some interaction with water, coiling of the hydrocarbon chains, or both. The size of hexane, as compared to the other hydrocarbons, could make it best suited to interact with the DTAC film at these low concentrations. As the surface concentration increases, it is reasonable to expect that the hydrocarbon portions of the surface-active agents will orient in a more perpendicular direction to the interface. This should improve the chances for the longer-chain hydrocarbons to interact with the exposed groups of the surface-active agents.

The preceding discussion considered only the interactions between the oil and the hydrocarbon group of the surface-active agent. It is important, however, not to neglect the possibility of an interaction between the polar group of the surface-active agent and the oil. Figures 5–7 show that, although the π-A curves of DPC, DTAC, and DEAC are quite similar at the air/water surface, differences between the 3 quaternary ammonium compounds at an oil/water interface do exist. The π-A curves for DPC are "compressed" to a greater extent than those for DTAC and DEAC at the oil/water interfaces, especially at the hexane/water interface. This effect may be due to a dipole-induced dipole interaction between the planer aromatic pyridinium ion and the oil, which is less possible in the case of DTAC or DEAC. Once again, the steric nature of the hexane molecule probably makes it best suited for this interaction.

It appears, therefore, that the nature of the oil phase influences adsorption of water-soluble substances, such as quaternary ammonium salts, and that the oil used in such studies must be considered in any discussion.

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Solubilizing Properties of Bile Salt Solutions I

Effect of Temperature and Bile Salt Concentration on Solubilization of Glutethimide, Griseofulvin, and Hexestrol

By THEODORE R. BATES, MILO GIBALDI and JOSEPH L. KANIG

Data on the micellar solubilization of the poorly water-soluble drugs, griseofulvin, hexestrol, and glutethimide, in 0–0.6 *M* aqueous solutions of the sodium salts of cholic, desoxycholic, taurocholic, and glycocholic acids at 3 temperatures are presented. Employing the pseudo two-phase model for micellar solubilization, the thermodynamic functions, ΔF^0, ΔS^0, and ΔH^0 of partitioning of the drug molecule between the aqueous phase and the micellar phase have been determined for hexestrol and griseofulvin. The physical-chemical ramifications and biological implications in these systems are considered.

AQUEOUS solutions of surfactants exhibit a more or less abrupt change in their physical properties over a narrow concentration range. This distinct change in properties is generally accepted to be due to the formation of oriented aggregates or micelles. The narrow surfactant concentration range at which micelles begin to form is referred to as the critical concentration for micelle formation or CMC. Among the more interesting properties of micellar solutions is their ability to solubilize water-insoluble materials.

Micellar solubilization has been defined by McBain (1) as "the spontaneous passage of solute molecules of a substance, insoluble in water, into an aqueous solution of a surfactant in which a thermodynamically stable solution is formed." This process essentially involves the diffusion of the added solubilizate molecules (*i.e.*, the water-insoluble material being solubilized) from the bulk phase into the surfactant micelle. The solubilized system is in a state of equilibrium.

Micellar solubilization has been broadly classified into 3 types (1–3). (*a*) *Nonpolar (nonspecific) solubilization:* the solubilizate is in-

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corporated into the hydrocarbon center of the micelle, away from the polar head groups. (*b*) *Polar-nonpolar (specific) solubilization:* the solubilizate is incorporated by penetration into the palisade layer of the micelle with the solubilizate molecule oriented in approximately the same manner as is the surfactant molecule in the micelle. (*c*) *Adsorption solubilization:* in this type of solubilization, the solubilizate is adsorbed onto the polar surfaces of the micelle.

In 1936, Hartley (4) predicted that bile salts, like soaps, should form micellar solutions above their CMC. Equivalent conductivity-concentration (5–7), freezing point (5), dye solubilization (8), and small angle X-ray scattering (9) experiments, as well as the solubilization of 20-methylcholanthrene (10), are some of the numerous investigations that conclusively demonstrate that colloidal aggregates form in conjugated and unconjugated bile salt solutions at a certain minimum concentration.

Numerous investigations have demonstrated the solubilizing properties of bile salts for water-insoluble materials. Verzar (11) showed in 1933 that bile salts were capable of solubilizing aniline, calcium carbonate, calcium phosphate, camphor, quinine, strychnine, paraldehyde, quinoline, and benzaldehyde. McBain and co-workers (8) studied the equilibrium solubility of the dye, Yellow AB, in 1% aqueous solutions of the sodium salts of cholic acid, desoxycholic acid, taurocholic acid, and dehydrocholic acid at 25°. Merrill and McBain (12) compared the solubilities of the dyes, Yellow AB and Orange OT, in 1% aqueous solutions of the same 4 bile salts used in the previously cited study (8). Ekwall (13) reviewed some of the solubilization work done by himself and co-workers with a wide variety of insoluble substances in bile salt solutions. This paper includes: (*a*) solubilization of various carcinogenic polycyclic hydrocarbons at 40° (10, 14, 15); (*b*) solubilization of *p*-xylene in solutions of sodium cholate and desoxycholate; (*c*) solubility of cholic and desoxycholic acids in aqueous solutions of their respective sodium salts (16); (*d*) solubilization of cholesterol in sodium desoxycholate, cholate, and taurocholate; and (*e*) solubilization of C_6–C_{18} fatty acids, lecithin and glyceryl monostearate, in 0.09 *M* sodium taurocholate solutions. Ekwall and Sjöblom (17–19) have studied the solubilization of various steroid hormones in bile salt solutions. The solubilization of the nonsteroidal synthetic estrogen, hexestrol, in 5, 10, and 20% solutions of sodium cholate, desoxycholate, dehydrocholate, and glycocholate at 40° was also considered by these investigators.

