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Mechanisms of Surfactant Effects on Drug Absorption

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Surface-active agents are one of the most important groups of adjuvants in pharmaceutical preparations. One or another of these agents has been used, for diverse reasons, in almost every type of dosage form. The widespread use of surfactants as well as their unique physical-chemical properties has prompted considerable interest in the possibile influence of these agents on drug absorption. Attention has been focused to inadvertent effects on drug absorption which may result from the inclusion of a surface-active agent in a given formulation as well as to the use of surfactants in a deliberate attempt to modify drug absorption.

The drug literature contains a number of reports which clearly demonstrate that surface-active agents can influence the rate and extent of absorption of certain drugs. However, enhancement as well as inhibition of the absorption and pharmacologic activity of drugs has been observed in the presence of surfactants. Many of these reports have been reviewed by Blanpin (1). A more recent compilation has been provided by Swisher (2) in a review primarily concerned with the environmental exposure levels and toxicity of surfactants. As noted by Levy (3), much of the difficulty in interpreting some of these studies has been due to the different types of effects which surface-active agents can exert. These effects include interaction with biologic membranes and modification of membrane permeability, interaction with the drug, interaction with the dosage form, and interaction with the organism itself resulting in a pharmacologic effect which may in turn influence drug absorption. These effects may be operative at the same time, some tending to enhance drug absorption, others tending to retard it, and the net effect dependent on the relative magnitude of each.

This article is intended as a selective, rather than exhaustive, review of the drug literature which illustrates the major mechanisms of surfactant activity affecting drug absorption. Several critical determinants of the rate and extent of drug absorption—*viz.*, drug solubility and dissolution rate, gastric emptying, and membrane permeation, are considered with respect to the influence of surface-active agents. Finally, attention is given to the role of physiologic surfactants in the gastrointestinal absorption of drugs.

ROLE OF DRUG SOLUBILITY AND DISSOLUTION RATE IN GASTROINTESTINAL ABSORPTION

When a drug is administered orally in solid form (tablet, capsule, or suspension) or intramuscularly as a pellet or suspension, one frequently finds that the rate of absorption is controlled by the slowest step in the following sequence:

$$\text{solid drug} \xrightarrow{\text{dissolution}} \text{drug in solution} \xrightarrow{\text{absorption}} \text{absorbed drug}$$

In many instances the slowest or rate-limiting step is found to be dissolution of drug in the fluids at the ab-

sorption site. When dissolution is the controlling step in the overall process, absorption is said to be dissolution rate limited. Since the rate-limiting step in the absorption process is the dissolution step, any factor influencing the rate of solution must influence also the rate of absorption. According to dissolution theory (4–6), two important parameters determining the dissolution rate of a solid in a given solvent are the solubility of the drug in the dissolution medium and the surface area of the drug exposed to the medium.

The molecules of many surface-active agents tend to aggregate in aqueous solutions when some bulk concentration, termed the critical micelle concentration (CMC), is exceeded. These aggregates, known as micelles, frequently demonstrate a marked tendency to associate with and solubilize organic and inorganic solutes. The phenomenon of micellar solubilization has been reviewed by Swarbrick (7) and is the subject of a recent text (8). In view of the relationship between solubility and dissolution rate as formulated in the Noyes-Whitney equation (4), the enhanced solubility of a drug in a micellar solution of surfactant should result in a proportional increase in the dissolution rate. While this proportionality is never realized in practice (because of the failure of the Noyes-Whitney relationship to account for changes in the effective diffusion coefficient of the drug), the increase in apparent solubility will usually result in an increase in dissolution rate.

The dissolution rate of a drug, regardless of dissolution mechanism, is always directly proportional to the effective surface area of the drug, *i.e.*, the surface area of drug available to the dissolution fluids. The relationship between surface area, dissolution rates, and gastrointestinal absorption rates has been reviewed by Levy (9) and, more recently, by Fincher (10).

The effective surface area of a drug is usually much smaller than the specific surface area which is an idealized *in vitro* measurement. Many drugs whose dissolution characteristics could be improved by particle size reduction are extremely hydrophobic and may resist wetting by gastrointestinal fluids. Therefore, the gastrointestinal fluids may come in intimate contact with only a fraction of the potentially available surface area. The effective surface area of hydrophobic drug particles can often be increased by the addition of a surface-active agent to the formulation, which functions to reduce the contact angle between the solid and the gastrointestinal fluids. Reduction in contact angle permits more intimate contact of drug and fluids, thereby increasing effective surface area and dissolution rate.

In 1948, Kellner *et al.* (11) studied the effect of polysorbate 80 (polyoxyethylene sorbitan monooleate) on the *in vivo* absorption of cholesterol. These workers reported that rabbits fed polysorbate 80 and cholesterol developed blood cholesterol levels that were two to three times as high as those obtained by feeding cholesterol alone. The reason suggested for this marked increase in cholesterol absorption in the presence of the surfactant was an improved emulsification of cholesterol in the intestinal tract and more efficient absorption. However, solubilization of cholesterol by polysorbate 80 and increased dissolution rate of the water-insoluble compound could also account for the observed effects.

Another example of a possible surfactant effect on drug absorption involving solubilization and increased dissolution rate is found in the work of Fuchs and Ingelfinger (12). These workers observed that sodium lauryl sulfate "hastened the appearance and increased the levels of vitamin A in the blood of human subjects." Krause (13) reported that incorporation of sodium lauryl sulfate in G-strophanthin pills resulted in an increase in absorption of the drug in dogs, guinea pigs, rabbits, and cats. The author postulated that an increase in solubility and a higher rate of dissolution of the drug in the presence of the surfactant were responsible for the increase in pharmacologic effect noted with the formulation.

In a study concerned with the oral absorption of spironolactone, Gantt *et al.* (14) found that when polysorbate 80 was administered with the steroid, gastrointestinal absorption was markedly improved. One explanation for the observed effects is an increase in the dissolution rate of the drug due to solubilization and/or wetting effects by the surfactant. However, changes in the formulation and manufacture of the dosage form upon incorporation of the surfactant may have also played a role in the enhanced absorption (15).

