

## Further Studies on the Solubilisation of Oestrogens

CLAUS BLOMQUIST and LARS SJÖBLOM

Department of Biochemistry, Åbo Akademi,  
Åbo, Finland

One of us (L.S.) has previously<sup>1-3</sup> studied the solubilisation of oestrone and oestradiol-17 $\beta$  in aqueous solutions of different association colloids. These investigations revealed that oestradiol-17 $\beta$ , which has hydroxyl groups at C<sub>3</sub> and C<sub>17</sub>, is solubilised in larger amounts than oestrone, which has a hydroxyl group at C<sub>3</sub> and an oxo group at C<sub>17</sub>. Furthermore, the solubilities of both oestrogens were found to be considerably lower than those of corticosteroids, androgens, and gestagens.<sup>3-8</sup>

In continuation of the systematic investigation of the solubilisation of steroids, the solubilities of six further oestrogens in aqueous solutions of an anionic and a non-ionic association colloid (sodium dodecyl sulphate and Tween 20) have been determined. The experimental technique was that described previously,<sup>1</sup> in which the solubility is determined by measuring the ultra-violet absorption.

The results obtained are illustrated by Figs. 1-2 and Table 1, in which the

Table 1. Maximum solubilisation powers of association colloids for different oestrogens. NaDS = sodium dodecyl sulphate (40°C), Tw 20 = Tween 20 (20°C).

Steroid	Moles steroid per mole micellar substance	
	NaDS	Tw 20
Oestrone	0.014	0.0068
Oestradiol-17 $\beta$	0.025	0.013
Oestradiol-17 $\alpha$	0.029	0.017
Oestriol	0.031	0.024
17 $\alpha$ -Ethinyl-oestradiol-17 $\beta$	0.13	0.18
Oestrone-3-acetate	0.15	0.046
Oestradiol-3-benzoate	0.018	0.010
Oestradiol-3,17-dipropionate	0.051	0.013

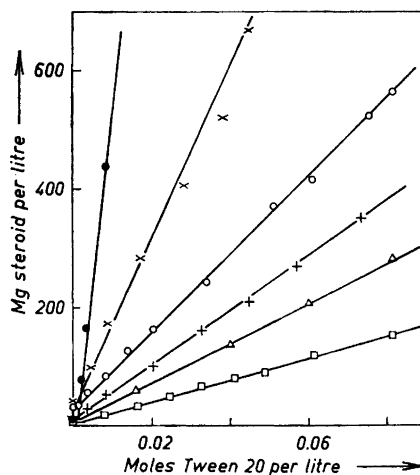


Fig. 1. The solubility of oestrogens in aqueous Tween 20 solutions at 20°C. Oestrone (□), oestradiol-17 $\beta$  (Δ), oestradiol-17 $\alpha$  (+), oestriol (○), 17-ethinyloestradiol (●), oestrone acetate (×).

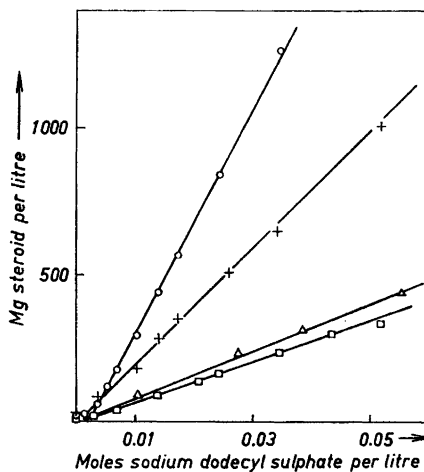


Fig. 2. The solubility of oestrogens in aqueous sodium dodecyl sulphate solutions at 40°C. Oestradiol-17 $\beta$  (Δ), oestradiol-3-benzoate (□), oestradiol-3,17-dipropionate (+), 17-ethinyloestradiol (○).

saturation capacities of the micellar substances for the oestrogens calculated from the slopes of the linear parts of the solubility curves are given. For comparison, previously obtained data for oestrone and oestradiol-17 $\beta$  are included. The data presented reveal the following.

