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INFLUENCE OF SURFACTANTS ON THE ABSORPTION OF DRUGS

By

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CHAPTER I

INTRODUCTION

Dioctyl sodium sulfosuccinate (DSS) and polaxalene (ethylene oxidepropylene oxide polymer, Pluronic F-68$^{(R)}$) are used frequently as anti-constipating agents. Combination of DSS and oxyphenistan has been shown to produce jaundice in several patients (132, 164), and it is suspected that DSS increases the absorption of oxyphenistan and the latter causes the hepatotoxicity. Recently (29) it has been found that DSS is itself absorbed and that it may enhance the absorption of oxyphenistan from the gastrointestinal tract.

DSS has been shown to promote the absorption of a poorly absorbed drug, phenol red, from the colon of rats (96). In the same study, polaxalene was shown not to have any appreciable effect on the absorption of phenol red from the colon. However, in this study only one concentration of the surfactants was used.

Since the action of surfactants on drug absorption is known to be concentration dependent and because more recent reports (98) have shown that phenol red, like most other drugs, is absorbed mainly from the small intestine, therefore a more detailed study of the effect of various concentrations of these surfactants on the absorption of phenol red was felt necessary. In addition, the effect of DSS on the peritoneal absorption was also carried out.

The effect of DSS on the absorption of a poorly soluble drug, sulfisoxazole, was also studied.

Biological Membranes

Membranes are structures of universal occurrence in nature. The existence of plasma membranes and other biologic membranes is a basic foundation of modern cellular biology, and the idea that internal membrane systems constitute the basic cytoskeleton and circulatory system of the cell is firmly established. Compartments are formed by the membranes, which differ qualitatively and quantitatively in the substances that they contain; from this it must be concluded that all membranes possess selective permeability (57).

At the end of the last century Overton (123) suggested that a submicroscopic lipid layer surrounds the cell and separates the cytoplasm from the extracellular space. This assumption was based in part on his finding that fat-soluble molecules readily penetrate into the cell. This dependence of the rate of penetration of a fat-soluble substance on its lipid solubility was later confirmed by the work of Collander (20). Subsequently, Gorter and Grendel (56) determined the surface area of erythrocytes and the area that would be occupied by the lipids extracted from the membrane and showed that the latter area was twice as large as the erythrocyte surface area. They concluded that erythrocyte surface is formed by a bimolecular lipid layer.

Danielli and Davson (22) in 1934 proposed a membrane model: a bimolecular leaflet composed of phospholipids on which protein molecules are adsorbed. They also pointed out that these protein molecules may impart structural solidity to the membrane which lipids alone could not achieve. The presence of a cross-linked network of polypeptide chains, if the adsorbed proteins were to unfold themselves on the surface of the cell, may perhaps account for the sieve-like properties of cell membranes. The concept of "pores" in the membranes was first postulated by Collander and Bürland (21), and the effective diameter of these pores has been measured indirectly in the red blood cells (160) and in the intestinal epithelium (46), and found to be approximately 4-8 Angstroms.

Since water-soluble ions, amino acids and sugars are adsorbed, the concept of "carriers" was postulated. Wilbrandt and Rosenberg (178) postulated that the carrier first combines with the substrate molecule and takes it across the membrane in some complex form which is lipophilic in nature; this complex then dissociates at the intracellular interface of the membrane and releases the substrate.

From studies of electron micrographs of certain membranes, Robertson (139) modified the model of Danielli and Davson in three ways: (a) the number of lipid layers was limited to two, (b) instead of being globular, the proteins were assumed to be spread on the lipid layer, and (c) the membrane was assumed to have an assymetric structure.

Benson (10) proposed a model in which the protein is largely globular and in the interior of the membrane to maximize hydrophobic interactions. The lipid molecules, however, are not arranged in a bilayer. Their fatty acid chains are individually intercalated into the folds of the protein chain, with the polar heads of the lipids at the exterior surfaces of the membrane in contact with the water. This structure generated a more or less uniform complex of lipoproteins so it is proposed that such complexes exist as morphological subunits held together by hydrophobic interactions in the plane of the membrane.

According to Singer (152), lipids and proteins of membranes are held together by non-covalent interactions and a steady state structure is assumed. Applying thermodynamic considerations we need to know whether the steady state structure of the membranes is the one with the lowest free energy. At the molecular level, the following general consequences of the thermodynamic considerations can be recognized: (1) In order to maximize hydrophillic interactions, essentially all the ionic, zwitterionic, and highly polar groups such as sugar residues, which are attached to both the lipids and the proteins in the membrane, should be in contact with the bulk aqueous phase. (2) Models must attempt to maximize hydrophobic interactions of the entire system of lipids and proteins in the membrane. This involves sequestering not only the fatty acid side chains of the lipids from contact with water, but to the maximum extent possible, the nonpolar amino acid residues of the proteins as well. The interior hydrophobic region of the membrane must be highly compact with very few holes or gaps of atomic dimensions or larger. (3) Potential hydrogen bond donor and acceptor groups of proteins which are sequestered from contact with water should form hydrogen bonds with one another to the maximum extent possible.

The major drawbacks in structural features of the Davson-Danielli-Robertson (D-D-R) Model are as follows: (1) The ionic heads of phospholipids are largely not in contact with the bulk phase (aqueous), but rather with the polar and ionic groups of the protein monolayers. To account for the fact that most membranes consist of proteins and lipids in a weight ratio considerably greater than unity, the spread protein must essentially completely blanket the ionic heads of the phospholipids. (2) The proteins and lipids interact with one another and the membrane is stabilized, primarily by electrostatic forces between their ionic groups. (3) The protein monolayers should exhibit a significant amount of β-conformation (antiparallel pleated sheet) when not in a random conformation. (4) A significantly large fraction of the nonpolar residues of the proteins must be exposed to the water. (5) In Robertson's work, evidence had been obtained only from myelin sheaths. (6) None of the evidence so far obtained for the bilayer form permits us to say whether the bilayer is continuous or interrupted. (7) None of the experiments are sufficiently sensitive and quantitative to prove whether 100 percent of the phospholipid is in the bilayer form. It is therefore not excluded that some significant fraction of the phospholipid (perhaps as much as 30 percent) is physically in a different state from the rest of the lipid.

It follows that this is not an arrangement which would lead to the lowest free energy of a membrane in its aqueous environment. The two most serious thermodynamic problems arise because (a) the nonpolar residues of the membrane proteins are largely exposed to, instead of being sequestered from contact with, water molecules, and (b) the ionic groups of the lipids and proteins are largely in contact with one another and out of contact with water; "the burying of the ionic groups in a lower polarity environment is very costly in free energy"

(152). The D-D-R Model, therefore, is thermodynamically unlikely. The function of lipids appears to be to disperse and "solubilize" membrane proteins by a kind of detergent action, in which the hydrophobic proteins of the lipids and proteins interact with one another.

In the case of Benson's Model, it appears that this is in too high a free energy state (but lower than that of D-D-R Model) to constitute the general pattern of organization of most of the lipids and proteins of membranes. Intercalating most of the nonpolar fatty acid chains among the polypeptide chains in the interior of the membrane should, by virtue of separating the polypeptide chains, prevent the formation of the maximum number of interpeptide hydrogen bonds (which the fatty acid chains cannot participate in), and this would be thermodynamically unsatisfactory.

There is substantial evidence that the major portion of the phospholipids is in bilayer form in a variety of intact membranes. The structures of the lipid in the membrane and of the lipid in isolated aqueous dispersion are closely similar. This conclusion is supported by X-ray diffraction and spin label studies on similar membrane preparations. So this bilayer character of membranes rules out models such as that of Benson.

Singer's Fluid Mosaic Model. Essentially on the basis of thermodynamic considerations, together with some experimental data on the conformation of the proteins in intact membranes, Lenard and Singer (152, 153) proposed a hydrophobic model for the organization of the lipids and the integral proteins (the property that distinguishes integral proteins from others is its capacity to interact with lipids in water solutions to form lipoprotein structures with lower free energy than the separated lipid and protein) of the membranes. This model is also called, lipid-globular protein mosaic model. The lipids and globular integral proteins are arranged in an alternating mosaic pattern throughout the membrane. The hydrophobic portions of the lipids and a large fraction of the nonpolar amino acid residues of the proteins are sequestered from contact with water, mainly in the hydrophobic interior of the membrane, while the ionic groups of the lipids and the charged residues of the proteins are both in direct contact with water, predominantly on the exterior surfaces of the membranes. The saccharide groups whether on glycolipids or glycoproteins also are exposed to the bulk aqueous phase of this arrangement. Mosaic appears to be a fluid or dynamic one and, for many purposes, is best thought of as a two-dimensional oriented viscous solution. This model is applicable to most functional biological membranes.

Oseroff et al. (122) recently have supported this model: that in most membranes the majority of the lipid is in the form of a fluid bilayer matrix, and that the membrane proteins are both loosely bound and, in some cases, deeply or transversely embedded in this matrix. Proteins and protein-lipid complexes are probably mobile, but it is not yet clear whether these motions result from passive diffusion or are actively controlled by the cell. Whether there exists a long range but random order is also unclear.

Based on the results from freeze cleaving and freeze etching the trilamellar "unit membrane" described by Robertson (138) is not justified. Experiments with labelled stearate bilayers (25) and examination of both halves of the fractured specimen (17, 173) have clearly established that the cleavage plane tends to follow the curvature of the cell surface but passes within the membrane matrix, probably in the midplane of the lipid bilayer. In most membranes the fracturing process exposes an array of particles; the density of the particles is greatest in functionally specialized membranes, such as, retinal discs and chloroplast lamellae; particles are completely absent in the myelin sheath. Other evidence that the intramembrane particles are due to proteins intercalated into a lipid bilayer comes from work with artificial membrane systems. Pure lipid vesicles are lamellar phases and show an absence of particles, but they appear with the addition of erythocyte (130) and sarcoplasmic reticulum proteins (104) or with rhodopisn (62) which definitely seemed the material of choice.

All these evidences point to the fact that Singer's model is the best model put forward so far.

Mechanisms of Drug Absorption

Absorption is the unidirectional movement of material from outside the body to the inside of the body. In man and other terrestrial mammals, the alimentary canal, the lungs, and the skin represent the most important sites of entry for exogenous material. At each of these sites, the movement of material takes place across epithelial tissues that serve as membranes or anatomical barriers. There are similarities in the overall processes of the translocation of materials at these seemingly diverse barriers; the mechanisms that serve to move materials across these membranes are those which serve to move substances across any biological barrier, i.e., passive diffusion, facilitated diffusion, active transport, and pinocytosis; the principles governing these transport mechanisms apply equally at each site; and a substance passes through the membrane and reaches the other side, the vascular system, the gastrointestinal, alveolar or integumental epithelium. On the other side of each of these barriers substances come in contact with fluids maintained at relatively constant composition, pH, and temperature, and having orderly biochemical interactions.

Passive diffusion. Passive or simple diffusion does not require expenditure of cellular energy; movement occurs in proportion to the physical forces available, and in the same directions as the electrochemical gradient (diffuse from a region of high concentration to a region of low drug concentration) (7, 84). Most pharmacologically important lipid-soluble drugs are transported across the biological membranes by this process.

Gastrointestinal epithelium, like other biological membranes, is essentially lipid in nature. Therefore, it permits the passive diffusion of lipid-soluble drugs but imposes a barrier to the diffusion of lipid-insoluble drugs. Since most drugs are weak acids or bases, they can exist in both the undissociated and dissociated forms in an aqueous environment. The degree of dissociation depends on the pH of the medium and the dissociation constant of the drug. Since it is the undissociated form of the drug that has greater lipid solubility and hence is the more readily absorbed form of the drug, the rate of gastrointestinal absorption of weakly acidic or basic drugs is related to the fraction of total drug in solution in un-ionized form. The rate of absorption is enhanced when pH conditions are changed so that this fraction is increased. These observations form the basis of the pH partition hypothesis (61, 144, 151), which relates the dissociation constant, lipid solubility, pH at the absorption site with the absorption characteristics of various drugs throughout the gastrointestinal tract.

Since the stomach has a low pH, weakly acidic drugs ($pKa > 2.5$) exist primarily in the un-ionized form and are readily absorbed. Conversely, weak bases exist primarily in the ionized form, and their gastric absorption is negligible. Partially dissociated (very weak) bases ($pKa < 2.5$) such as antipyrine and caffeine are absorbed to some extent. Additional evidence that it is mainly the non-ionized form of the drug which crosses gastric epthelium was provided by Schanker et al. (145) who demonstrated it by the reversal of the absorption pattern when the gastric contents were made alkaline (pH 8.0). Many basic compounds become undissociated at this pH and show an increased absorption rate; conversely, acidic compounds become more ionized and show a decreased rate of absorption. The preferential permeability of the gastric mucosa to the non-ionized form of drugs is further emphasized by the predictable manner in which drugs distribute between gastric juice and plasma. Thus, at a steady state (constant plasma levels), basic drugs are more concentrated in gastric juice than in plasma, and acidic drugs are more concentrated in plasma than in gastric juice (151).

The pH of the fluid of the small intestine is approximately 6.5 and is quite high compared to the stomach, but it behaves like the stomach in the absorption of drugs. Most weak acids and bases are readily absorbed; stronger and more highly ionized acids and bases are more slowly absorbed; and completely ionized quaternary ammonium compounds and sulfonic acids are very slowly absorbed. Since pH is high in the intestine, the absorption of weak bases is favored over weak acids, since a large fraction of the drug is in un-ionized form. Still the absorption of weak acids with pKa values greater than 3 is quite rapid. The large mucosal surface area available for absorption in the small intestine appears to decrease the need for a large fraction of the drug to be present in the un-ionized form. For example, salicylic acid (pKa $\simeq$3) exists almost completely in the ionized form in the intestine, but the drug is relatively well absorbed from this site (149). The influence of pH on the intestinal absorption of weak acids and bases in rats has been shown by Brodie (16) and Kakemi et al. (64). Acidification of the intestinal fluids increases the fraction of the drugs present in the un-ionized form and the absorption rate of weak acids, and decreases the fraction of the drug in the un-ionized form and the absorption rate of weakly basic drugs.

It has been suggested by Hogben and Schanker (61, 145) that the pH of the bulk contents of the gastrointestinal tract is not a true index of the pH on the absorbing surface. It is proposed that a zone with an effective or virtual pH of 5.3, possibly located at the surface of the intestinal epithelium, determines the degree of ionization of drugs as they approach the boundary to be absorbed.

The pattern of drug absorption in the colon is very similar to that in the small intestine. However, this is not the prime site of absorption for most drugs.

Gastrointestinal absorption of drugs is influenced not only by the degree of ionization but also by lipid solubility in the un-ionized form. An indication that lipid solubility is the physical property governing the passage of un-ionized molecules across the gastric epithelium has been provided by the barbiturates and the lipid-water partition coefficients of their un-ionized form (148). Kakemi et al. (66) also have shown similar correlation of partition coefficients with absorption rate of various barbiturates. With a homologous series of compounds this correlation of lipid solubility and absorption rate is very significant, but the ability to make predictions concerning an expected degree of absorption of an individual compound on the basis of its partition coefficient is indeed very limited. Despite this fact, it is in general true that compounds which are highly soluble in lipids are rapidly absorbed and those that are relatively less lipid soluble are more slowly absorbed when no specialized mechanism is involved in their absorption.

Facilitated diffusion. Like passive diffusion this does not require cellular energy, and movement occurs only with the electrochemical gradient. It differs from passive diffusion in that the physico-chemical properties of the constituents of the membrane and those of solute are insufficient to describe the kenitics of the process. Therefore, the additional concept of temporary combination of the solute with a chemical structure or site, "carrier," of the membrane must be evoked to explain the total phenomenon (84). There is increasing evidence that the protein present in the membrane plays an important role not only in structure but in function, and that proteins are intimately involved in membrane transport (125, 152, 153, 175).

Active transport. This process, while resembling facilitated diffusion in that the process requires the additional concept of carrier mediated passage across the membrane, is clearly distinguished from facilitated and passive diffusions by the fact that a net flux, or movement, of the solute must occur in a direction opposite to that of the electrochemical gradient of the absorbed species. This active transport requires an energy source for the work to be done in moving the solute "uphill". This process permits the cell not only to control the rate at which substances move into, or out of, its environment, as does facilitated diffusion, but also to control the concentration of the specific substances inside and outside the cell. Pardee (125) and Whitman and Wheeler (175) have reviewed the progress made to isolate these "carriers" in active transport processes.

The various active transport processes known to exist in the gastrointestinal tract are primarily associated with the absorption of nutrients and food digestion products (35, 146). Evidence of drugs absorbed by an active transport mechanism appears to be limited to those agents that bear close similarity to normal body constituents. For example, certain serine and threonine derivatives of nitrogen mustard and derivatives of uracil and thiamine (e.g., 5-fluoracil) have been shown to be transported across the intestinal epithelium against concentration gradients by processes that transport the parent amino acids and pyrimidines, respectively.

Pinocytosis. This transfer mechanism also requires the expenditure of cellular energy. It differs from active transport in that the transport of a solute, or particulate matter, is not mediated by combination with a carrier but by the local invagination of the cell membrane and subsequent budding off of a vesicle containing the permeant. The poisoning by botulin toxins and allergic reactions resulting from the ingestion of offending proteins are well known examples in humans.

CHAPTER II

SURVEY OF LITERATURE

Rarely a drug is used in the form of a pure substance. In pharmaceutical products a drug is generally combined with different types of adjuvants. Surfactants are one of the most important groups of the adjuvants used for diverse reasons in almost every dosage form including liquids, semisolids, and solids.

Since these agents could affect the integrity of the biological membranes, it has been thought that they might be effective absorption promoters. However, it has been found that enhancement as well as inhibition of absorption takes place when surfactants are added to a drug. Blanpin (13) and Ritschel (136) have reviewed and summarized many of these reports. As noted by Levy (85), the difficulty in interpreting some of these studies probably has been due to different types of effects which surfactants can exert. Briefly, these effects include interaction with biologic membranes and change of 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. Several of these effects may be operative at the same time, some tending to enhance drug absorption, others tending to retard it, and the net effect being dependent on the relative magnitude of each. Gibaldi and Feldman (49) have also recently reviewed the major mechanisms of surfactant activity.

