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SURFACE BEHAVIOUR OF OESTRADIOL-17β

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SUMMARY

The adsorption of [^{3}H]oestradiol-17β from its aqueous solutions has been measured in the range 0–10μg/ml.

It is found that the first adsorbed molecules are parallel to the interface and occupy 100 Å^2. Those adsorbed in the range 6–10 μg/ml occupy 21 Å^2. They are presumably associated.

When the adsorption occurs in the presence of a synthetic lecithin monolayer, the molecular area is equal to 16 Å^2.

Surface tension measurements of the solutions of oestradiol-17β and a parallel study of their fluorescence have been performed. No association of the hormone molecules has been observed in bulk.

It is concluded that surfaces and liquid monolayers may favour molecular association of the oestradiol-17β.

INTRODUCTION

A large number of biological processes take place at interfaces; transport across membranes, enzymatic reactions, etc. In this light, we have undertaken the study of the surface behaviour of the oestrogenic hormone oestradiol-17β (1,3,5(10)-oestratriene-3,17β-diol), whose physiological importance no longer needs demonstration.

MATERIALS AND METHODS

All solutions were prepared with triple-distilled water. Oestradiol-17β was supplied by Ikapharm, Ramath Gan (Israel), 2, 4, 6, 7 [^{3}H]oestradiol of particularly high specific activity (110 Ci/mmol) was furnished by NEN (Boston, U.S.A.). The aqueous solutions of oestradiol-17β used were obtained by evaporating an alcoholic solution of oestradiol-17β and taking the dry extract up in several ml of 1 M NaOH, then neutralized with 1 M HCl. The DL-β,γ-dipalmitoyl-α-lecithin was supplied by Fluka (Switzerland).

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The measurements of surface radioactivity were performed with the aid of a gas-flow Geiger Müller counter having an ultra-thin window [1]. Counting of surface radioactivity was begun immediately after the sweeping of the surface [2] or after the spreading of lecithin monolayers (see section 2 of Results).

The measurements of the surface potential were performed according to a technique previously described [3]. Surface tensions were measured by the method of Wilhelmy using an electro-balance (C.I. Electronics).

The fluorescence of oestradiol solutions was measured using a FICA 55 spectrofluorimeter.

RESULTS

(1) *Adsorption of oestradiol at the surface of its solution*

The amount of adsorbed oestradiol at the surface of its solution has been measured.

In Fig. 1, we plot the surface density of the hormone at equilibrium as a function of its concentration in solution. The curve obtained shows two plateaux. The first corresponds to a surface density of 0.456 mg/m^2 or 100 Å^2/molecule. The second corresponds to a surface density of 2. 12 mg/m^2 or 21 Å^2/molecule.

The dimensions of the oestradiol molecule, such as we could calculate with the use of a molecular model, are 13. 5 Å $\times$ 7. 5 Å $\times$ 5.5 Å. A molecule adsorbed at the

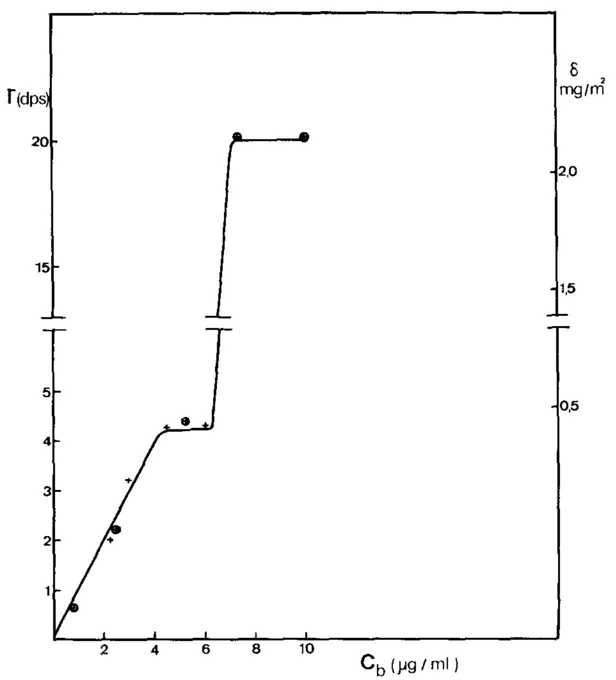

Fig. 1. Adsorption of oestradiol at the air/water interface. Γ, counting rate of the adsorbed film; δ, surface density; c_b, concentration in bulk. Room temperature.

surface can thus occupy a maximal area of 101 Å^2 if the major axis is parallel to the surface, and a minimal area of 41 Å^2 when the major axis is perpendicular to the surface.

It seems, from these calculations, that the first plateau corresponds to the presence at the surface of a monolayer of molecules having their major axis parallel to the interface, the two polar groups thereby being in contact with the aqueous phase. Thus, the second plateau must correspond to the presence of aggregates of molecules at the surface or multilayers.

In order to verify the surface orientation of these molecules, the surface potentials ΔV of the films were measured for two solutions whose concentrations correspond respectively to 'lying down' monolayers and to multilayers, i.e. 2.75 μg/ml and 7 μg/ml. The values found were 50 and 140 mV, respectively. Using these results and the simple Helmholtz expression for the surface potential:

$$\Delta V = 4\pi \left(\frac{\mu}{\varepsilon}\right) \delta$$

where μ is the dipole moment of the molecule and ε the dielectric constant at the surface, it was found that the effective molecular dipole moment (μ/ε) was equal to 0.14 D for the 'lying-down' molecules.

