(43) N. K. Jerne and D. A. Nordin, *Science*, **140**, 405 (1963).
(44) T. Igarashi, M. Okada, S. Kishimoto, and Y. Yamamura, *Immunology*, **28**, 37 (1975).
(45) E. M. Uyeki, R. S. Klassen, and V. Llacer, *Proc. Exp. Biol. Med.*, **132**, 1140 (1969).
(46) A. J. Crowle and C. C. Hu, *Proc. Soc. Exp. Biol.*, **127**, 190 (1968).
(47) A. J. Crowle, C. C. Hu, and A. Patruce, *J. Allergy*, **42**, 140 (1968).
(48) B. Baggett, I. H. Hall, R. G. Boelgi, K. H. Palmer, and M. Wall, *Fertil. Steril.*, **21**, 68 (1970).
(49) I. Hall, G. L. Carlson, G. S. Abernethy, and C. Piantadosi, *J. Med. Chem.*, **17**, 1253 (1974).
(50) J. G. Wilson, in "First Fertility Conference on Congenital Malformations," General Principles in Experimental Teratology, Lippincott, Philadelphia, Pa., 1961, pp. 187–194.
(51) C. W. Emmens, *Methods Horm. Res.*, **2**, 61 (1970).
(52) D. A. Brodie and B. J. Chase, *J. Gastroenterol.*, **53**, 604 (1967).
(53) S. A. Lang, B. D. Johnson, E. Cohen, A. E. Sloboda, and E. Greenblatt, *J. Med. Chem.*, **19**, 1404 (1976).
(54) G. W. Snedecor, "Statistical Methods," Iowa State College Press, Ames, Iowa, 1956.
(55) E. Arrigoni-Mantelli, "Inflammation and Anti-inflammatories," Spectrum, New York, N.Y., 1977.
(56) Y. H. Chang, *Annu. Rep. Med. Chem.*, **11**, 138 (1976).
(57) O. J. Mellbye, J. B. Natvig, T. E. Michaelson, and H. M. Hoyeraal, in "The Immunological Basis of Connective Tissue Disorders," L. G. Silvestri, Ed., North-Holland Publishing, Amsterdam, The Netherlands, 1975.
(58) P. Davies and R. J. Bonney, *Annu. Rep. Med. Chem.*, **12**, 152 (1977).
(59) E. Munthe and J. B. Natvig, *Scand. J. Immunol.*, **1**, 217 (1972).
(60) D. Hamerman, in "The Immunological Basis of Connective Tissue Disorders," L. G. Silvestri, Ed., North-Holland Publishing, Amsterdam, The Netherlands, 1975, p. 17.
(61) D. Hurwitz, N. E. Cremer, F. Quismorio, E. H. Lennette, and G. J. Friou, *J. Rheum. (Suppl.)*, **1**, 120 (1974).
(62) A. C. Allison, *Ann. Rheum. Dis.*, **32**, 283 (1973).
(63) P. Stastny, *Arthritis Rheum.*, **16**, 572 (1972).
(64) J. B. Peter, T. Konen, K. Stenepel, and C. Cardin, *ibid.*, **14**, 179 (1971).

ACKNOWLEDGMENTS

The authors acknowledge financial support from the following sources: University of North Carolina Research Council Grant VF475 awarded to I. H. Hall, NIH Grant CA-17625 and American Cancer Society Grants CH-19A and CH-19B awarded to K. H. Lee, and the Research Corporation of America grant and the American Chemical Society grant awarded to T. G. Waddell.

Simultaneous Solubilization of Steroid Hormones II: Androgens and Estrogens

B. LUNDBERG ˣ, T. LÖVGREN, and B. HEIKIUS

Received March 1, 1978, from the *Department of Biochemistry and Pharmacy, Åbo Akedemi, Porthansgatan 3, 20500 Åbo 50, Finland.* Accepted for publication October 18, 1978.