Bile salts have been shown also to play an important role in the physiological processes of digestion and absorption of dietary lipids. The most modern theory for fat digestion and absorption is the one proposed by Borgström (20). According to his theory, the breakdown products of fat digestion (*i.e.*, fatty acids and monoglycerides) are absorbed across the intestinal mucosa from a mixed micellar solution composed chiefly of fatty acids, monoglycerides, and conjugated bile salts. In connection with this theory of fat absorption, several *in vitro* investigations have appeared in the literature demonstrating the marked solubilizing ability of conjugated bile salts for fatty acids and monoglycerides (21–24).

Although considerable evidence has appeared in the literature concerning the effects of bile salts on endogenous materials, little work has been done to determine the effects of bile salts on drug molecules. Accordingly, little consideration has been given to the possibility that insoluble drugs may be absorbed by a mechanism involving preliminary solubilization of the drug by the bile salt micelles normally present in the intestine. This consideration prompted an extensive physicochemical investigation of the solubilization of 3 water-insoluble pharmaceuticals in dilute bile salt solutions as well as some of the factors which may influence the extent of their solubilization.

This paper reports some of the findings on the effect of temperature and bile salt concentration and structure on the degree of solubilization of the water-insoluble drugs, griseofulvin, hexestrol, and glutethimide, in dilute aqueous solutions of the sodium salts of cholic, desoxycholic, taurocholic, and glycocholic acids.

THEORY

Micellar solubilization of a poorly water-soluble material can be treated as a process in which the poorly water-soluble material is partitioned between an aqueous phase and a micellar phase formed by the surfactant above its CMC (1). The partition coefficient associated with this process is expressed by the equation:

$$K = \frac{[D_M]}{[D_{NM}]} \qquad \text{(Eq. 1)}$$

where $[D_M]$ is the concentration of drug in the micelle and $[D_{NM}]$ is the concentration of drug in the nonmicellar phase.[1] The brackets denote concentrations expressed in terms of the individual phase volumes, rather than the total volume of the system.

Multiplying the numerator and denominator of Eq. 1 by the total volume of the system, V (*i.e.*, $V_{NM} + V_M$), yields the expression,

$$K = \frac{(D_M)}{(D_{NM})} \cdot \frac{V_{NM}}{V_M} \qquad \text{(Eq. 2)}$$

[1] It was assumed that the activity coefficient of the drugs in the nonmicellar phase closely approximated the activity coefficient in the micellar phase.

where the parenthesis denote concentrations expressed in terms of total volume. V_{NM} and V_M represent the nonmicellar and micellar volumes, respectively.

Expressing Eq. 2 in terms of micellar volume yields

$$K = \frac{(D_M)}{(D_{NM})} \cdot \frac{(V - V_M)}{(V_M)} \qquad \text{(Eq. 3)}$$

However, V_M/V may be represented as M, where M is defined as the volume fraction of surfactant (25, 26). Substituting this relationship into Eq. 3 gives the expression,

$$K = \frac{(D_M)}{(D_{NM})} \cdot \frac{(1 - M)}{(M)} \qquad \text{(Eq. 4)}$$

Assuming M is small as compared to the total volume (25, 26) then $(1 - M)$ is approximately equal to unity and Eq. 5 is obtained.

$$K = \frac{(D_M)}{(D_{NM})} \cdot \frac{1}{M} \qquad \text{(Eq. 5)}$$

Above the CMC of a surfactant an equilibrium exists between monomers and micellar aggregates. The solution is saturated with respect to monomers, and further addition of surfactant molecules results in further aggregation. It has been theorized that additional molecules of surfactant produce an increase in the number rather than in the size of the micelles. Accordingly, micellar volume is a direct function of surfactant concentration. Over a limited range, this is considered to be a reasonable approximation (4, 27). This concept may be expressed as Eq. 6.

$$\frac{C_1}{C_2} = \frac{M_1}{M_2} \qquad \text{(Eq. 6)}$$

where C is the molar concentration of the surfactant. The subscripts, 1 and 2, refer to different concentrations above the CMC of the surfactant.

Inserting the relationship between C and M, as expressed in Eq. 6, into Eq. 5 yields the final equation:

$$\Delta D_M = K D_{NM} \Delta C \qquad \text{(Eq. 7)}$$

It can be seen readily from the form of Eq. 7 that a plot of (D_M) *versus* C should yield a straight line above the CMC of the surfactant. The slope of this linear plot divided by (D_{NM}) will give the value of K, from which the thermodynamic constants can be calculated.