Kakemi *et al.* (16) studied the rectal absorption of sulfonamides in the presence of several nonionic surface-active agents. In experiments where sulfisoxazole was administered in the form of suspensions containing varying concentrations of polysorbate 80, it was found that the blood level after 1 hr. increased with increasing concentrations of surface-active agent up to a maximum polysorbate 80 concentration of 20%, the concentration of surfactant which completely solubilized the excess drug. Comparison of relative drug solubility in surfactant solutions and relative blood levels indicates that an 18-fold increase in sulfisoxazole solubility in the presence of 20% polysorbate 80 results in a threefold increase in initial blood level compared to the level following the administration of the control suspension.

A number of studies have attempted to quantitate the relationship between drug solubility in micellar solutions and dissolution rate. Bates *et al.* (17) reported substantial increases in the dissolution rates of griseofulvin and hexestrol in micellar solutions of bile salts. Bates *et al.* (18) have also shown that physiologic concentrations of lysolecithin, a phospholipid, produce marked increases in the solubility and dissolution rate of hexestrol, dienestrol, and griseofulvin. The results of these studies are in general agreement with the Noyes-Whitney relationship (4). However, in each case, because of the extremely low solubilities of the drugs, dissolution was followed over a concentration range which was above the saturation solubility of the drug in water.

Dissolution studies conducted under "sink conditions" (*i.e.*, drug concentration in solution does not exceed 10 to 20% of saturation solubility in buffer) in micellar solutions clearly show that dissolution rate is not proportional to the apparent solubility of the drug. This was first demonstrated by Higuchi (5), who found that the ratio of dissolution rate of benzocaine in polysorbate 80 solution to that without the surfactant was substantially lower than the ratio predicted by the Noyes-Whitney theory. These results have been sup-

ported by the findings of Parrott and Sharma (19), Gibaldi *et al.* (20), and Elworthy and Lipscomb (21).

In 1964, Higuchi (22) presented a theoretical analysis pertinent to the influence of interacting colloids, such as micelles, on mass transport. Higuchi concluded that dissolution rate (d.r.) in micellar solution under sink conditions could be described by

$$\text{d.r.} = \frac{DC_s}{h} + \frac{D_mC_m}{h} \qquad \text{(Eq. 1)}$$

where D and D_m are the diffusion coefficients for the free drug and micelle-solubilized drug species, respectively, C_s is the solubility of the drug, C_m is the solubility increase due to solubilization, and h is the effective diffusion layer thickness. The influence of solubilization is determined by calculating the ratio (R) of dissolution rate in surfactant solution to that in pure solvent and by assuming that certain constants, such as h, have essentially the same value under each experimental condition. It follows that

$$R = \frac{DC_s + D_mC_m}{DC_s} \qquad \text{(Eq. 2)}$$

Rewriting Eq. 2 according to Singh *et al.* (23):

$$R = \frac{D_{\text{eff.}}C_t}{DC_s} \qquad \text{(Eq. 3)}$$

where C_t is the total solubility of the drug in the surfactant solution $D_{\text{eff.}} = (DC_s + D_mC_m)/C_t$. According to Eq. 3, at any degree of micellar solubilization the larger the molecular size of the micelle-drug species formed upon interaction, the smaller will be the influence of solubilization on the dissolution rate. Hence the potential influence of enhanced solubility on dissolution rate in a micellar solution is offset by the expected small diffusion coefficient of the micelle-drug species. In fact, it is plausible to consider that in some cases $D_{\text{eff.}} \cong DC_s/C_t$ and that the surfactant would have virtually no effect on dissolution rate. Higuchi (22) further predicted that the magnitude of effects of interacting colloids on dissolution rate will approach that on solubility only when the drug concentration in solution approaches or exceeds the solubility in pure solvent. The larger effects on dissolution rate predicted above saturation solubility are evident in the reports of Bates *et al.* (17, 18) and Wurster and Polli (24).

Gibaldi *et al.* (20) studied the effect of micellar solutions of a nonionic surfactant on the dissolution rate of benzoic acid using the rotating-disk and static-disk methods. Their results suggested that Eq. 3 was not applicable to all dissolution systems since the influence of the surfactant on dissolution rate was substantially greater in the static-disk method studies than in the rotating-disk method studies. The role of hydrodynamics in assessing the influence of colloidal solubilizers on dissolution rate was subsequently quantified by Singh *et al.* (23). These workers found that the influence of surfactants on dissolution rate using the stirred and static-disk methods could be described by Eqs. 4 and 5, respectively:

$$R = \frac{(D_{\text{eff.}})^{2/3}C_t}{D^{2/3}C_s} \qquad \text{(Eq. 4)}$$

$$R = \frac{(D_{\text{eff.}})^{1/2}C_t}{D^{1/2}C_s} \qquad \text{(Eq. 5)}$$

Equation 3 described the situation reasonably well only when dissolution occurred under conditions of apparent laminar flow provided by a propeller-driven stirrer apparatus.

These findings were confirmed by Gibaldi *et al.* (25) in their studies on the dissolution of benzoic and salicylic acids in micellar solutions of polyoxyethylene lauryl ether. Their results also suggest that the influence of micellar solubilization on the dissolution rate of drugs from conventional dosage forms will be significantly greater than that predicted by diffusion layer theory (Eq. 3).

While the influence of micellar solubilization on dissolution rate has been studied rather extensively, the effect of low concentrations (below the CMC) of surface-active agent on the dissolution of drugs from powders and other solid dosage forms has been given limited attention. Finholt and Solvang (26) studied the dissolution of phenacetin powder dusted on the surface of 0.1 *N* HCl containing low concentrations of polysorbate 80. An increase in the polysorbate 80 concentration from 0 to 0.01% causes a significant increase in the dissolution rate. A linear relationship was observed when the time required to dissolve 10% of the powder was plotted as a function of the surface tension of the test solution. The effect of polysorbate 80 on the dissolution rate of phenacetin is caused mainly by its ability to reduce the contact angle between the powder and the dissolution medium.

More recently, Weintraub and Gibaldi (27) studied the influence of premicellar concentrations of a nonionic surfactant and certain physiologic surfactants on the dissolution rate of drugs from powders and from commercial dosage forms. Polyoxyethylene lauryl ether and lysolecithin enhanced the dissolution rate of powdered salicylic acid in 0.1 *N* HCl, and sodium glycocholate increased the dissolution rate of powdered salicylamide in pH 6.0 buffer. In each case the effect principally involved a "wetting" phenomenon rather than solubilization. Both the nonionic ether surfactant and lysolecithin enhanced the dissolution rate of aspirin from a tablet dosage form but were without effect on the dissolution rate of the drug from a capsule dosage form. Good correlation was found between surface tension lowering and the dissolution rate of aspirin from the tablet.