Abstract □ The simultaneous solubilization of some androgens and estrogens in aqueous polysorbate 40, tetradecyltrimethylammonium bromide, and sodium lauryl sulfate was studied. The solubilizations of estradiol and testosterone were independent of each other in all three association colloids. However, if the estrogen component was ethinyl estradiol, the solubilization was dependent on the addition order. The estrogen precipitates more readily than testosterone in polysorbate 40 and tetradecyltrimethylammonium bromide, but the opposite is true in sodium lauryl sulfate. The simultaneous solubilizations of methyltestosterone or ethisterone with the estrogens tested were different from those of testosterone. The solubilization behavior of the steroids is discussed, starting with the pseudophase model and different solubilization loci. Results indicated that the free energy change of micellar binding, ΔG_b, decreases with increased steroid polarity. The simultaneous solubilization cannot be predicted by ΔG_b but may be explained by differences in the solubilization mechanism.

Keyphrases □ Solubilization—androgens and estrogens in various association colloids □ Androgens—solubilization in various association colloids □ Estrogens—solubilization in various association colloids

The solubilization of poorly soluble drugs is of great pharmaceutical interest. The surfactants used may be important in drug bioavailability (1).

The solubilization of steroid hormones by aqueous solutions of surfactants was reviewed previously (2). More recent reports (3–7) indicated that solubilization continues to be of interest.

A recent report from this laboratory (8) dealt with the simultaneous solubilization of estrogens and C_{21}-steroids in aqueous solutions of association colloids. The poorly soluble estrogen, estradiol, solubilized independently of the C_{21}-steroids, whereas the solubilization of ethinyl estradiol was independent of corticosterone and hydrocortisone but dependent on the presence of progesterone and desoxycorticosterone (21-hydroxyprogesterone).

This report deals with the dissolution behavior of estrogens and androgens simultaneously solubilized in aqueous solutions of three association colloids chosen as representatives of nonionic, cationic, and anionic types.

EXPERIMENTAL

Materials—Purification methods and the tests of purity of the steroid hormones and the association colloids were described previously (8). The association colloids used were sodium lauryl sulfate[1], tetradecyltrimethylammonium bromide[2], and polysorbate 40[3].

Solubilization Experiments—The solubility studies were carried out as previously described (8). The procedures were: saturation of the solution of association colloid with the first steroid and quantitation of solubilized steroid, saturation with the second steroid, and, finally, UV spectroscopic quantitation of both solubilized steroids. Special notice was paid to complete equilibration of the solutions. The solubilization temperatures were 20° for tetradecyltrimethylammonium bromide and polysorbate 40 and 40° for sodium lauryl sulfate.

The UV absorbance of the steroid solutions was recorded at around

[1] Koch-Light Laboratories.
[2] K & K Laboratories.
[3] Tween 40, Atlas Chemical Industries.

0022-3549/79/0500-0542$01.00/0

Table I—Influence of the Addition Order on Solubilization Capacities of Surfactants for Hormonal Steroids

Order of Addition of Steroid		Moles of Steroid per Mole of Surfactant[a]					
		I		II		III	
First	Second	First	Second	First	Second	First	Second
Testosterone	Estradiol	0.027	0.013	0.13	0.068	0.20	0.025
Estradiol	Testosterone	0.013	0.027	0.068	0.13	0.025	0.20
Testosterone	Ethinyl estradiol	0.027	0.18	0.12	0.25	0.04	0.13
Ethinyl estradiol	Testosterone	0.063	0.027	0.078	0.024	0.065	0.18
Methyltestosterone	Ethinyl estradiol	0.046	0.18	0.13	0.25	—	—
Ethinyl estradiol	Methyltestosterone	0.18	0.046	0.27	0.12	—	—
Ethisterone	Estradiol	—	—	0.005	0.068	—	—
Estradiol	Ethisterone	—	—	0.068	0.005	—	—

[a] I = polysorbate 40, II = tetradecyltrimethylammonium bromide, and III = sodium lauryl sulfate.

280 nm for the estrogens and around 240 nm for the androgens. The molar absorptivity of the steroids in different colloid solutions was investigated. The steroids did not affect the molar absorptivity of each other.

RESULTS

The simultaneous solubilization of the estrogens estradiol and ethinyl estradiol and the androgens testosterone, methyltestosterone, and ethisterone in the three association colloids tetradecyltrimethylammonium bromide, polysorbate 40, and sodium lauryl sulfate was investigated. If excess estradiol was added to a solution saturated with testosterone, or *vice versa*, the steroids were solubilized in all three association colloids as if they had been added independently. The amount of steroids solubilized increased linearly with the concentration of association colloid.