It is currently believed that surfactants influence the rate and extent of drug absorption through one or more of the following mechanisms: (1) effect on drug solubility and dissolution rate, (2) effect on gastric emptying time and intestinal motility, (3) surfactant and drug interactions, and (4) change in permeability of membranes. The literature reports pertinent to these mechanisms are discussed below.

Effect of Surfactants on the Solubility and Dissolution Rate of Drugs

When a drug is administered orally in solid dosage form it dissolves in the fluids around it; the drug in the solution form is then absorbed by different processes, depending upon its own nature and the conditions around it (e.g., pH, food, surfactant, etc.). For drugs which have limited solubility in the body fluids at the absorption site, the dissolution rate of the drug becomes the rate-limiting step in their absorption. If the dissolution process at the solid-liquid interface is rate limited by diffusion from the very thin layer of saturated solution at the solid surface into the bulk liquid, then Noyes-Whitney (equation 1) and Noyes-Nernst (equation 2) equations are valid:

$$\frac{dC}{dt} = K(C_s - C) \qquad (1)$$

and

$$\frac{dC}{dt} = \frac{SD}{Vh}(C_s - C) \qquad (2)$$

where dC/dt is the dissolution rate of a drug, C_s is its saturated concentrations, C is the concentration at the time t during dissolution, S is the surface area of the solid, D is the diffusion coefficient, h is the thickness of the diffusion layer, and V is the volume of dissolution medium (77).

According to dissolution theory, two important parameters determining the rate of dissolution of a solid in a given solvent system are: the solubility of the drug in that system, and the surface area of the drug exposed to it. It can readily be seen from equations (1) and (2) that, for relatively water-insoluble drugs, the solubility term C_s is one of the most important factors governing the rate of dissolution. Therefore, any change in the dissolution media which will effectively increase C_s should, in theory, increase the dissolution rate of the drug. One means by which this can be, and is accomplished, is by the addition of a surfactant to the dissolution media.

The mechanisms by which surfactants increase the dissolution rate of a relatively water-insoluble drug are by decreasing the interfacial tension between the drug and the dissolution medium, thus allowing the latter to wet the drug more completely and/or by means of micellar solubilization. The latter mechanism involves the incorporation of the water-insoluble material into micelles (aggregates composed of monomers of the surfactant) formed above the critical micelle concentration (CMC) of the surfactant (8). The enhanced solubility of a drug in a micellar solution of surfactant should result in a proportional increase in the dissolution rate. While exact proportionality is never realized in practice (because of the failure of the Noyes-Whitney equation 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 (49).

The dissolution rate of a drug, regardless of the dissolution medium, is always directly proportional to the effective surface area of the drug available to the dissolution medium. 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 drugs can often be increased by the addition of a surfactant to the formulation, which functions to reduce the interfacial tension between the solid and the gastrointestinal fluids. Reduction in interfacial tension permits more intimate contact of drug and fluids, thereby increasing effective surface area and dissolution rate (49).

Importance of dissolution rate in the absorption of drugs is reflected in the observation by Wagner et al. (170) that blood levels after oral administration of four different commercial brands of warfarin showed a strong correlation with their in vitro dissolution rates. Bass et al. (6) have shown that availability of tetracycline is decreased almost 50 percent from commercial capsules when 2 gm of sodium bicarbonate was administered at the same time. When tetracycline was dissolved prior to administration, no differences in availability were observed with or without the concomitant administration of sodium bicarbonate, indicating that the dissolution rate step is involved in the decreased absorption of tetracycline capsules. The biologic availability of several commercial digoxin tablets of known low and high therapeutic potencies and of digoxin dissolved in alcohol was studied in digitalized patients and compared with the dissolution rates of the tablets (161). Biologic availability was estimated by measuring the area beneath the plasma digoxin curve. The bioavailability of the "low potency" tablet was markedly inferior to that of the "high potency" tablet, which did not differ significantly from that of digoxin in alcoholic solution. A significant correlation between bioavailability and the dissolution rate was found. Lindenbaum and Butler (92) have also studied the bioavailability of digoxin tablets in humans after single 0.5 mg doses and in the steady state after 8-10 days of drug administration. Excellent correlations were observed between dissolution rate and bioavailability. Variation in digoxin bioavailability appears to result from differences in the rate at which the tablets go into solution in the gastrointestinal tract. The U.S.P. XVIII interim revision has recently changed the requirements from a disintegration test to a dissolution rate test for digoxin tablets based on the above findings on bioavailability data.

Kellner (79) showed that higher blood cholestrol levels in rabbits could be achieved by administering cholestrol and polysorbate 80 than by administering cholestrol alone. The reason for this marked increase in cholestrol absorption could be the increased dissolution rate of the water-insoluble compound. Allawala and Riegelman (2) found the activity of solubilized iodine preparations, using polyoxyethylene glycol nonylphenal as the solubilizing agent, also to be controlled by the concentration in the aqueous phase which, in turn, depends on the relative concentrations of iodine and surfactant and the distribution of iodine between the micellar and aqueous phases. Fuchs and Ingelfinger (47) found that sodium lauryl sulfate hastened the appearance and increased the levels of vitamin A in the blood of human subjects. It has been reported that the absorption of carotene (a precursor of vitamin A) is more rapid when solubilized in solutions of polysorbate 80 than when administered orally or intramuscularly in oil (167). Sobel (157, 158) has revealed improved absorption of vitamin A itself when in solubilized form; the transfer of the vitamin to the milk of nursing mothers is superior in such case. Kakemi et al. (69) also have shown that the absorption rate of vitamin A from the rat intestine is increased in the presence of polysorbate 80. Münzel observed that vitamins A, D and E were absorbed more effectively from sterile surfactant solutions than from oily solution on parenteral administration (112). Sodium lauryl sulfate improved the blood levels of griseofulvin (which is poorly and irregularly absorbed) (134). The polyene antibiotic, amphotericin B, is poorly soluble in water at neutral pH. A solubilized preparation, employing sodium deocycholate as solubilizer gives better absorption and less pain than when administered intramuscularly as suspension (31). The percutaneous absorption of esterone is enhanced when the steroid is solubilized in a surfactant. Using the potency of an oily solution injected subcutaneously as having an activity equal to 1.0, Sjöblöm (155) found the percutaneous activity in female mice of solubilized preparations containing moderate concentrations of surfactants to be 0.36 ± 0.02 compared to a value of 0.13 ± 0.02 when solutions of esterone in oil were used. Sjöblöm (156) has also conducted studies on estradial-17 and found similar results.

Span 60 (sorbitan monostearate) and Atlas G-2164 (polyoxyethylene propylene monostearate) have been shown to increase the absorption of sulfathiazole from a lanolin-petrolatum base (150). It is not possible to decide whether this is due to a solubilization effect or a simple miscibility effect. However, it is known that surfactants increase the solubility of soluble sulfonamides in ointment bases. Whitworth and Becker (176) have studied the effect of Arlacel 83 (Span 83 or sorbitan sesquioleate) on the solubility of sulfacetamide sodium and sulfathiazole sodium in liquid petrolatum and cottonseed oil bases. Arlacel 83 increased the diffusion of both drugs from the cottonseed oil base; from the petrolatum base the highest concentrations of the surfactant decreased the diffusion process. It is obvious that the solubility of a drug in the base is an important factor. Solubilization of the drug in the bases will increase the saturation of the drug in the base, and will tend to promote its diffusion from the base, hence increase the absorption (31). Incorporation of sodium lauryl sulfate in G-strophanthin tablets resulted in an increase in absorption both rectally and orally in dogs, guinea pigs, rabbits, and rats. This is attributed to an increase in solubility and a higher rate of dissolution of the drug in the presence of the surfactant (36). G-strophanthin in a dose of 1 mg/kg, which has no effect on guinea pigs when given rectally, actually exhibits a toxic effect on addition of sodium lauryl sulfate (136).

Addition of hydrophilizing agents to tablet preparations leads indirectly to improved absorption, since the surfactants used not only lead, as a result of the improved wetting, to faster dissolution and hence to faster release of the drug from the preparation, but can also give better distribution (135). Chodkowska and Krowozynski also found the enhancing effects on absorption when surfactants were included into a tablet formulation (18).

In some cases a surfactant may inhibit the absorption of a compound by formation of a less soluble compound. Hudson et al. (63) reported that sitosteral and cholestrol formed a 1:1 mixed crystal or solid solution, which has a solubility only one-third that of cholestrol in methanol, and a reduced solubility compared with cholestrol in aqueous sodium oleate or sodium deoxycholate solutions. This may possibly explain the hypocholestremic effects of sitosteral in human beings. Solubilization of salicylic acid (91), normally a well absorbed drug, led to a decrease in activity resulting from a lower level of absorption.

Gantt et al. (48) studied the influence of polysorbate 80 on the absorption of spironolactone when administered orally and found that the former markedly improved the absorption of the spironolactone; increase in dissolution rate due to solubilization and/or wetting effects is one explanation of the observed effects. However, changes in the formulation and manufacture of the dosage form upon incorporation of the surfactant may have been a factor in enhanced absorption. A study was performed by Cid and Jaminet (19) concerning the effect of some surfactants, such as polysorbate 80, on the gastrointestinal absorption of aspirin in man. A significant increase in blood levels of the salicylate was observed after administration of aspirin tablets containing the surfactant.

The behavior of sulfisoxazole in surfactant solutions has been studied by Kakemi et al. (72). When sulfisoxazole was administered in the form of suspensions containing varying concentrations of polysorbate 80, it was found that the blood levels after one hour increased with increasing concentrations of the surfactant up to a maximum polysorbate 80 concentration of 20 percent, the concentration which completely solubilized the excess drug. Comparison of relative drug solubility in surfactant solutions and relative blood levels indicates that 18-fold increase in sulfisoxazole solubility in the presence of 20 percent polysorbate 80 resulted in a threefold increase in initial blood levels compared to the level following the administration of the control suspension. Above 20 percent concentration of polysorbate 80, the rectal absorption decreased due to the entrapment of the drug in the micelles.

Surfactants are often used as emulsifiers and solubilizers in oily base suppository formulations. Their addition is presumed to affect the drug absorption to some extent from rectum. Kakemi et al. (75) have investigated the effect of various types of surfactants on rectal absorption of sulfisoxazole from cocoa butter. Blood levels of sulfisoxazole were, in general, increased with increasing concentrations of surfactant, but decreased with higher concentrations. Surfactants accelerated the release of the drug from the base to the medium, but, on the other hand, surfactants reduced the absorption rate of the drug from aqueous solution as noted earlier (72). The use of lipophilic Spans, either alone or in combination with hydrophilic Tweens, increased the release of active ingredient, aminopyrine, from suppositories (78).

Similarly, Parrott (126) has found that aspirin was rapidly released from suppositories of polyethylene glycol and polyoxyethylene sorbitan monostearate base. Hanks et al. (58) also investigated the influence the surfactants, polysorbates and sodium lauryl sulfate, on the release and activity of chloramphenical from the suppositories prepared with polyethylene glycol 1500. Optimal activity and release were obtained with sodium lauryl sulfate. Lowenthal and Borzellaca (97) observed faster absorption with Tween 61 in the investigation of the rectal absorption of salicylic acid from various suppositories bases. It has also been shown in the case of barbiturates that the rectal absorption is increased by the addition of non-ionic surfactants (43).

As mentioned before, relatively water-insoluble drugs are solubilized by the process of micellization. Micelles containing a drug, when surrounded by the biological fluids, are able to release the drug, depending upon the partitioning behavior of the un-ionized species of the drug. Water-insoluble drugs, solubilized by micellization, are better absorbed than when they are administered in the form of a suspension or a solid dosage form, because of the slow dissolution rate. The phenomenon of micellar solubilization from the latter dosage form has been reviewed by Swarbrick (164) and Mulley (111). Elworthy et al. (31) have published a book on this subject. Numerous examples have been cited of the pharmaceutical applications of micellar solubilization.

A number of studies have attempted to quantitate the relationship between drug solubility in micellar solutions and dissolution rates. Taylor and Wurster (166) found that sodium dodecyl sulfate substantially increased the rate of solution of prednisolone. Bates et al. (8) reported substantial increases in the dissolution rates of griseofulvin and hexestrol in micellar solutions of bile salts. Bates et al. (9) have also shown that physiologic concentrations of lysolecithin produced marked increases in the solubility and dissolution rates of hexestrol, dienestrol, and griseofulvin. Kuroda (83) noted that the poor rate of dissolution of benzoic acid is improved in the presence of polysorbates 20 or 80. Parrott and Sharma (127) have also found increase in the dissolution rate of benzoic acid in the presence of surfactants: tyloxapol, polysorbate 80, sodium lauryl sulfate and polaxakol. These surfactants only slightly improved the dissolution rate below their critical micelle concentration (CMC) due to improved wetting of surface. But at concentrations exceeding the CMC, the dissolution rate increased with increasing concentrations of the surfactants. Elworthy and Lipscomb (32) studied the dissolution rate of griseofulvin in water and aqueous solutions of four non-ionic surfactants and found that the latter increase the dissolution rate of the drug. Gibaldi et al. (52) studied the influence of polyoxyethylene [23] lauryl ether, a non-ionic surfactant, on the dissolution rate of benzoic acid and salicylic acid and found that the dissolution rate was increased in the micellar solution. Influence of surfactant micelles (polyoxyethylene lauryl ether) on the dissolution of salicylic acid from constant-surface pellets has been studied (51) and the rate of dissolution was found to increase.

While the influence of micellar solubilization on dissolution rate has been studied extensively, the effect of low concentrations (below the CMC) of surfactants on the dissolution of drugs from powders and other solid dosage forms has been given limited attention. Finholt and Solvang (44) studied the dissolution of phenacetin powder sprinkled on the surface of 0.1N hydrochloric acid containing low concentrations of polysorbate 80. An increase in the polysorbate 80 concentration from zero to 0.01 percent causes a significant increase in the dissolution rate. The effect of polysorbate 80 on the dissolution rate of phenacetin is caused mainly by its ability to reduce the interfacial tension between the powder and the dissolution medium. Prescott et al. (131) have also studied the effect of polysorbate 80 on the absorption of phenacetin in humans given in the forms of suspensions and tablets. In separate experiments individuals received phenacetin as a fine suspension with and without polysorbate 80, a medium suspension, and a coarse suspension. They found striking differences in the plasma concentrations of phenacetin depending on particle size administered. The highest values were observed with the fine particles suspended with polysorbate 80, followed in decreasing order by fine, medium, and coarse particles. They concluded that particle size is an important factor in the absorption of phenacetin, and also that absorption is apparently enhanced by polysorbate 80.

Weintraub and Gibaldi (174) have studied the influence of premicellar concentration of a non-ionic surfactant (polyoxyethylene lauryl ether) and a physiologic surfactant (lysolecithin) on the dissolution rate of drugs from powders and from commercial dosage forms. Polyoxyethylene lauryl ether and lysolecithin increased the dissolution rate of powdered salicylic acid, and sodium glycocholate increased the dissolution rate of powdered salicylamide. In each case the effect principally involved a "wetting" phenomenon rather than solubilization. Both the non-ionic 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.

Effect of Surfactants on the Gastric Emptying Time and Intestinal Motility

In view of the qualitative and quantitative differences between the absorption properties of the stomach and intestine, any delay in the transfer of a drug from stomach to intestine may affect the absorption rate and, thereby, the onset of therapeutic activity. For example, a weak base such as codeine will be absorbed mainly from the small intestine rather than from the stomach, and any delay in gastric emptying will tend to delay the onset of analgesia. Slow gastric emptying can also affect the biologic availability of drugs that are unstable in gastric fluids, the extent of degradation being proportional to the time during which such drugs are exposed to low pH or gastric enzymes (85). The effect of pharmaceutical formulation ingredients on the dissolution rate of weakly acidic drugs often will be most noticeable while the drugs are in the stomach, where ordinarily they dissolve relatively slowly. Such differences may disappear when the drugs reach the small intestine, where dissolution is more rapid and less affected by differences in properties of the dosage forms (87).

Delay in gastric emptying, on the other hand, can also cause increased absorption of a drug; an example has been provided by the work of Levy and Jusko (88). They found that administration of riboflavin in human subjects after a test meal increased the urinary recovery of the vitamin. Riboflavin is apparently absorbed by a specialized process high in the jejumum. 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. These workers postulated that since a meal reduces the rate of gastric emptying, the rate at which riboflavin reaches the absorption site is also reduced, resulting in an increase in the absorption of the vitamin.

Surfactant may influence gastric emptying rate by direct action on the stomach. Hardt (59) found that when sodium lauryl sulfate was introduced in certain doses in solid or solution form into the stomachs of dogs, it produced complete inhibition of normal gastric motility for periods up to 90 minutes.

Surfactants may also influence gastric emptying rate and intestinal transit by physically altering the viscosity of the gastrointestinal fluids. Okuda et al. (121) studied the effects of non-ionic surfactants on the intestinal absorption of vitamin B_{12}. They found that three of the surfactants studied enhanced the gastrointestinal absorption of the vitamin when the surfactants were administered undiluted in high doses. This enhancing effect of polysorbate 80, polysorbate 85 (polyoxyethylene sorbitan trioleate), and G-1096 (polyoxyethylene sorbitan trioleate) 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 thus increased 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. Dioctyl sodium sulfosuccinate (DSS) retards gastric emptying (94) and has an inhibitory effect on gastric secretions, even when administered in relatively low doses. Inhibition of gastric secretions occurs when the drug is administered intraduodenally, but there is no effect when contact of the drug is limited to the lumen of the stomach or when it is administered parenterally. It has been suggested by the author (94) that the inhibitory effect of DSS is mediated by a hormone released from the intestinal mucosa, e.g., enterogastrone. Inhibition of gastric motility in the dog following introduction of certain detergents into the gastric pouch was found by Necheles and Sporn (115).

The influence of bile salts on gastrointestinal motility has also been studied. Pannett and Wilson (124) have reported 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. Saski (140, 141) reported the effects of orally administered bile salts on the motility of the rabbit gastrointestinal tract. It was found that the effects of bile salts on gastric motility were extremely variable, with a slight increase in motility noted at low doses and a small decrease in motility at higher dosage levels of bile salts. The effect of bile salts on intestinal motility also was of a small order of magnitude (140). The bile salts usually produced a small increase in motility (141). Feldman et al. (38) have reported the effect of orally administered bile salts on gastric emptying in the rat. Using phenol red as a marker substance, it was found that sodium deoxycholate and sodium taurodeoxycholate significantly decreased gastric emptying of the phenol red solution. Gastric emptying of phenol red in control animals was found to proceed by apparent first order kinetics, but a very different kinetic pattern was observed upon administration of bile salt. Sodium deoxycholate and sodium taurodeoxycholate also produced a large net secretion of fluids into gastric pouch for at least one hour after stomach intubation. It is proposed that the resulting increase in gastric volume is the immediate cause for the decreased rate of gastric emptying (41). Mayersohn et al. (103) investigated the gastrointestinal absorption of riboflavin and flavin mononucleotide (FMN) under controlled conditions and after oral administration of 600 mg of sodium deoxycholate. Increased urinary excretion levels suggest an unusually prolonged absorption of riboflavin which may be due, in part, to a decrease in gastric emptying time.

