THE INTERFACIAL ACTIVITY OF STEROID HORMONES AND SYNTHETIC ESTROGENS*

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INTRODUCTION

The ability of a hormone to concentrate at interfaces is a property which should be taken into account in considering the distribution of the hormone throughout the organism, its eventual localization at various sites, and perhaps its mechanism of action[1]. In the case of unmodified steriod hormones this property has been studied only to a slight extent[2], although considerable attention has been devoted to derivatives of these compounds[3,4,5,6].

The present work originated with the observation that steroid hormones were adsorbed at a variety of interfaces between polar and non-polar media such as glass-hydrocarbon, water–siliconed glass, water–air, and water–hydrocarbon interfaces. Some of these observations have been made before: DOISY, HUFFMAN, THAYER AND DOISY[7] have noticed that steroids are adsorbed by glass; while BERGMEYER AND DIRSCHERL[2] have shown that the interfacial tension of water–benzene and water–olive oil systems is lowered by testosterone, testosterone propionate, estradiol-17β and deoxycorticosterone acetate. But in neither of these cases was the concentration of steroid at the interface determined.

Of the various interfaces mentioned above, the most suitable for quantitative study was considered to be the water–hydrocarbon interface, because it was found from preliminary experiments that definite saturation values of interfacial concentration could be attained with the steroids at this interface, permitting molecular area determinations to be made.

METHODS AND MATERIALS

Interfacial concentrations were derived, as will be described later, from measurements of interfacial tension. Interfacial tensions were determined with a du Nouy tensiometer (Central Scientific Co., Model 70535), using a platinum ring of 6 cm circumference. The error in these measurements was of the order of $\pm$ 0.5 dynes/cm. The measurements were conducted in a room in which the temperature was controlled at 24 $\pm$ 1° C.

Hormone concentrations were determined, within $\pm$ 5%, by measuring the ultraviolet absorption of the solutions, using a Cary Spectrophotometer. The highest concentrations employed were near, and in some cases probably beyond, the saturation values at 24° C.

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The following materials were used: estriol (CIBA); hydrocortisone (The Upjohn Co.); testosterone (Chas. Pfizer & Co., Inc.); deoxycorticosterone (The Upjohn Co.); progesterone (The Glidden Co.); diethylstilbestrol (The Matheson Co.). These compounds were used without further purification.

The hydrocarbon phase for all experiments which are reported quantitatively here, consisted of *n*-heptane. Phillips "Pure" grade *n*-heptane was used as starting material. This was further purified by treatment with concentrated sulfuric acid and alkaline permanganate[8], after which it was distilled, the 97–99° C fraction being collected. Distilled water, redistilled through all-glass apparatus, was used for all measurements.

THEORY

The following symbols are used:
s, w, and h refer to solute, water and hydrocarbon respectively
Γ_i: interfacial concentration of component i (moles/cm^2)
τ: thickness of interfacial layer
γ: interfacial tension (dynes/cm)
γ_0: interfacial tension of pure solvents (dynes/cm)
$\pi = \gamma_0 - \gamma$: force in interface
μ_i: chemical potential of component i
c_i^j: concentration of component i in phase j
x_i^j: mole fraction of component i in phase j

The symbols Y_w and Y_h are used for brevity. They are defined by the equations

$$Y_w = \frac{x_s^w\, x_h^h - x_s^h\, x_h^w}{x_w^w\, x_h^h - x_w^h\, x_h^w}$$

$$Y_h = \frac{x_s^h\, x_w^w - x_s^w\, x_w^h}{x_w^w\, x_h^h - x_w^h\, x_h^w}$$

V_i: partial molar volume of component i in interfacial layer
A: area per molecule of solute in interfacial layer (A^2)
$R = 8.314 \cdot 10^7$ ergs/mole °K: gas constant
T: absolute temperature (°K)

By definition,

$$x_s^w + x_w^w + x_h^w = 1 \qquad (1)$$

$$x_s^h + x_w^h + x_h^h = 1 \qquad (2)$$

In this section we shall be concerned with the equations that apply to a solute at a water–hydrocarbon interface, or more generally, at a liquid–liquid interface. We shall adopt GUGGENHEIM'S definition of interfacial concentration at a plane interface[9], according to which Γ_i is the number of moles of component i per unit area of a layer of thickness τ which encloses the inhomogeneous interfacial region. It should be observed that this definition implies that $\Gamma_i \gtrless 0$. As shown by GUGGENHEIM, at constant temperature and pressure the interfacial concentrations satisfy the relation

$$-\mathrm{d}\gamma = \Gamma_s\, \mathrm{d}\mu_s + \Gamma_w\, \mathrm{d}\mu_w + \Gamma_h\, \mathrm{d}\mu_h\,, \qquad (3)$$

which is identical to the equation derived by GIBBS[10] with a somewhat different definition of Γ_i.