ROLE OF GASTRIC EMPTYING AND INTESTINAL TRANSIT IN DRUG ABSORPTION

The rate at which a drug leaves the stomach may have a profound influence on the overall rate and extent of drug absorption. For example, a weak base such as quinine will be absorbed primarily from the small intestine rather than from the stomach. Slow gastric emptying can also affect the biological availability of drugs that are unstable in gastric fluids.

An example of the effect of gastric emptying on drug absorption can be found in the work of Levy and Jusko (28). These workers studied the absorption of riboflavin in human subjects and found that administration of the vitamin after a test meal increased the urinary recovery

Table I—Influence of Bile Salts on Gastric Emptying of Phenol Red in the Rat

Test Solution	No. of Animals	Gastric Emptying,[a] % ± 1 *SD*
Control	5	76 ± 3
Sodium taurodeoxycholate		
26 m*M*	5	80 ± 5
50 m*M*	5	67 ± 10
100 m*M*	5	49 ± 8
Sodium deoxycholate		
5 m*M*	3	69 ± 4
10 m*M*	1	56
26 m*M*	4	17 ± 5

[a] Results expressed as percent of dose emptied 0.5 hr. after gastric intubation.

of the vitamin. Riboflavin is apparently absorbed by a specialized process high in the jejunum (28). Since the absorption process for riboflavin is capacity limited, the rate at which the vitamin passes the absorption site may have an influence on the overall extent of absorption. The authors postulate that a meal reduces the rate of gastric emptying and in turn the rate at which riboflavin reaches the site of specialized transport. This in turn results in an increase in the gastrointestinal absorption of the vitamin.

Varga (29) studied the effect of route of administration on the LD_{50} of chloroquine in rats. He found that there was a marked difference between the LD_{50} of chloroquine after gastric intubation (1080 mg./kg.) and intestinal intubation (210 mg./kg.). The difference in LD_{50} was attributed to the delayed gastric emptying resulting from a pharmacologic effect of chloroquine since little absorption of the drug occurs from the stomach.

Surface-active agents, particularly polyoxyethylene derivatives, may influence gastric emptying rate and intestinal transit by physically altering the viscosity of the gastrointestinal fluids. Levy and Jusko (30) have shown that an increase in the viscosity of the gastrointestinal fluids can decrease the absorption rate of certain drugs by retarding the diffusion of drug molecules to the absorbing membranes and by reducing gastrointestinal transit. Okuda *et al.* (31) studied the effects of nonionic surfactants on the intestinal absorption of vitamin B_{12}. These workers found that three of the surfactants studied enhanced the gastrointestinal absorption of the vitamin when the surfactant was administered undiluted in high doses. The enhancing effect of polysorbate 80, polysorbate 85 (polyoxyethylene sorbitan trioleate), and G-1096 (polyoxyethylene sorbitan hexaoleate) was postulated to be due to the formation of a highly viscous mass in the gastric and intestinal lumen which resulted in a delay in gastric emptying and an enhancement in the gastrointestinal absorption of vitamin B_{12}.

A surfactant may also exert a specific pharmacologic effect on the gastrointestinal tract which may influence drug absorption. For example, Lish (32) reported that dioctyl sodium sulfosuccinate, an anionic agent, inhibits the rate of propulsion of a test meal through the gastrointestinal tract of the rat. The effect was ascribed to a slowing of gastric emptying by the surfactant. The mechanism of action was thought to be mediated by a substance (or substances) formed after contact of the intestinal mucosa with the surface-active agent. In a recent study, Necheles and Sporn (33) found that there was an inhibition of gastric motility in the dog following introduction of certain detergents into the gastric pouch.

The influence of bile salts on gastrointestinal motility and transit has been the subject of several reports. Pannett and Wilson (34) first reported in 1921 that the addition of a small quantity of sodium taurocholate to a test meal is followed by an abnormally rapid evacuation of the stomach contents. They also found an increase in the secretion of acid in the presence of the bile salt. In a detailed study, Sasaki (35, 36) reported the effects of orally administered bile salts on the motility of the rabbit gastrointestinal tract. Sasaki (35) found that the effects of bile salts on gastric motility were extremely variable, with a slight increase in motility noted at low doses of bile salts and a small decrease in motility at higher dose levels. The effect of bile salts on intestinal motility (36) also was of a small order of magnitude. The bile salts usually produced a small increase in motility.

Recently, Feldman and Gibaldi (37) reported on the effect of orally administered bile salts on gastric emptying in the rat. Using phenol red as a marker substance, these workers found that both sodium deoxycholate and its taurine conjugate, sodium taurodeoxycholate, significantly decreased gastric emptying of the phenol red test solution (Table I). A significant difference was observed, however, in the relative influence of the bile salts on gastric emptying. The unconjugated bile salt produced results comparable to the conjugated derivative at about 0.1 to 0.2 the dose of the latter. Further studies (38) as to the influence of sodium deoxycholate on gastric emptying as a function of time after administration indicate that the bile salt markedly alters the pattern of gastric emptying. Gastric emptying of intubated phenol red in control rats proceeds in an apparent first-order manner with a half-life of about 13 min. In rats given sodium deoxycholate, an initially rapid gastric emptying phase, followed by a transition region where little emptying occurs, is noted over the 1st hour. The 2nd hour after intubation is characterized by an exponential decline in phenol red in the stomach, with a half-life of 36 min.

Intubation of sodium deoxycholate and sodium taurodeoxycholate also results in a large net secretion of fluids into the gastric pouch for at least 1 hr. after administration. The increase in gastric secretion and volume in the presence of bile salts offers an explanation for the observed decrease in gastric emptying. Hunt and MacDonald (39) have shown that as the volume of a test meal increases, the percentage of the gastric contents leaving the stomach per unit time becomes smaller. In addition, these workers noted that distension of the intestine reduces the coordinated propulsive activity of the gastric antrum as well as the duodenum. Thus, it appears likely that the reduced gastric emptying and transit rate through the proximal intestine induced by the administration of bile salts are related to the increase in the volume of fluid secreted into the gastric pouch.

