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Simultaneous Solubilization of Steroid Hormones I: Estrogens and C_{21} Steroids

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Abstract □ The simultaneous solubilization of some estrogens and C_{21} steroids in aqueous polysorbate 40, tetradecyltrimethylammonium bromide, and sodium lauryl sulfate was studied. The less soluble estrogen estradiol was solubilized independently of the C_{21} steroids. The micellar solubilities of ethinyl estradiol and both corticosterone and hydrocortisone were independent of the presence of each other while the solubility of 11α-hydroxyprogesterone was enhanced by ethinyl estradiol. The solubilizations of ethinyl estradiol and the two C_{21} steroids, progesterone and 21-hydroxyprogesterone, were dependent on each other so that a varying amount of the steroid solubilized first was precipitated by an excess of the second steroid. If saturated solutions of the two steroids were mixed, no precipitation occurred. A possible mechanism for the simultaneous solubilization of steroids and its relation to structure are discussed.

Keyphrases □ Steroid hormones, various—simultaneous solubilization in aqueous surfactants □ Estrogens, various—simultaneous solubilization in aqueous surfactants □ Solubilization, simultaneous—various steroid hormones in aqueous surfactants

Micellar solubilization of drugs in aqueous solutions is well documented, and pharmaceutical systems have utilized surfactants for many years (1–3). Steroid hormones often have low aqueous solubility (3), and surfactants have been used to increase it. As early as 1944, it was noted that bile salts enhance the water solubility of steroid hormones (4). Since that time, the effect of steroid structure on solubilization and the maximum solubilization of steroids in solutions of surfactants have been investigated (5–9). Later reports described the micellar solubilization of testosterone (10–17) and the solubilization of steroids by lysophosphatidylcholine (18).

This study investigated the simultaneous solubilization of estrogens and C_{21} steroids in aqueous solutions of sodium lauryl sulfate, tetradecyltrimethylammonium bromide, and polysorbate 40. The study was undertaken to determine whether the steroids can be incorporated independently in the micelles as if separate loci for solubilization are involved or if an interaction occurs between the steroids that influences their solubility and can be related to chemical structure.

EXPERIMENTAL

Materials—The steroid hormones[1] were used as received after their melting points were found to be in good agreement with published values. Sodium lauryl sulfate[2] was purified by recrystallization from alcohol. Tetradecyltrimethylammonium bromide[3] and polysorbate 40[4] were used as received.

Solubilization Experiments—Solubilities were determined by equilibration of several concentrations of the aqueous surfactant with the steroids, followed by spectrophotometric analyses of suitably diluted aliquots as described previously (6). To two series of 5-ml ampuls, each containing the surfactant of known concentration, a sufficient amount of estrogen, *e.g.*, estradiol, or C_{21} steroid, *e.g.*, progesterone, was added to ensure an excess at equilibrium. The ampuls were closed and shaken mechanically in a thermostat at 20° (40° for sodium lauryl sulfate) for 72 hr until equilibrium was reached.

The contents of the ampuls then were filtered[5] or centrifuged to remove the undissolved steroid. The UV absorbance of the steroids was used to calculate the amount solubilized. To the estrogen-saturated surfactant solutions, an excess of C_{21} steroid was added; to the C_{21} steroid-saturated solutions, an excess of estrogen was added. The ampuls again were closed and shaken mechanically for 72 hr until equilibrium was reached. The undissolved steroid was removed, and the UV absorbance of the solutions was used to calculate steroid concentrations.

The UV absorbance of the solutions was recorded at around 280 nm for the estrogens and 240 nm for the C_{21} steroids with a spectrophotometer[6], using silica cells of 10- and 1.0-mm path length. Reference solutions containing known amounts of steroid were prepared in all surfactant solutions investigated to ascertain the possible influence of the solvent on the absorbance and for calculation of the molar absorptivity of the steroids. The simultaneous solubilization of two steroids in the same surfactant solution did not affect the molar absorptivity of each steroid.

RESULTS AND DISCUSSION

When various concentrations of polysorbate 40, tetradecyltrimethylammonium bromide, and sodium lauryl sulfate were saturated first with progesterone and then with estradiol and *vice versa*, the results were the same as if the solubilization had been done independently. The micelles of the colloids can solubilize the two steroids simultaneously without affecting their micellar solubility. In all cases, the amount of steroids solubilized increased linearly with the surfactant concentration. The amount of solubilized steroid can be calculated from the solubilization capacities measured previously (2, 3).