The free energy of partitioning (ΔF^0) may be calculated from

$$\Delta F^0 = -2.3RT \log K \qquad \text{(Eq. 8)}$$

The heat of partitioning (ΔH^0) can be determined with the aid of the relationship

$$\frac{\delta \log K}{\delta(1/T)} = \frac{-\Delta H^0}{2.3R} \qquad \text{(Eq. 9)}$$

by plotting log K at various temperatures *versus* $1/T$ (°K.). From the slope of this linear plot, ΔH^0 can be obtained. This method of acquiring ΔH^0 requires that ΔH remain reasonably constant over the temperature range studied.

The change in entropy (ΔS^0) associated with this process of solubilization would follow from the equation

$$\Delta S^0 = \frac{\Delta H^0 - \Delta F^0}{T} \qquad \text{(Eq. 10)}$$

EXPERIMENTAL

Materials.—Hexestrol,[2] griseofulvin,[3] and glutethimide[4] were used as recieved. The pure bile salts, sodium cholate,[5] sodium desoxycholate,[5] sodium glycocholate,[6] and sodium taurocholate[6] were dried *in vacuo* for 36 hr. prior to use.

Equilibration.—The solubility of each drug was measured in a series of aqueous solutions containing various concentrations of the individual bile salts. Hexestrol and griseofulvin were studied at 27, 37, and 45°. The solubility of glutethimide was determined at 27, 32, and 37°.

In each case an excess amount of drug was added to bile salt solution contained in suitably sealed tubes. The tubes were placed in a shaker-incubator[7] and equilibrated for periods usually not less than 1 week's duration. Equilibrium was determined by repetitive sampling.

Assay Procedure.—Each time the tubes were sampled the shaker was turned off to allow most of the excess solid to settle to the bottom of the tubes. The supernatant liquid then was filtered through a filter (Millipore, 0.45 M pore size) to insure that no undissolved solid was present in the sample taken for analysis. To eliminate any temperature differential during the filtration and sampling steps, precautions were taken to maintain the filtration equipment and pipets at the same temperature as that employed for the equilibrium experiments.

Aliquots of the clear drug solutions were diluted with anhydrous reagent methanol, and the drug concentration was determined spectrophotometrically using a Beckman DB recording spectrophotometer. Methanol–water (10:1) served as the blank for hexestrol and griseofulvin, and methanol–water (8:1) was employed for glutethimide. The peak absorbance of griseofulvin and hexestrol (in 1:10 water–methanol solvent mixture) at 292 mμ and 278 mμ, respectively, and glutethimide (in 1:8 water–methanol solvent mixture) at 257.6 mμ was used to prepare Beer's law plots. In the dilutions required for spectrophotometric analysis, no shifts in absorbance maxima were observed as a result of the presence of surfactant. However, the bile salts do absorb slightly at the wavelength of maximum absorbance of these drugs. Therefore, the absorbance of varying concentrations of the bile salts, at the 3 previously mentioned wavelengths, was plotted *versus* bile salt concentration, and a calibration curve was thus constructed.

To determine the amount of drug that had been solubilized, the absorbance value corresponding to the concentration of bile salt in the final dilution

[2] Obtained from Gallard-Schlesinger Chemical Mfg. Co. New York, N. Y.

[3] Generously supplied by Schering Co., Bloomfield, N. J. Marketed as Fulvicin.

[4] Generously supplied by Ciba Pharmaceutical Co., Summit, N. J. Marketed as Doriden.

[5] Obtained from Mann Research Laboratories, Inc., N. Y., special enzyme grade.

[6] Obtained from Southeastern Biochemicals, Morristown, Tenn. Reported to be 98–99% pure by thin-layer chromatography.

[7] Gyratory incubator shaker, model G-25, New Brunswick Scientific Co., N. J.

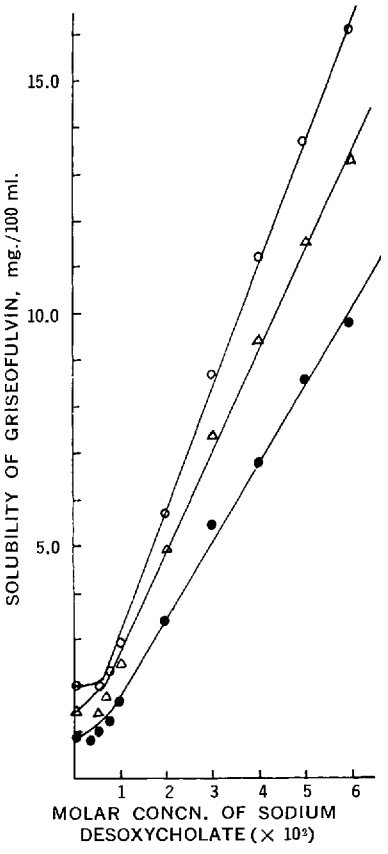

Fig. 1.—Solubility of griseofulvin as a function of sodium desoxycholate concentration and temperature. Key: ●, 27°; △, 37°; ○, 45°.

was subtracted from the observed absorbance values, and the corrected absorbance values were converted to concentrations by the use of the Beer's law plots.