From the Gibbs-Duhem equations for the homogeneous phases,

$$x_s^w\, \mathrm{d}\mu_s + x_w^w\, \mathrm{d}\mu_w + x_h^w\, \mathrm{d}\mu_h = 0$$

$$x_s^h\, \mathrm{d}\mu_s + x_w^h\, \mathrm{d}\mu_w + x_h^h\, \mathrm{d}\mu_h = 0\,,$$

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it follows that

$$d\mu_w = - Y_w \, d\mu_s$$
$$d\mu_h = - Y_h \, d\mu_s .$$

After rearranging, Eqn. 3 then becomes

$$\Gamma_s - Y_w \Gamma_w - Y_h \Gamma_h = - \frac{d\gamma}{d\mu_s}, \qquad (4)$$

the right-hand side of which is determined experimentally as a function of μ_s.

In general, two more equations relating Γ_s, Γ_w and Γ_h are necessary in order to solve for these three quantities. Such equations must usually be selected on the basis of some more or less arbitrary hypotheses concerning the structure of the interfacial region. In the present case, however, it is possible to eliminate Γ_w and Γ_h from Eqn. 4 without recourse to auxiliary hypotheses, as the terms involving these quantities can be shown to be negligible.

In order to do this, the reasonable assumption is made that the thickness of the inhomogeneous interfacial region will be less than 50 A. If this figure is taken as the maximum value for τ, the maximum values of Γ_w and Γ_h can then be calculated from the equation

$$\Gamma_i \text{ (max)} = \frac{\tau}{V_i} .$$

Taking $V_w = 0.018$ l/mole and $V_h = 0.157$ l/mole (for *n*-heptane), we arrive at the results:

$$0 \leqq \Gamma_w < 28 \cdot 10^{-9} \text{ moles/cm}^2$$
$$0 \leqq \Gamma_h < 3.2 \cdot 10^{-9} \text{ moles cm}^2.$$

The limits of Y_w and Y_h can be determined from the experimental data. In all the cases that will be considered,

$$10^{-7} < x_s^w < 10^{-5}$$
$$10^{-8} < x_s^h < 10^{-3}$$

From data[11,12] on the solubility of heptane in water, and water in heptane,

$$x_h^w < 10^{-4}$$
$$x_w^h < 10^{-3}$$

Using Eqns. 1 and 2, and the definitions of Y_w and Y_h, the limits are then found to be:

$$10^{-7} < Y_w < 10^{-5}$$
$$10^{-8} < Y_h < 10^{-3}$$

When these values are combined with those for Γ_w and Γ_h, it is found that

$$0 \leqq Y_w \Gamma_w < 2.8 \cdot 10^{-13} \text{ moles/cm}^2$$
$$0 \leqq Y_h \Gamma_i < 32 \cdot 10^{-13} \text{ moles/cm}^2$$

These quantities are much smaller than the error involved in the experimental determination of $d\gamma/d\mu_s$, so that with no sensible loss in accuracy, Eqn. 4 can be reduced to

$$\Gamma_s = - \frac{d\gamma}{d\mu_s} . \qquad (5)$$

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If the activity coefficients of the solute are constant, the chemical potential can be expressed in terms of the concentrations, and Eqn. 5 then becomes

$$\Gamma_s = -\frac{1}{RT}\frac{d\gamma}{d\ln c_s^w} = -\frac{1}{RT}\frac{d\gamma}{d\ln c_s^h}. \quad (6)$$

Once Γ_s is known, the area, A, per molecule of solute in the interfacial layer is given by

$$A = \frac{1.66 \cdot 10^{-8}}{\Gamma_s}, \quad (7)$$

where A is in square angstroms if Γ_s is in moles/cm^2.

RESULTS

The results of interfacial tension measurements for various hormones are given in Fig. 1. For convenience, the concentrations have been plotted on a logarithmic scale. The curves have been drawn to fit the points as smoothly as possible, except at high concentrations, where the best linear fit has been determined by a least squares calculation of γ on $\ln c_s$. For these calculations, the last four experimental points of curves 2 and 4 were used, the last five points of curves 3 and 6, and the last eight of curve 5. Curve 1 does not have a linear portion.