The micellar solubility of ethinyl estradiol is within the same range as that of progesterone in ionic surfactants and is considerably larger in nonionic surfactants (2, 3, 9). With progesterone and ethinyl estradiol as the estrogen component, the solubilization no longer occurred independently. The steroid added first to saturate the colloid solution pre-

[1] Fluka AG, Switzerland.

[2] Koch-Light Laboratories.
[3] K & K Laboratories.
[4] Tween 40, Atlas Chemical Industries.
[5] Schleicher & Schüll.
[6] Beckman DU-2.

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Table I—Influence of the Addition Order on Solubilization Capacities of Surfactants for 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
Progesterone	Estradiol	0.037	0.013	0.16	0.068	0.24	0.025
Estradiol	Progesterone	0.013	0.037	0.068	0.16	0.025	0.24
Progesterone	Ethinyl estradiol	0.0015	0.18	0.0032	0.27	0.024	0.13
Ethinyl estradiol	Progesterone	0.034	0.037	0.068	0.13	0.052	0.24
Ethinyl estradiol	11α-Hydroxyprogesterone	0.18	0.028	0.27	0.20	0.13	0.33
11α-Hydroxyprogesterone	Ethinyl estradiol	0.026	0.18	0.15	0.27	0.31	0.13
Estradiol	17α-Hydroxyprogesterone	0.037	0.0072	0.16	0.043	0.24	0.090
17α-Hydroxyprogesterone	Estradiol	0.0072	0.037	0.043	0.16	0.090	0.24
Estradiol	21-Hydroxyprogesterone	0.037	0.11	0.16	0.43	0.24	0.38
21-Hydroxyprogesterone	Estradiol	0.11	0.037	0.43	0.16	0.38	0.24
21-Hydroxyprogesterone	Ethinyl estradiol	0.039	0.18	0.099	0.27	0.076	0.13
Ethinyl estradiol	21-Hydroxyprogesterone	0.032	0.11	0.068	0.43	0.052	0.38
11,21-Dihydroxyprogesterone	Ethinyl estradiol	0.12	0.18	0.60	0.27	0.42	0.13
Ethinyl estradiol	11,21-Dihydroxyprogesterone	0.18	0.12	0.27	0.60	0.13	0.42
Hydrocortisone	Ethinyl estradiol	0.057	0.18	—	—	—	—
Ethinyl estradiol	Hydrocortisone	0.18	0.057	—	—	—	—

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

cipitated during the saturation with the second steroid. This reaction was confirmed by visual inspection of the ampuls, which showed a milky precipitate upon addition of the second steroid. The amount of precipitate increased at higher colloid concentrations.

When a progesterone-saturated solution of polysorbate 40 was equilibrated with an excess of ethinyl estradiol, 96% of the solubilized progesterone precipitated while the estrogen component was solubilized maximally (Fig. 1).When the saturation was done in the opposite order (Fig. 2), 81% of the ethinyl estradiol precipitated and progesterone was solubilized maximally. At polysorbate 40 concentrations above 25 m*M*, progesterone solubilization tended to increase in the presence of the second steroid (Fig. 1).

If an excess of both steroids was added at the same time, progesterone was solubilized maximally while the micellar solubility of ethinyl estradiol dropped to 19% of its maximal value, in agreement with the result obtained when it was added as the first component (Figs. 1 and 2). When equal volumes of polysorbate 40 solutions equilibrated with an excess of progesterone and ethinyl estradiol were mixed and shaken for 48 hr, no precipitation occurred and the absorbance of the steroids in solution dropped to exactly half of the value before mixing.

In tetradecyltrimethylammonium bromide, 98% of the solubilized progesterone precipitated on addition of an excess of ethinyl estradiol, which, in turn, was solubilized maximally (Fig. 3). Saturation in the opposite sequence resulted in a 75% precipitation of ethinyl estradiol, but progesterone reached only 83% of its maximal saturation (Fig. 4). The solubilization loci in the micelles contained some ethinyl estradiol even in the presence of excess progesterone. In fact, ethinyl estradiol was more soluble in tetradecyltrimethylammonium bromide solutions than progesterone (2, 3). In spite of this fact, upon simultaneous addition of an excess of the two steroids, progesterone was solubilized maximally while ethinyl estradiol reached only 25% saturation (Figs. 3 and 4), the same level reached as when ethinyl estradiol was added first.