Surfactants and Drug Interactions

A significant change in the ability of a drug to permeate a biologic membrane may result from an interaction of the drug molecule with the surfactant to form a molecular complex. 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 coefficients, 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. In such cases, the fraction of drug in the complex, which is in equilibrium with the non-complexed drug, will be in an essentially nonabsorbable form and the effective concentration of drug will be less than the total concentration.

In simple solutions the antibacterial activity of the phenol is logarithmically related to its concentration, but in solutions containing surfactants at concentrations high enough to form micelles, the activity is not related to the overall concentration in solution because the micelles compete with free water for phenol so that only part of it is available for interaction with bacteria. The effect may be regarded as a partition phenomenon, so that the concentration of the phenol in aqueous phase depends on the "partition coefficient" of the phenol and the ratio of the volume of the micellar "phase" to the aqueous "phase" (111).

Below the CMC the activity of phenols is dramatically increased by the surfactants. This seems to be due to the increased uptake of the phenol on the biologic membrane by the high surface activity of a loose complex between the phenol and the surfactant. The permeability of the bacterial surface may also be increased in the presence of surfactant. In systems where the concentration of surfactant is kept constant (above CMC) and the concentration of phenol is increased (i.e., capacity of the system is fixed) one would expect activity to increase regularly since both micelles and aqueous "phase" are being progressively saturated with the phenol. This expectation has been proved by the work of Berry and Briggs (11), who have shown that all phases in a system like this will be in equilibrium and the concentration of phenol "free" in the aqueous phase will increase in the same proportion as that in the micelles. A growth inhibition study of hexetidine (bis-1, 3β-ethylhexyl-5-amino-5-methyl [hexabydropyrinidine]) carried out in the presence of pluronic F-68, L64 and L62 showed that the activity was enhanced below the CMC of pluronic F-68 and L64 (pluronic L62 had no effect at any concentrations) and above the CMC the activity of hexetidine was reduced by the former compounds (142).

Another example of interaction of surfactant with the drugs is that of iodophors. For many purposes solutions containing 1 to 2 percent of iodine are used, solubilized by a suitable proportion of the surfactant, usually the non-ionic type. Allawala and Riegelman (2) have shown that the proportion of iodine solubilized changes proportionately with the concentration of the surfactant (nonyl phenol polyglycol ether-Antarox A-400). The same authors found that the activity of solubilized iodine depended on the amount in the free solution which is controlled by the proportion of iodine to the surfactant and the "distribution coefficient" of iodine between micelles and the aqueous phase.

Preservatives, like the p-hydroxybenzoates, have far greater activity in aqueous solutions of surfactants below and up to their CMC, but activity decreases dramatically beyond this point; same phenomenon takes place as described above. Bolle and Mirimanoff (14) were the first to point out the reduced effect of methyl-p-hydroxybenzoate in the presence of several structurally different non-ionic surfactants. De Navare (27) followed up this work and in 1957 remarked that practically all non-ionic surfactants based on ethylene and propylene oxide condensed with each other or with fatty esters, alcohols or acids, inactivate all the preservatives considered at that time suitable for drug and cosmetic preparations. This has been confirmed by other workers (171). Non-ionic surfactants have been found also to reduce the effect of quaternary ammonium compounds (137). Activity of organic mercury compounds is also markedly reduced in the presence of non-ionic surfactants (171). The inactivating effects of non-ionic surfactants on the preservatives are of greater significance than those of other types of surfactants because not only is the order of their inactivation much higher, but, unlike the other surfactants, non-ionics have comparatively no growth-inhibiting properties, making the necessity for adequate preservation of systems containing them much greater.

Wedderburn (172) has summarized the evidence relevant to establishing the mechanism or mechanisms by which these preservatives are inactivated in relation to their interaction with non-ionic surfactants. Micellar solubilization and the formation of molecular complexes of the type described by Higuchi et al. (60) have both been proved as being responsible for their inactivation. While complexation is thought to be important in some systems, Evans (34) has suggested that the most important factor is micellar solubilization when the non-ionic surfactant concentration exceeds the CMC. The antimicrobial activity of preservatives in such systems has been shown to be directly related to the concentration of free unbound preservative (172).

Cationic forms of chlopromazine, promethazine, tetracaine, and methylrosaniline, and anions such as naphthalene sulfonate were bound to polysorbate 80, the degree of interaction being sufficient to suggest that the non-ionic might have considerable influence on the stability and availability of ionic drugs in formulations (107).

Studies of the effects of neomycin and kanamycin upon intestinal absorption in normal but obese humans have shown that both antibiotics produce steatorrhea and are able to reduce their own intestinal absorption due to the formation of insoluble, nonabsorbable precipitates with bile salts (37). Kakemi et al. (65) studied the effect of sucrose esters and other surfactants on the absorption of various drugs using perfusion technique on the rat small intestine. Tetracycline, sulfanilamide, isoniazid, and salicylic acid were used to test drugs and sodium lauryl sulfate, benzethonium chloride, polysorbate 80, sucrose monostearate, and sucrose distearate were the surfactants used. It was found that ionic nature of the surfactants substantially influenced the absorption; rate of absorption of tetracycline was accelerated by sodium lauryl sulfate, benzethonium chloride, and sucrose esters; polysorbate 80 showed a marked reduction in the absorption of salicylic acid and tetracycline; benzethonium chloride reduced the absorption of salicylic acid and sucrose esters within different concentrations tested did not reduce the absorption of all the drugs tested. These were explained due to the formation of complexes or other forms of interactions and/or by the correlation of partition coefficients.

Malone et al. (99) found a significant increase in the pharmacological activity of reserpine in mice after oral administration of the drug as a solid dispersion in deoxycholic acid. A correlation between the composition of the reserpine-deoxycholic acid dispersion and biologic activity was also noted in that an increase in the deoxycholic acid : reserpine ratio resulted in an increase in blepharoptotic activity. Gibaldi et al. (49) suggested, on the basis of dissolution rate studies of this system (deoxycholic acid : reserpine), that particle size reduction of reserpine in the dispersions, leading to increased dissolution and absorption rate, and possibly increased availability of reserpine, is likely to be a major factor in the enhancement of pharmacologic activity. DeCato et al. (26) have also studied the reserpine-bile salts coprecipitates absorption in mice. It was shown that intravenous administration of reserpine acetate and oral deoxycholic acid showed no increase in blepharoptotic potency relative to intravenous reserpine acetate alone. Since this experiment eliminated the physico-chemical interactions within the gastrointestinal tract, the authors have concluded that the potentiation of reserpine taken orally as reserpine-bile salts coprecipitates is by physico-chemical rather than physiopharmacological means.

Riegelman and Crowell (133) studied the effects of surfactants on the rectal absorption of iodoform, tri-iodophenol, and iodide in rats. Polysorbate 80 and sodium lauryl sulfate were found to decrease the rectal absorption rate of iodoform and tri-iodophenol, but to increase the absorption rate of iodide. The decrease in rectal absorption rate of iodoform and tri-iodophenol 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 mucosal surface. Since iodide ion is lipid insoluble, it would not be expected to be incorporated into the surfactant micelles.

Retardation of iodoform and tri-iodophenol 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 micellar phase and the aqueous phase is constant, independent of drug concentrations, 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 (91) studied the effect of micellar solutions of polysorbate 60 on the absorption of ethanol and salicylic acid from the rat gastric pouch. They found that in the presence of 2 percent polysorbate 60 the absorption of salicylic acid was decreased from 50 percent in one hour to 33 percent in one hour, 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 surfactant. Kakemi et al. (72) studied the effect of various non-ionic surfactants on the rectal absorption of sulfonamides from solutions in the rat. At concentrations of the surfactant above the CMC a reduction in the absorption rates of the sulfonamides was observed due to entrapment of drugs in micelles. Ionized sulfonamides are poorly solubilized in the micelles, but it is the un-ionized form which is biologically active and the un-ionized form has distribution coefficient in favor of the surfactant micelles than the biological fluid. They also noted that the ionized moiety of these drugs is also able to be absorbed to a certain extent. Moroshita et al. (110) also found that the absorption from rat intestine of sulfonamides in the anionic form was quite significant. Yamada and Yamamoto (182) 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 polysorbate 80 solution. It is obvious that this technique can detect only irreversible effects on membrane permeability. Matsumoto (101) 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 (141) studied the effect of micellar solutions of tyloxapol, a non-ionic surfactant, on the transfer of hydrocortisone across 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. Utsumi (168) found that sodium lauryl sulfate formed a complex with benzoylthiamine disulfide (BTDS) through ionic and hydropholic interaction, and decreased the absorption rate of BTDS from rat intestine. But it was found later by the same authors that a system containing both sodium lauryl sulfate and sodium glycocholate reversed the decrease in absorption rate of BTDS up to around the control level. By determining the saturation solubility of BTDS it was found that its solubility in 0.1% sodium lauryl sulfate solution was remarkably reduced by mixing sodium glycholate in this solution. In this binary surfactant mixture an increase in the CMC value with decreasing partition coefficient for BTDS in the micelles was found in proportion to the increase in the ratio of sodium glycocholate and sodium lauryl sulfate. The authors have concluded from this that sodium glycocholate contributes to creating new mixed micelles having a different partition coefficient from the drug (BTDS) than from those of sodium lauryl sulfate micelles. The formation of mixed micelles and the ensuing increase in the amount of BTDS out of micelles are evidenced to be responsible for the sodium glycocholate effect which cancels the inhibitory action of sodium lauryl sulfate in the absorption of BTDS.

Influence of Surfactants on the Permeability of Membranes

A number of substances have been found to 'interact' with biological membranes and thereby alter permeability or transport characteristics. Windsor and Cronheim (180) reported that heparin and sulfopolyglycine, normally very poorly absorbed from the gastrointestinal tract, were absorbed to an appreciable extent when administered together with the chelating agent, ethylenediamine tetra-acetic acid (EDTA). Schanker and Johnson (147) also observed an increase in the in vivo intestinal absorption of mannitol, inulin, a quaternary ammonium compound, and sulfanilic acid (all lipid insoluble compounds) in the presence of EDTA. Tetracycline has also been shown to interact with the biologic membrane mediated through calcium ions (67, 113). Tetracycline increased the absorption of sulfanilic acid and sulfaguanidine (114). Nadai et al. (114) have examined the histological changes occurring in the rat small intestine (using light microscope and scanning electron microscope) after administration of EDTA, which has absorption enhancing effect on the poorly absorbable drug. Marked separation of epithelial cells was observed but histological changes were not observed as viewed with light microscope. The role of calcium in maintaining the integrity of the intestinal membrane was affected by the chelating agent and the removal of calcium ion from the intestinal mucosa caused such marked shedding of epithelial cells.

Surfactants may also be capable of modifying the properties of biologic membranes. Perhaps the earliest report of the effect of surfactant on drug activity is that of Billard and Dieulafe (12), who noted that the toxic effect of curare injected intraperitoneally into guinea pigs could be increased by the addition of low concentrations of soap and decreased by high concentrations. Alexander and Trim (1) reported that the penetration of hexylresorcinal into Ascaris lumbricoides can be affected by cetyltrimethyl ammonium bromide (CTAB) in two different ways. Below the CMC the surfactant increased the penetration of the drug, and at concentrations above CMC, the penetration of the drug was reduced. Effect of surfactant below CMC is due possibly to the increased permeability of the membrane surface. Levy et al. (89) studied the effect of polysorbate 80 on the absorption of a number of barbiturates across the goldfish membranes. The absorption rate of barbiturates was found to increase significantly in the presence of low concentrations (below CMC) of the surfactant and to decrease at higher concentrations of the surfactant. Their further studies (86) showed that the increase in absorption rate of secobarbital at the concentrations of 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 non-micellar polysorbate: secobarbital complex. The same authors (3) have also shown that pre-micellar concentrations of polysorbate 80 enhance the absorption and exsorption of 4-aminoantipyrine across the goldfish membranes. Gibaldi et al. (53) studied the influence of sodium taurodeoxycholate on the pharmacologic effect (overturn time) of pentobarbital and ethanol in goldfish. They found that bile salt significantly potentiated the pharmacologic effect. Ethanol can diffuse through the 'pores' of the membrane, while the barbiturate must diffuse across the lipoidal barrier; the non-ionic surfactant might have a specific effect on the lipoid content of the cell membrane and thus change its permeability. Further studies (117) indicated that the bile salt exerts an all-or-none effect on the uptake of 4-aminoantipyrine in goldfish; an alteration in membrane permeability was observed above a certain bulk concentration but below the CMC of the surfactant. Whitworth and Yantis (177) found an increase in the absorption of salicylic acid across the external membranes of the frog in the presence of 0.1% polysorbate 80. The effect of polysorbate 80 on the biological activity of chlorpromazine hydrochloride in solution was investigated by Florence (45) using the goldfish. Below a certain critical concentration the activity was enhanced in unbuffered drug solutions, but above this concentration the activity was diminished, possibly due to some association between surfactant micelles and drug molecules.

Human cornea has also been used to study the effect of surfactants on absorption. In such a study (100) 0.1% solutions of non-ionic surfactants were used. The permeability of the cornea was measured by following the movement of the fluorescein dye. Only three surfactants (Tween 20, Brig 35, and Brig 58) had any marked effect on the absorption of fluorescein. Tween 20 was the only compound that safely increased the permeability of the corneal epithelium; the other two agents produced eye irritation.

Nickel salts compounded with anionic surfactants caused edema, but non-ionic and cationic surfactants did not show this effect. This could be due to an acanthotic effect (modification of the prickle cell layer [stratum germinativum] of the skin) or to the denaturation of the epidermal proteins by the anionic surfactants, allowing nickel salts to penetrate skin and cause eczema (169). Scala et al. (143) in a study of the percutaneous absorption of ionic surfactants found that alkylbenzene sulfonates 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. Kay (78) found that the permeability of the Ehrlich-Lettre ascites carcinoma cells was increased greatly in the presence of polysorbate 80 as shown by the uptake of Lissamine green dye. Percutaneous absorption was measured by immersing the hind foot of a mouse in a drug solution, then extracting the absorbed drug. It was shown that polysorbate 80 increased pyrrolnitrin absorption when a surfactant concentration of 0.01% and 0.1% was used.

Appel (5) found that simultaneous feeding of sodium lauryl sulfonate and inulin to rats results in up to a tenfold increase in urinary excretion over control values. The enhanced urinary excretion of urine may be the result of an increase in intestinal permeability to inulin in the presence of the surfactant. Mori et al. (109) found that rats and hamsters fed polysorbate 20 showed increased gastrointestinal absorption of iron. However, Brise (15) 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 the absorption of iron in hamsters and rats in the presence of polysorbate 20 by Mori et al. may have been due to some 'toxic' action of the surfactant. The absorption of barium chloride ingested by cats was promoted by both polysorbate 20 and sodium lauryl sulfate at low concentrations and inhibited at high concentrations (159).

Suzuiki et al. (162) found increased permeability, as measured by a circulating dye, at the site of an intracutaneous injection of various non-ionic surfactants. Authors postulated that this increase was mainly due to the wetting and solubilizing effects of the surfactants on lipid structure of the capillary wall. Matsuzawa et al. (102) studied the effect of some non-ionic surfactants on the muscular absorption of enduracidin. Addition of the surfactant remarkably promoted the absorption of enduracidin from the muscles of the rats. They postulated that since the effects of surfactants are considered to be due to their interaction with both the biological membrane and the drug, therefore it is reasonable to suppose that one of the enhancing effects observed could be attributed to the surface tension lowering of the biological membrane, which allows the ready passage of the antibiotic through the muscles.

Penzotti and Mattocks (128) found an increase in the rate of peritoneal dialysis of urea and creatinine in rabbits in the presence of surfactants. It was found that the order of magnitude of effects decreased in the following manner: cationic $>$ anionic $>>$ non-ionic. It appears that the mechanism involves a change in the permeability of the peritoneal membrane.

Lish and Weikel (96) studied the effects of surfactants on the absorption of an anionic dye, phenolsulfonphthalein (PSP) from the colon of rats. Both sodium lauryl sulfate and dioctyl sodium sulfosuccinate, but not the non-ionic Pluronic F-68, increased the absorption of PSP. None of the surfactants studied had any effect on the absorption of the cationic dye, methyl violet. Engel and Riggi (33) studied the effects 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-300 (an alkyl aryl sulfonate) resulted in an increase in heparin absorption over that observed when heparin was administered alone. They also reported enhanced heparin absorption in the presence of 0.4% sodium taurocholate. The authors postulated that the increase in heparin absorption is due to an effect of the surfactant on the intestinal mucosa. Kakemi et al. (69) studied the absorption of solubilized vitamin A (in surfactant) from the rat intestine. Contrary to the notion that the only form in which drug can penetrate the membrane is the free form of the drug, both vitamin A acetate and vitamin A alcohol, entrapped almost completely in the micelles, were absorbed fairly well from the small intestine, but not as from the large intestine. It was demonstrated that in the very early stages of absorption, vitamins solubilized in micelles are absorbed onto the membrane, which is favored by the surfactant, and this results in a local build-up of concentration of the vitamins. This process makes the drug transport through the membranes. Kakemi et al. (70) studied the absorption of drugs from oil in water emulsions from the rat large intestine. It was found that in the case of poorly oil-soluble drugs the absorption was increased when administered in the form of emulsion; but in case of drugs of low lipophilicity the absorption was inhibited when emulsion volume was kept constant in both cases.

