fact under more rigorous conditions.

Table I.—Secobarbital Absorption by Goldfish from a Stirred and an Unstirred Medium[a]

Concn. of Polysorbate 80, w/v	Stirring	Time of Death,[b] min.	Ratio of Times of Death, With Polysorbate: Without Polysorbate
0	no	19.0 ± 5.7	1.5
2	no	28.6 ± 7.4	
0	yes	13.8 ± 2.8	1.5
2	yes	20.8 ± 3.9	

[a] 0.020% sodium secobarbital in 0.05 *M* Tham buffer, pH 5.9. [b] Mean of 5 animals ±1 standard deviation; determined at room temperature (28 ± 1°).

of secobarbital and polysorbate 80, and the absorption enhancing effect of polysorbate 80 itself, the model shown in Scheme I is believed to represent the over-all effect of the surfactant.

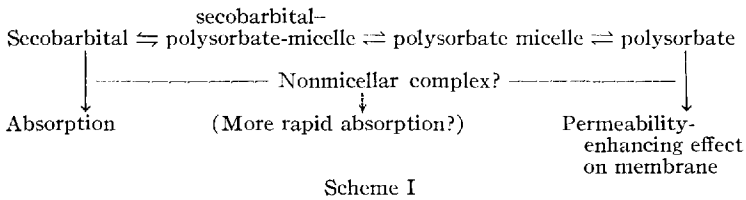
The possibility that the more rapid absorption of secobarbital in the presence of low concentrations of polysorbate 80 is mediated by a nonmicellar secobarbital–polysorbate 80 complex will be considered in a subsequent paragraph. Disregarding this possibility, a kinetic model based on an equation derived previously (4, 5) would consist of the following relationships.

No polysorbate present: $k = L/(CT_L)$,
Polysorbate concentrations below CMC: $k' = L/(CT_L)$
Polysorbate concentrations above CMC: $k' = L/(C_f T_L)$

where k and k' represent the "normal" and the "enhanced" absorption rate constant, respectively; L is the lethal dose of the drug; C and C_f are the concentrations of total and free drug, respectively; T_L is the time of death, and CMC is the critical micelle concentration. This model may be verified by showing that the value of k' remains constant over a wide concentration range of polysorbate 80.

The apparent and corrected constants for secobarbital absorption in the presence of various concentrations of polysorbate 80 are listed in Table II. The corrected constants are based on the concentration of free secobarbital as determined by equilibrium dialysis. The low concentration of polysorbate (0.01%) is approximately the critical micelle concentration (14). Binding of secobarbital at this concentration was either absent or very slight (<5%). The values of k' were reasonably constant at 0.01, 1.0, and 2.0% polysorbate 80 concentrations and were appreciably greater than k. These results verify, within the limits of experimental accuracy, the kinetic model presented in the preceding paragraph. The data show also that the effect of the surfactant above the CMC represents the net result of absorption-enhancing and retarding effects, as has been suggested previously by one of the authors (2).

The question arises why polysorbate 80 enhances the absorption of secobarbital but has no such effect on the absorption of ethanol. It was thought possible that ethanol itself decreases the surface tension of the solution so much that addition of polysorbate 80 would have no appreciable additional effect. This possibility was ruled out experimentally (Table III). Moreover, it was found that



Scheme I

TABLE II.—RATE CONSTANTS[a] FOR SECOBARBITAL ABSORPTION IN GOLDFISH

Concn. of Polysorbate 80, % w/v	Concn. of Sodium Secobarbital, % w/v	Free Drug, %	Time of Death,[b] min.	Apparent[c] k L. Gm.$^{-1}$ min.$^{-1}$ × 10^6	Corrected[d] k L. Gm.$^{-1}$ min.$^{-1}$ × 10^6
0	0.020	100	23.2		8.19
0.010	0.020	>95	16.6		11.4
1.0	0.020	50	29.3	6.48	13.0 } k'
2.0	0.020	36	46.2	4.11	11.4

[a] $k = L/(CT_L)$, where L = 0.038 mg./Gm. body weight. [b] Mean value, based on 10 animals; determined at 20 ± 1°. [c] Based on total secobarbital concentration. [d] Based on free secobarbital concentration.

the surface tensions of secobarbital–polysorbate 80 and ethanol–polysorbate 80 solutions were quite similar at any given surfactant concentration. Alexander and Trim (3) have suggested that the enhanced absorption of a drug in the presence of surfactant concentrations below the CMC may be due to formation of a nonmicellar complex of increased interfacial activity which augments the amount of drug on the surface of the biologic membranes. No evidence for an association of secobarbital and polysorbate 80 at concentrations of 0.005 and 0.01% of the latter was obtained from solubility, equilibrium dialysis, and ultraviolet absorption data. However, it is possible that such interactions may yet be found by other, more sensitive methods.

Another possible reason for the difference in the effect of polysorbate 80 on the absorption of ethanol and secobarbital, respectively, can be related to the different routes of absorption of these 2 drugs. Ethanol can diffuse across membranes through pores, while secobarbital diffuses through the lipoid barrier (4). Polysorbate 80 in 0.01% concentration had no significant effect on the absorption of other low molecular weight alcohols but increased significantly the absorption of another barbiturate (Fig. 3). This lends support to the theory that polysorbate 80 could have a specific effect on the permeability of the lipoid barrier portion of the biological membrane. There is some direct and biochemical evidence which suggests such an effect (15).