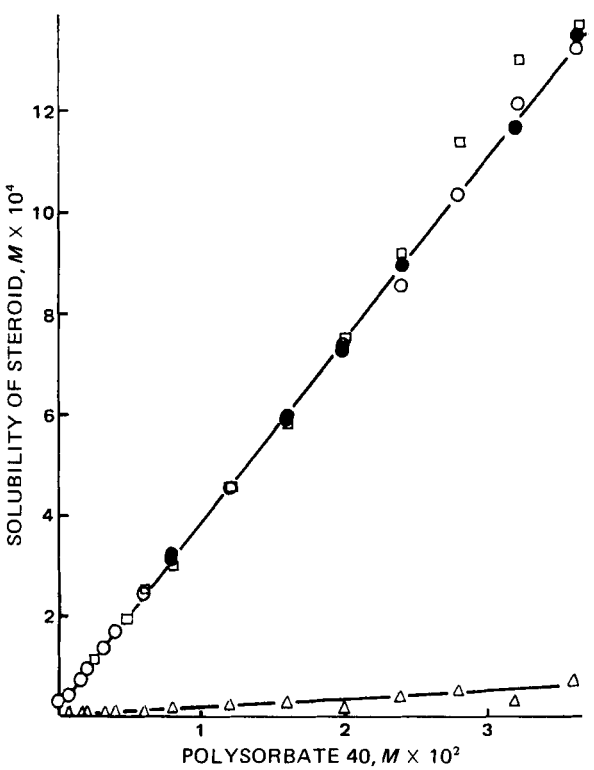

Figure 1—*Solubility of progesterone in aqueous solutions of polysorbate 40. Key:* ○, *progesterone only;* △, *progesterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and progesterone second; and* ●, *progesterone and ethinyl estradiol at the same time.*

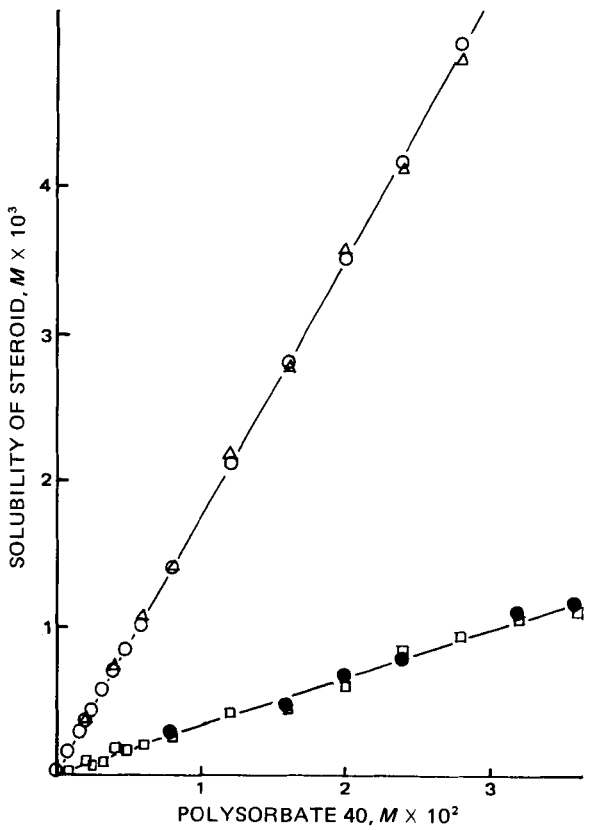

Figure 2—*Solubility of ethinyl estradiol in aqueous solutions of polysorbate 40. Key:* ○, *ethinyl estradiol only;* △, *progesterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and progesterone second; and* ●, *progesterone and ethinyl estradiol at the same time.*

Table II—Solubilization Capacities of Surfactants for Progesterone and Ethinyl Estradiol when the Steroids Were Added at the Same Time

Steroid	Moles of Steroid per Mole of Surfactant[a]		
	I	II	III
Progesterone	0.037	0.16	0.24
Ethinyl estradiol	0.034	0.068	0.016

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

In a solution of the anionic colloid, sodium lauryl sulfate, 90% of the solubilized progesterone precipitated on saturation with ethinyl estradiol, which was solubilized maximally (Fig. 5). If, in turn, an excess of progesterone was added to solutions previously saturated with ethinyl estradiol, 60% of the estrogen component precipitated while progesterone reached a maximal saturation level. At higher colloid concentrations, progesterone solubility tended to increase (Fig. 5).