Davis and Kreutler (24) studied the effect of surfactants on the absorption of water-soluble substances from rats. Using labelled cyanocobalamin (^{57}Co) both gastric and intestinal absorption were markedly increased by the addition of Brij 98 (polyoxyethylene-20-olelyl ether) to the aqueous solution of the vitamin. Absorption of cephaloridine and cephalothin was promoted both in ligated stomach and ligated small intestine. Using cholestrol monolayers Gillan and Florence (54) have found that, in case of non-ionic surfactants, where the surfactant has long hydrophile chains (i.e., > 5 ethylene oxide units) rather than a single ethylene oxide chain or several short ethylene oxide chains, drug absorption rates would not increase. This indicates that the effectiveness of the surfactant is due to the ease with which the surfactant molecule penetrates lipid membranes.

> Bile salts have long been implicated in the intestinal absorption of fat and other nutrients. They are known to have an important role in the emulsification of water-insoluble, long-chain triglycerides and in stimulating the action of pancreatic lipase, resulting in a mixture consisting of fatty acids, monoglycerides, diglycerides, and triglycerides. 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 (49).

Bile salts have also been shown to be involved in the absorption of vitamins A, D, and K. Several reports have shown the effect of bile salts on the absorption of cholestrol; exogenous bile salts also enhance the absorption of cholestrol (49). A recent report has shown that bile salts increased about 25 percent the absorption of labelled dietary cholestrol fed to germ-free rats (177). Longemann and Dobbins (49) found that intraperitoneal injections and large oral doses of sodium taurocholate enhanced the absorption of calcium by the rat. Seyfried and Lutz (49) reported that the absorption of tetraiodophenolphthalein from intestine was greatly diminished in the absence of bile salts. Pekanmaki and Sabin (49) reported that the absence of bile from the intestine of cats reduced the absorption of phenolphthalein. Davenport (23) found that bile salts are capable of increasing the permeability of the gastric mucosa as judged by hydrogen ion flux. When sodium deoxycholate was administered 30 minutes prior to a dose of riboflavin, a 50-80 percent increase in total urinary recovery was found. Two mechanisms were postulated by the authors: (a) reduction in gastric emptying time, and (b) increase in the permeability of gastric mucosa (103). Meli et al. (105) reported that endogenous bile influences the rate of intestinal absorption of an estrogen (ethynylestradiol-6,7-^{3}H-3-cyclopertyl ether) in rats. The rate of absorption of estrogen was considerably lower in biliary-cannulated rats than in control animals. Since the steroid is relatively water insoluble, the presence of bile salts might have increased the solubility of the drug in the intestinal lumen and thereby enhanced the dissolution and absorption rate. Levels of conjugated bile salts normally found in the proximal intestine alter the permeability of everted rat intestine to salicylate (39), salicylamide (38), riboflavin (43), and several other drugs. Nightingale et al. (116) studied the effect of bile flow on the absorption of sulfadiazine. They showed that bile flow is an important factor in sulfadiazine absorption from the intestinal loops. Feldman et al. (40) studied the influence of sodium deoxycholate on the absorption of phenol red in the rat with different methods. Each of the methods provided evidence that the bile salt markedly enhanced the absorption of phenol red by altering the permeability of the intestinal membranes. Kakemi et al. (73) studied the influence of sodium taurocholate and sodium glycocholate on the absorption of the same drug (phenol red). They postulated that there are three likely mechanisms by which bile salts can affect drug absorption from the rat small intestine; first, the loss of thermodynamic activity of a drug due to the formation of a micellar complex; second, the local concentration build-up effect, such as accumulation on the absorptive surface; third, the direct effect on the permeability of the intestinal mucosa. Kakemi et al. (74) also studied the influence of these bile salts on the intestinal absorption of sulfaguanidine. They postulated that the enhancement of absorption of drug was caused by the direct action of bile salts to the structure of the absorptive surface.

Some drugs like tetracycline and dextromethorphan (42, 129) themselves possess surface activity and influence their own absorption, e.g., due to the anion's contribution to the surface activity in case of dextromethorphan salts (42).

Evidence has accumulated which suggests that a drug's affinity for the intestinal wall may be an important factor in its transport across. In the study (71) of absorption of barbiturates a discrepancy from the pH-partition hypothesis was noted. This discrepancy was correlated significantly with the in vitro binding to mucusal preparations of rat small intestine. The sorption of a surfactant also takes place onto the intestinal wall and can also effect the absorption of drugs. Nogami et al. (120) studied the sorption of ionic surfactants (sodium lauryl sulfate and cetyltrimethyl ammonium bromide) into the intestinal tissue of the rats. Kakemi et al. (68) studied the absorption of certain ion-pair complexes of some pharmaceutical amines with sodium lauryl sulfate and sodium saccharin. It was found that the enhancement of absorption of these drugs could better be related to the binding behavior of these drugs to the rectal mucosal preparations. Some authors, in order to confirm that the binding to the mucosa is an important factor in the absorption from small intestine, studied the binding of fifteen drugs with mucosal homogenates. Their experiments indicate that the binding of drug (both ionized and un-ionized) to the mucosa of the small intestine is important in absorption of drugs. They also mentioned that while absorption from rectum is consistent with pH-partition hypothesis, it is not so in case of small intestine. Suzuiki et al. (163) studied the absorption of quinine and chlorpheniramine from the gut given with various anionic agents (including sodium lauryl sulfate) which form ion-pair complexes. It was found that the increase of the drug binding to the absorptive membrane was responsible for the enhancement of the drugs present in the form of an ion-pair complex. The affinity of surfactants for hydrophobic surfaces and for interfaces in general leads one to expect that they will have a profound influence on the behavior or condition of cell surfaces, which are predominantly hydrophobic in nature. Many cationic detergents have a non-specific disrupting effect on the cells of bacteria and tissue, thus precluding their systemic use. Nissim (119) studied the effect of feeding cationic, anionic, and non-ionic surfactants on the histology of the mouse gastrointestinal tract. He found marked pathological changes when the ionic surfactants were fed to mice but no effects when non-ionic surfactants were tested. However, there is evidence that non-ionic polysorbate 80 disrupts membrane structure but there is a rapid reconstitution of cell membrane material after treatment (80). Triton X-100, an alkylphenyl polyether, totally disrupts lysosomes, mitochondria, and erythrocytes (28). Taylor (165) studied the effects of cetyltrimethyl ammonium bromide on transport and metabolism in the small intestine of rat. The surfactant was found to produce no histological damage to everted rat intestine sacs at lower concentrations of the surfactant. But at higher concentrations, concomitant with the mucosal damage, there was an inhibition of the transport of glucose, methionine and water. Nissim (118) had also found increased absorption of glucose with various surfactants at low concentrations. Kozlick and Mosinger (80) had observed that low nontoxic doses of sodium lauryl sulfate increased the rate of absorption of glucose administered to rabbits. Kakemi et al. (72) also found that cationic surfactants, cetyltrimethyl ammonium bromide and benzethonium chloride, caused an increase in the absorption of sulfisoxazole from the rat rectum and pointed out the resemblance of this work to those of Nissim and Taylor. Aoki et al. (4) studied the effects of surface active agents (ionic and non-ionic) on intestinal absorption of drugs using three methods, (a) circulation of the drug with surfactant, (b) perfusion with surfactant solution followed by perfusion of drug solution free from surfactant, and (c) feeding experiments of surfactant solutions, followed by circulation of drug solution. It was found that due to the solubilizing action and degeneration of the surfactants on the mucus membrane, the absorption of ionized species was decreased more compared to its normal absorption, but the absorption of un-ionized species of the drug was not decreased in the presence of surfactants, rather it was increased. The other effect observed was decreased absorption of drugs due to complexation with surfactants (non-ionics had least action in this respect). Nadai et al. (113) found that sodium lauryl sulfate produced pronounced changes in the gross appearance of the mucosal surface of the small intestine of the rat and this is invariably accompanied by the increased changes in permeability. Morphological changes were associated conceivably with the solubilization of the lipid components of the membrane such as lipoproteins.

CHAPTER III

OBJECTIVES OF RESEARCH

In spite of the fact that DSS and polaxalene are used widely in medicine as fecal softeners very few reports are available of their effects on absorption of drugs. The purpose of this study was to find the effects of DSS and polaxalene on the absorption of a poorly absorbed drug (phenol red) and a poorly soluble drug (sulfisoxazole). Although Lish (96) has studied the effects of DSS and polaxalene on the absorption of phenol red, his study was limited to only rat colons and only to one concentration of the surfactants. Since the action of surfactants on the absorption of drugs is known to be concentration dependent and since phenol red, like most other drugs, is absorbed mainly from the upper gastrointestinal tract, a more detailed study of the effects of DSS and polaxalene on the absorption of phenol red and sulfisoxazole was deemed necessary.

The objectives of this study are the following:

(1) Determination of the effects of the surfactants on the absorption of phenol red and sulfisoxazole from intact rat.

(2) Determination of the effects of the surfactants on the absorption of phenol red from rat small intestinal loops.

(3) Determination of the effects of DSS on the absorption of phenol red from peritoneal cavity of the rat.

CHAPTER IV

EXPERIMENTAL

Materials

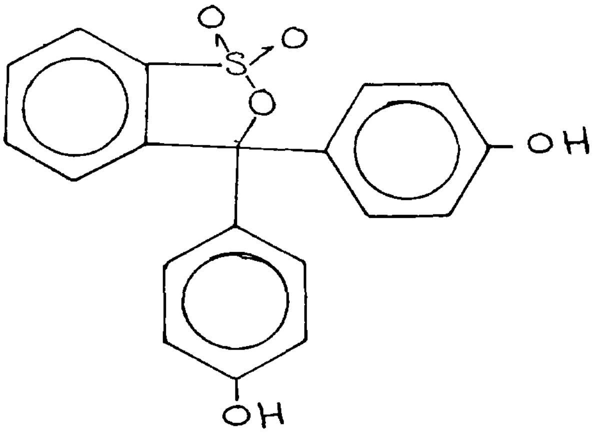
The following materials were used in these experiments:

Phenolsulfonphthalein (phenol red)
J. T. Baker, Analytical Grade
Dioctyl Sodium Sulfosuccinate (DDS)
E. H. Sargent and Company
Polaxalene, "Polyoxyethylene polyoxypropyline polymer"
(Pluronic F-68)
Wyandote Chemicals, Wyandote, Michigan
Sulfisoxazole (U.S.P.)
Hoffman LaRoche, New Jersey
Octanol-1 (n-Octanol)
J. T. Baker, Analytical Grade

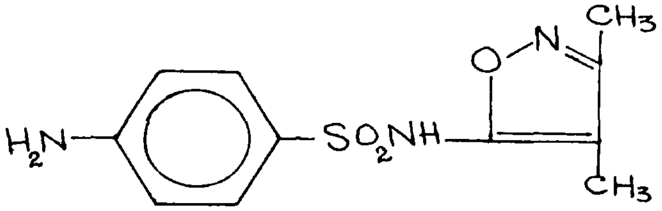
Procedures

Influence of DSS and Polaxalene on the Gastrointestinal Absorption of Phenol Red

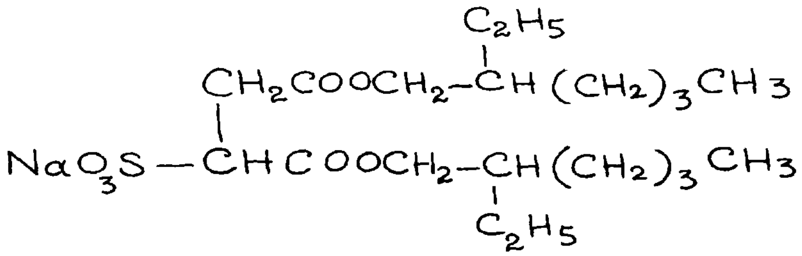
Intestinal loop. Male Sprague Dawley rats, weighing between 195 and 330 grams, were starved for 18-20 hours with water allowed ad libitum. The rats were anesthetized with ether and a midline incision



Phenol Red

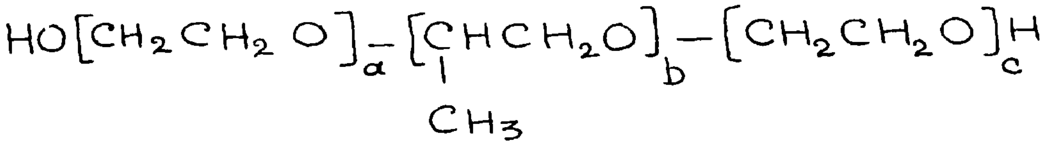


Sulfisoxazole



$$\begin{array}{l} CH_2COOCH_2-CH(C_2H_5)(CH_2)_3CH_3 \\ | \\ NaO_3S-CHCOOCH_2-CH(C_2H_5)(CH_2)_3CH_3 \end{array}$$

Dioctyl Sodium Sulfosuccinate



$$HO[CH_2CH_2O]_a-[CH(CH_3)CH_2O]_b-[CH_2CH_2O]_cH$$

Polaxalene

Figure 1

Drug Formulas

was made to expose the abdominal cavity. A small incision was made in the small intestine about 0.5-1 cm from the pyloric end and a hypodermic needle (22 gauge) with a blunt end was inserted into the incision and secured tightly with a silk suture. Another small incision was made in the small intestine about 0.5-1 cm from the ileo-cecal junction and this portion of the small intestine was kept outside the abdominal cavity. About 3 ml of normal saline was placed in the peritoneal cavity to hydrate the intestine. Normal saline (50 ml) was pushed slowly through the small intestine until the washings became clear of the particulate matter. The rats were kept under anesthesia for 20-25 minutes, before closing the ileo-cecal end, to allow for absorption of any residual liquid.

Exactly 5 ml of phenol red solution (0.75 mg/ml) in distilled water or in various concentrations of the surfactant solution was injected into the intestinal loop from the pyloric end. The needle was then taken out, making sure that no solution came out and the suture was tightly secured at the same time. The abdominal cavity was closed with sutures and the animal was left for 3 hours. During this time the animal had recovered from anesthesia and was kept in a cage. The animal was sacrificed after the 3-hour period; the whole small intestine was removed and washed with normal saline to get rid of any blood on its surface. Then it was homogenized in a Waring blender with a little water (96) and transferred to a 500 ml volumetric flask. To this was added the calculated volume of 95% ethanol needed to make the final solution 70% in ethanol content. The blender was rinsed with distilled water and the washings were added to the volumetric flask and then the volume was made up to 500 ml with distilled water. The solution was left for 30 minutes to allow for precipitation of proteins. Then about 35 ml of this solution was centrifuged at 7,000 r.p.m. for 20 minutes. Then 25 ml of the clear supernatant solution was pippetted out and transferred to a 50 ml volumetric flask. Then 1 ml of 2N NaOH was added and the volume was made up to 50 ml with 70% ethanol.

The absorbance of this solution was measured at 560 mµ against 70% ethanol in a Bausch & Lomb Spectronic 600 spectrophotometer.

The percent of phenol red absorbed was calculated by measuring the difference in absorbancy between the original solution diluted in the same manner (5 ml to 1,000 ml) and the intestinal homogenate solution.

This method for calculating the percent absorption of phenol red runs a higher risk of experimental error compared to a direct measurement. However, extreme care was taken in introducing the same volume of drug solution into the intestinal loop each time. Furthermore, the study deals with the differences between phenol red solution and phenol red solutions containing surfactants, so that any experimental error due to the techniques was expected to be constant.

Gastric intubation. Male Sprague Dawley rats weighing between 195 and 300 grams were starved for 18-20 hours, with water allowed ad libitum. The experiments were performed in a cross over fashion, i.e., each rat serving as its own control.

Each rat received a control dose of 2 ml of phenol red solution in distilled water (0.75 mg/ml) by gastric intubation (by means of a

Tygon tube). The animal was then kept in a metabolic cage, and water was allowed ad libitum, but food was withheld. The urine was collected for a period of 24 hours.

After a recovery period of three days, the same rat received 2 ml of phenol red solution (0.75 mg/ml) in one of the different concentrations of the surfactants studied. The urine was collected for a period of 24 hours as mentioned before.

Half the number of animals first received control doses of the drug, followed by a dose of the drug with the surfactant after the recovery period. While in the other half number of animals, the procedure was reversed, i.e., first a dose of the drug was given with the surfactant followed by a control dose of the drug after the recovery period.

The pH of the urine was adjusted to 10.00 with 2N sodium hydroxide solution and the volume of the sample was made up to 50 ml. A portion of the sample was filtered through Millipore filter (0.45 μ) and its phenol red content was determined by measuring its absorbance against a blank prepared in the same manner as the sample, at 560 mμ in a spectrophotometer (38).

Using a standard curve for phenol red, the percent of the dose absorbed by the rat was calculated.

Influence of DSS on the Peritoneal Absorption of Phenol Red

Male Sprague Dawley rats weighing between 210 and 380 grams were starved for 18-20 hours with water allowed ad libitum. The experiments were performed in a cross over fashion, i.e., each rat serving as its own control.

Each rat received a control dose of 0.5 ml of phenol red solution in distilled water (0.75 mg/ml) by intraperitoneal injection. The animal was kept in a metabolic cage, and water was allowed ad libitum but food was withheld. The urine was collected for a period of 24 hours. After a recovery period of three days the same rat received a dose of 0.5 ml of phenol red solution (0.75 mg/ml) in one of the different concentrations of DSS in distilled water. The urine was again collected for a period of 24 hours as mentioned before.

The content of phenol red in the urine was determined as described previously under "Gastric intubation", but the volume of urine was made up to 100 ml.

Effect of DSS and Polaxalene on the Equilibrium Solubility of Phenol Red at 37°C.

Excess amounts of phenol red powder were placed in 25 ml ampules and then 10 ml of various solutions of the surfactants were added to the ampules. The ampules were sealed and rotated in a Metabolyte shaker maintained at 37°C. After equilibrium was achieved (approximately 3 days), an aliquot was filtered through a Millipore filter (0.45 μ). One ml of this filtered solution was diluted to 500 ml after adding 2N sodium hydroxide to adjust pH to 10.0. The absorbance of this solution was measured at 560 mμ in a spectrophotometer.

Effect of DSS on the Apparent Partition Coefficient of Phenol Red between Octanol-1 and Surfactant Solution

Solutions of phenol red (0.05%) were prepared in phosphate buffer[1] (M/100 and pH 6.0) or in the same buffer containing various concentrations of DSS. Ten ml of buffered phenol red solution was added to an equal volume of octanol-1, previously saturated with the buffer solution, in a 50 ml Erlenmeyer flask. The contents were equilibrated at 37°C for two days. After separation by centrifugation (at 11,000 r.p.m., for 30 minutes, and at 37°C) an aliquot (1 ml) of the aqueous phase was rendered alkaline (pH 10.0) with sodium hydroxide solution and diluted to 250 ml with distilled water. The absorbancy of this solution was measured at 560 mμ and the apparent partition coefficient was calculated from the decrease in the concentration of phenol red in the aqueous phase.