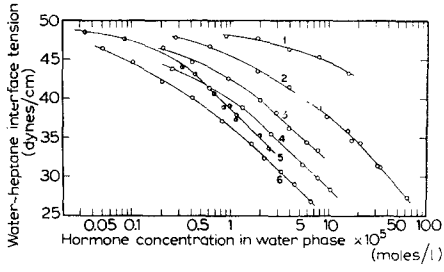

Fig. 1. Variation of interfacial tension of water–heptane interface with logarithm of concentration of hormone in solution. Curve 1: estriol. Curve 2: hydrocortisone. Curve 3: testosterone. Curve 4: deoxycorticosterone. Curve 5: diethylstilbestrol. Curve 6: progesterone. The interfacial tension of the interface between pure water and heptane is 50.0 dynes/cm. Temperature: 24 ± 1° C.

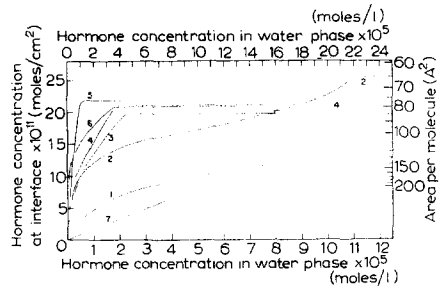

Fig. 2. Variation of interfacial concentration of hormone with concentration of hormone in solution. Curves 1 and 2 refer to the upper scale, while curves 3, 4, 5, 6 and 7 refer to the lower scale. Curves 1 to 6 are for a water–heptane interface at 24 ± 1° C. Curve 7 is for a water–air interface at 21 ± 1° C. Curve 1: estriol. Curve 2: hydrocortisone. Curve 3: testosterone. Curve 4: deoxycorticosterone. Curve 5: diethylstilbestrol. Curve 6: progesterone. Curve 7: testosterone.

From the slopes ($d\gamma/d\ln c_s^w$) of these curves, given by the least squares calculation for the linear portion, and determined graphically at lower concentrations, the interfacial concentration of hormone is obtained by means of Eqn. 6. The results of these calculations are given in Fig. 2. The ordinate on the right-hand side of Fig. 2 is given in terms of the area per molecule of solute, as calculated from Eqn. 7. In the discussion we shall be particularly concerned with the areas corresponding to the linear sections of the curves, and these values have therefore been collected in Table I, together with the standard errors derived from the least-squares calculation.

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TABLE I

AREA PER MOLECULE AT CONSTANT, MAXIMUM INTERFACIAL CONCENTRATION

Hormone	*Area ± (A^2) std. error*
Testosterone	84 ± 4
Progesterone	79 ± 2
Deoxycorticosterone	79 ± 2
Hydrocortisone	65 ± 3
Diethylstilbestrol	6 ± 4

Force-area (πA) curves are given in Fig. 3. As $\gamma_0 = 50.0$ dynes/cm at 24° C, the force, π, is given by the equation $\pi = 50.0 - \gamma$. The accuracy of the areas given in this figure, as of those in Fig. 2, is difficult to estimate except where the areas remain constant. This difficulty arises mainly because one cannot determine uniquely the curve that best fits the experimental points of Fig. 1. Except as indicated in Table I, the areas are certainly not reliable to better than ± 5%.

In Figs. 1 and 2, the concentration of solute in the water phase only has been given. The corresponding concentration in the heptane phase can be calculated from

$$c_s^h = kc_s^w$$

where k is the distribution coefficient. At 24° C, k was found to have approximately the following values for the various hormones: estriol < 0.05; hydrocortisone 0.015; testosterone 4.2; deoxycorticosterone 5.1; diethylstilbestrol 2.3; progesterone 160.

The results given so far apply to a water–heptane interface. With testosterone, measurements of interfacial tension were also carried out for a water-cyclohexane interface. Within experimental error, the resulting curve of interfacial concentration as a function of concentration in the water phase is identical to that given in Fig. 2 for testosterone at a water–heptane interface.

Also with testosterone, determinations were made of concentration at the water-air interface. These results will not be taken up in the discussion, but for purposes of comparison they are included in Fig. 2.