When an excess of both components was added simultaneously, progesterone reached complete saturation while ethinyl estradiol solubility was 12% of its maximal value (Figs. 5 and 6). With simultaneous addition, ethinyl estradiol solubility in a sodium lauryl sulfate solution was considerably lower than if it were added first and progesterone second. This result was probably influenced by the micellar solubility of the steroids (2, 3, 9). Ethinyl estradiol is less soluble than progesterone in sodium lauryl sulfate while the opposite is true of tetradecyltrimethylammonium bromide and polysorbate 40 solutions.

Ethinyl estradiol was solubilized maximally in tetradecyltrimethylammonium bromide solutions if 11α-hydroxyprogesterone was the second steroid. Ethinyl estradiol solubilization did not depend on the addition order, and it obviously was not forced out from the micellar phase by 11α-hydroxyprogesterone. The two other surfactants behaved similarly in respect to these two steroids. 11α-Hydroxyprogesterone solubility increased about 19% when it was added to an ethinyl estradiol-saturated tetradecyltrimethylammonium bromide solution. Evidently, ethinyl estradiol affected the micellar volume of the colloid and more 11α-hydroxyprogesterone was solubilized. Similar results were obtained in solutions of the other two colloids, although the increase in solubility was considerably smaller.

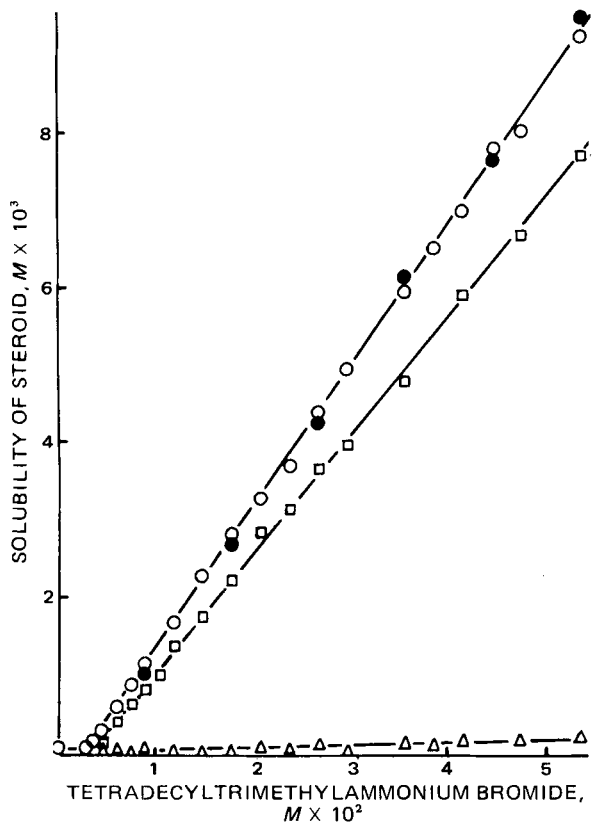

Figure 3—*Solubility of progesterone in aqueous solutions of tetradecyltrimethylammonium bromide. Key: ○, progesterone only; △, progesterone first and ethinyl estradiol second; □, ethinyl estradiol first and progesterone second; and ●, progesterone and ethinyl estradiol at the same time.*