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[1] Clark and Lubs Phosphate Buffer.

Influence of DSS on the Gastrointestinal Absorption of Sulfisoxazole

Gastric intubation. Male Sprague Dawley rats weighing between 200 and 240 grams were starved for 18-20 hours, with water allowed ad libitum. The experiments were performed in a cross over fashion, i.e., each rat serving as its own control.

Each rat received a control dose of 5 mg of sulfisoxazole in the form of a suspension in distilled water. Preparation of a drug suspension was done in situ by placing the drug into the barrel of a dry 5 ml syringe, adding 2 ml water to it and intubating the resulting suspension. An additional 1.5 ml of water was added in small increments to ensure complete delivery of the drug from the syringe. The animal was then kept in a metabolic cage, water was allowed ad libitum but food was withheld. The urine was collected for a period of 24 hours.

After a recovery period of three days the same rat received the same amount of the drug suspended in 2 ml of one of the various concentrations of the DSS solution. A 1.5 ml of water was added in small increments to the syringe to ensure complete delivery of the drug. The urine was collected for a period of 24 hours as mentioned before.

Half the number of animals first received control dose followed by a dose of the drug with the surfactant after the recovery period. While in the other half number of animals, the procedure was reversed, i.e., first a dose of the drug was given with the surfactant, followed by a control dose of the drug after the recovery period.

Urinary analysis for sulfisoxazole. The total urinary excretion of the sulfisoxazole (free, as well as acetylated portion) by the modified Bratton and Marshal method (30).

Urine was diluted to exactly 100 ml and a 10 ml aliquot was pippetted out in a 100 ml volumetric flask, to which was added 10 ml of 0.5 N hydrochloric acid. This mixture was heated on a steam bath for one hour to hydrolyze the acetylated portion of the excreted drug. After cooling, 10 ml of a 0.1% solution of sodium nitrite, freshly prepared, was added and shaken thoroughly. After 6 minutes, 10 ml of a 0.5% solution of ammonium sulfamate was added and shaken thoroughly. The pH of the resulting solution was checked and adjusted to a value of approximately 1.3-1.4 with 1 N hydrochloric acid. Six minutes after the addition of ammonium sulfamate, 10 ml of a 0.1% solution of N-1-Naphthylethylenediamine dihydrochloride was added. The volume was adjusted to 100 ml and a portion of it was filtered through a Millipore filter (0.45 µ). The absorbance of this solution was read at 533 mµ against a blank prepared in the same manner.

The amount of sulfisoxazole absorbed was calculated by means of a standard curve.

Effect of DSS on the Solubility of Sulfisoxazole at Room Temperature

Sulfisoxazole (100 mg) was placed in a 20 ml test tube and 5 ml of various concentrations of DSS solutions, prepared in M/5 phosphate buffer, were added to it. The tube was shaken at a medium speed on a vortex mixer for exactly 5 minutes at room temperature. It was then filtered through a Millipore filter (0.45 µ). One ml of this filtered solution was properly diluted with a phosphate buffer of pH 7.5 (M/5). Absorbance of this solution was read against a blank (phosphate buffer) at 252 mµ.

Statistical Analysis of Results

To find out the significance of the effect of various concentrations of surfactants a student *t* test for the significant difference in the means was performed. An analysis of variance in conjunction with an *F* test was also performed. This analysis involves an extension of the pooled variance technique and the calculation of a variance ratio. This is in contrast to the ratio of the difference between means to the standard error of the difference required by the *t* test. This analysis answers the same question as a student *t* test does for the difference between two means and is used as a test of significance for two or more groups of data.

Table 1

Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loop

Phenol Red

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.668	0.640	4.19
2	0.668	0.635	4.94
3	0.563	0.540	4.08
4	0.563	0.540	4.08
5	0.563	0.537	4.62
6	0.573	0.545	4.89
Arithmetic Mean	0.5996	0.5728	4.4667
Standard Deviation		0.0502	0.4006
Standard Error		0.0205	0.1635

Table 2

Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loop

Phenol Red + 0.25% DSS (12.5 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.595	0.505	6.72
2	0.595	0.470	21.00
3	0.595	0.500	16.00
4	0.595	0.475	20.17
5	0.595	0.460	22.67
6	0.595	0.485	18.50
Arithmetic Mean		0.4825	17.5100
Standard Deviation		0.0175	5.7544
Standard Error		0.0071	2.3500

$t = 5.5387$ (significant difference at $p < .001$ when compared to control)

Table 3

Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loop

Phenol Red + 0.5% DSS (25 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.590	0.390	33.88
2	0.590	0.380	35.59
3	0.590	0.360	38.98
4	0.590	0.340	42.37
5	0.590	0.185	68.64
6	0.590	0.160	72.68
7	0.590	0.130	77.96
8	0.590	0.120	79.66
9	0.590	0.380	35.59
Arithmetic Mean		0.2717	53.9278
Standard Deviation		0.1188	20.1223
Standard Error		0.0396	6.7074

t = 5.9444 (significant difference at $p < .001$ when compared to control)

Table 4

Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loop

Phenol Red + 1.0% DSS (50.0 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.600	0.353	41.177
2	0.600	0.140	76.67
3	0.600	0.140	76.67
4	0.600	0.115	80.83
5	0.600	0.108	82.00
6	0.600	0.095	84.17
Arithmetic Mean		0.1585	73.5845
Standard Deviation		0.0969	16.1574
Standard Error		0.0396	6.6000

t = 10.476 (significant difference at $p < .001$ when compared to control)

Table 5

Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loop

Phenol Red + 1.5% DSS (75.0 mg)

Rat	Absorbance at 560 mμ Phenol Red Solution	Absorbance at 560 mμ Intestinal Homogenate	Percent Dose Absorbed
1	0.590	0.380	35.59
2	0.590	0.340	42.37
3	0.590	0.305	48.30
4	0.590	0.265	55.08
5	0.590	0.270	54.24
6	0.590	0.185	68.64
Arithmetic Mean		0.2908	50.7033
Standard Deviation		0.0676	11.4632
Standard Error		0.0276	4.6807

t = 9.8739 (significant difference at $p < .001$ when compared to control)

Table 6

Influence of DSS on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 0.5% DSS (10 mg).

	Control		DSS	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.260	5.420	0.365	7.609
2	0.290	6.045	0.410	8.547
3	0.210	4.378	0.350	7.296
4	0.280	5.837	0.470	9.798
5	0.216	4.503	0.360	7.505
6	0.220	4.586	0.382	7.963
7	0.230	4.795	0.400	8.338
8	0.190	3.961	0.430	8.964
9	0.200	4.169	0.465	9.693
Arithmetic Mean	0.2329	4.8548	0.4036	8.4125
Standard Deviation	0.0356	0.7413	0.0442	0.9223
Standard Error	0.0118	0.2471	0.0147	0.3074

$t = 9.6147$ (significant difference at $p < .001$)

Table 7

Influence of DSS on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 1.0% DSS (20 mg).

Rat	Control		DSS	
	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.340	7.088	0.480	10.006
2	0.210	4.378	0.310	6.462
3	0.270	5.628	0.414	8.630
4	0.310	6.462	0.530	11.049
5	0.212	4.419	0.580	12.091
6	0.217	4.524	0.580	12.091
7	0.300	6.254	0.740	15.426
8	0.261	5.441	0.740	15.426
9	0.210	4.378	0.434	9.047
Arithmetic Mean	0.2589	5.3969	0.5342	11.1364
Standard Deviation	0.0497	1.0355	0.1443	3.0085
Standard Error	0.0165	0.3452	0.0481	1.0028

$t = 5.8987$ (significant difference at $p < .001$)

Table 8

Influence of DSS on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 1.5% DSS (30 mg).

	Control		DSS	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.230	4.795	0.388	8.088
2	0.244	5.086	0.500	10.423
3	0.238	4.961	0.495	10.319
4	0.247	5.149	0.558	11.632
5	0.204	4.253	0.480	10.006
6	0.220	4.586	0.605	12.612
7	0.190	3.961	0.500	10.423
8	0.190	3.961	0.565	11.778
9	0.190	3.961	0.690	14.384
Arithmetic Mean	0.2170	4.5237	0.5312	11.0739
Standard Deviation	0.0240	0.4999	0.0858	1.7889
Standard Error	0.0079	0.1666	0.0186	0.5963

t = 9.6859 (significant difference at $p < .001$)

Table 9

Influence of DSS on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 2.0% DSS (40 mg).

Rat	Control		DSS	
	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.245	5.107	0.351	7.317
2	0.215	4.482	0.385	8.026
3	0.195	4.065	0.365	7.609
4	0.165	3.440	0.325	6.775
5	0.190	3.961	0.362	7.546
6	0.195	4.065	0.400	8.338
7	0.135	2.814	0.300	6.254
8	0.210	4.378	0.490	10.215
9	0.240	5.003	0.560	11.673
Arithmetic Mean	0.1978	4.1461	0.3931	8.1948
Standard Deviation	0.0350	0.7198	0.0823	1.7156
Standard Error	0.0115	0.2399	0.0274	0.5718

t = 8.8382 (significant difference at $p < .001$)

Table 10

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 0.01% DSS (0.05 mg).

	Control		DSS	
Rat	Absorbance at 560 mµ	Percent Dose Absorbed	Absorbance at 560 mµ	Percent Dose Absorbed
1	0.340	56.702	0.410	68.376
2	0.315	53.533	0.380	63.373
3	0.405	67.542	0.435	72.545
4	0.345	57.536	0.415	69.210
5	0.405	67.542	0.422	70.377
Arithmetic Mean	0.3620	60.3711	0.4124	68.7763
Standard Deviation	0.0409	6.8152	0.0204	3.4026
Standard Error	0.0182	3.0479	0.0091	1.5217

t = 4.4960 (significant difference at $p < .02$)

Table 11

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 0.05% DSS (0.25 mg).

	Control		DSS	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.332	55.368	0.390	65.041
2	0.240	40.025	0.320	53.367
3	0.320	53.367	0.370	61.705
4	0.355	59.204	0.385	64.207
5	0.305	50.8651	0.360	60.037
Arithmetic Mean	0.3104	51.7657	0.365	60.8714
Standard Deviation	0.0434	7.2373	0.0278	4.6427
Standard Error	0.0194	3.2367	0.0124	2.0763

$t = 6.8164$ (significant difference at $p < .005$)

Table 12

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 0.10% DSS (0.50 mg).

Rat	Control		DSS	
	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.310	51.699	0.350	53.370
2	0.295	49.197	0.320	53.367
3	0.325	54.200	0.358	59.704
4	0.260	43.360	0.310	51.699
5	0.320	53.366	0.330	55.034
Arithmetic Mean	0.3020	50.3648	0.3336	55.6348
Standard Deviation	0.0261	4.3569	0.0201	3.3554
Standard Error	0.0117	1.9485	0.0090	1.5006

$t = 4.6560$ (significant difference at $p < .01$)

Table 13

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 0.50% DSS (2.50 mg).

	Control		DSS	
Rat	Absorbance at 560 mµ	Percent Dose Absorbed	Absorbance at 560 mµ	Percent Dose Absorbed
1	0.300	50.031	0.250	41.693
2	0.280	46.696	0.237	39.525
3	0.243	40.525	0.165	27.517
4	0.290	48.364	0.243	40.525
5	0.355	59.2037	0.310	51.699
Arithmetic Mean	0.2936	48.9639	0.2410	40.1918
Standard Deviation	0.0405	6.7584	0.0516	8.6004
Standard Error	0.0181	3.0225	0.0231	3.8463

$t = 8.1494$ (significant difference at $p < .005$)

Table 14

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 1.00% DSS (5.00 mg).

	Control		DSS	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.370	61.705	0.210	35.022
2	0.310	51.699	0.200	33.354
3	0.320	53.367	0.175	29.185
4	0.320	53.367	0.195	32.520
5	0.360	60.037	0.280	46.695
Arithmetic Mean	0.3360	56.0350	0.2120	35.3554
Standard Deviation	0.0272	4.5058	0.0401	6.6865
Standard Error	0.0122	2.0151	0.0179	2.9904

t = 8.9141 (significant difference at $p < .001$)

Table 15

Influence of DSS on the Peritoneal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Intraperitoneal Administration of 0.375 mg dose of Phenol Red with and without 1.50% DSS (7.5 mg).

	Control		DSS	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.293	48.864	0.124	20.680
2	0.350	58.370	0.160	26.683
3	0.340	56.702	0.170	28.351
4	0.367	61.205	0.218	36.356
5	0.360	60.037	0.200	33.354
Arithmetic Mean	0.3420	57.0356	0.1744	29.0848
Standard Deviation	0.0292	4.8750	0.0365	6.0838
Standard Error	0.0130	2.1802	0.0163	2.7208

$t = 24.8031$ (significant difference at $p < .001$)

Table 16

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops

Phenol Red

Rat	Absorbance at 560 mμ Phenol Red Solution	Absorbance at 560 mμ Intestinal Homogenate	Percent Dose Absorbed
1	0.668	0.640	4.19
2	0.668	0.635	4.94
3	0.563	0.540	4.08
4	0.563	0.540	4.08
5	0.563	0.537	4.62
6	0.573	0.545	4.89
Arithmetic Mean	0.5996	0.5728	4.4667
Standard Deviation		0.0502	0.4006
Standard Error		0.0205	0.1635

Table 17

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops

Phenol Red + 1% Polaxalene (50 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.575	0.547	4.87
2	0.575	0.555	3.48
3	0.575	0.560	2.68
4	0.575	0.552	4.00
5	0.575	0.550	4.35
6	0.575	0.551	4.17

Arithmetic Mean	0.5525	3.9250
Standard Deviation	0.0045	0.7599
Standard Error	0.0018	0.3102

t = 1.5445 (no significant difference when compared to control)

Table 18

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops

Phenol Red + 2% Polaxalene (100 mg)

Rat	Absorbance at 560 mµ Phenol Red Solution	Absorbance at 560 mµ Intestinal Homogenate	Percent Dose Absorbed
1	0.570	0.540	5.26
2	0.570	0.540	5.26
3	0.570	0.550	3.51
4	0.570	0.547	4.03
5	0.570	0.550	3.51
6	0.570	0.555	2.70
Arithmetic Mean		0.547	4.045
Standard Deviation		0.006	1.0329
Standard Error		0.0024	0.4217

t = 0.9323 (no significant difference when compared to control)

Table 19

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops

Phenol Red + 3% Polaxalene (150 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.590	0.580	1.69
2	0.590	0.580	1.69
3	0.590	0.580	1.69
4	0.590	0.585	0.85
5	0.590	0.585	0.85
6	0.590	0.575	3.36
Arithmetic Mean		0.5808	1.4348
Standard Deviation		0.0038	1.1066
Standard Error		0.0015	0.4518

t = 6.8038 (significant difference at $p < .001$ when compared to control)

Table 20

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops

Phenol Red + 5% Polaxalene (250 mg)

Rat	Absorbance at 560 mμ		Percent Dose Absorbed
	Phenol Red Solution	Intestinal Homogenate	
1	0.590	0.585	0.85
2	0.590	0.585	0.85
3	0.590	0.585	0.85
4	0.590	0.570	3.39
5	0.590	0.565	4.24
6	0.590	0.465	4.24
Arithmetic Mean		0.5758	2.4023
Standard Deviation		0.0102	1.7284
Standard Error		0.0042	0.7057

t = 2.8466 (significant difference at $p < .01$ when compared to control)

Table 21

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 0.5% Polaxalene (10 mg).

	Control		Polaxalene	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.140	2.918	0.250	5.212
2	0.295	6.150	0.330	6.879
3	0.260	5.420	0.200	4.169
4	0.320	6.671	0.220	4.586
5	0.340	7.088	0.340	7.088
Arithmetic Mean	0.2710	5.6494	0.2680	5.5868
Standard Deviation	0.0791	1.6487	0.0638	1.3299
Standard Error	0.0354	0.7373	0.0285	0.5948

t = 0.0818 (no significant difference)

Table 22

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 1.5% Polaxalene (30 mg).

Rat	Control		Polaxalene	
	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.220	4.586	0.200	4.169
2	0.260	5.420	0.160	3.335
3	0.240	5.003	0.240	5.003
4	0.140	2.918	0.200	4.169
5	0.224	4.670	0.270	5.628
6	0.170	3.544	0.180	3.752
7	0.256	5.337	0.200	4.169
Arithmetic Mean	0.2157	4.4968	0.2071	4.3182
Standard Deviation	0.449	0.9355	0.0368	0.7680
Standard Error	0.0170	0.3536	0.0139	0.2903

t = 0.4047 (no significant difference)

Table 23

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 2.5% Polaxalene (50 mg).

	Control		Polaxalene	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.260	5.420	0.220	4.586
2	0.170	3.544	0.280	5.837
3	0.310	6.462	0.250	5.212
4	0.310	6.462	0.220	4.586
5	0.280	5.837	0.270	5.628
6	0.200	4.169	0.286	5.962
Arithmetic Mean	0.2550	5.3159	0.2543	5.3015
Standard Deviation	0.0582	1.2138	0.0293	0.6097
Standard Error	0.220	0.4588	0.0111	0.2304

t = 0.0201 (no significant difference)

Table 24

Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red

Total Urinary Excretion of Phenol Red in Individual Rats after Gastric Intubation of Phenol Red (1.5 mg dose) with and without 5.0% Polaxalene (100 mg).

	Control		Polaxalene	
Rat	Absorbance at 560 mμ	Percent Dose Absorbed	Absorbance at 560 mμ	Percent Dose Absorbed
1	0.280	5.837	0.170	3.544
2	0.260	5.420	0.230	4.795
3	0.180	3.752	0.100	2.085
4	0.190	3.961	0.200	4.169
5	0.290	6.045	0.180	3.752
Arithmetic Mean	0.2400	5.0031	0.1760	3.6690
Standard Deviation	0.0515	1.0731	0.0483	1.0063
Standard Error	0.0230	0.4799	0.0216	0.4500

$t = 2.7142$ (significant difference at $p < .05$)

Table 25

Effect of DSS on the Apparent Partition Coefficient of Phenol Red between Octanol-1 and Surfactant Solution

Concentration of DSS %	Absorbance of Aqueous Phase at 560 mμ		Apparent Partition Coefficient Octanol-1/DSS Solution
	Before Partitioning[a]	After Partitioning[b]	
0.	0.325	0.297	0.086
0.01	0.319	0.294	0.078
0.10	0.320	0.296	0.075
0.25	0.329	0.305	0.073
0.50	0.319	0.295	0.075
1.00	0.320	0.298	0.069
1.50	0.332	0.306	0.078

[a] Average of two samples.