RESULTS AND DISCUSSION

Effect of Bile Salt Type and Concentration on Solubilization.—The solubilization curves for Griseofulvin in varying concentrations of sodium desoxycholate solutions at 27, 37, and 45° are shown in Fig. 1. These data are representative of the type of curves obtained with sodium cholate, sodium glycocholate, and sodium taurocholate solutions. It can be seen from each of these curves that the solubility of griseofulvin increases linearly with bile salt concentration, after a certain minimum concentration of bile salt has been exceeded, *i.e.*, the CMC. The CMC values for the 4 bile salts employed in this study, as determined from the solubilization of griseofulvin and hexestrol, respectively, at 37°, are: sodium cholate (0.014, 0.016), sodium desoxycholate (0.005, 0.010), sodium taurocholate (0.008, 0.014), and sodium glycocholate (0.010, 0.015). These values are in good agreement with those obtained by other investigators employing various solubilizates (9, 13). It should be borne in mind that CMC values determined from solubilization data are only approximate, since the presence of solubilized material may exert an effect on the process of micelle formation (1, 2).

The slope of the linear portion of the solubilization curve, after the CMC, is termed the saturation ratio or capacity, *i.e.*, the ratio of micellar drug to micellar bile salt. The saturation ratios, expressed as moles of solubilized drug per mole of bile salt as well as the inverse of these ratios for griseofulvin in each of the 4 bile salts employed in this study, are presented in Table I. The saturation ratio at 27 and 37° in decreasing order are: cholate ≥ desoxycholate > glycocholate ≥ taurocholate. This sequence also indicates the order of solubility of griseofulvin in a particular concentration of these colloidal electrolytes. At 45° the order of cholate and desoxycholate is reversed, suggesting that at this higher temperature sodium desoxycholate micelles have a higher affinity for griseofulvin than do sodium cholate micelles.

Figures 2 and 3 show the solubility of the synthetic estrogenic hormone, hexestrol, in sodium glycocholate and sodium taurocholate solutions at 27, 37, and 45°. Similar curves were obtained in sodium cholate and sodium desoxycholate solutions. The saturation ratios as well as the inverse of these ratios are listed in Table II. As with griseofulvin, the solubility of hexestrol at all 3 temperatures increases linearly with bile salt concentration above the CMC. The saturation ratios for hexestrol are many times greater than those of griseofulvin, illustrating the well-established fact that the structure of the solubilizate is a critical factor in governing the extent or degree of micellar solubilization (1, 2). The saturation ratios for hexestrol, in decreasing order, at any one temperature, are: glycocholate ≥ taurocholate > cholate > desoxycholate. Thus, in the case of hexestrol, the conjugated bile salt micelles (*i.e.*, sodium glycocholate and sodium taurocholate) show a greater affinity for the solubilizate molecules than do the unconjugated bile salt micelles. The reverse was found to be true for the solubilization of griseofulvin.

Representative solubilization curves for glutethimide in 0–0.06 *M* solutions of sodium cholate at 27, 32, and 37° are shown in Fig. 4. The corresponding saturation ratios (moles of solubilized glutethimide per mole of bile salt) and the inverse of these ratios are reported in Table III. The 32°-

Table I.—Maximum Solubilizing Power of Bile Salts for Griseofulvin

Solubilizer	Saturation Ratio[a] × 10³, moles of Griseofulvin/mole of Solubilizer			Inverse of Saturation Ratio, moles of Bile Salt/mole of Griseofulvin		
	27°	37°	45°	27°	37°	45°
Water	4.59×10^{-4}	7.14×10^{-4}	10.2×10^{-4}	...	...	...
Sod. cholate	5.36	6.18	6.80	187	162	147
Sod. desoxycholate	4.68	6.18	7.54	214	162	133
Sod. taurocholate	3.77	4.90	6.15	265	204	163
Sod. glycocholate	3.85	5.13	6.29	260	195	159

[a] Slope of linear portion of solubilization curve determined by the method of least squares.

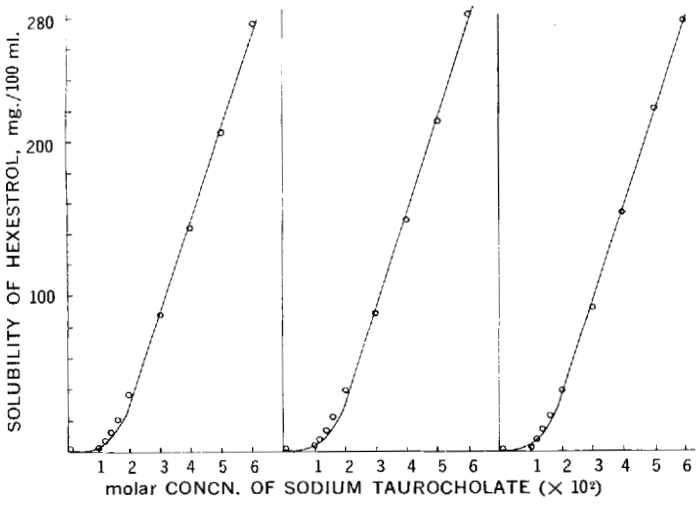

Fig. 2.—Solubility of hexestrol as a function of sodium taurocholate concentration at 27° (left), 37° (middle), and 45° (right).