When ethinyl estradiol was used as the estrogen component together with testosterone, the solubilization was dependent on the order of addition. If excess ethinyl estradiol was added to a saturated solution of testosterone in polysorbate 40, the two steroids solubilized independently (Fig. 1*a*). However, if the addition was done in the opposite order, the micellar solubilization of ethinyl estradiol dropped to 35% of its maximal value while testosterone was solubilized almost maximally (Fig. 1*b*). If excesses of both steroids were added at the same time, testosterone was solubilized maximally while the micellar solubility of ethinyl estradiol dropped to the same order of magnitude (~30%) as if it were added first.

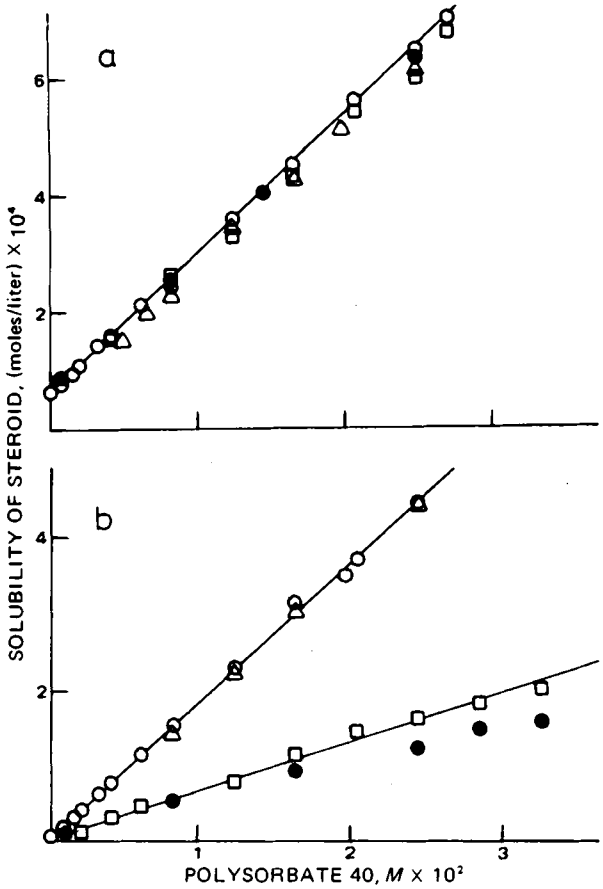

Figure 1—(a) *Solubility of testosterone in aqueous solutions of polysorbate 40. Key:* ○, *testosterone only;* △, *testosterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and testosterone second; and* ●, *testosterone and ethinyl estradiol at the same time.* (b) *Solubility of ethinyl estradiol in aqueous solutions of polysorbate 40. Key:* ○, *ethinyl estradiol only;* △, *testosterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and testosterone second; and* ●, *testosterone and ethinyl estradiol at the same time.*

In tetradecyltrimethylammonium bromide, 8% of the solubilized testosterone precipitated on addition of excess ethinyl estradiol which, in turn, reached 93% of its maximal saturation (Fig. 2*a*). If the saturations were done in the opposite order, 71% of the solubilized ethinyl estradiol precipitated while testosterone reached only 72% of its maximal saturation (Fig. 2*b*).

In a solution of sodium lauryl sulfate, the simultaneous solubilization of testosterone and ethinyl estradiol was strikingly different from that in polysorbate 40 and tetradecyltrimethylammonium bromide. Eighty percent of the solubilized testosterone precipitated on addition of excess ethinyl estradiol while the estrogen component solubilized maximally (Fig. 3*a*). If the saturation were carried out in the opposite direction, 50% of the ethinyl estradiol precipitated and testosterone was solubilized almost maximally (Fig. 3*b*).

Methyltestosterone showed a different solubility behavior from testosterone. In polysorbate 40, the solubilities of methyltestosterone and ethinyl estradiol were independent of each other (Fig. 4). In the opposite direction, 17% of methyltestosterone precipitated and the estrogen component reached 93% of its maximal value (Fig. 4).

Ethisterone was also studied. This androgen hormone has the lowest micellar solubility of all steroids tested. It was solubilized independently of estradiol in tetradecyltrimethylammonium bromide.

The solubilization capacities of the three surfactants for the different steroids were calculated (Table I).