[b] Average of three samples.

Table 26

Effect of Polaxalene on the Equilibrium Solubility of Phenol Red at 37° C

Concentration of Polaxalene (Percent)	Absorbance at 560 mμ[a]	Solubility of Phenol Red (gm/litre)[b]
0	0.370	1.156
0.5	0.378	1.181
1.0	0.437	1.366
2.0	0.448	1.400
3.0	0.486	1.519
4.0	0.494	1.544
5.0	0.500	1.563

[a] Average absorbancies of six samples.

[b] Calculated by the following equation:

Absorbancy = e x concentration (mg/litre)

e = 0.16, as determined by the method of least squares from the standard curve.

Factor of dilution: 1 to 500 ml.

Table 27

Effect of DSS on the Equilibrium Solubility of Phenol Red at 37° C

Concentration of DSS (Percent)	Absorbance at 560 mμ[a]	Solubility of Phenol Red (gm/litre)[b]
0	0.370	1.156
0.25	0.424	1.325
0.50	0.480	1.500
0.75	0.515	1.609
1.00	0.558	1.744
1.25	0.578	1.806
1.50	0.590	1.844
2.00	0.639	2.166

[a] Average absorbancies of six samples.

[b] Calculated by the following equation:

Absorbance = e x concentration (mg/litre)

e = 0.16, as determined by the method of least squares from the standard curve.

Factor of dilution: 1 to 500 ml.

Table 28

Influence of DSS on the Gastrointestinal Absorption of Sulfisoxazole

Total Urinary Excretion of Sulfisoxazole in Individual Rats after Gastric Intubation of Sulfisoxazole (5 mg) with and without 0.1% DSS (2 mg).

	Control		DSS	
Rat	Absorbance at 533 mμ	Dose Absorbed mg	Absorbance at 533 mμ	Dose Absorbed mg
1	0.410	3.417	0.430	3.583
2	0.420	3.500	0.450	3.750
3	0.360	3.000	0.382	3.183
4	0.380	3.167	0.415	3.458
5	0.415	3.458	0.433	3.608
6	0.495	4.125	0.475	3.958
7	0.410	3.147	0.450	3.750
8	0.390	3.250	0.420	3.500
9	0.440	3.667	0.455	3.792
10	0.420	3.500	0.480	4.000
Arithmetic Mean	0.414	3.450	0.439	3.658
Standard Deviation	0.036	0.304	0.029	0.245
Standard Error	0.011	0.096	0.009	0.078

$t = 3.8048$ (significant difference at $p < .005$)

Table 29

Influence of DSS on the Gastrointestinal Absorption of Sulfisoxazole

Total Urinary Excretion of Sulfisoxazole in Individual Rats after Gastric Intubation of Sulfisoxazole (5 mg) with and without 0.5% DSS (5 mg).

	Control		DSS	
Rat	Absorbance at 533 mµ	Dose Absorbed mg	Absorbance at 533 mµ	Dose Absorbed mg
1	0.380	3.167	0.475	3.958
2	0.450	3.750	0.465	3.875
3	0.485	4.042	0.520	4.333
4	0.450	3.750	0.480	4.000
5	0.490	4.083	0.560	4.667
6	0.305	3.208	0.520	4.333
7	0.420	3.500	0.460	3.833
8	0.450	3.750	0.500	4.167
9	0.395	3.292	0.510	4.250
10	0.410	3.417	0.520	4.333
Arithmetic Mean	0.431	3.596	0.501	4.175
Standard Deviation	0.039	0.329	0.031	0.260
Standard Error	0.012	0.104	0.010	0.082

t = 5.275 (significant difference at $p < .001$)

Table 30

Influence of DSS on the Gastrointestinal Absorption of Sulfisoxazole

Total Urinary Excretion of Sulfisoxazole in Individual Rats after Gastric Intubation of Sulfisoxazole (5 mg) with and without 1.0% DSS (10 mg).

Rat	Control		DSS	
	Absorbance at 533 mμ	Dose Absorbed mg	Absorbance at 533 mμ	Dose Absorbed mg
1	0.440	3.667	0.570	4.750
2	0.430	3.583	0.540	4.500
3	0.380	3.167	0.520	4.333
4	0.410	3.147	0.540	4.500
5	0.430	3.583	0.570	4.750
6	0.360	3.000	0.515	4.292
7	0.420	3.500	0.585	4.875
8	0.360	3.000	0.535	4.458
9	0.390	3.250	0.530	4.417
Arithmetic Mean	0.405	3.380	0.545	4.542
Standard Deviation	0.031	0.257	0.024	0.203
Standard Error	0.010	0.085	0.008	0.067

$t = 21.7398$ (significant difference at $p < .001$)

Table 31

Effect of DSS on the Solubility of Sulfisoxazole at Room Temperature

Concentration of DSS (Percent)	Absorbance at 252 mμ
0.00	0.196[a]
0.01	0.211
0.05	0.211
0.10	0.212

[a] Average of three samples.

CHAPTER V

DISCUSSION

Phenol red, the drug selected for this study, serves as a good model to study the effects of the two medicinally used surfactants, dioctyl sodium sulfosuccinate (DSS) and polaxalene, on the absorption of poorly absorbed drugs.

Phenol red is a weak acid, with a molecular weight of 354 and a pKa of 7.9 (106). It is used as an indicator, in acid-base titrations, changing its color in the pH range of 6.0 to 7.5. Using the Henderson-Hasselbach equation, one can calculate that the degree of ionization of phenol red at α pH of 5.3 (the virtual pH at the absorption site in the intestine) (61, 145) is only 0.25 percent. However, it may generate the strong benzenesulfonic acid group by acid hydrolysis of the sulfalactone ring at very low pH (≤ 2). These suggest that the poor absorption of phenol red through small intestine can be attributed to low lipid/water partition coefficient of the un-ionized form, while the poor absorption through the stomach can be attributed to the degree of ionization. This account of the poor absorpability of phenol red was suggested by Lien (93), and was confirmed by our results, that phenol red has very low partition coefficient (Octanol-1/water) as shown in Table 25.

Intestinal perfusion studies in rats have shown that phenol red is equally well absorbed in the proximal as well as distal regions of the small intestine (108). In the 0.5 to 20 mg per litre concentration range phenol red absorption from the gastrointestinal tract of the rat occurs by passive diffusion (81). At lower concentrations, there is appreciable contribution by the specialized transport process, which can be inhibited by p-aminohippuric acid (82).

Influence of DSS on the Absorption of Phenol Red

Absorption from Intestinal Loops

The intestinal loop, being a well defined section of the gastrointestinal tract of the rat, is well suited for the study of the absorption of drugs and the influence of the surfactants on their absorption. In this technique, the animal's blood supply remains intact and, hence, the absorption profiles obtained are more realistic. In addition, it is possible to control the initial concentration of the surfactant present within the intestinal lumen, when compared to intact rat.

The results obtained from the intestinal loops are presented in Tables 1 to 5. The percent of phenol red dose absorbed in the control studies was about 4.5 percent. This value is in good agreement with the value of 5.6 percent reported by Feldman et al. (40).

The intestinal loop experiments indicate a dose-dependent effect of DSS on the intestinal absorption of the phenol red. The relative rate of absorption of phenol red is plotted against surfactant concentration, as shown in Figure 2. All the concentrations of DSS studied, however, showed a highly significant increase in the absorption of phenol red in the presence of DSS. This significance was determined by the student's *t* test for independent (unpaired) data

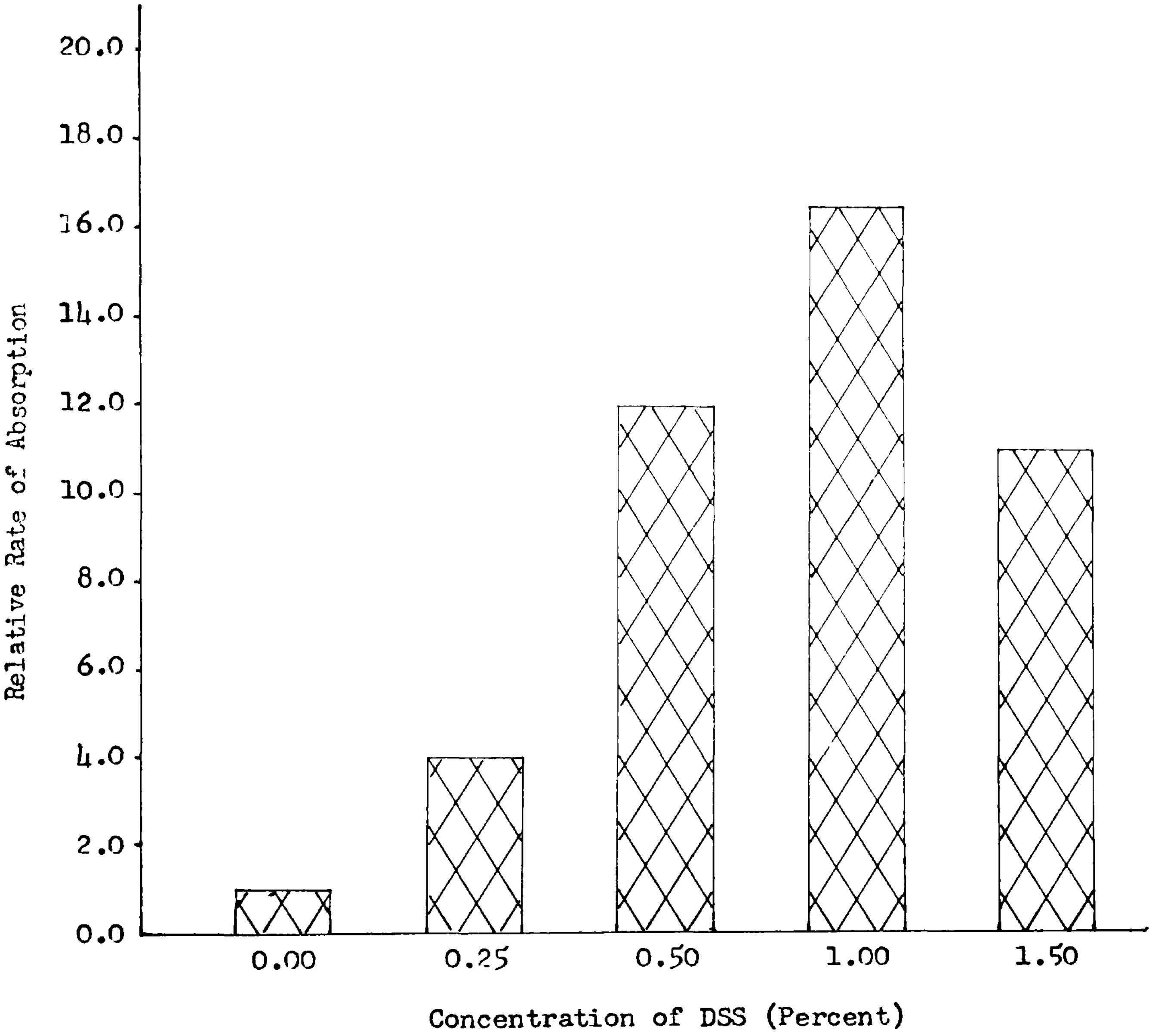


Figure 2. Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops.

(at $p < .001$). For example, at 0.25% concentration of DSS the percentage of absorption increased from a control value of 4.4 to 17.5 percent. Higher concentrations of the surfactant increased the absorption of phenol red even more, up to a maximum of 73.58 percent in the presence of 1% DSS. This represents about 16-fold increase in phenol red absorption compared to control studies. A higher concentration of DSS (1.5%) showed a smaller increase in absorption than 1% DSS, but nevertheless much more than the control.

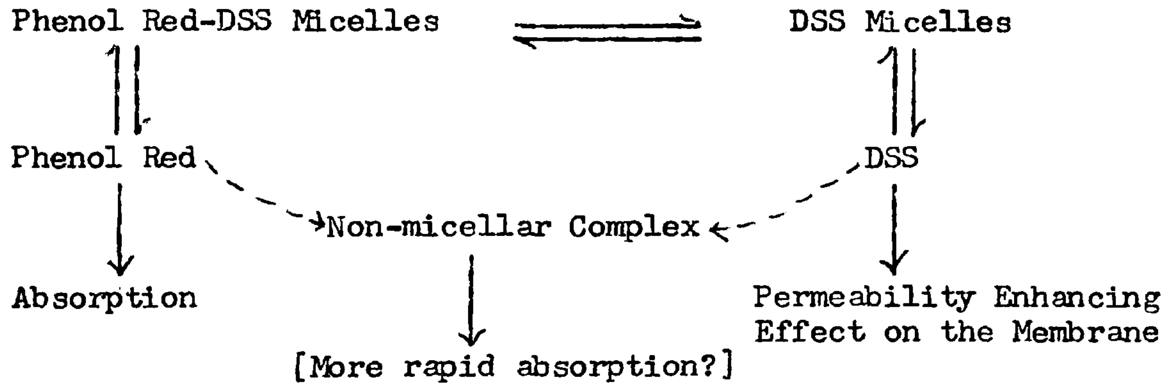
Surfactants can modify drug absorption by one or more mechanism or mechanisms. Briefly, they may act on the biological membrane or on the drug. In addition, some surfactants may also have some pharmacological properties specific to their particular chemical structure and not related to their surfactant properties in general. More than one of these mechanisms may be operative at the same time, the magnitude of each being dependent on the concentration of the surfactant. Surfactants can thus exert a two-phase effect which is a function of concentration. Below the critical micelle concentration (CMC), absorption of drugs may be enhanced due to better contact of the solution with the biologic membrane. There also may be a direct effect of the surfactant on the permeability of the biologic membrane. Above the CMC, a portion of the drug molecules may become "entrapped" in the surfactant micelles and, as such, be unavailable for absorption. The net effect (absorption enhancement or retardation) depends to some degree on the relative magnitude of interaction between the drug and the surfactant. The absorption retarding effect usually predominates at higher concentrations, because a larger fraction of the drug is bound in the micelles.

The enhancement of phenol red absorption from its solutions by DSS could be the result of two possible mechanisms. First, a change in the physiochemical properties of the drug due to the presence of the surfactant that could lead to enhanced absorption, and second, an alteration in the permeability of the membrane could also lead to increased absorption.

A significant change in the ability of the drug to permeate the intestinal membrane may result from an interaction of phenol red with DSS to form a complex. Such a complex could have an increased affinity for the intestinal lipoidal membrane and result in enhanced drug absorption. The magnitude of interaction of phenol red with DSS was determined by solubility studies (Table 26 and Figure 7). The results obtained show that above the CMC of DSS [0.11%] (179), the solubility of phenol red increases, indicating micellar complexation. The possibility that such a complex has a higher partition coefficient and, hence, results in enhanced absorption seems unlikely on the basis of our studies of the effect of DSS on the partition coefficient of phenol red and shown in Table 25. Both premicellar and postmicellar concentrations had no significant effect on the partition coefficients. However, one must keep in mind that these in vitro studies do not necessarily represent what is happening at the biological membrane in vivo.

On the basis of our results (intestinal loop and solubility studies) it can be said that the overall effect of DSS on the phenol red absorption may represent the sum of two effects: modification of the permeability of the membrane, and micellar complexation of the drug. The results obtained from the influence of the concentration of DSS on the phenol red absorption (Tables 1 to 4) suggest that the former effect (i.e., permeability change allowing for increased absorption) predominates up to a concentration of 1% DSS. Above this concentration the micellar entrapment of the drug molecules starts to predominate and cause a decrease in the absorption of the phenol red. Several authors have found that other surfactants also show a similar concentration dependent activity (72, 89, 91, 133).

The effect of various DSS concentrations on the absorption of phenol red could be summarized in the following scheme, similar to the one proposed by Levy (89).


Phenol Red-DSS Micelles
DSS Micelles
Phenol Red
DSS
Non-micellar Complex
Absorption
Permeability Enhancing Effect on the Membrane
[More rapid absorption?]

Our results seem to indicate that DSS, an anionic surfactant, is preferentially absorbed at the membranes and has conceivably a direct effect by disrupting the highly ordered structure of the gastrointestinal epithelium, thus changing its permeability and causing an increase in the absorption of phenol red. Nissim (118) and Nadai (113) have reported that similar ionic surfactants (sodium lauryl sulfate, in particular) caused a disruption of the gastrointestinal epithelium and thus promoted drug absorption through them.

Recently Dujone and Shoemaker (29) have found that combination of DSS and oxyphenistan produced cytotoxicity in the liver cell cultures due to increased uptake of oxyphenistan by the cells. They have also found in the same study that DSS, contrary to the previous belief, is itself absorbed from the gastrointestinal tract of the humans and the rats.

Based on our findings and those of others, it is our conclusion that DSS changes the permeability of the gastrointestinal epithelium and results in its own absorption as well as in increasing the absorption of other drugs, such as phenol red.

Absorption of Phenol Red in the Intact Rat

Although the in situ intestinal loop is a useful technique for routine exploratory investigation of adjuvants, like surfactants on drug absorption, it has some deficiencies, e.g., the animal has been surgically manipulated and, therefore, is not under normal physiological conditions. The importance of many factors that may influence drug absorption, such as gastric emptying, intestinal motility and the direct effect of drugs on the gastrointestinal tract, can be assessed only by means of in vivo studies, in the intact animals. Hence, it was of interest to consider the absorption of phenol red, and the effect of the surfactants on its absorption, in the intact rat. Furthermore, it was also of interest to determine the correlation between absorption from in situ intestinal loop technique and absorption under normal physiological conditions in the intact rat.

Tables 6-9 show urinary recovery of phenol red expressed as percent of dose after oral administration of 2 ml of 0.75 mg/ml solution in the presence of various concentrations of DSS. The ratio of the amount of phenol red excreted with and without DSS is plotted against increasing concentrations of DSS as a histogram in Figure 3.