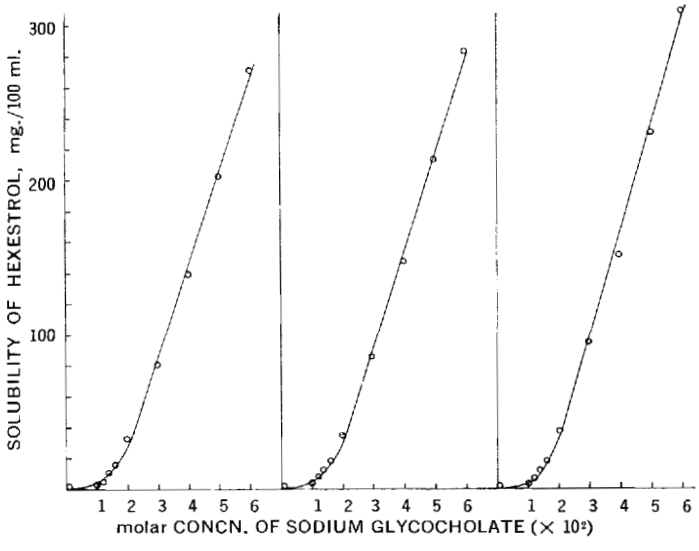

Fig. 3.—Solubility of hexestrol as a function of sodium glycocholate concentration at 27° (left), 37° (middle), and 45° (right).

temperature study was included since at 45° the solubilization curve for glutethimide was not linear, suggesting a possible change in the solubilization mechanism at this higher temperature. All experiments were performed in duplicate, and reasonable replication was obtained. Thus, spurious experimental factors were ruled out as contributing to the nonlinearity. In addition, no change in the U.V. spectra of glutethimide was observed. The saturation ratios in decreasing order are: desoxycholate > cholate ≥ taurocholate > glycocholate.

The differences in the order of the saturation ratios obtained in the various bile salt solutions with each drug used in this study are probably due to differences in the arrangement of the bile salt molecules in the micelle as well as differences in the degree and/or type of interaction between the drug molecule and the bile salt micelle. The penetration of the solubilizate may alter significantly the actual organization, shape, and even the size of the resultant bile salt micelle (13). This would explain not only the differences found in the affinity between these bile salts and the individual drug, but also the differences found in the saturation ratio se-

Table II.—Maximum Solubilizing Power of Bile Salts for Hexestrol

Solubilizer	Saturation Ratio[a] × 10³, moles of Hexestrol/mole of Solubilizer 27°	37°	45°	Inverse of Saturation Ratio, moles of Bile Salt/mole of Hexestrol 27°	37°	45°
Water	4.66×10^{-4}	6.66×10^{-4}	9.32×10^{-4}	...	...	...
Sod. cholate	187	195	197	5.35	5.13	5.08
Sod. desoxycholate	164	167	179	6.10	5.99	5.59
Sod. taurocholate	220	225	223	4.55	4.44	4.48
Sod. glycocholate	221	231	251	4.52	4.33	3.98

[a] Slope of linear portion of solubilization curve determined by the method of least squares.

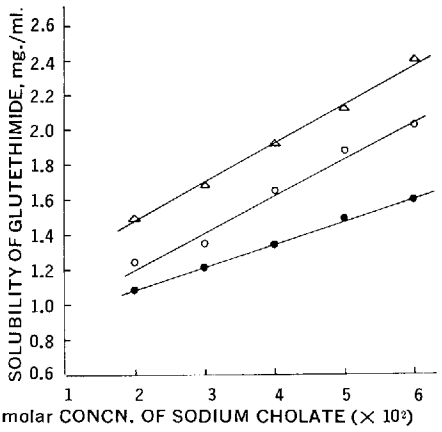

Fig. 4.—Solubility of glutethimide as a function of sodium cholate concentration and temperature. Key: ●, 27°; ○, 32°; △, 37°.

quences of these bile salts and each of the drugs. Unfortunately, at present, very little is known about the structures of the bile salt micelles. However, they are thought to be considerably different from the classical spherical micelles formed by the more common surfactants. A detailed explanation for these observed differences must await a more complete understanding of the molecular arrangement of the bile salt micelles, as well as knowledge of the nature of the interactions that are important in causing micellar aggregation of both the unconjugated and conjugated bile salts.

It can readily be seen from both the saturation ratio and inverse saturation ratio data, presented in Tables I–III, that the solubilities of hexestrol, glutethimide, and griseofulvin in the same bile salt differ greatly. For these solubilizates, the solubilities increase in the following order: griseofulvin < glutethimide < hexestrol. It would appear, based on the higher saturation ratios observed for both hexestrol and glutethimide as compared to griseofulvin, that they are being incorporated predominately into the "palisade layers" of the bile salt micelle as would a relatively polar solubilizate molecule (1–3). On the other hand, griseofulvin is probably more closely associated with the hydrocarbon region of the micelle, similar to a nonpolar solubilizate molecule (1–3).

As previously indicated, knowledge of the exact structure of the bile salt micelles is lacking. In view of this insufficiency, the mechanism by which the molecules of the solubilized substances are incorporated in the micelles must remain speculative until further investigations in this area. It should also be noted that the chemical nature, size, and structure of the solubilized molecules influence in various ways the arrangement of the surfactant molecules in the micelle (9, 13). Experiments presently being conducted in our laboratories, dealing with the effects of amphiphilic and nonpolar additives and inorganic electrolyte on the solubilization characteristics of these drugs should yield an insight to the location of the solubilized drug molecules in the bile salt micelle.