DISCUSSION

Solubilization was defined as the transfer of cosolute (X) from the pure state, either crystalline (c) or liquid (l), to micelles (9). The process must be distinguished from micellar binding, which involves the transfer of cosolute from an aqueous (aq) solution into the micelle (m). The equilibrium between X_{aq} and X_m may be governed by the distribution law, or nonlinear isotherms (plot of $[X_m]$ against $[X_{aq}]$) are obtained for micellar binding (9). To determine the micellar constants from solubility measurements alone, the micellar binding has to be governed by the distribution law. If the law is obeyed, saturation solubilities may be used for determination of the partition constant K_p (9).

The linear relationship between surfactant concentration and quantity of solubilized steroid in unsaturated systems is indicative of solubility governed by the distribution law. The linearity was confirmed for a

Table II—Free Energy Change, ΔG_b, of Micellar Binding of Hormonal Steroids in Aqueous Solutions of Surfactants

Steroid	ΔG_b, J mole^{-1}		
	I[a]	II	III
Testosterone	14,322	18,217	19,285
Ethisterone	14,583	19,254	20,594
Estradiol	16,256	20,353	17,874
Ethinyl estradiol	21,258	22,263	20,452

[a] See footnote *a*, Table I.

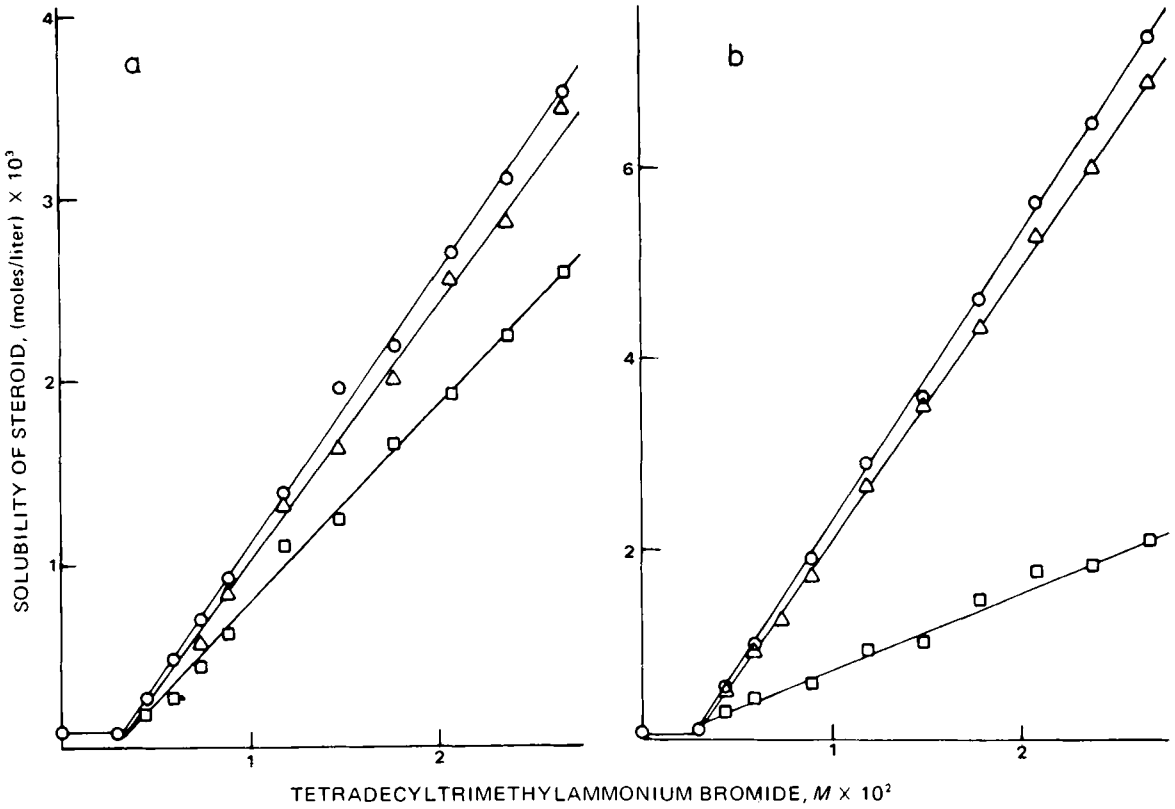

Figure 2—(a) *Solubility of testosterone in aqueous solutions of tetradecyltrimethylammonium bromide. Key:* ○, *testosterone only;* △, *testosterone first and ethinyl estradiol second; and* □ *ethinyl estradiol first and testosterone second.* (b) *Solubility of ethinyl estradiol in aqueous solutions of tetradecyltrimethylammonium bromide. Key:* ○, *ethinyl estradiol only;* △, *testosterone first and ethinyl estradiol second; and* □, *ethinyl estradiol first and testosterone second.*