The percent of phenol red absorbed in control animals was, on the average, about 5 percent of the dose administered. This value is in good agreement with the intestinal loop experiment and the value of 3.6 percent reported by Feldman and Gibaldi (40). The results show that for all surfactant concentrations employed there was a significant ($p < .001$) increase in the gastrointestinal absorption of phenol red as manifested by increased urinary excretion of phenol red in 24 hours. For example, the coadministration of 0.5% DSS (10 mg) and phenol red increased urinary excretion from about 5.3 percent to 8.4 percent, about a twofold increase. Higher concentrations of DSS increased the urinary excretion even more. Again, a maximum effect was seen to be at 1 percent to 1.5 percent (20-30 mg) level of DSS. Higher concentrations started to cause a lesser increase in the urinary excretion.

It is significant to note that the amount of DSS given to intact rats varied from 10 to 40 mg per rat ; or, on the average, approximately 50 to 200 mg per kg body weight of rat. Maximum effect was observed at a dose of about 100 mg/kg of DSS (1% concentration). This 100 mg per kg dose of DSS was found to be the ED_{50} for the fecal hydrating effect of DSS in the rats (95).

Although there were quantitative differences, in the extent of absorption, between the intestinal loop and intact rat experiments, nevertheless, the concentration dependent activity of the surfactant (DSS) in the intact rat followed more or less the same pattern as

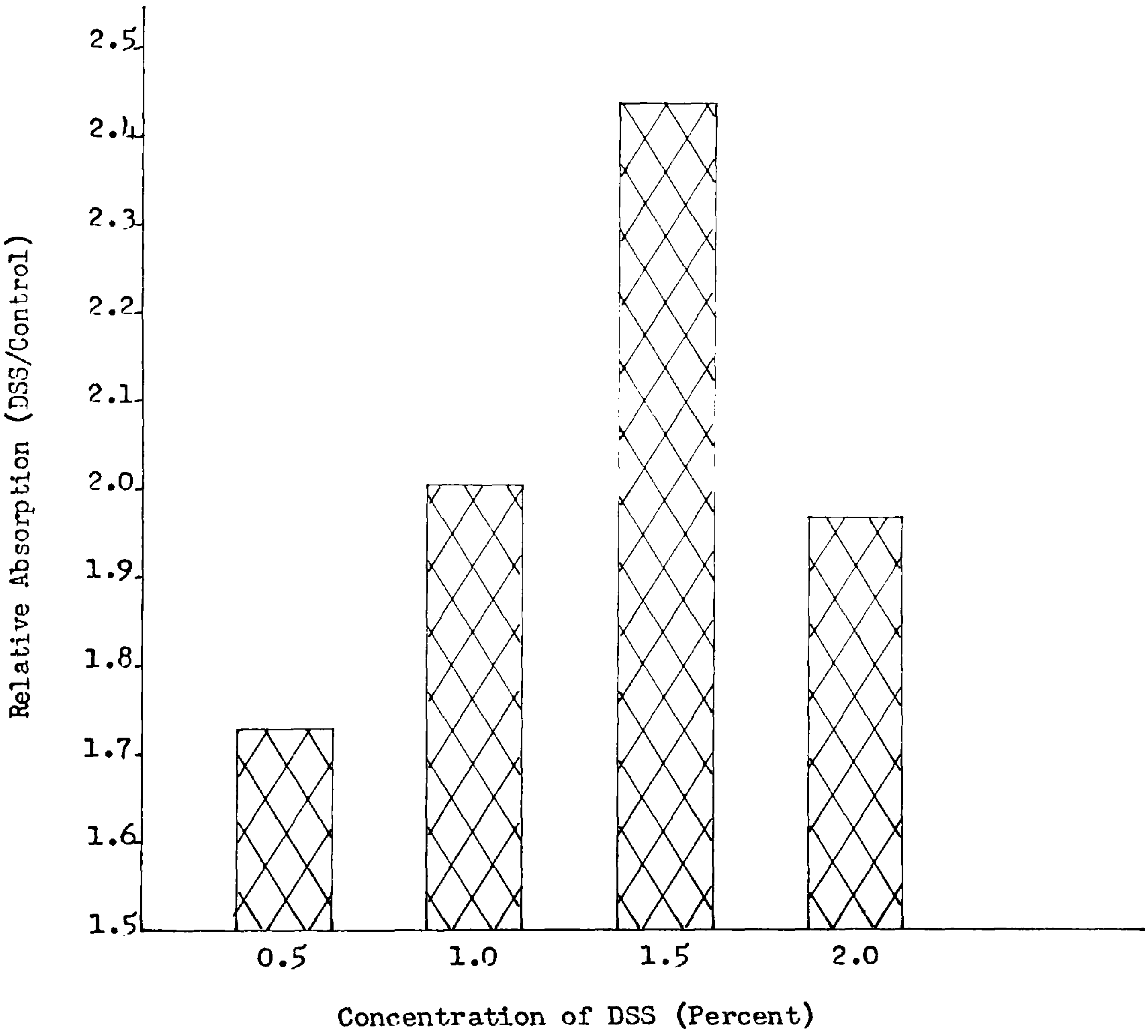


Figure 3. Influence of DSS on the Gastrointestinal Absorption of Phenol Red - Gastric Intubation.

observed in the intestinal loop. These quantitative differences are to be expected in view of the differences in the effective drug and surfactant concentrations at the absorption site and due to differences in the techniques.

The results of the intestinal loop and intact rat studies both seem to indicate that DSS promotes the absorption of phenol red, through a mechanism that involves a direct action of the surfactant on the membrane permeability. However, in the intact rat, being a more complex system physiologically, other possible additional mechanisms could also play a role in promoting absorption. For example, DSS has been found to inhibit the rate of propulsion of a dye meal through the gastrointestinal tract of the rat, chiefly by slowing the gastric emptying rate (94). Such a delay in the gastrointestinal transit rate also could be an added factor in promoting the absorption of the drug.

Absorption of Phenol Red from the Peritoneal Membrane

In view of our findings that DSS changes the permeability of the gastrointestinal epithelial membrane and thus causes an increase in the absorption of a poorly absorbable drug, phenol red, it was of interest to determine whether DSS will have a similar effect on different membrane (peritoneal membrane).

The results of the influence of DSS on the absorption of phenol red from the peritoneal cavity are shown in Tables 10-15 and Figure 4. The percentage of phenol red absorbed from the peritoneal cavity in the control studies was on the average about 54 percent of the amount of the dose administered. This is approximately tenfold the absorption

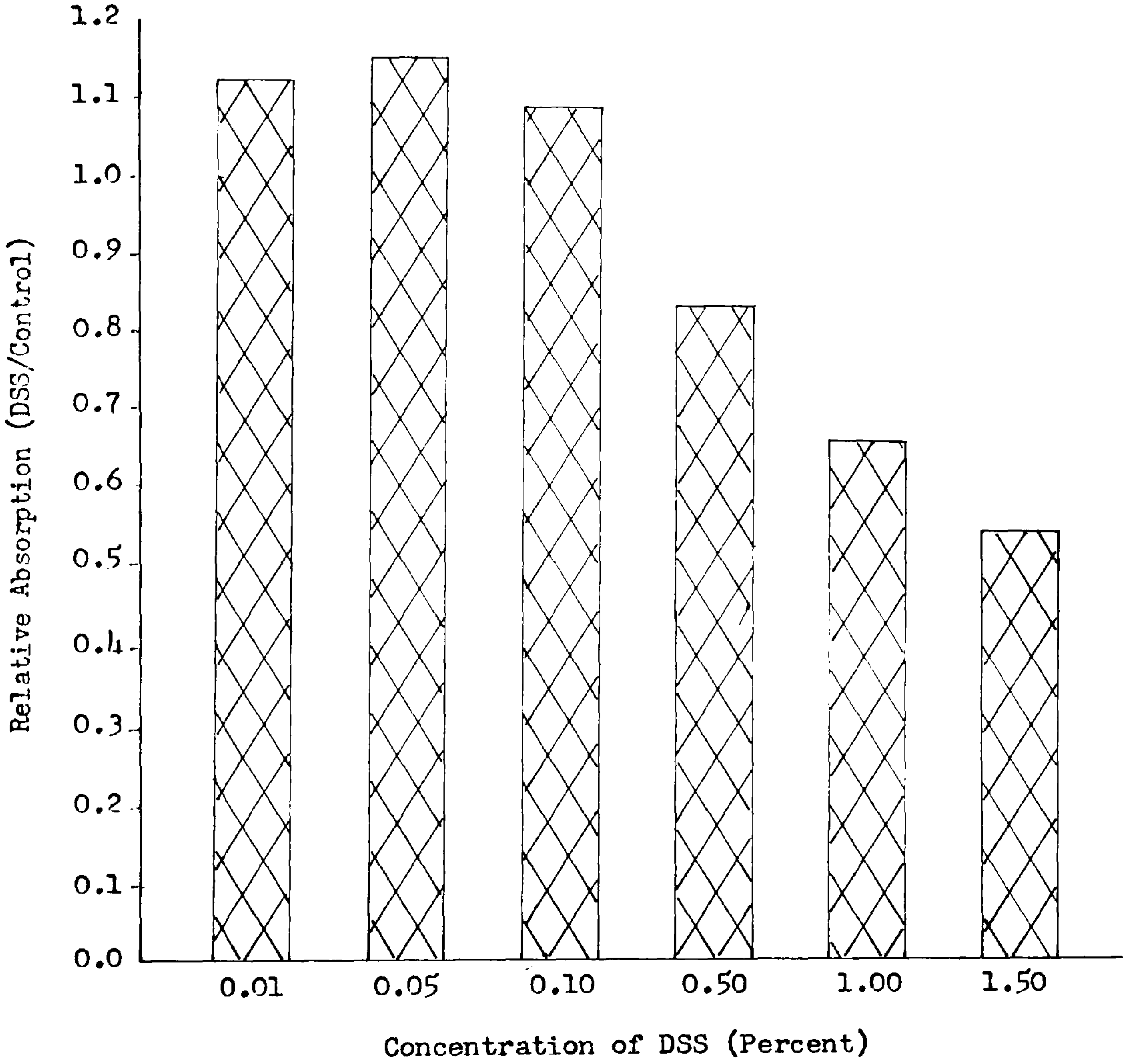


Figure 4. Influence of DSS on the Peritoneal Absorption of Phenol Red.

from the gastrointestinal tract. It is known that peritoneal membrane is more permeable to drugs than gastrointestinal epithelium and our results are in accord with this fact and are in close agreement with the findings of Feldman et al. (40), that phenol red was absorbed about tenfold more from the peritoneal cavity compared to the absorption from the gastrointestinal tract of the rat. The concomitant administration of DSS and phenol red resulted in a significant ($p < .01$) increase in the absorption of phenol red up to 0.1% DSS. Above this concentration of DSS the absorption of phenol red started to decrease.

The concentration dependent activity of the surfactant qualitatively follows the same pattern as found in absorption of phenol red from both the intestinal loop and the intact rat studies. The extent in the increase of absorption was, however, less in the case of peritoneal absorption, and this was to be expected because of the differences in nature of the two membranes.

The results are in accord with the following mechanism: first, below the CMC, the surfactant (DSS) potentiated the absorption of phenol red through direct action on the biological membranes, and second, above the CMC the drug is entrapped in the micelle and is not readily available for absorption, hence causes a decrease in absorption of the phenol red.

This mechanism is basically similar to the one for the effect of DSS on the absorption of phenol red from the intestinal loops and in intact rats. However, in the case of peritoneal absorption study, smaller amount of the surfactant resulted in an increase in the absorption of phenol red. These differences stem from the fact that, in case of gastrointestinal absorption, the surfactant can interact with mucus and/or other components of the intestinal fluids, thus reducing the effective concentration at the absorption site.

Influence of Polaxalene on the Absorption of Phenol Red

Intestinal Loops

The results obtained from the intestinal loops are shown in Tables 16-20. The effect of concentration of the surfactant on the relative rate of absorption of phenol red is plotted as a histogram in Figure 5.

Unlike DSS, polaxalene did not cause any significant increase in the absorption of phenol red. However, at higher concentrations the surfactant caused a significant decrease in absorption. According to our results it seems that polaxalene does not change the permeability of the intestinal membrane and, hence, there was no increase in absorption. These findings are in agreement with the findings of Lish and Weikel (96), that 1% polaxalene did not change the absorption of phenol red from rat colon. It is also in agreement with the lower toxicity of non-ionic surfactants compared to ionic surfactants (118). Other authors (91, 128, 182) have also shown that other non-ionic surfactants do not promote absorption of drug solutions from gastrointestinal tract. Levy et al. (90) found that polysorbate 80 (a non-ionic surfactant) had no apparent effect on the absorption of salicylate, salicylamide, and 4-aminoantipyrine from their solutions from in situ rat small intestine.

Retardation of phenol red absorption at the higher polaxalene

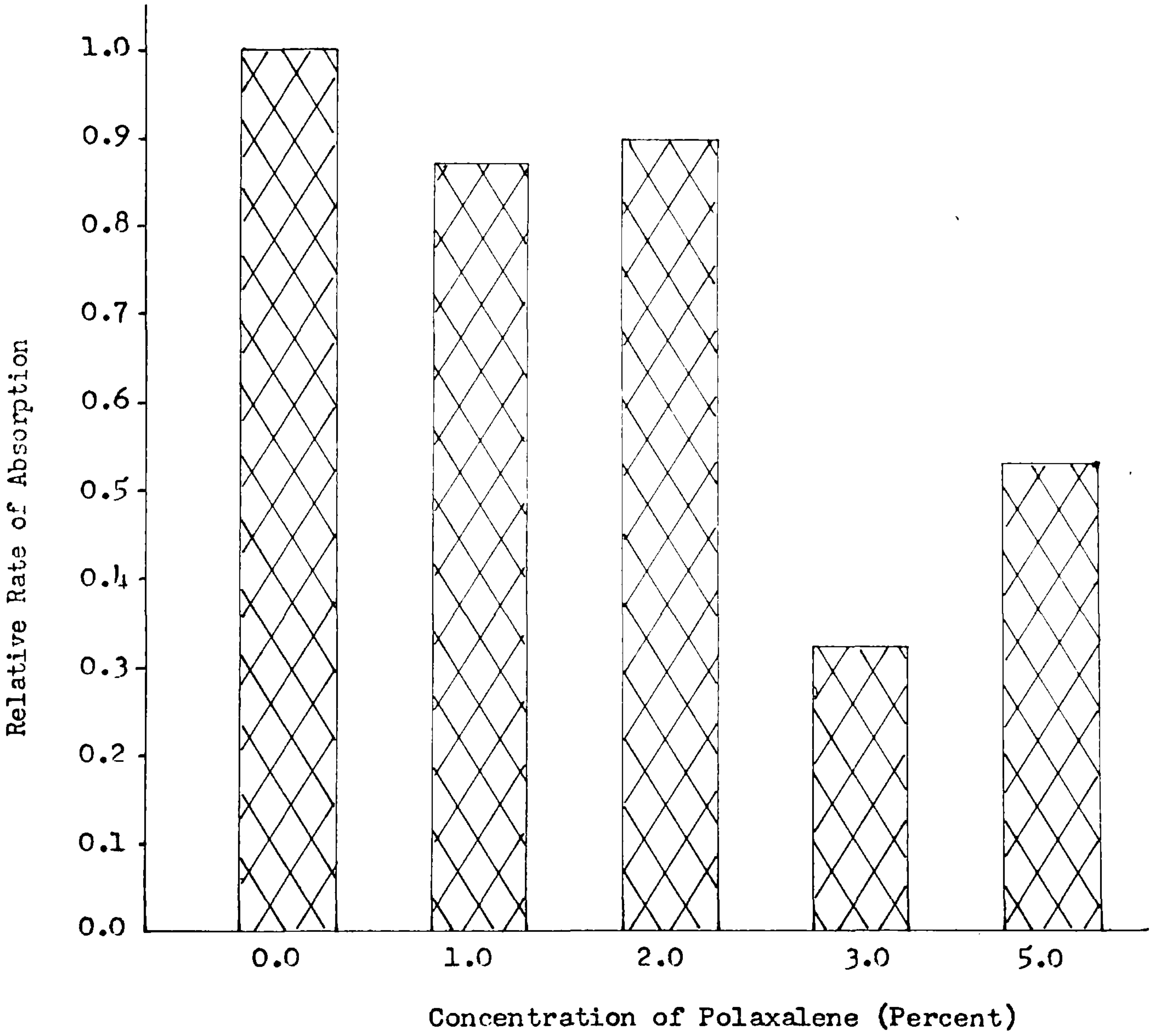


Figure 5. Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Intestinal Loops.

concentrations is due to interaction between the surfactant and drug, or, in other words, due to entrapment of the drug in the micelles. Table 27 and Figure 8 show that phenol red is solubilized by the micelles of the surfactant. Since the drug in the micellar phase is unavailable for absorption, the effective concentration of drug is less than the apparent concentration of drug; therefore, a decrease in absorption is observed. Many authors (72, 89, 91, 133) have found that higher concentrations of non-ionic surfactants do decrease drug absorption.

Gastric Intubation

The influence of polaxalene on the absorption of phenol red from the intact rat is shown in Tables 21-24. The concentration dependent activity of the surfactant is shown in Figure 6. The ratio of the amount of phenol red excreted in the urine with and without polaxalene is plotted against increasing amounts of polaxalene.

The results are similar to those obtained in the study of intestinal loops where no increase in absorption was noticed at lower concentrations of the surfactant, while at higher concentrations there was a decrease in absorption. The decrease in absorption of phenol red at higher concentrations is due to the entrapment of the drug in the micelles of the surfactant.