Effect of Temperature on Solubilization.—Temperature is an important factor which has a varying effect on the extent of micellar solubilization. The structure of the surfactant and/or the solubilizate will dictate whether there is an effect and whether it is positive or negative.

Inspection of the saturation ratio values for griseofulvin, presented in Table I, shows that as the temperature of the system increases from 27 to 45° there is a corresponding increase in the degree of micellar solubilization. This positive temperature effect is observed in all of the bile salt solutions studied. Similar temperature effects were observed for both hexestrol (Table II) and glutethimide (Table III). However, temperature appears to have less of an influence on the solubilization of hexestrol in comparison to the other 2 solubilizates.

A positive temperature effect is contrary to the theory proposed for micellar solubilization in solutions of fatty acid soap-type surfactants (3). According to this theory, in a homologous series of fatty acid soaps, below 25°, as the temperature is increased, the CMC of the surfactant decreases, due to an increase in the entropy of the structured water molecules around the hydrocarbon portion of the surfactant molecule. As the temperature is increased above 25°, the kinetic motion of the surfactant molecules in the micelle is enhanced and overshadows this entropy effect. The thermal motion causes a slight increase in the CMC of the surfactant and thus increases the difficulty with which micelles form. Based on these facts, one would expect that at higher temperatures solubilization should decrease.

Although a number of studies have been conducted to examine the effect of temperature on the solubilization process in typical ionic surfactant solutions, relatively few temperature studies have been conducted in bile salt solutions. Merrill and McBain (12) observed a positive temperature coefficient (*i.e.*, the ratio of the saturation ratio at a higher temperature to that at a lower temperature exceeded unity) for the dye Yellow AB in 0.1 *N* sodium desoxycholate solutions. Hofmann observed a similar temperature effect on the solubilization of azobenzene in sodium glycochenodesoxycholate solutions, but observed no effect on the solubilization of the monoglyceride, 1-monoolein (24).

Table III.—Maximum Solubilizing Power of Bile Salts for Glutethimide

Solubilizer	Saturation Ratio[a] × 10³, moles of Glutethimide/mole of Solubilizer			Inverse of Saturation Ratio, moles of Bile Salt/mole of Glutethimide		
	27°	32°	37°	27°	32°	37°
Water	7.13×10^{-2}	8.38×10^{-2}	9.94×10^{-2}	...	...	...
Sod. cholate	59.8	96.2	104	16.7	10.4	9.62
Sod. desoxycholate	103	119	163	9.71	8.40	6.13
Sod. taurocholate	61.2	100	108	16.3	10.0	9.26
Sod. glycocholate	54.3	92.0	71.8	18.4	10.9	13.9

[a] Slope of linear portion of solubilization curve determined by the method of least squares.

TABLE IV.—PARTITION COEFFICIENTS FOR GRISEOFULVIN, HEXESTROL, AND GLUTETHIMIDE BETWEEN THE MICELLAR AND NONMICELLAR PHASE AT 3 TEMPERATURES

Solubilizer	Partition Coefficient, $K \times 10^{-3}$ 27°	32°	37°	45°
		Griseofulvin		
Sod. cholate	11.7	...	8.66	6.67
Sod. desoxycholate	10.2	...	8.66	7.39
Sod. taurocholate	8.21	...	6.86	6.03
Sod. glycocholate	8.39	...	7.18	6.17
		Hexestrol		
Sod. cholate	398	...	294	211
Sod. desoxycholate	352	...	251	192
Sod. taurocholate	472	...	338	240
Sod. glycocholate	475	...	347	269
		Glutethimide		
Sod. cholate	0.840	1.15	1.05	...
Sod. desoxycholate	1.45	1.42	1.64	...
Sod. taurocholate	0.859	1.20	1.09	...
Sod. glycocholate	0.762	1.10	0.722	...

Since micellar solubilization is closely related to micelle formation, one possible explanation for the positive temperature effects observed in the present study is that as the temperature of the system is increased the CMC of the bile salt decreases. However, values determined from the solubilization curves for the individual bile salts show that the CMC values of the bile salts are not significantly altered in the temperature range employed in this study. Thus, the observed temperature effects cannot be explained on the basis of CMC values.

A more plausible explanation has been proposed by McBain and Hutchinson (1). According to these investigators, it may be assumed that the principal effect of temperature is to "change the solubility of the solubilizate in the micelle." Hofmann (24), in his investigations of the effect of temperature on the solubilization of azobenzene and 1-monoolein in sodium glycochenodesoxycholate solutions at 23 and 37°, states that, "the higher saturation ratio observed for the former solubilizate at 37° need not indicate any change in the state of micellar aggregation." This view is in agreement with Hofmann's observations that 1-monoolein had the same saturation ratio at 23 and 37°, thereby indicating that temperature has little effect on the micellar organization.

