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# The Spectrophotometric Determination of Steroid Hormones Solubilized in Aqueous Solutions of Association Colloids *

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In a previous paper [1] we reported on the maximum solubilities of a number of $\Delta^4$-3-ketosteroids in aqueous solutions of certain association colloids. The solubility values were determined polarographically. This method can, however, be utilized only for a limited group of steroid hormones and hence it was found necessary to investigate the applicability of other methods for the accurate determination of steroid hormones solubilized in colloid solutions. The measurement of the ultra-violet absorption was found suitable for this purpose. This procedure made possible the determination of the hormone contents of association colloid solutions directly without previous separation of the components by laborious extractions as in the polarographic method. The present paper describes the spectrophotometric method and gives saturation values for hormones in aqueous sodium lauryl sulphate solutions as determined with this method.

## EXPERIMENTAL

Experiments were conducted to determine $\alpha$-oestradiol, desoxycorticosterone, testosterone, testosterone propionate, and cortisone-21-hemi-succinate spectrophotometrically in aqueous solutions of sodium lauryl sulphate. The ultra-violet absorption spectra of progesterone and desoxycorticosterone acetate in sodium lauryl sulphate solutions were also recorded. Oestradiol possesses a maximum absorption at about 2 800 Å due to its phenolic ring; the other hormones investigated show a maximum at about 2 400 Å which is due to a ketonic group conjugated with a double bond. The aqueous hormone solutions were prepared in the manner earlier described [2,3]. The measurements were carried out with a Beckman Model DU Quartz Spectrophotometer using 1.00 or 0.10 cm cells. The respective pure colloid solutions were used as blanks. Determinations were made both on solutions containing known amounts of the hormones and on saturated solutions.

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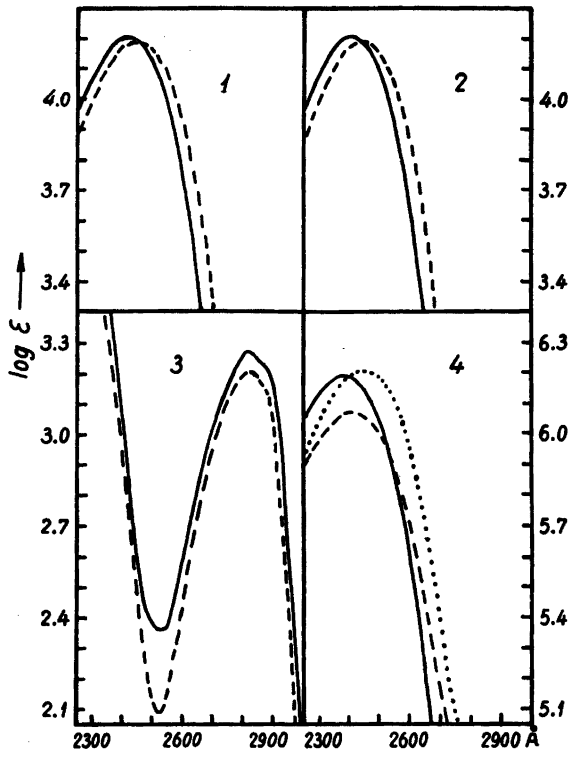


*Fig. 1. The ultra-violet absorption of 1. testosterone, 2. progesterone, 3. oestradiol, and 4. cortisone-21-hemi-succinate in ethanol (————), in aqueous sodium lauryl sulphate solution (- - - - -), and in water (· · · · ·).*

## RESULTS AND DISCUSSION

In aqueous sodium lauryl sulphate solutions the hormones investigated show absorption curves that are similar to those recorded in ethanol (Fig. 1). Except in the case of oestradiol there is, however, observed a shift toward longer wavelengths. A similar shift of the absorption spectrum has previously been observed in the case of solubilized polycyclic aromatic hydrocarbons [4]. In all cases the molar extinction is somewhat lower in sodium lauryl sulphate solutions than in ethanol. The absorption at the maximum increases linearly with the hormone concentration and permits a direct quantitative determination of the amounts of hormones solubilized in sodium lauryl sulphate solutions.