Oestradiol-17 $\alpha$  is solubilised in larger amounts than oestradiol-17 $\beta$  both by sodium dodecyl sulphate and by Tween 20 solutions. This indicates that the  $\alpha$ -orientation of the hydroxyl group at C<sub>17</sub> favours the solubilisation. Oestriol, which has hydroxyl groups at C<sub>3</sub>, C<sub>16</sub>, and C<sub>17</sub>, is solubilised in larger amounts than the oestradiols. However, in sodium dodecyl sulphate solutions the difference is rather small, whereas in Tween 20 solutions the solubilisation of the three natural oestrogens is roughly proportional to the number of hydroxyl groups in the steroid molecule. In general, these results confirm the previous suggestion<sup>2</sup> that the solubilisation of oestrogens depends on the number of hydrophilic substituents in the steroid molecule.

On introduction of an ethynyl group at C<sub>17</sub>, rather surprising results were obtained. 17 $\alpha$ -Ethynyl-oestradiol is solubilised in some ten times greater amounts than the unsubstituted oestradiol both by sodium dodecyl sulphate and by Tween 20 solutions. The introduction of an ethynyl group at C<sub>17</sub> thus increases the micellar solubility to values of the same order of magnitude as those reported for different corticosteroids.<sup>8</sup> As the corticosteroids also possess a two-carbon substituent at C<sub>18</sub>, this seems to indicate that a short side chain at this position enhances the solubilisation of the steroids, perhaps by penetrating between the hydrocarbon chains of the micelles. The previously noted fact<sup>8</sup> that esterification of a hydroxyl group at C<sub>21</sub> markedly decreases the solubilisation supports the assumption that this part of the steroid molecule is of great importance for the solubilisation mechanism involved.

The results obtained with different esterified oestrogens are not unambiguous. Oestrone-3-acetate is up to ten times more soluble than the unesterified oestrone

in colloid solutions. However, esterification of the hydroxyl group at C<sub>3</sub> in oestradiol-17 $\beta$  with benzoic acid results in a decrease in the micellar solubility. When both hydroxyl groups of oestradiol-17 $\beta$  are esterified with propionic acid, the solubility in the non-ionic colloid solutions remains unaffected, but that in the anionic colloid solutions slightly increases. These findings indicate that esterification of hydroxyl groups directly attached to the steroid skeleton does not necessarily decrease the micellar solubility as does the esterification of a hydroxyl group at C<sub>21</sub>.

When the saturation capacities of sodium dodecyl sulphate solutions are compared with those of Tween 20 solutions, it is noted that they are of the same order of magnitude for all unesterified oestrogens. However, the saturation capacities of the non-ionic colloid for esterified oestrogens as well as for corticosteroids and androgens<sup>8-9</sup> are considerably lower than those of ionic colloids. This suggests that different mechanisms may be involved in the solubilisation of steroids by different types of association colloids.

Systematic investigations with the aim of clarifying further the mechanism of solubilisation of steroids are in progress.

*Acknowledgement.* The authors are indebted to the *State Commission for Natural Sciences* for financial support.

1. Sjöblom, L. *Acta Acad. Aboensis, Math. Phys.* **20** (1956) No. 14.
2. Sjöblom, L. *Ibid.* **21** (1958) No. 7.
3. Ekwall, P. and Sjöblom, L. *Acta Chem. Scand.* **3** (1949) 1179.
4. Ekwall, P. and Sjöblom, L. *Acta Endocrinol.* **4** (1950) 179.
5. Ekwall, P., Lundsten, T. and Sjöblom, L. *Acta Chem. Scand.* **5** (1951) 1383.
6. Ekwall, P., Sjöblom, L. and Olsen, J. *Ibid.* **7** (1953) 347.
7. Nylander, A. *Farm. Notisblad* **62** (1953) 183.
8. Sjöblom, L. and Sundblom, N.-O. *Acta Chem. Scand.* **18** (1964) 1996.
9. Sjöblom, L. and co-workers. *To be published.*

Received November 18, 1964.