The possibility exists that oral administration of bile salts may influence the absorption of drugs admin-

istered concurrently by modifying the pattern of gastric emptying. For example, the data of Mayersohn *et al.* (40) suggest that the increased absorption of riboflavin in man observed upon coadministration of sodium deoxycholate may be due, in part, to a decrease in gastric emptying. Administration of riboflavin with sodium deoxycholate results in both a pronounced increase in the peak urinary excretion rate of flavins as well as a marked delay in the occurrence of the peak rate compared to control studies (Fig. 1). A reduced rate of gastric emptying would result in prolonged and more complete absorption of the vitamin since absorption is limited to the proximal intestine and occurs *via* a "saturable" process (28, 41).

FACTORS INFLUENCING DRUG PERMEATION OF BIOLOGIC MEMBRANES

Two possible ways come to mind by which the rate of drug transfer from solution across biologic membranes may be altered. First, a change in the physical-chemical properties of the drug due to the presence of an additive could reduce or enhance the rate of transport and, second, an alteration in the permeability of the membrane could also influence transfer rate. Each of these possibilities is developed further in the subsequent paragraphs.

1. Drug–Adjuvant Interactions—A significant change in the ability of a drug to permeate a biologic membrane may result from an interaction with another molecule. A molecular complex consists of constituents held together by weak forces such as hydrogen bonds. This type of interaction is usually reversible, provided that the complex is sufficiently soluble in the biologic fluids. The properties of drug complexes, including solubility, molecular size, diffusiveness, and lipid–water partition coefficient, can differ significantly from the properties of the respective free drugs. These differences are responsible for the fact that many drug complexes cannot penetrate biologic membranes and, therefore, have no biologic activity (42). In such cases, the fraction of drug in the complex, which is in equilibrium with the noncomplexed drug, will be in an essentially nonabsorbable form and the effective concentration of drug will be less than the total concentration.

Surfactants may also interact with drug molecules and affect the gastrointestinal absorption of these compounds. Riegelman and Crowell (43–45) studied the effects of surfactants on the rectal absorption of iodoform, triiodophenol, and iodide in rats. Polysorbate 80 and sodium lauryl sulfate were found to decrease the rectal absorption rate of iodoform and triiodophenol, but to increase the absorption rate of iodide. The decrease in rectal absorption rate of iodoform and triiodophenol was attributed to micellar complexation of the drugs, while the increase in iodide absorption rate was postulated to be due to a cleansing action of the surfactant on the intestinal mucosa surface. Since iodide ion is lipid insoluble, it would not be expected to be incorporated into the surfactant micelles. Retardation of iodoform and triiodophenol absorption in the presence of micellar concentrations of the surfactants is in accord with the following model: (*a*) a micellar solution consists of two phases; (*b*) the partition ratio of drug between the micellar phase and the aqueous phase is constant, independent of drug concentration; and (*c*) absorption of the drug incorporated in the micelle is negligible. Since the drug in the micellar phase is unavailable for absorption, the effective concentration of the drug is less than the apparent concentration, and a decreased absorption rate is observed.

Levy and Reuning (46) studied the effect of micellar solutions of polysorbate 60 (polyoxyethylene sorbitan monostearate) on the absorption of ethanol and salicylic acid from the rat gastric pouch. These workers found that in the presence of 2% polysorbate 60 the absorption of salicylic acid was decreased from 50% in 1 hr. to 33% in 1 hr., while ethanol absorption remained unchanged. The observed effect was due to a decrease in activity of salicylic acid as a result of micellar complexation. The absorption of ethanol (which would not be incorporated into the surfactant micelles) was unaffected by the presence of the surfactant.

Kakemi *et al.* (16) studied the effect of various nonionic surface-active agents on the rectal absorption of sulfonamides from solution in the rat. At concentrations of the surfactant above the CMC, a reduction in the absorption rate of the sulfonamides was observed due to "entrapment" of drug in micelles. The experimental results were in agreement with the limiting case of the following theoretical equation, assuming that the solubilized drug was not absorbed to any appreciable extent.

$$A_T = \frac{A_f}{1 + K_m S} + \frac{A_m K_m S}{1 + K_m S} \qquad \text{(Eq. 6)}$$

where A_T, A_f, and A_m represent the absorption rates of both free and micelle-solubilized drug, free drug, and solubilized drug, respectively; S is the surfactant concentration (g./100 ml.); and K_m is a distribution constant equal to [drug-micelle]/[free drug]·[S].

Yamada and Yamamoto (47) found similar effects of micellar solutions of polysorbate 80 on the intestinal absorption of salicylamide in the perfused rat small intestine. Also, they observed no apparent effect of polysorbate 80 on the mucosal membrane, as determined by permeability experiments with salicylamide before and after a prolonged perfusion of the intestine with a

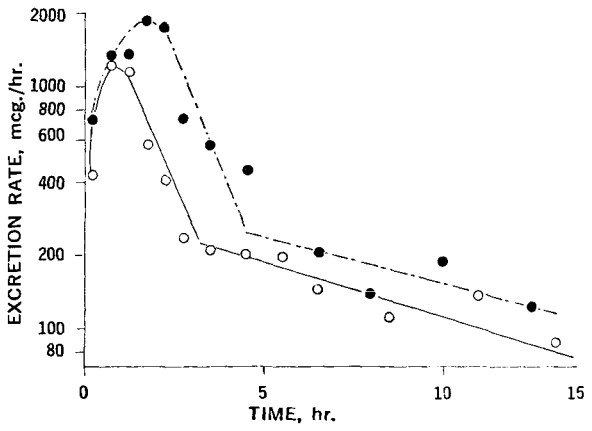


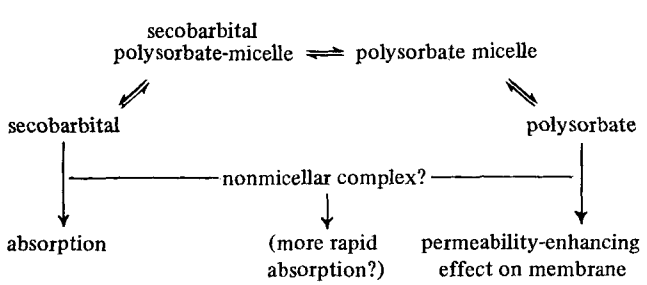
Figure 1—*Urinary excretion rate of apparent riboflavin after oral administration of 30 mg. riboflavin (Subject W.J.). Key: ○, control, and ●, 600 mg. sodium deoxycholate.*

polysorbate 80 solution. This technique, however, can only detect irreversible effects on membrane permeability. Matsumoto (48) offered essentially the same mechanism of micellar solubilization and a corresponding decrease in free drug concentration to explain the effect of polysorbate 80 on the intestinal absorption of sulfisoxazole in the rat. Saski (49) studied the effect of micellar solutions of tyloxapol, a nonionic surface-active polymer, on the transfer of hydrocortisone across the everted rat intestine. Drug transfer rate was inversely proportional to the surfactant concentration and the viscosity of the solution tested. The data suggest that the membrane is impermeable to the drug-micelle species.