Our spectrophotometric determinations on saturated solutions show that the solubilities of the hormones in sodium lauryl sulphate solutions increase linearly with the colloid concentration, at least in the concentration range

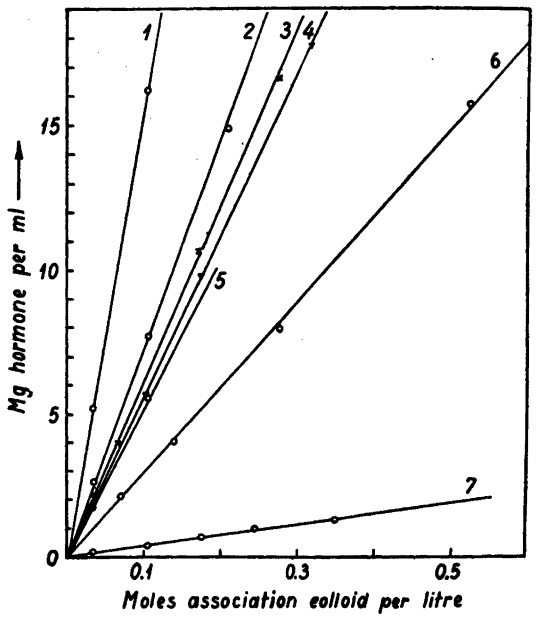


*Fig. 2. The solubilities of some steroid hormones in aqueous solutions of sodium lauryl sulphate: 1. desoxycorticosterone, 2. testosterone propionate, 3. testosterone, 4. progesterone 5. cortisone-21-hemi-succinate, 6. desoxycorticosterone acetate, 7. oestradiol.*

investigated. The solubility curves are shown in Fig. 2 which also includes two solubility curves determined earlier with the polarographic method (desoxycorticosterone acetate and progesterone).

From the linear solubility curves we have calculated the saturation capacities of the micellar substance for the respective hormones. These values are

*Table 1. Maximum solubilizing power of sodium lauryl sulphate for steroid hormones.*

| Hormone | Moles hormone per mole micellar sodium lauryl sulphate | Moles micellar sodium lauryl sulphate per mole solubilized hormone |
|---|---|---|
| Desoxycorticosterone | 0.47 | 2.1 |
| Testosterone | 0.21 | 4.7 |
| Testosterone propionate | 0.21 | 4.7 |
| Progesterone | 0.18 | 5.5 |
| Cortisone-21-hemi-succinate | 0.12 | 8.5 |
| Desoxycorticosterone acetate | 0.08 | 12.5 |
| Oestradiol | 0.015 | 65.8 |

collected in Table 1. From these figures it is seen that desoxycorticosterone is the most soluble of the hormones in sodium lauryl sulphate solutions. The solubilities of testosterone, testosterone propionate, and progesterone are of the same order, whereas desoxycorticosterone acetate and cortisone-21-hemi-succinate are somewhat less soluble. The solubility of oestradiol is only about a tenth of that of the other hormones investigated *. If the lauryl sulphate micelles are assumed to contain 100 molecules of colloid-forming substance, the number of molecules oestradiol per micelle is about 1 to 2, and that of the other hormones about 10 to 20 molecules. The degree of dispersion of the hormones in aqueous sodium lauryl sulphate is thus fairly high. Of the hormones investigated, cortisone-21-hemi-succinate is the most soluble in pure water, 0.2 mg per ml; its solubility in water is, however, only about a tenth of that in a 1 per cent (0.034 *M*) sodium lauryl sulphate solution.

The new results confirm that the solubilities of the steroid hormones in association colloid solutions are of a magnitude quite different from the solubilities of the polycyclic aromatic hydrocarbons with 3 to 5 rings [4]. In spite of their larger molecules the solubilities of the hormones studied except oestradiol are thus 100 to 500 times greater than that of methylcholanthrene which resembles most closely the hormones in structure. Oestradiol has an intermediate position with a solubility about 10 times greater than methylcholanthrene. As previously pointed out [1], it seems probable that the solubilization of steroid hormones involves an interaction between the hydrophilic groups of the hormones and the colloids.

The spectrophotometric method for direct determination of steroid hormones in association colloid solutions is applicable in all cases where the solvent itself does not possess any appreciable absorption at the wavelengths in question. If the solvent has a strong absorption at these wavelengths, extraction procedures similar to those described earlier [1] must be performed before the spectrophotometric determination can be conducted.

## SUMMARY

A direct spectrophotometric determination of steroid hormones solubilized in aqueous association colloid solutions is possible in many cases. The maximum solubilities of a number of steroid hormones in aqueous sodium lauryl sulphate solutions have been determined spectrophotometrically.

* Of the same order as the solubility of oestradiol is that of oestrone which is the subject of a special study by one of us[5].

## REFERENCES

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