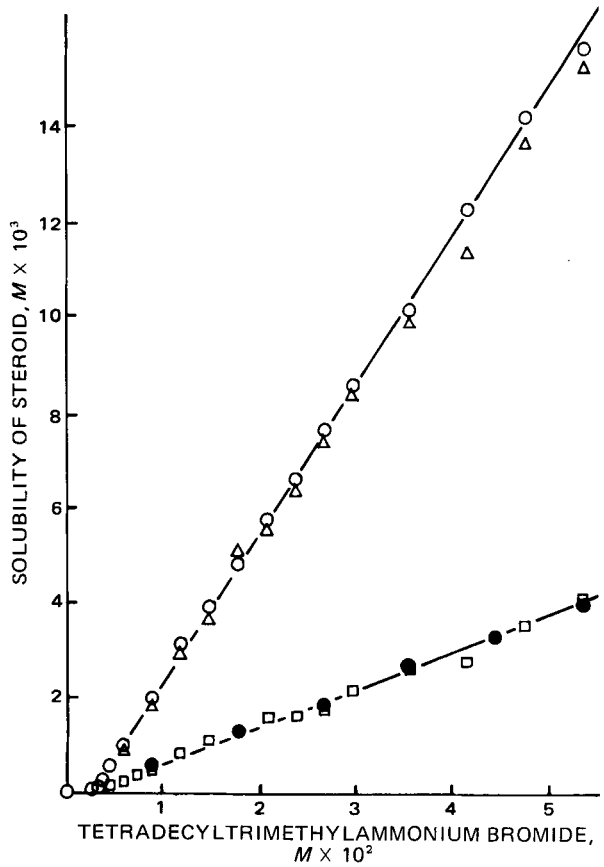

Figure 4—*Solubility of ethinyl estradiol in aqueous solutions of tetradecyltrimethylammonium bromide. Key: ○, ethinyl estradiol only; △, progesterone first and ethinyl estradiol second; □, ethinyl estradiol first and progesterone second; and ●, progesterone and ethinyl estradiol at the same time.*

The micellar solubility of 17α-hydroxyprogesterone and 21-hydroxyprogesterone (11-desoxycorticosterone) in tetradecyltrimethylammonium bromide was tested by adding estradiol as the first or second component. No deviation was found based on the addition order. The steroids were solubilized independently.

Obvious deviations again were obtained in solutions of all three surfactants if ethinyl estradiol was the estrogen component and 21-hydroxyprogesterone was the second steroid. In polysorbate 40, an excess of ethinyl estradiol precipitated about 65% of the solubilized 21-hydroxyprogesterone and reached maximal solubility. If the opposite order of addition was used, an excess of 21-hydroxyprogesterone precipitated 82% of the solubilized ethinyl estradiol and reached maximal micellar solubility. In tetradecyltrimethylammonium bromide solutions, 77% of the solubilized 21-hydroxyprogesterone precipitated when ethinyl estradiol was added second while 75% of the ethinyl estradiol precipitated when the addition order was reversed. When an excess of ethinyl estradiol was added to a 21-hydroxyprogesterone-saturated sodium lauryl sulfate solution, 80% of the first added component precipitated. In the opposite case, 60% of the ethinyl estradiol precipitated.

Compared to 21-hydroxyprogesterone, corticosterone has an additional hydroxyl group at C-11. When corticosterone and ethinyl estradiol were used as the steroids, no deviation from normal micellar solubility was observed independent of the addition order and the surfactant used. The presence of a hydroxyl group at C-11 seems to be important for solubilization independent of ethinyl estradiol. Hydrocortisone (11,17,21-trihydroxyprogesterone), which was tested only in polysorbate 40, showed a similar behavior—*viz.*, the steroids were solubilized independently.

The solubilization capacities of the surfactants for the steroids were calculated from all experiments (Tables I and II).

The results of the present investigation clearly demonstrate that steroids can be solubilized according to different mechanisms, although the exact mechanism has not been defined. Previous investigations showed

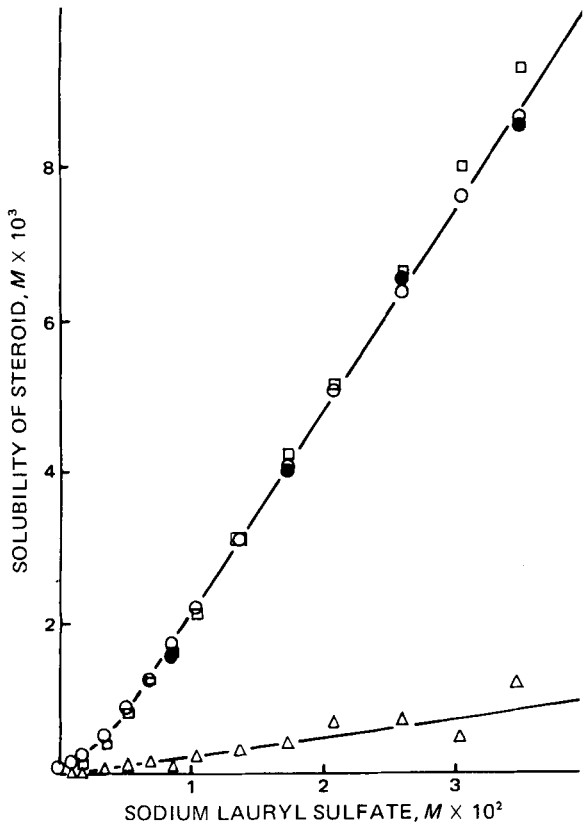

Figure 5—*Solubility of progesterone in aqueous solutions of sodium lauryl sulfate. Key:* ○, *progesterone only;* △, *progesterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and progesterone second; and* ●, *progesterone and ethinyl estradiol at the same time.*