2. Membrane Permeability—A number of substances have been found to "interact" with biologic membranes and thereby alter permeability or transport characteristics. For example, Schanker and Johnson (50) and Windsor and Cronheim (51) have shown that the chelating agent, ethylenediaminetetraacetic acid, can increase the *in vivo* intestinal absorption of a number of lipid-insoluble compounds in the rat. The depletion of calcium from the intestinal membranes by the chelating agent was suggested as the reason for these results.

Surfactants may also be capable of modifying the properties of biologic membranes. Alexander and Trim (52) reported that the penetration of hexylresorcinol into *Ascaris lumbricoides* can be affected by ionic surfactants in two different ways. Surfactant concentrations below the CMC increased the penetration of hexylresorcinol into the worm. These workers suggested that a complex between hexylresorcinol and surfactant monomer was responsible for the increase in concentration of the drug at the membrane surface. They did not consider the possibility that the site of action of the surfactant could be the membrane itself and that the increased penetration of the drug in the presence of the surfactant could be due to alteration of membrane permeability. At concentrations of surfactant above the CMC, the absorption of hexylresorcinol was reduced due to the formation of an unabsorbable micelle-drug complex.

Levy *et al.* (53) studied the effect of a nonionic surfactant, polysorbate 80, on the absorption of a number of barbiturates across the goldfish membranes. The absorption rate of the barbiturates was found to increase significantly in the presence of low concentrations (below the CMC) of surfactant and to decrease at higher concentrations of the surfactant. Scheme I was proposed to explain the results. Further studies by Levy and Anello (54) showed that the increase in absorption rate of secobarbital at concentrations of



Scheme I

polysorbate 80 below the CMC was due to an increase in the permeability of the biologic membranes rather than to formation of a more rapidly absorbed nonmicellar polysorbate–secobarbital complex. Anello and Levy (55) have also shown that premicellar concentrations of polysorbate 80 enhance the absorption and exsorption of 4-aminoantipyrine across the goldfish membranes. Gibaldi and Nightingale (56) studied the influence of sodium taurodeoxycholate on pharmacologic effect (time required to produce overturn) of pentobarbital and ethanol in goldfish. These workers found that the bile salt significantly potentiated the pharmacologic effect, presumably by modifying membrane permeability. Further studies (57) indicated that the bile salt exerts an all-or-none effect on the uptake of 4-aminoantipyrine in goldfish; an alteration in membrane permeability is observed above a certain bulk concentration but below the CMC of the surfactant. Whitworth and Yantis (58) found an increase in the absorption of salicylic acid across the external membranes of the frog in the presence of 0.1% polysorbate 80.

In a study of the percutaneous absorption of ionic surfactants, Scala *et al.* (59) found that the salt of a long-chain fatty acid, an alkylbenzene sulfonate, and dodecyltrimethyl ammonium chloride alter skin permeability as they diffuse into and through the skin. When nicotinate and thiourea were placed in the surfactant solution, the rate of diffusion of these compounds was found to increase with time, similar to the diffusion characteristics of the surfactants themselves. In the absence of surfactant, nicotinate and thiourea showed linear diffusion which remained constant over the time period studied. In a study of the effects of polysorbate 80 on the *in vitro* metabolism of Ehrlich-Lettre ascites carcinoma cells, Kay (60) found that the permeability of the cells was increased greatly in the presence of the surfactant as shown by the uptake of a dye, Lissamine green.

Appel *et al.* (61) found that simultaneous feeding of sodium lauryl sulfonate and inulin to rats results in up to a 10-fold increase in urinary inulin excretion over control values. The enhanced urinary excretion of inulin may be the result of an increase in intestinal permeability to inulin in the presence of the surface-active agent.

Mori *et al.* (62) reported that rats and hamsters fed polysorbate 20 (polyoxyethylene sorbitan monolaurate) showed increased gastrointestinal absorption of iron. However, Brise (63) in a later study reported that there was no effect of polysorbate 20 on iron absorption in man. He further postulated that the increase in absorption of iron in hamsters in the presence of polysorbate 20 observed by Mori *et al.* may have been due to some "toxic" action of the surfactant.

Suzuki *et al.* (64) found increased capillary permeability, as measured by a circulating dye, at the site of an intracutaneous injection of various nonionic surfactants. The authors concluded that this increase was mainly due to the wetting and solubilizing effects of the surfactants on the lipid structure of the capillary wall. Penzotti and Mattocks (65) found an increase in the rate of peritoneal dialysis of urea and creatinine in rabbits in the presence of surface-active agents. It was found that the order of magnitude of effects decreased in the following manner: cationic > anionic ≫ nonionic.

The possible mechanisms of action were not discussed, but it appears likely that the mechanism may involve a permeability alteration of the peritoneal membrane.

Nissim (66) studied the effect of feeding cationic, anionic, and nonionic surface-active agents on the histology of the mouse gastrointestinal tract. He found marked pathological changes when the ionic surfactants (cationic and anionic) were fed to mice but no effects when nonionic surfactants were tested. Taylor (67) studied the effects of cetyltrimethylammonium bromide on transport and metabolism in the small intestine of the rat. The surfactant was found to produce no histological damage to everted rat intestine sacs at concentrations of 10^{-4} *M*, but caused considerable injury to the mucosa at concentrations of 5×10^{-4} *M* and 10^{-3} *M*. Concomitant with the mucosal damage by the higher concentrations of the surfactant was an inhibition of the everted intestinal transport of glucose, methionine, and water.