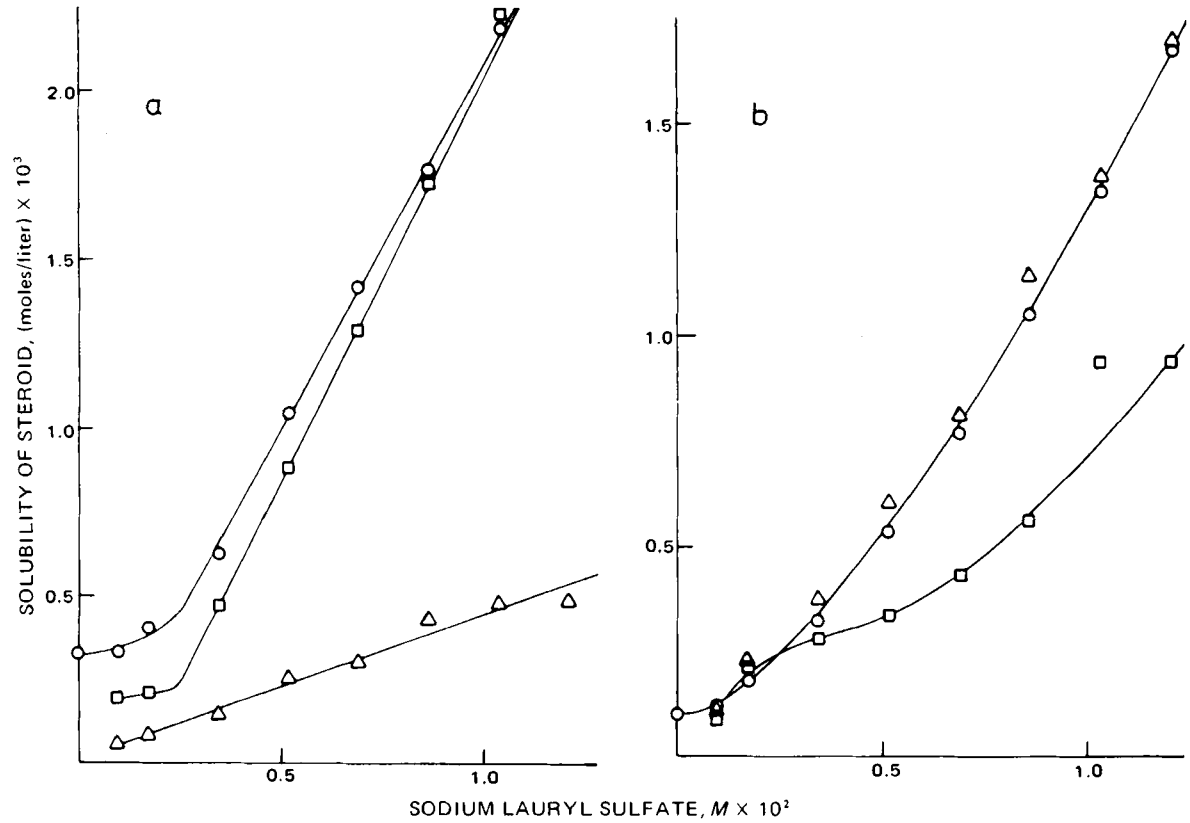

Figure 3—(a) *Solubility of testosterone in aqueous solutions of sodium lauryl sulphate. Key:* ○, *testosterone only;* △, *testosterone first and ethinyl estradiol second; and* □, *ethinyl estradiol first and testosterone second.* (b) *Solubility of ethinyl estradiol in aqueous solutions of sodium lauryl sulfate. Key:* ○, *ethinyl estradiol only;* △, *testosterone first and ethinyl estradiol second; and* □, *ethinyl estradiol first and testosterone second.*