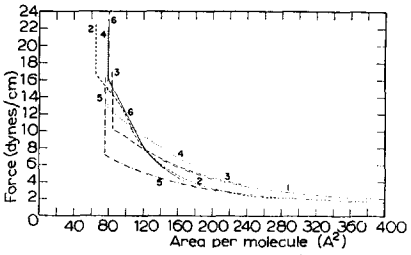

Fig. 3. Force-area curves for hormones at water–heptane interface. Curve 1: estriol. Curve 2: hydrocortisone. Curve 3: testosterone. Curve 4: deoxycorticosterone. Curve 5: diethylstilbestrol. Curve 6: progesterone. Temperature: 24 ± 1° C.

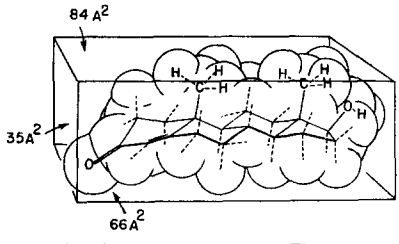

Fig. 4. Model of testosterone. The approximate cross-sectional areas (± 10%) are represented by the rectangular parallelepiped. The same approximate dimensions apply to progesterone, deoxycorticosterone and hydrocortisone.

DISCUSSION

In Fig. 1 it can be seen that, within the accuracy of the measurements, the curves for all the hormones except estriol become linear at high concentrations. From Eqn. 5,

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this linear portion corresponds to a constant value of the interfacial concentration of hormone, a point which is clearly illustrated in Fig. 2. It is reasonable to assume that when the interfacial concentration reaches such a constant, maximum value, the hormone molecules in the interface are tightly packed; and that there should therefore be some relation between the area per molecule at the interface, and the dimensions of the molecule.

Comparison of the data in Table I with the dimensions for the testosterone molecule given in Fig. 4 immediately suggests such a relation, since the experimentally determined area for testosterone is the same as that of the largest cross-section of the model. If the molecules are tightly packed, the only way to account for this fact is to suppose that the testosterone molecules in the interface form a monomolecular layer, and that the molecules lie more or less flat in this layer. Within the accuracy to which the dimensions of the model can be determined, these conclusions apply equally well to progesterone and deoxycorticosterone.

The molecules of testosterone, progesterone and deoxycorticosterone all consist of a large hydrocarbon nucleus, with a polar group attached at each end. Consequently, as the polar groups would be predominantly attracted to the water phase, while the hydrocarbon nucleus would be attracted to the heptane phase, one might have expected that these molecules would lie more or less flat in the interface.

In contrast to the areas for the three hormones just considered, the area determined for hydrocortisone corresponds closely to that of the next largest cross-sectional area of the model, *i.e.*, to that area which the molecule would occupy if it were lying "on edge" in the interface. Although there are many ways in which the molecule could occupy such an area, it is reasonable to suppose that it actually is on edge, with carbon 11 towards the water phase, as in this position all the polar groups can be in the water phase while at the same time most of the hydrocarbon nucleus is in the heptane phase.

The steroid molecules considered so far are quite rigid. On the other hand the molecule of the synthetic estrogen, diethylstilbestrol, is somewhat flexible. For this reason, the cross-sectional areas of this molecule cannot be established from models in an unambiguous fashion. However, the area determined experimentally for diethylstilbestrol ($76 A^2$) is roughly equal to the area the molecule would occupy with its largest cross-section parallel to the interface. In this position both hydroxyl groups would be near the interface, and the planes of the conjugated rings would be parallel to the interface.

The general behaviour of the hormones over the whole concentration range is best discussed with reference to the force-area curves of Fig. 3. The curves for all the hormones (except estriol, with which it was not possible to attain high enough concentrations) consist of two well-defined regions: at high interfacial forces there is a region of constant area, corresponding, in the terminology adopted by ADAM[13] for insoluble surface films, to a condensed film of very low compressibility. As shown in the preceding paragraphs, in this region the molecules probably form a closely packed monomolecular layer. At lower forces the curves correspond to a gaseous type of film, again using ADAM's terminology. The molecules here are separated from one another, but interact considerably as evidenced by the fact that at the lowest forces the curves are still far from the theoretical curve for a perfect gas ($\pi A = 400$; *cf.* reference 13), even if a correction is made for the fraction of the total area occupied by the molecules

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themselves. The transition from one type of film to another appears to be quite abrupt. However, it is possible that there are important details in the transition region which have been smoothed out, owing to the relatively low accuracy of the procedure by which the curves were obtained. Only one of the curves, that for hydrocortisone, has a clearly defined inflection point, a feature which also distinguishes the hydrocortisone curve in Fig. 2. It seems likely that the inflection is caused by the hydrocortisone molecule lying more or less flat in the interface when the available area per molecule is large, and then being tilted up on edge as the area decreases.