Lish and Weikel (68) studied the effects of surface-active agents on the absorption of an anionic dye, phenol red, from the colon of the anesthetized rat. Anionic surfactants were found to enhance greatly absorption of the dye while a nonionic surfactant had no effect. None of the surfactants studied influenced the absorption of a cationic dye, methyl violet.

Matsuzawa *et al.* (69) found that the addition of nonionic surfactants (polysorbate 80 and a series of polyoxyethylene derivatives of hydrogenated castor oil) to solutions of the antibiotic, enduracidin hydrochloride, resulted in an increase in absorption of the antibiotic from the femoral muscles of the rat. The mechanism of action was not elucidated, but there is a strong possibility that the surfactant may have altered the permeability of the muscle to the antibiotic.

Engel and Riggi (70, 71) studied the effect of surfactants on the intestinal absorption of heparin in the rat. They found that intraduodenal administration of heparin with either sodium lauryl sulfate, dioctyl sodium sulfosuccinate, or G-3300 (an alkyl aryl sulfonate) resulted in an increase in heparin absorption over that observed when heparin was administered alone. These workers also reported enhanced heparin absorption in the presence of 0.4% sodium taurocholate. The authors postulate that the increase in heparin absorption is due to an effect of the surfactant on the intestinal mucosa.

Davenport (72) has reported that bile salts are capable of increasing the permeability of the gastric mucosa as judged by hydrogen-ion flux. The influence of an unconjugated bile salt, sodium deoxycholate, on the absorption of phenol red in the rat was recently studied by Feldman *et al.* (73) using three different techniques to assess absorption—*viz.*, urinary excretion after oral administration to intact animals, loss of drug from *in situ* intestinal loops, and transfer of drug across the isolated everted intestine. Each method showed that the bile salt markedly enhances the absorption or transfer rate of phenol red across the gastrointestinal membranes. The studies in intact animals indicated that the effect of sodium deoxycholate on gastrointestinal permeability was reversible.

Few studies of surfactant effects on drug absorption in man have been reported. Mayersohn *et al.* (40) found that when 600 mg. sodium deoxycholate is administered 30 min. prior to a 30-mg. dose of riboflavin, there is a 50 to 80% increase in total urinary recovery of apparent riboflavin. A similar but less marked enhancement was observed when the same dose of flavin mononucleotide was given with the bile salt. It is likely that change in the permeability of the gastrointestinal membranes is the principal mechanism of these effects, although in the case of riboflavin a reduction in gastric emptying may also play a role.

PHYSIOLOGIC SURFACTANTS AND DRUG ABSORPTION

Interest in the possible existence of a surfactant(s) in gastric fluid has been stimulated by the recent work of Finholt and Solvang (26). Samples of gastric juice obtained from patients under examination for diseases of the stomach showed low surface-tension values (38 to 47 dynes/cm.) and marked wetting activity as judged by powder dissolution studies. The rates of dissolution of powdered phenacetin in diluted gastric juice and in diluted HCl at the same pH and adjusted to the same surface tension with polysorbate 80 were similar but markedly faster than the rates observed in diluted HCl alone. Hence, the dissolution of hydrophobic drugs in the stomach may be facilitated by the presence of physiologic wetting agents.

The source of this surface activity in gastric fluids is not known, nor is it known whether or not gastric secretions themselves manifest surface activity. The presence of surface-active compounds in the gastric juice of some *Crustacea* was established by Vonk (74). Van den Oord *et al.* (75) reported that no bile salts could be detected in extracts of crab gastric juice. However, material with emulsifying properties was isolated; later, these compounds were shown to be fatty acylsarcosyltaurines (76). Further work by Van den Oord (77) suggested that the emulsifiers occurring in the gastric juice of the crab are of endogenous origin. Unfortunately, similar studies with human gastric juice are not available.

A possible source of the surface activity observed in human gastric fluid may be contamination from the duodenum. Reflux of duodenal contents would result in the presence of conjugated bile salts and lysolecithin, both highly surface active, in the gastric fluid. This possibility is strengthened by a recent report of Rhodes *et al.* (78) on the concentration of bile acids in the human stomach upon fasting and after a test meal.

Gastric fluid from normal fasting subjects contained a mean bile acid concentration of 0.08 ± 0.03 (*SE*) m*M*. The levels observed after a test meal were essentially the same. On the other hand, gastric fluids from fasting subjects with gastric ulcers contained a mean bile acid concentration of 0.65 ± 0.34 (*SE*) m*M*. For gastric ulcer patients, in contrast to normal subjects, the concentration of bile acids after food was always greater than the immediate preprandial value. These findings indicate that a small amount of reflux of intestinal contents into the stomach occurs normally and that the reflux of bile is increased considerably in patients with gastric ulcers. Since Finholt and Solvang (26) obtained gastric juice from patients under examination for diseases

Table II—Influence of Bile on Sulfadiazine Absorption from Intestinal Loops in the Rat

Experimental Condition	No. of Animals	% Absorbed in 3 hr. ± 1 *SD*
Controls	6	43 ± 8
Sham bile duct ligation	6	44 ± 7
Bile duct ligation	10	26 ± 17
Choleresis	6	63 ± 8

of the stomach, the extrapolation of their results to normal gastric fluid requires further investigation.

One of the most important groups of surfactants present in man is the bile salts. Bile is chiefly composed of conjugated bile salts, cholesterol, calcium, and lecithin, a phospholipid (79). There are six bile salts present in man—*viz.*, the taurine and glycine conjugates of deoxycholic acid, chenodeoxycholic acid, and cholic acid, present as the sodium salts. Endogenous bile salts represent the end products of cholesterol metabolism in the liver and are stored in the gall bladder after conjugation in the liver until required for digestion. The conjugation of bile salts by the liver with glycine and taurine is an essential physiologic process, since unconjugated bile salts are insoluble at the relatively low pH (6.3–6.6) present in the upper portion of the small intestine. Although the bile salts secreted into the intestinal lumen are, for the most part, conjugated, free acids may be present in the feces due to cleavage of the conjugated bile salts by microorganisms present in the large intestine. The concentrations of conjugated bile salts in the upper jejunum of the fasting human are above the critical micelle concentration and range between 5 and 10 m*M* (80). During fat digestion, concentrations of conjugated bile salts in the order of 40 m*M* are commonly found in the jejunum (81).