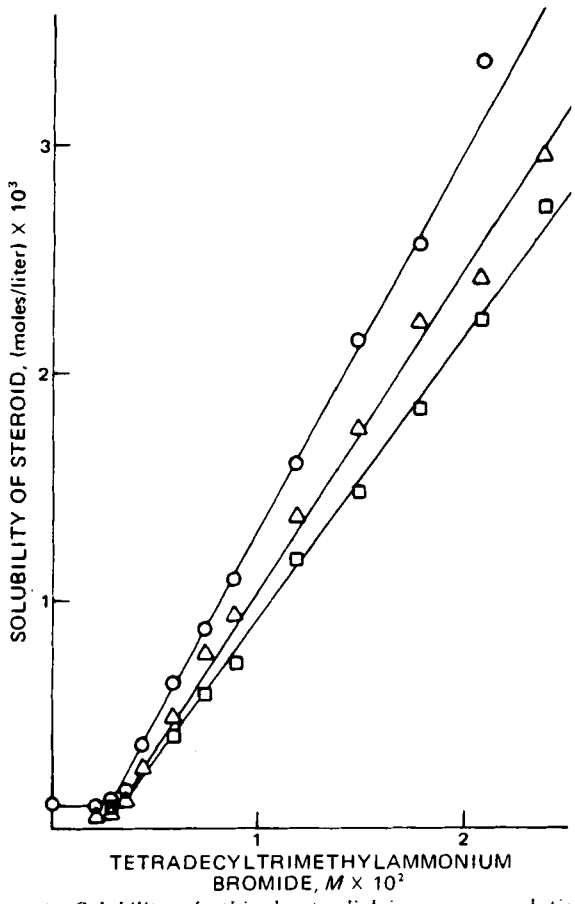

Figure 4—*Solubility of ethinyl estradiol in aqueous solutions of tetradecyltrimethylammonium bromide. Key:* ○, *methyltestosterone only;* △, *methyltestosterone first and ethinyl estradiol second; and* □, *ethinyl estradiol first and methyltestosterone second.*

number of steroids by dialysis experiments (10). Thus, the partition constant between micellar and nonmicellar steroids can be defined by (9, 11):

$$K_p = \frac{[X^s_m]}{[X^s_{aq}]} \qquad \text{(Eq. 1)}$$

where K_p is the partition constant, $[X^s_m]$ is the saturation solubility of steroid in the "micellar phase," and $[X^s_{aq}]$ is the saturation solubility of steroid in water.

Although the two-phase model is inappropriate for describing monomer–micelle equilibria, it is useful for solubilizing systems. Accordingly, Eq. 2 (9, 11) can be used to calculate the free energy change of micellar binding ΔG_b (J mole^{-1}):

$$\Delta G_b = -RT \ln K_p \qquad \text{(Eq. 2)}$$

By starting with the values of the water solubility of steroids (12) and solubilization capacities, ΔG_b values of steroids solubilized separately and simultaneously were calculated (Table II). The ΔG_b values differed considerably.

The introduction of an ethinyl group into the 17 α-position of estradiol decreased the ΔG_b value greatly while the effect on testosterone was minor. This apparent contradiction can be explained by the effect of the ethinyl group on the polarity of the steroid molecule, increasing the net dipole moment of estradiol but reducing that of testosterone (13). Thus, ΔG_b depends on the balance of hydrophilicity between the ends of the molecule, which determine the orientation of the molecules in the micelle.

These results clearly indicate that ΔG_b decreases with increased steroid polarity in contradiction to an earlier study (10). This result can be explained by different solubilization mechanisms; nonpolar species are solubilized by the hydrocarbon core while the polar ones penetrate the palisade layer of the micelle. Furthermore, the location of the cosolute in the palisade layer can be characterized as deep or short penetration (14). Presumably, the polar steroids decrease the interfacial energy by orientating themselves in the palisade layer with the polar groups at the micelle–water interface. The solubilization of nonpolar species is determined by the volume of the hydrocarbon core of the micelle (15).

The fact that testosterone and estradiol can be simultaneously solubilized independently of each other is consistent with the idea that steroids can be solubilized in different loci. Testosterone has a larger net dipole moment (13) and is likely to penetrate the palisade layer while estradiol is solubilized in the hydrocarbon core. This assumption is supported by observations indicating that testosterone is associated with the polar part of the micelle (16).