Thermodynamic Evaluation.—The results of this investigation are consistent with the hypothesis that temperature primarily influences the "solubility" of the solubilizate molecule in the bile salt micelle. Therefore, one would expect that as the temperature of the system is increased so should the degree of interaction between the solubilizate molecule and the bile salt molecules comprising the micelle. In view of this consideration, it was decided to determine the thermodynamics of these bile salt–drug systems so that an appreciation for the magnitude of the energies involved in the process of micellar solubilization in bile salt solutions could be obtained.

In order to determine the thermodynamic constants associated with the solubilization of griseofulvin and hexestrol in bile salt solutions, a pseudo two-phase model was selected. According to this model, the solubilizate molecule is partitioned between an aqueous phase and a micellar phase. This partitioning is similar to that observed for a poorly water-soluble drug between a nonpolar solvent and water. The partition coefficient, K, associated with this process was determined by the use of Eq. 7. The values obtained for griseofulvin, hexestrol, and glutethimide at 3 temperatures are listed in Table IV. The magnitude of these values shows that the poorly water-soluble drugs are preferentially partitioned to the micellar phase.

The data in Table IV also indicate that a decrease in the partition coefficients of both griseofulvin and hexestrol occurs with an increase in temperature. No definite conclusion could be made for the effect of temperature on partition coefficient for glutethimide. A similar decrease in partition coefficient with temperature was observed by Rippie and coworkers (25), in considering the solubilization of a methylprednisolone-21-hemiester in aqueous solutions of polysorbate 80. These investigators partially attribute this negative effect to micellar size changes. In conducting this investigation it was decided to consider the phenomenon on the basis of thermodynamic factors. The heats of solution of

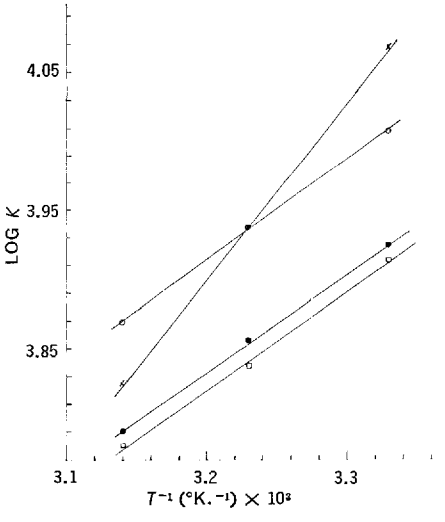

Fig. 5.—Plots of log K *vs.* $1/T$ for griseofulvin. Key: ●, sodium glycocholate; □, sodium taurocholate; ○, sodium desoxycholate; ×, sodium cholate.

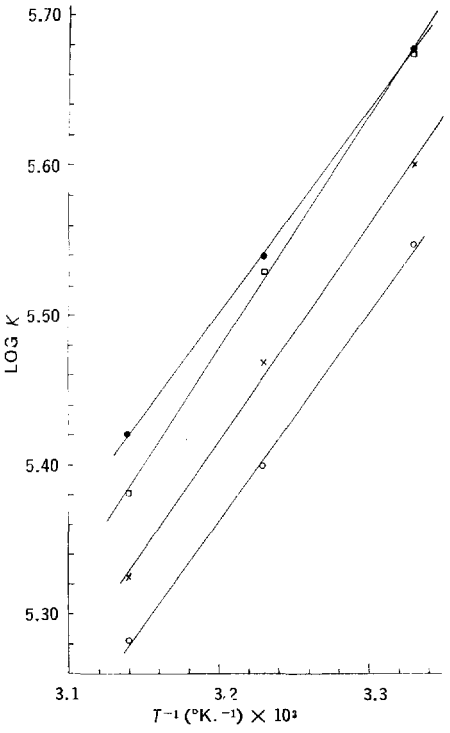

Fig. 6.—Plots of log K *vs.* $1/T$ for hexestrol. Key: ●, sodium glycocholate; □, sodium taurocholate; ○, sodium desoxycholate; ×, sodium cholate.

griseofulvin and hexestrol in water and in each of the bile salt solutions was determined from the slope of a Clausius-Clapeyron-type plot (*i.e.*, a plot of log solubility *versus* $1/T$). The heats of solution ($\Delta H_{soln.}$) in the bile salt micelle ranged from −2.5 to −5.0 Kcal./mole for griseofulvin and from approximately zero to −1.4 Kcal./mole for hexestrol, as compared to −7.3 Kcal./mole for griseofulvin and −8.4 Kcal./mole for hexestrol in water. This indicates that the solubility of the drugs in the aqueous phase is affected by temperature more than it is in the bile salt solutions, resulting in the observed decrease in the partition coefficient.

According to Eq. 9, a plot of log K *versus* $1/T$ should be linear. From the slope of such a plot the standard enthalpy change, ΔH^0, associated with this partitioning process can be obtained. Typical van't Hoff-type plots for griseofulvin (Fig. 5) and hexestrol (Fig. 6) show the excellent linearity observed over the temperature range studied. Treatment of the glutethimide data in a similar manner produced plots which were nonlinear. This suggests the possibility that glutethimide is solubilized by a different mechanism than are hexestrol and griseofulvin.