Bile salts have long been implicated in the absorption of fat from the small intestine. They are known to have an important role in the emulsification of water-insoluble, long-chain triglycerides and in stimulating the hydrolytic action of pancreatic lipase, resulting in a mixture consisting of fatty acids, monoglycerides, diglycerides, and triglycerides (82). A powerful emulsifying agent is also formed by removal of a fatty acid moiety from a molecule of lecithin by a pancreatic phospholipase to give lysolecithin (83).

The ability of bile salts to solubilize lipid material has also been known for many years. The two lipids that are appreciably solubilized by the bile salt micelles are the lipolysis products—*viz.*, monoglycerides and fatty acids (82). Hofmann and Borgstrom (84) have shown that the presence of monoolein in a bile salt micelle greatly increases its ability to solubilize saturated fatty acids. Feldman and Borgstrom (85) have recently shown that small amounts of diglyceride and triglyceride can also enter such micelles. Fatty acids and monoglycerides contained in the micelles are delivered to the brush border of the mucosal cells of the proximal small intestine, where they are absorbed by a process which is not understood at present. The bile salts, however, are not appreciably absorbed from the proximal intestine and become available for further solubilization of fat digestion products. Intestinal motility eventually carries the bile salts to the ileum where they are absorbed by an active transport process (86).

Bile salts have also been shown to be involved in the absorption of materials other than fats. Bernhard *et al.* (87) showed that in the biliary fistula rat less than 1% of a dose of vitamin A was absorbed. However, if a solution of sodium cholate or taurocholate was introduced into the duodenum, the absorption of vitamin A was increased significantly. Greaves (88) has also reported that bile salts are essential for the intestinal absorption of vitamin K in the rat. Adequate absorption of vitamin D by the rat was shown by Greaves and Schmidt (89) to require bile. When the bile duct was implanted into the colon, the animals absorbed little or none of the vitamin. Oral administration of deoxycholic acid greatly improved absorption of the vitamin in these rats. Taylor (90) confirmed the need for bile for adequate vitamin D absorption in dogs. Heymann (91) also found that dogs did not absorb crystalline vitamin D_2 when bile was not present in the small intestine.

Several reports have appeared in the literature (92–94) as to the importance of bile salts in the intestinal absorption of cholesterol. These studies also showed that the addition of exogenous bile salts enhances the absorption of this sterol.

Lengemann and Dobbins (95) found that intraperitoneal injections and large oral doses of sodium taurocholate enhanced the absorption of calcium by the rat, provided that bile was allowed to flow freely into the small intestine. Seyfried and Lutz (96) reported that the intestinal absorption of tetraiodophenolphthalein is greatly diminished in the absence of bile. Simultaneous administration of bile or bile salts increases the absorption of this substance. Pekanmaki and Salmi (97) found that the absence of bile from the intestine of cats reduced the absorption of free phenolphthalein but had no influence on the amount of absorbed phenolphthalein glucuronide, a water-soluble conjugate. The effect of the endogenous bile on free phenolphthalein absorption was attributed to the solubilizing effect of bile salts on the free drug.

Recently, Meli *et al.* (98) reported that endogenous bile influences the rate of intestinal absorption of ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether in rats. The rate of absorption of the estrogen was considerably lower in biliary-cannulated rats than in control animals. Since the steroid is relatively water insoluble, it is reasonable to consider that the presence of bile salts increased the solubility of the drug in the intestinal lumen and thereby enhanced the dissolution and absorption rate.

Nightingale *et al.* (99) studied the absorption of sulfadiazine in aqueous suspension from proximal intestinal loops in the rat under four experimental conditions —*viz.*, in control animals with intact bile flow, in bile duct-ligated animals, in sham-ligated animals, and in animals where bile flow was stimulated by intraperitoneal administration of sodium dehydrocholate. The results are shown in Table II and clearly indicate that bile flow is an important factor in sulfadiazine absorption from intestinal loops. The mechanism by which bile enhanced sulfadiazine absorption is probably due to micellar solubilization, resulting in an increase in the

dissolution rate of the drug. This possibility is supported by *in vitro* solubility studies.

As noted, conjugation of free bile acids with glycine and taurine results in a considerable lowering of the pKa. Consequently, the conjugated bile acids exist in the intestinal lumen almost entirely as negatively charged ions. In the presence of amphipathic cations, bile salts behave as typical amphipathic anions and an insoluble molecular complex is the frequent result.

Neomycin, a tetrasaccharide with two positively charged amine groups, precipitates glycine and taurine conjugates of dihydroxy and trihydroxy bile salts from aqueous solution (100). Addition of kanamycin, a cationic disaccharide antibiotic, to human bile also results in precipitation (101). Formation of an insoluble complex between a drug and a component of the luminal contents will probably result in decreased absorption of the drug. The poor oral availability of certain drugs such as streptomycin may well be due to bile salt interaction.

Although the detergent properties of bile salts are well recognized, their potential for altering or regulating membrane permeability in the intestine has been given little attention. It is perhaps significant that the ileum, which is the site of active transport of conjugated bile salts, is the region of the small intestine in which the mucosal cells have the shortest life span (102).

Levels of conjugated bile salts in the order of 10^{-2} *M*, comparable to the levels normally found in the proximal intestine, alter the permeability of the everted rat intestine to salicylate (103), salicylamide (104), riboflavin (40), and several other drugs. The salicylate data are shown in Table III. There is a greater than twofold increase in the steady-state transfer rate of salicylate when micellar concentrations (>5 m*M*) of taurodeoxycholate are initially present in the mucosal solution. There are no significant differences between the transfer rates observed at different sodium taurodeoxycholate concentrations above the CMC. Mucosal solutions containing 1 and 5 m*M* taurodeoxycholate produced considerably smaller changes in membrane permeability, an increase of 20–40% over control steady-state transfer rates. Incubation of the intestinal segments in drug-free mucosal solution containing 10 m*M* bile salt followed by the determination of salicylate transfer from bile salt–free drug solution yielded transfer rates essentially equivalent to those observed when 10 m*M* taurodeoxycholate was initially present with the drug.

The data in Table III suggest that the bile salt effect is mediated *via* two different mechanisms. One mechanism appears to be operative at concentrations of sodium taurodeoxycholate below or about the CMC and results in small increases in salicylate transfer rates. A second mechanism, which results in large increases in the permeability of the intestinal membrane, appears to be operative at sodium taurodeoxycholate concentrations above the CMC.