The simultaneous solubilization of steroids cannot be predicted by the free energy of micellar binding (Table II). Other factors obviously influence the behavior of the steroids in the micellar solution.

Ethinyl estradiol has a more negative ΔG_b than testosterone but is solubilized only to ~30% of its maximal value, while testosterone is solubilized maximally. This contradiction may be explained by differences in the solubilization mechanism. Both ethinyl estradiol and testosterone are rather polar and are supposed to be solubilized in the palisade layer. However, the less polar testosterone is most likely to be solubilized by deep penetration while ethinyl estradiol could be solubilized by short penetration. Thus, the solubilization loci of the two steroids partly overlap. During simultaneous solubilization, a new state of equilibrium for the micelle has to be considered. Testosterone does not precipitate in polysorbate 40 and tetradecyltrimethylammonium bromide solutions when excess ethinyl estradiol is added because testosterone is more deeply embedded in the micelle. On the other hand, ethinyl estradiol precipitates on addition of testosterone because the microenvironment of the micelle is changed in an unfavorable direction for solubilization of ethinyl estradiol.

From the present data, the enthalpy and entropy contributions to the free energy of solubilization cannot be deduced, but some hypothetical conclusions can be drawn. If the micelle is assumed to be in liquid state (17), the partial molar entropy of solubilization is given by:

$$\Delta S^\circ_s = (S_s - S_l)_{p,t} + (S_l - S_c)_{p,t} \qquad \text{(Eq. 3)}$$

where S_s, S_l, and S_c are the entropies in the solubilized, liquid, and crystalline states, respectively. The transfer from the crystalline to liquid state implies an increase in entropy, which is assumed to be almost the same for all steroids. Hence, the entropy change of the transfer from pure liquid to micelle is most important and is dependent on the type of micelle and the interaction between the surfactant and cosolute.

A similar change in enthalpy can be expected for the steroids dissolving in the micellar core. A different enthalpy change is observed for molecules solubilized at the micelle surface. This factor, in addition to the entropy change, may be important in the simultaneous solubilization of steroids.

REFERENCES

(1) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 425 (1969).

(2) L. Sjöblom, in "Solvent Properties of Surfactant Solutions," K. Shinoda, Ed., Dekker, New York, N.Y., 1967, p. 189.

(3) K. J. Simons and C. T. Rhodes, *Pharmazie*, **26**, 623 (1971).

(4) N. H. Choulis, *ibid.*, **28**, 376 (1973).

(5) L. H. Loh, N. H. Choulis, and C. T. Rhodes, *Can. J. Pharm. Sci.*, **8**, 93 (1973).

(6) L. S. C. Wan and P. F. S. Lee, *ibid.*, **10**, 69 (1975).

(7) Y. W. Chien and H. J. Lambert, *Chem. Pharm. Bull.*, **23**, 1085 (1975).

(8) T. Lövgren, B. Heikius, B. Lundberg, and L. Sjöblom, *J. Pharm. Sci.*, **67**, 1419 (1978).

(9) P. Molyneux and C. T. Rhodes, *Kolloid-Z. Z. Polym.*, **250**, 886 (1972).

(10) B. W. Barry and D. J. D. El Eini, *J. Pharm. Pharmacol.*, **26**, 87 P (1974).

(11) K. J. Humphreys and C. T. Rhodes, *J. Pharm. Sci.*, **57**, 79 (1968).

(12) P. Kabasakalian, E. Britt, and M. D. Yudis, *ibid.*, **55**, 642 (1966).

(13) M. M. Gale and L. Saunders, *Biochim. Biophys. Acta*, **248**, 466 (1971).

(14) S. Riegelman, N. A. Allawala, M. K. Hrenoff, and L. A. Strait, *J. Colloid Sci.*, **13**, 208 (1958).

(15) B. A. Mulley, in "Advances in Pharmaceutical Sciences," H. S. Bean, A. H. Beckett, and J. E. Carless, Eds., Academic, New York, N.Y., 1964.

(16) A. L. Thakkar and N. A. Hall, *J. Pharm. Sci.*, **57**, 1394 (1968).

(17) K. Shinoda and T. Soda, *J. Phys. Chem.*, **67**, 2072 (1963).