Fig. 2 shows the results of the measurement of the surface tension of solutions of non-radioactive oestradiol at various concentrations. The surface density, δ, of the adsorbed film is given by the slope of the tangent to the curve of Fig. 2 : $\delta = -(d\gamma/kT d\ln c)$.

The increase in slope and δ occurs between 6 and 7 μg/ml. This range is identical to that at which the increase in the measured surface density occurs (Fig. 1).

It is important to make sure that the association of adsorbed oestradiol molecules is a surface phenomenon and that it does not already exist in the solution. To do this, the fluorescence emitted by oestradiol solutions prepared under the same con-

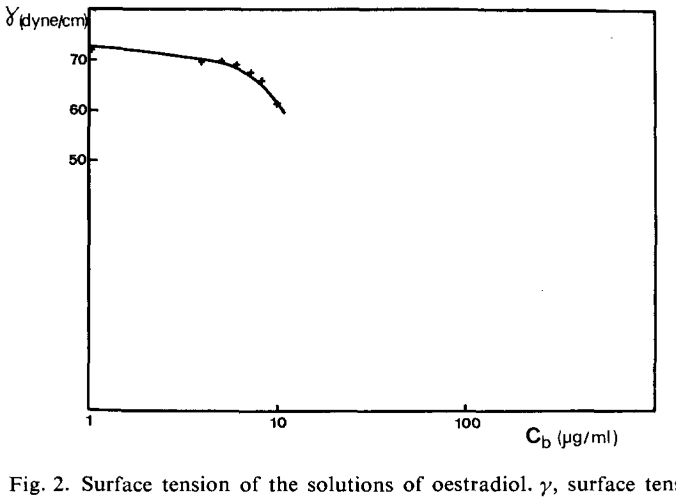

Fig. 2. Surface tension of the solutions of oestradiol. γ, surface tension; c_b, concentration of the solution.

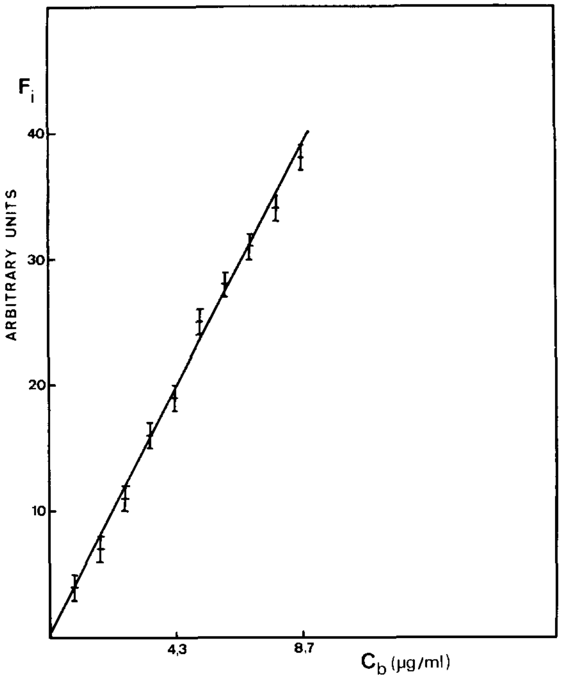

Fig. 3. Variation of the emission fluorescence intensity F_i of oestradiol with the steroid concentration in bulk c_b. Excitation wavelength, 280 nm; emission wavelength, 310 nm.

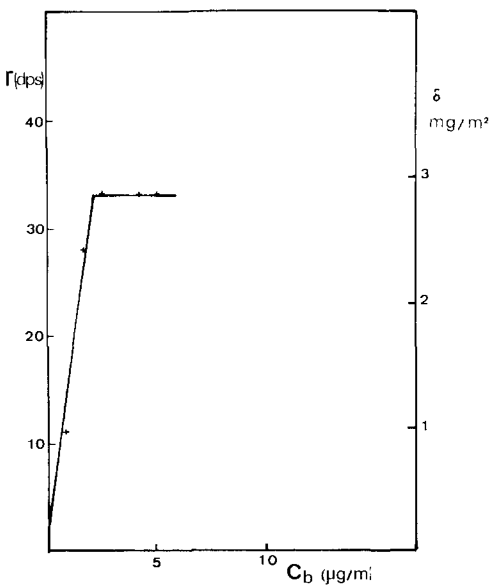

Fig. 4. Effect of lecithin on the adsorption of oestradiol. Surface density of lecithin = $3 \cdot 10^{14}$ molecules/cm^2. c_b, concentration of oestradiol in solution; Γ, counting rate of the adsorbed oestradiol.

ditions has been measured. The results obtained (Fig. 3) show that the intensity of emitted light is rigorously proportional to the concentration of oestradiol throughout the range of concentrations studied.

In conclusion, it does seem that it is the interface which concentrates and orientates the molecules in such a way that they associate.

(2) *Adsorption of oestradiol by spread monomolecular layers of lecithin*

The quantity of oestradiol adsorbed by a spread monolayer of lecithin has also been measured. The surface density of lecithin was equal to $3 \cdot 10^{14}$ mol/cm^2. This system is a model of the biological membrane.

The results are shown in Fig. 4, where the surface density of the hormone is plotted as a function of its concentration in solution. Only one plateau is found, at 2μg/ml, corresponding to a molecular area of 16 Å^2/molecule, or to multilayers.

CONCLUSION

This study shows the considerable importance of interfaces in the behaviour of a molecule of great biological interest, oestradiol-17β.

The role of the interface is to concentrate, orient and even to favour the association of molecules of the hormone and, eventually, to precipitate the hormone on the monolayers of lecithin.

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