Values for the standard free energy changes (ΔF^0) and entropy changes (ΔS^0) associated with the solubilization process were determined with the aid of Eqs. 8 and 10, respectively. The values of ΔF^0, ΔH^0, and ΔS^0 for the solubilization of griseofulvin in the 4 individual bile salts are presented in Table V, and those for hexestrol in Table VI.

The negative ΔF^0 values obtained is indicative of the spontaneity of the solubilization process. The standard enthalpies, ΔH^0, for each drug in all of the bile salts are quite similar. However, there is a significant difference between the ΔH^0 values for hexestrol and for griseofulvin. This indicates that the enthalpy function is more dependent on the nature of the drug molecule than on the nature of the bile salt molecule. The negative enthalpy changes obtained are consistent with the hypothesis that micellar solubilization is an exothermic process (1, 25, 26).

It has been proposed that because of the loss of freedom experienced by the drug molecule in going from the aqueous phase to the micellar phase a negative entropy change should accompany the solubilization process (26). The small positive entropy values obtained in this investigation indicate that other factors must be taken into consideration. A possible explanation for the positive entropy values is that loss of water structure in the system counterbalances the restriction placed on the drug molecule when it is solubilized by the bile salt micelle.

Biological Implications.—A comparison of the equilibrium ratio of the amount of drug solubilized by a 0.04 M bile salt solution to that in water for the 3 solubilizates at 37° shows that the ratios in decreasing order are: hexestrol > griseofulvin > glutethimide (Table VII). A bile salt concentration of 0.04 M is considered to be the approximate

Table V.—Standard Thermodynamic Functions for Griseofulvin in Bile Salt Solutions

Bile Salt	ΔH^0,[a] Kcal./mole	ΔF^0, Kcal./mole 300°K.	310°K.	318°K.	ΔS^0, Entropy units 300°K.	310°K.	318°K.
Sod. cholate	−5.9	−5.61	−5.61	−5.59	−1	−1	−1
Sod. desoxycholate	−3.4	−5.53	−5.61	−5.66	+7	+7	+7
Sod. taurocholate	−3.2	−5.40	−5.47	−5.53	+7	+7	+7
Sod. glycocholate	−3.2	−5.42	−5.50	−5.54	+7	+7	+7

[a] Slope of the linear plot of log K *vs.* $1/T$ determined by the method of least squares.

Table VI.—Standard Thermodynamic Functions for Hexestrol in Bile Salt Solutions

Bile Salt	ΔH^0,[a] Kcal./mole	ΔF^0, Kcal./mole 300°K.	310°K.	318°K.	ΔS^0, Entropy units 300°K.	310°K.	318°K.
Sod. cholate	−6.6	−7.73	−7.80	−7.79	+4	+4	+4
Sod. desoxycholate	−6.4	−7.65	−7.70	−7.73	+4	+4	+4
Sod. taurocholate	−7.1	−7.83	−7.88	−7.87	+2	+3	+2
Sod. glycocholate	−6.2	−7.83	−7.90	−7.93	+5	+6	+5

[a] Slope of the linear plot of log K *vs.* $1/T$ determined by the method of least squares.

molarity of the total bile salts present in the small intestine during fat absorption (21, 24, 28). These values show that the bile salts display a significant effect in increasing the solubility of these poorly water-soluble drugs.

TABLE VII.—RATIOS OF THE SOLUBILITIES OF HEXESTROL, GRISEOFULVIN, AND GLUTETHIMIDE IN 0.04 *M* SOLUTIONS OF CONJUGATED AND UNCONJUGATED BILE SALTS TO THAT IN WATER AT 37°

Bile Salt	Drug		
	Hexestrol	Griseofulvin	Glutethimide
Sod. cholate	122	5.7	1.6
Sod. desoxycholate	139	6.7	2.1
Sod. taurocholate	148	5.6	1.6
Sod. glycocholate	147	5.7	1.5

Drug absorption across the gastrointestinal barrier takes place almost exclusively from a solution of the drug (29). Therefore, the drug must be in solution before it can be absorbed. In the case of extremely water-insoluble drugs, dissolution of the drug usually becomes the slow rate-determining step in the absorption process.

Poorly water-soluble drugs will partition between the aqueous phase and a liquid phase, with a relatively large lipid–water partition coefficient. From a physicochemical point of view, one can draw an analogy between these poorly water-soluble drugs and a dietary lipid. Based on this analogy it would be interesting to speculate that the gastrointestinal tract handles these drugs in the same manner as it handles dietary lipids. In view of the *in vitro* evidence it is quite conceivable that relatively water-insoluble drugs may be absorbed by a mechanism involving preliminary solubilization of the drug by bile salt micelles present in the small intestine.

Relative dissolution rate studies indicate that bile salts significantly increase the dissolution rates of griseofulvin and hexestrol over that in water (30). These findings serve to strengthen further the possibility that physiologic surfactants play an important role in the dissolution step of the absorption process.

Lecithin and cholesterol, which are normal components of bile, as well as fatty acids and monoglycerides, which are the normal breakdown products of fat digestion, have been shown to form mixed micelles with the conjugated bile salts in the small intestine. Extensive studies currently are being conducted to determine the effects of these, as well as other additives, on the degree of solubilization of water-insoluble drugs under conditions simulating those existing in the human small intestine during fat digestion and absorption.

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