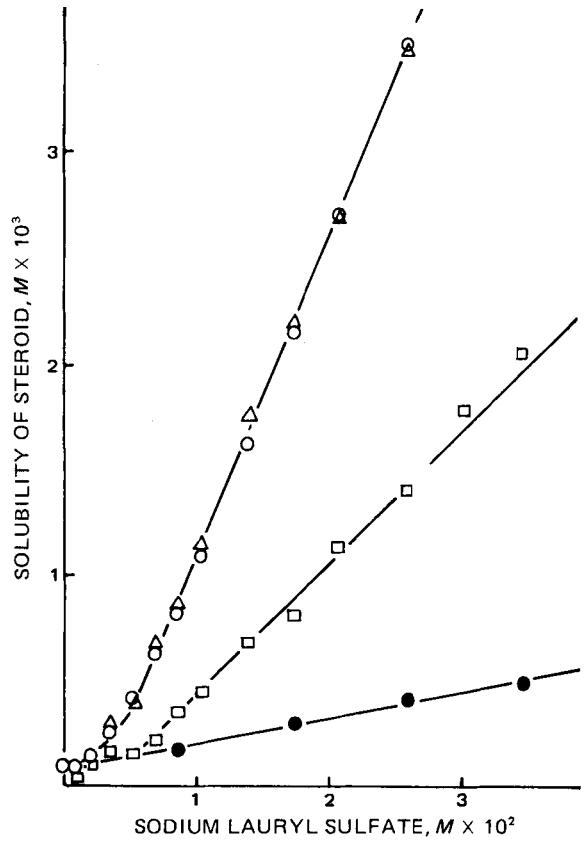

Figure 6—*Solubility of ethinyl estradiol in aqueous solutions of sodium lauryl sulfate. Key:* ○, *ethinyl estradiol only;* △, *progesterone first and ethinyl estradiol second;* □, *ethinyl estradiol first and progesterone second; and* ●, *progesterone and ethinyl estradiol at the same time.*

that the solubilization capacities of ionic surfactants are generally of the same order of magnitude for different steroids, except for the estrogens, while nonionic surfactants have lower capacities (2, 3). For estrogens, the difference is rather small. This finding could also indicate that estrogens are solubilized in a different way compared to the other steroids. The whole steroid molecule determines its micellar solubility (2).

In the present study, the pairs estradiol and progesterone, ethinyl estradiol and 11α-hydroxyprogesterone, estradiol and 17α-hydroxyprogesterone, estradiol and 21-hydroxyprogesterone, ethinyl estradiol and corticosterone, and ethinyl estradiol and hydrocortisone were solubilized independently of one another and most likely by different mechanisms. However, ethinyl estradiol affected the solubilization of progesterone and 21-hydroxyprogesterone, which appeared as a precipitation of the first added steroid from the steroid-saturated solution as the second steroid was added in excess. The same type of mechanism of solubilization probably was involved. The first added steroid was in equilibrium between the micellar and the nonmicellar phases as the excess of undissolved steroid was removed.

In the case where the steroid solubilization mechanism was the same, the equilibrium of the first added steroid was disturbed in the presence of an excess of the second steroid. As a consequence, a new equilibrium had to be reached, including both steroids, and the first steroid had to precipitate partly. When an excess of both steroids was added simultaneously, an equilibrium was reached that usually corresponded to the conditions observed when progesterone was added as the second component.

Apparently, all three surfactants favored progesterone. It is difficult to explain this observation. The entropy for progesterone solubilization by nonionic surfactants was shown to be positive (19). The configuration entropy of the solubilized molecules increases because of the breakup of the water structure surrounding nonpolar groups, which thus controls solubilization. Introduction of an ethinyl group to the D ring of estradiol increases the net dipole moment of the molecule (18). As a consequence, the solubilization is enhanced (2, 18) and influences the mechanism of solubilization of C_{21} steroids, except those with a hydroxyl group at C-11. The position of a substituent is thus important for solubilization.

Work on the simultaneous solubilization of steroid hormones is in progress, and the equilibria will be discussed considering thermodynamic arguments.

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