Influence of DSS on Sulfisoxazole Absorption

Sulfisoxazole (U.S.P.) is a weak acid, with a pKa of 4.62 and is a poorly soluble drug. It is used in human beings for urinary tract infections. It is available both as tablets and suspensions. Although

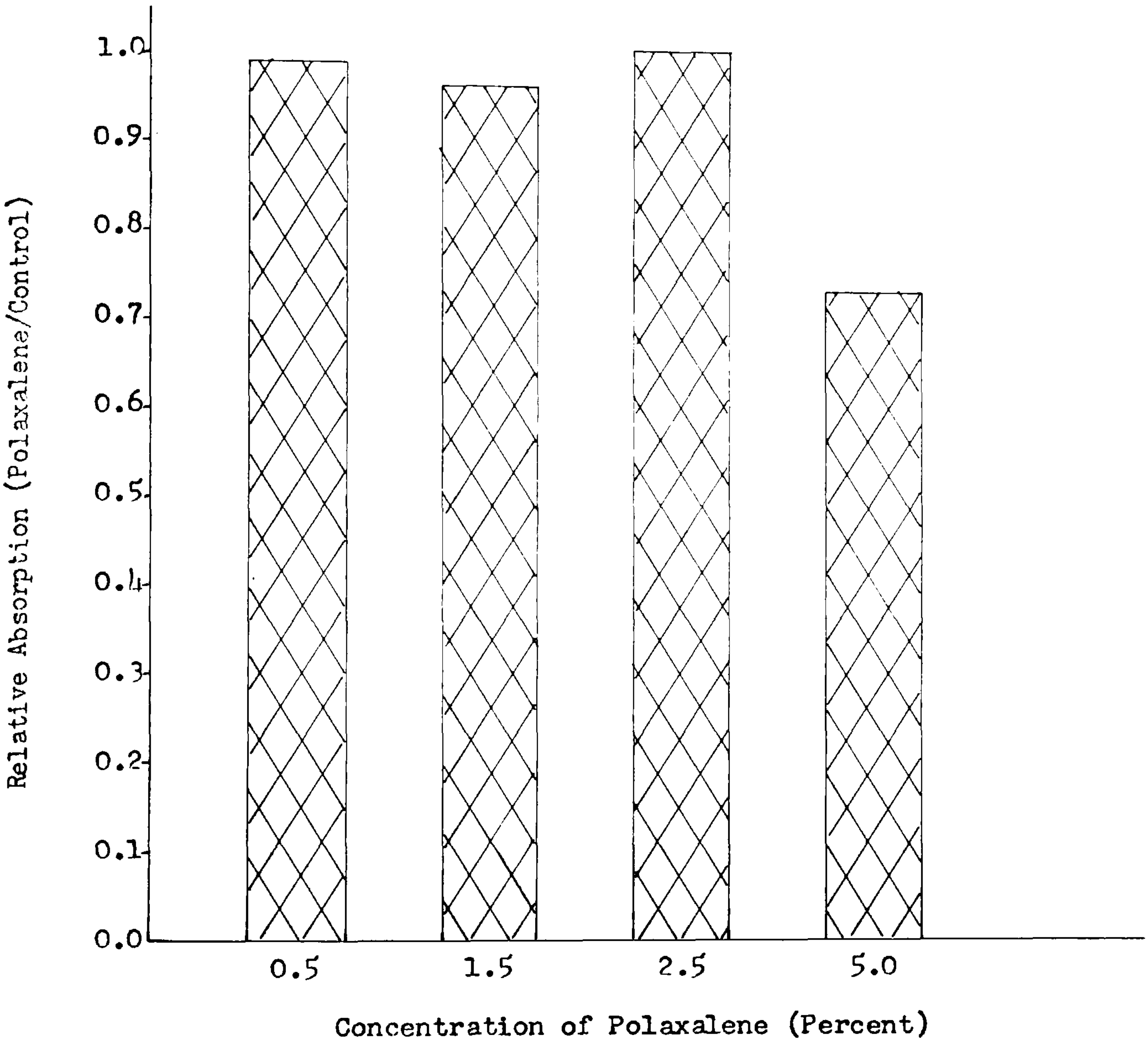


Figure 6. Influence of Polaxalene on the Gastrointestinal Absorption of Phenol Red - Gastric Intubation.

sulfisoxazole is well absorbed from solutions, its absorption from the solid dosage forms is dissolution rate limited and the U.S.P. now requires a test of dissolution for the tablets as an indication for its biologic availability. It was of interest to find out if DSS has any effect on its gastrointestinal absorption and the information obtained could be valuable for the proper formulation of both tablets and suspensions of this drug.

Results of absorption studies for the intact rat are presented in Tables 28-30. In control rats about 70 percent of the dose administered is absorbed in 24 hours. The concomitant administration of various concentrations of DSS in the same suspension of the drug significantly increased absorption.

When a drug is administered orally in solid form, 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 the dissolution of the drug at the absorption site. Since dissolution step is the rate limiting step, therefore any factor influencing the dissolution rate will influence its absorption. The mechanisms by which surfactants increase the dissolution rate of a relatively water-insoluble drug are by decreasing the interfacial tension between the drug and the dissolution medium and/or by means of micellar solubilization.

To obtain an indication of the ability of DSS to solubilize sulfisoxazole, the solubility study was performed and the results are

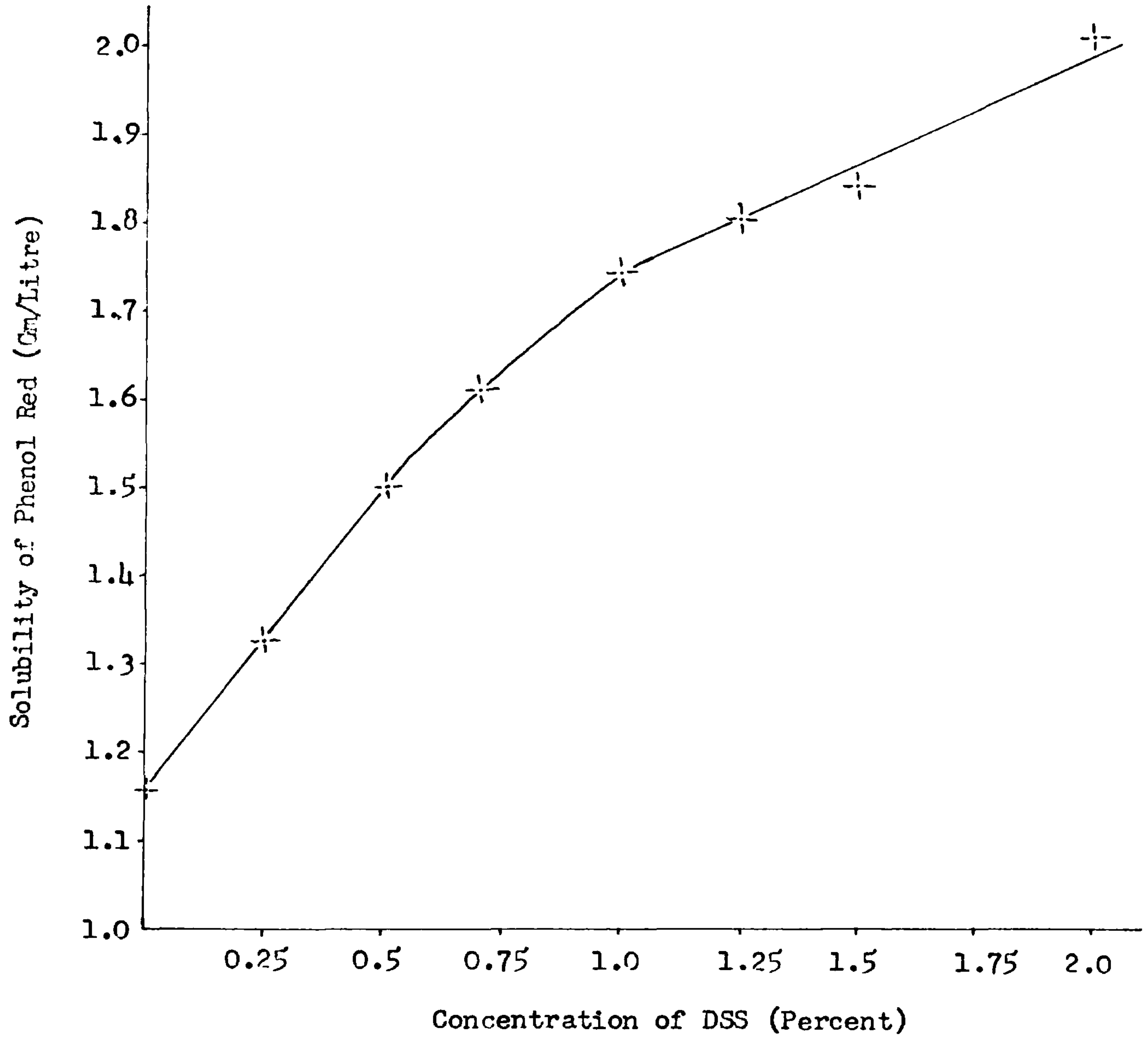


Figure 7. Effect of DSS on the Equilibrium Solubility of Phenol Red at 37°C.

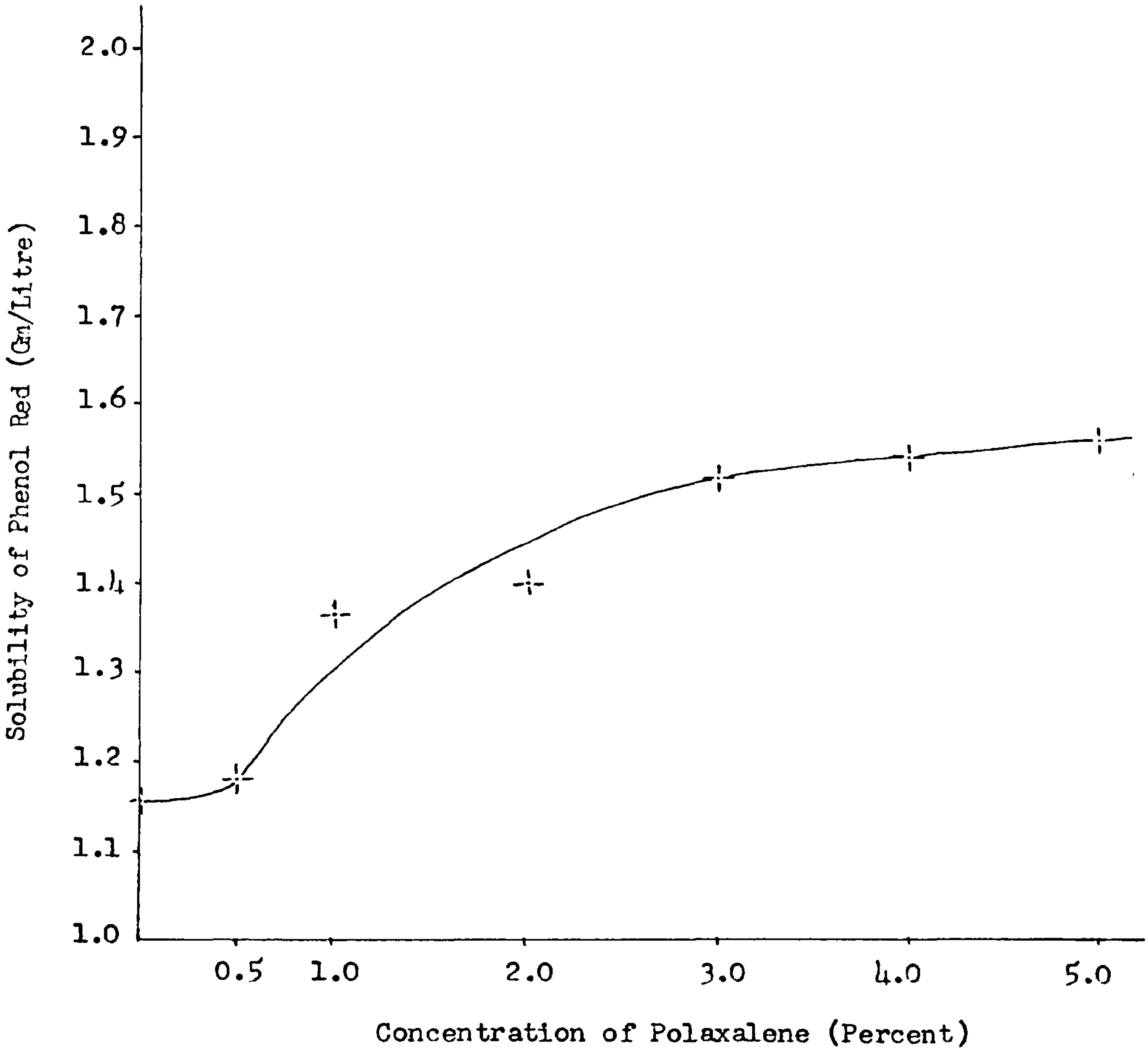


Figure 8. Effect of Polaxalene on the Equilibrium Solubility of Phenol Red at 37°C.

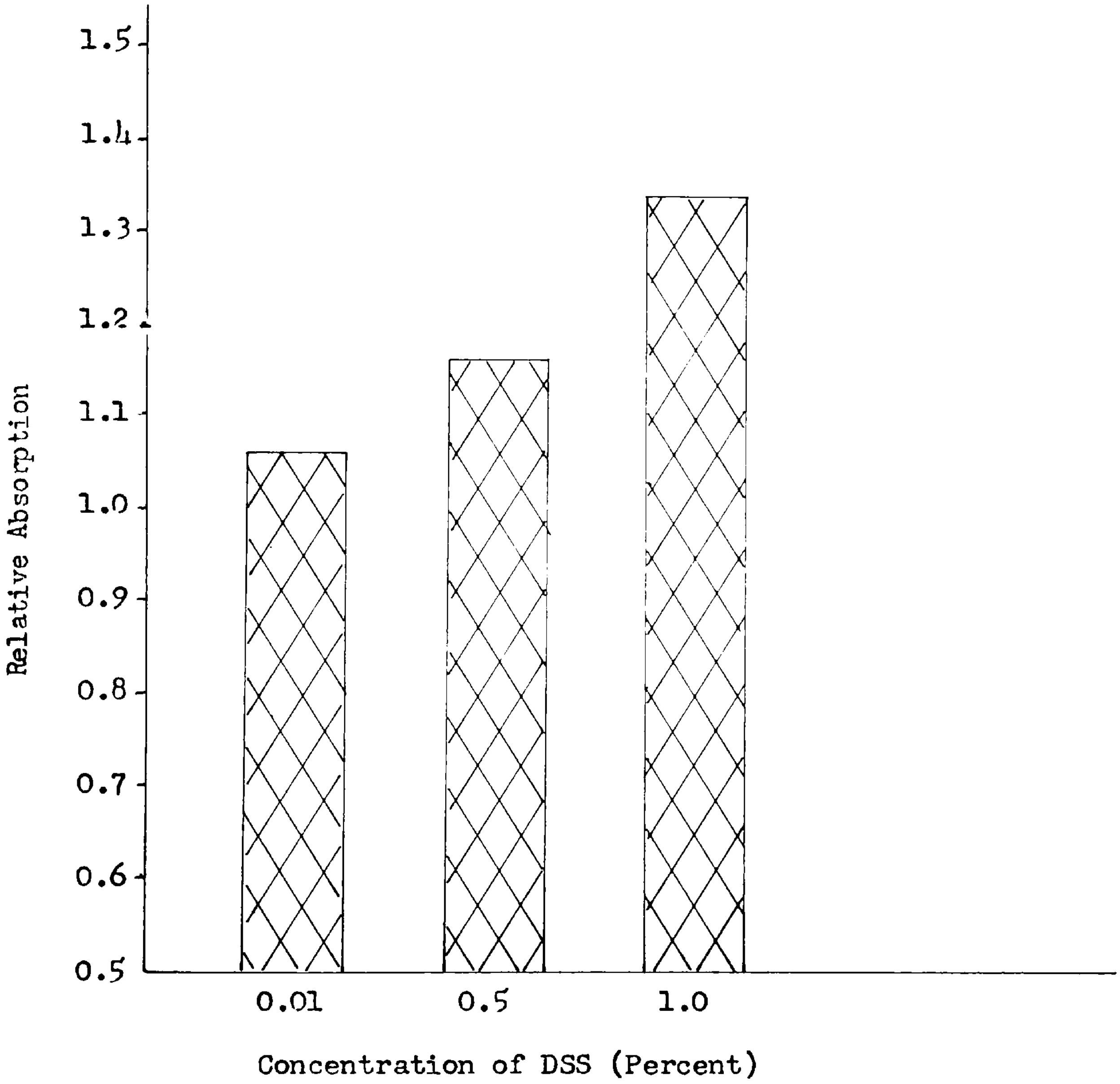


Figure 9. Influence of DSS on the Gastrointestinal Absorption of Sulfisoxazole - Gastric Intubation.

shown in Table 31 and Figure 9. It was found that the premicellar concentrations of DSS slightly increased the solubility (about 8%) of sulfisoxazole.

The fact that the solubility of sulfisoxazole was only increased about 8 percent, while its absorption from the gastrointestinal tract increased by about 30 percent in the presence of DSS leads us to believe that the enhancement of absorption is due to the sum of the following two effects: (a) the surfactants increase the effective surface area of the drug through a wetting effect and result in enhancement of dissolution and absorption, and (b) a direct effect on the permeability of the membranes resulting in an increased absorption of the drug. Other authors (8, 43, 52, 97, 127, 166) have also found surfactants to increase the absorption of poorly soluble drugs by the same mechanisms.

Biopharmaceutical Implications of the Study

DSS was found to increase the absorption of phenol red, a poorly absorbable drug. Our results indicate that the presence of DSS with another poorly absorbable drug, oxyphenistan, could have been the reason for the latter's hepatotoxicity. The combination of DSS in the same dosage form with other drugs not intended for absorption should be carefully reconsidered.

Unlike DSS, polaxalene, which is another medicinally used surfactant, did not increase drug absorption. Therefore, when a fecal-softener is formulated with other laxatives, not intended for absorption, polaxalene represents a better choice than DSS.

CHAPTER VI

SUMMARY

A poorly absorbable drug, phenol red, and a poorly water-soluble drug, sulfisoxazole, were selected to study the effects of the two medicinally used surfactants, dioctyl sodium sulfosuccinate and polaxalene, on the absorption of former drugs.

Two techniques, the in situ intestinal loop and gastric intubation of intact rat, were utilized to study this effect. The solubility method of analysis was employed to detect micellar complexation. The partition coefficients (Octanol-1/water) of phenol red in the absence and presence of various concentrations of DSS were determined. The effect of DSS on peritoneal absorption of phenol red was also studied.

DSS was found to increase the gastrointestinal absorption of both phenol red and sulfisoxazole. It also increased the peritoneal absorption of phenol red in the rat. Its effect was concentration dependent. The enhancement of drug absorption by this surfactant, DSS, is postulated to be due to a direct effect of surfactant on the biological membranes. The decrease in absorption at higher concentrations is postulated to be due to micellar entrapment of the drug by the DSS micelles.

Polaxalene was found not to increase the gastrointestinal absorption of phenol red. But its higher concentrations decreased the absorption of phenol red due to micellar entrapment.

Biopharmaceutical implications of the study were discussed.

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