The possibility that monomeric and micellar species of bile salt affect the biologic membrane in a significantly different manner is supported by recent studies on bile salt transfer in isolated jejunal loops of the rat. Dietschy (105) reports that at concentrations below the CMC the rate of passive transfer of sodium taurocholate increased

Table III—Effect of Sodium Taurodeoxycholate (STDC) on Mucosal-to-Serosal Steady-State Transfer of Salicylate[a] Across the Everted Rat Small Intestine at pH 6.0

	No. of Intestinal Segments	Transfer Rate ± *SD*, mcg./min.
Control	19	40 ± 5
STDC		
100 m*M*	2	90 (87, 93)
50 m*M*	4	89 ± 10
10 m*M*	11	87 ± 10
5 m*M*	6	57 ± 9
1 m*M*	7	48 ± 7
Incubation		
Control	4	40 ± 4
STDC, 10 m*M*	4	85 ± 7

[a] Mucosal salicylate concentration maintained essentially constant, 2 mg./ml. Serosal salicylate concentration never exceeded 0.25 mg./ml.

linearly with increasing mucosal concentrations of bile salt. According to Dietschy (105), "since the concentration of monomer becomes almost constant as the total concentration of bile acid is raised to and beyond the CMC, the rate of passive diffusion similarly should reach a limiting value at this same point if bile acid monomer were the only species diffusing passively across the bowel wall." In fact, the permeability coefficient of the membrane actually increased at taurocholate concentrations above the CMC. "Micellar" taurocholate appears to move across the intestinal membrane twice as fast as the monomeric form. Based on the authors' findings, one need not conclude that micellar transport of taurocholate proceeds at a faster rate than monomer transport or indeed that it occurs at all. It is possible that micellar concentrations of bile salt in contact with the mucosa significantly enhance the permeability of the membrane toward the monomeric species.

The latter possibility is further supported by Nogami *et al.* (106) who studied the sorption of surface-active agents by isolated rat intestine. The sorption of sodium lauryl sulfate and cetyltrimethylammonium bromide, with time, followed first-order kinetics at bulk concentrations below the CMC and zero-order kinetics at concentrations exceeding the CMC. This change in order is consistent with a mechanism involving penetration of

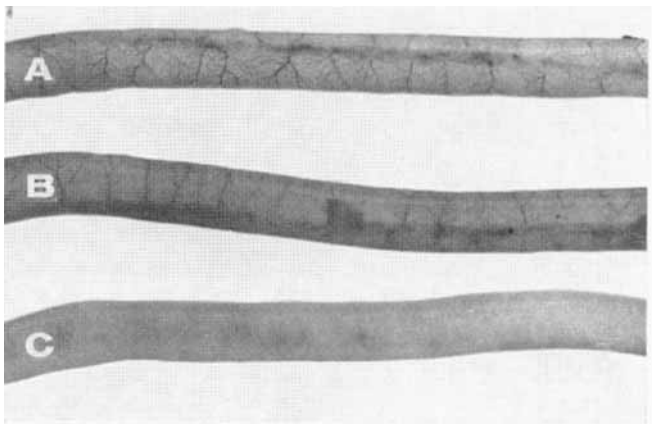


Figure 2—*Segments of everted rat small intestine after 1-hr. incubation. Key: A, physiologic buffer; B, buffer with 10 m*M *sodium taurodeoxycholate and 5 m*M *egg lecithin; and C, buffer with 10 m*M *sodium taurodeoxycholate.*

Table IV—Influence of Egg Lecithin, Oleic Acid, and Glyceryl Monooleate (GMO) in Modifying the Effect of 10 m*M* Sodium Taurodeoxycholate (STDC) on the Transfer Rate of Salicylate Across the Everted Rat Small Intestine at pH 6.0

Incubation Media	No. of Intestinal Segments	Mean Transfer Rate ± *SD*, mcg./min.
Control	6	30 ± 3
STDC	15	78 ± 8
STDC + 5 m*M* lecithin	4	55 ± 9[a]
STDC + 10 m*M* lecithin	4	51 ± 3[a]
STDC + 3 m*M* oleic acid + 1 m*M* GMO	4	72 ± 5[a]
STDC + 6 m*M* oleic acid + 4 m*M* GMO	2	64 (63, 66)

[a] Results significantly different from STDC alone ($p < 0.05$, Student's *t* test, method of paired comparisons).

the monomer but not the micellar species. However, in each case the observed zero-order rate constant was larger than the zero-order rate constant calculated from the results of experiments using premicellar concentrations. Hence, the micellar species appear to alter the permeability of the tissues for the monomeric species.

When the intestinal tissue is incubated in solutions exceeding 5 m*M* bile salt concentration, the changes in permeability are invariably accompanied by pronounced changes in the gross appearance of the mucosal surface (Fig. 2). These observations suggest the possibility that micellar concentrations of sodium taurodeoxycholate produce pronounced changes in membrane structure, conceivably by solubilizing lipid components of the membrane such as phospholipids. These observations also raise an intriguing question as to why such toxic effects are not observed in the intact animal where the intestinal concentration of conjugated bile salts is comparable to that used in the *in vitro* studies.

The micellar species in the proximal intestine normally contains a significant amount of lecithin in addition to the conjugated bile salts. During digestion the micelle will also contain considerable amounts of fat digestion products. Feldman and Gibaldi (107) compared the effect of micellar solutions of pure bile salt and bile salt with lecithin or fat digestion products on the permeability of the everted rat intestine to salicylate. Their results are shown in Table IV. Addition of either phospholipid (egg lecithin) or fat digestion products (oleic acid and glyceryl monooleate) to 10 m*M* solutions of sodium taurodeoxycholate resulted in a protective effect on the everted intestinal membrane. There was a significant decrease in the transfer rate of salicylate after exposure of the everted intestine segment to 10 m*M* sodium taurodeoxycholate containing either phospholipid or fat digestion products when compared to the transfer rates in 10 m*M* sodium taurodeoxycholate alone. As shown in Fig. 2, egg lecithin also protected the intestinal mucosa from the gross histological effects of the bile salt. Similar protective effects were observed with the fat digestion products. The results of this study may explain why conjugated bile salts are highly "toxic" to intestinal tissue *in vitro* but are apparently innocuous *in vivo*.

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