It has been pointed out already that the hormones dealt with here are adsorbed not only at a water–heptane interface, but at a variety of other interfaces between polar and non-polar media. So one may reasonably conclude that they would be adsorbed to some extent at any polar–nonpolar interface, with an orientation similar to that which they adopt at the water–heptane interface. In this regard it is interesting to note that almost all known steroid hormones and synthetic estrogens have structures of the general type described above; *i.e.*, they consist of a large hydrocarbon nucleus with polar groups at each end, and sometimes additional polar groups at other positions. These hormones would therefore also be expected to adsorb at interfaces between polar and non-polar media, and to lie flat or on edge in the interface.

Certain conjectures can then be made with respect to the general behaviour of these compounds in biological systems. Under conditions where the interfacial regions between polar and non-polar media constitute a significant part of the total volume, a large fraction of any hormone present is likely to be adsorbed at the interfaces at equilibrium, leaving only small amounts free in solution. In fact, at the lowest concentrations, at which physiological levels would presumably be approached, the ratio of adsorbed to free hormone would become greatest, as can be seen from the shape of the curves in Fig. 2. Furthermore, the adsorbed hormone molecules would necessarily be brought into interaction with other compounds that also are adsorbed, and one might therefore expect that in some circumstances they would be able to take part in, or to influence, processes that occur at the interfaces[14].

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SUMMARY

From measurements of interfacial tension, the interfacial concentrations at water–heptane interfaces have been determined for testosterone, progesterone, deoxycorticosterone, hydrocortisone, diethylstilbestrol and estriol.

In all cases save that of estriol, the interface becomes saturated at concentrations in the water phase that range from $5 \cdot 10^{-7}$ (for diethylstilbestrol) to $2 \cdot 10^{-4}$ moles/l (for hydrocortisone).

From the experimentally determined areas occupied by the molecules at the interface, it is concluded that testosterone, progesterone, deoxycorticosterone and diethylstilbestrol lie more or less flat in the interface, while hydrocortisone lies on edge. These orientations are those which would be expected from the structures of the compounds.

The force-area curves at large forces correspond to condensed films, and at lower forces to imperfect gaseous films.

The applicability of these results to other steroid hormones and synthetic estrogens is considered, and the biological implications are discussed.

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SUR LA PRÉSENCE DE CRÉATINE CHEZ LES INVERTÉBRÉS ET SA SIGNIFICATION BIOLOGIQUE

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On a longtemps admis qu'à de rares exceptions près, chez certains Echinodermes et Protochordés[1,2], la créatine et son dérivé phosphorylé, la phosphocréatine, étaient biochimiquement caractéristiques des Vertébrés, comme l'arginine et la phosphoarginine l'étaient des Invertébrés. De nombreuses exceptions ne tardèrent cependant pas à se manifester. Dès 1946, de la créatine a été isolée des testicules d'oursin et d'étoile de mer et caractérisée dans ceux de plusieurs ascidies, de Protochordés et d'Annélides marins[3]. Le muscle de certains Annélides s'est par ailleurs montré renfermer un phosphagène se comportant comme la phosphocréatine lors de son hydrolyse acide en présence de molybdate[4] et l'identification de la créatine dans les tissus de certains Vers marins[5], puis celle de la phosphocréatine chez ces mêmes animaux[6], ont été réalisées par la suite. Enfin, la créatine a été caractérisée chez un Alcyonnaire[7] et chez des Spongiaires[8].

Certains auteurs[9,10,4,11], ayant interprété la présence de la créatine chez des Invertébrés comme un indice de leur rattachement à des classes de transition entre Vertébrés et Invertébrés, il nous a paru intéressant de préciser la répartion de la créatine et de l'arginine chez ces derniers et d'étudier dans quelle mesure cette répartition pouvait correspondre au degré d'évolution des organismes envisagés. Nous avons donc entrepris de contrôler au moyen de techniques d'identification rigoureuses les résultats déjà acquis, obtenus pour la plupart dans des conditions n'offrant pas de garantie de spécificité (réactions à l'α-naphtol-diacétyle ou à l'α-naphtol-hypobromite sur des extraits tissulaires entre autres), et de les étendre, afin de définir le rôle biologique de la créatine chez les Invertébrés.

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