TABLE III.—EFFECT OF POLYSORBATE 80 ON SURFACE TENSION OF DRUG SOLUTIONS

Drug[a]	Polysorbate 80 Concn., % w/v	Surface Tension,[b] dynes/cm.
0.02% Sod. secobarbital	none	62.9
same	0.010	40.5
same	0.020	39.5
same	1.0	38.7
same	2.0	38.4
5% Ethanol	none	55.4
same	0.010	41.5
same	0.020	40.8
same	1.0	38.7
same	2.0	39.2

[a] Dissolved in 0.05 M Tham, adjusted with HCl to pH 5.9 at 20°. [b] Mean of 4 determinations, obtained at 20 ± 1°.

Figure 3 shows also the results of another experiment with ethanol on a larger number of fish. The purpose of this experiment was to establish definitely that 0.01% polysorbate 80 had no effect on ethanol absorption. The results obtained with *n*-octanol, also depicted in Fig. 3, cannot be interpreted readily because the data were quite variable and since there was some evidence of formation of mixed micelles.

In summary, it appears that the absorption-enhancing effect of polysorbate 80 may be due to the formation of a nonmicellar drug–surfactant complex, or that it may represent a direct action of the surfactant on the lipoid barrier portion of the biologic membrane. The present data do not permit distinction between these possibilities. It is reasonable to assume that an effect of the surfactant on biologic membranes should be time-dependent, yet the kinetic analysis (which assumes a time-independent effect) gives no such indication. However, the time course of the permeability-

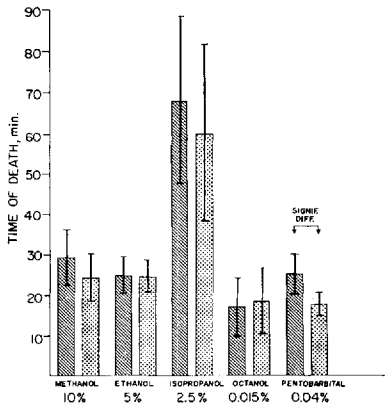


Fig. 3.—Effect of 0.010% polysorbate 80 on the time of death of goldfish immersed in solutions of various drugs in 0.05 M Tham (pH 5.9, 20°). Ethanol data are mean of 26 fish each, all others are the mean of 10 fish each. Vertical bars indicate ±1 standard deviation. Key: ▨, without polysorbate; ▩, with polysorbate 80, 0.01%.

enhancing effect could be such that most of the effect is elicited within a few minutes. Alternatively, the surfactant may promote better interfacial contact and thus increase the effective surface area of the membrane. Studies are now being initiated to determine if immersion of the fish in surfactant solutions for various periods of time will affect the rate of absorption of secobarbital upon subsequent immersion of the fish in secobarbital solutions without surfactant. This should establish whether or not the surfactant promotes drug absorption by modifying the barrier properties of the biologic membranes. However, the present investigation has shown already that the effect of polysorbate 80 on drug absorption is a function of the drug and of surfactant concentrations, and that an effect of polysorbate 80 concentrations above the CMC can represent the net result of absorption enhancement and retardation.

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Comparative Absorption of Micronized and Nonmicronized Medroxyprogesterone Acetate in Man

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A specific, sensitive method has been developed for the analysis in urine of microgram quantities of the principal urinary metabolite of medroxyprogesterone acetate. The method consists essentially of the following steps: hydrolysis with β-glucuronidase, extraction with chloroform, Florisil column chromatography, thin-layer silica gel chromatography, and measurement of either ultraviolet absorption or fluorescence resulting from sulfuric acid treatment. The method has been used to compare the gastrointestinal absorption of medroxyprogesterone acetate from tablets containing either 10 mg. of micronized or nonmicronized medroxyprogesterone acetate. The 8-hr. excretion of metabolite following oral ingestion of the tablets by normal adult humans was employed as the measure of absorption. The increased metabolite output, resulting from the tablet prepared from micronized medroxyprogesterone acetate, was very highly significant ($p < 0.001$). Ten subjects in a crossover study excreted an average of 2.23 ± 0.19 (S.E.M.) times as much metabolite in 8 hr. after ingesting the micronized formulation as they did after ingesting the nonmicronized one.

THIS STUDY was undertaken to develop an analytical method for the purpose of determining whether a tablet prepared from 10 mg. of micronized medroxyprogesterone acetate[1] (I) would afford a significant increase in absorption compared to a tablet prepared from nonmicronized material. Since medroxyprogesterone acetate has very low solubility in water (~0.3 mg./100 ml. at 37°), its gastrointestinal absorption may be limited by its gastrointestinal dissolution rate; reducing its particle size, therefore, might be expected to increase its physiologic availability (1–4). Helmreich and Huseby (5), who employed doses of 50–200 mg. of medroxyprogesterone acetate, have already noted that particle size reduction might influence its absorption efficiency.

Helmreich and Huseby (6) identified the principal urinary metabolite of medroxyprogesterone acetate as 6β,17α,21-trihydroxy-6-methyl-pregn-4-ene-3,20-dione,17-acetate (II). Others (7) have reported it to be the 21-acetate (III). This metabolite, which is excreted in the human as a glucuronide, accounts for approximately one-half of the total drug-related material excreted in the urine (5). The 24-hr. urinary output in the human was found to range from about 4–8% of a 200-mg.

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[1] Marketed as Provera by The Upjohn Co., Kalamazoo, Mich.