The Interaction between
Steroid Hormones and Lipid
Monolayers on Water

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ABSTRACT The interaction of progesterone, testosterone, androsterone, and
etiocholanolone with insoluble lipid films (cholesterol and saturated hydro-
carbons containing either alcohol, ester, acetamide, phosphate, amine, or
carboxyl groups) was studied. In addition to surface pressure and surface
potential measurements of the surface films, radioactive tracers were used to
measure the concentration of adsorbed steroid in the lipid films. In general,
steroids form mixed films with the insoluble lipid films. Compression of the
insoluble lipid films to their most condensed state leads to complete ejection of
adsorbed steroid from the surface in all cases except with the amine, for which
a small amount of steroid is still retained in the surface. Interactions between
the steroids and insoluble lipids are primarily due to van der Waals or disper-
sion forces; there were no significant contributions from dipole-dipole inter-
actions (except possibly with the amine). Specific interactions between cho-
lesterol and the soluble steroids were not observed. Evidence suggests that low
steroid concentrations influence structure of lipid films by altering the hydra-
tion layer in the surface film. In contrast to a specific site of action, it is pro-
posed that steroid hormones initiate structural changes in a variety of
biological sites; this model of steroid action is consistent with the ubiquity of
many steroid hormones.

INTRODUCTION

There are essentially two general theories for describing the molecular action
of steroid hormones (1, 2). One theory emphasizes the importance of cellular
membranes as diffusion barriers; the hormone is pictured as either reacting
with the diffusible metabolite (3, 4) or altering the structure of the membrane
in such a way that the specific permeability of the metabolite is affected (5).
The other theory postulates a direct effect of the hormone on enzymatic
activity (6, 7) or on the regulation of enzyme synthesis (8). Implicit in both theories is the concept that a specific interaction occurs between the "target site" (the enzyme or membrane) and the steroid hormone which results in a structural or conformational change in the system and a new level of physiological activity. Evidence in support of these theories is indirect and hence largely inconclusive. For example, steroid hormones have been reported to bind to proteins (9), nucleic acids (10), and coenzyme components (11), to cite just a few of the model systems which have been studied. However, it is difficult to draw any conclusions from these studies, beyond the fact of the binding itself, because the concept of binding has not yet been established as a meaningful aspect of the physiological response to hormones. It would seem that a study of the interaction of steroid hormones with a chemically well-defined model system which can undergo a chemical reaction or a change in physical state would provide useful insights into the nature of steroid hormone interactions in biological systems.

We have therefore investigated some of the general characteristics of steroid interactions with monomolecular films of insoluble lipid molecules on water. Lipid films were selected because they form well-defined physical states on water (12). Thus, it was thought that a study of steroid-lipid film interactions would provide some insights into possible effects of steroids on the physical properties of structured biological systems. In particular, we were interested in establishing whether steroid hormones interact selectively with various polar and ionic groups in the monolayer, and whether this binding affects the physical state of the lipid films.

A recent study of the interaction of steroid hormones with monooleadecyl phosphate monolayers on water has demonstrated that low concentrations ($10^{-6}$-$10^{-7}$ M) of steroids in the substrate markedly alter the mechanical properties of the phosphate films (13). Other monolayer studies suggested that steroids do not strongly associate with lipid films (14, 15), although they may affect the hydration layer of the monolayer (16). In the present work the scope of these earlier studies was broadened, and we have examined the influence of each of the steroids shown in Fig. 1 on surface properties of a group of lipid compounds which include the phosphate, carboxyl, amino, amido, ester, and alcohol radicals. Since we were primarily concerned with general characteristics of steroid interactions, steroids were selected without regard to their specific physiological properties, but rather because they represent a spectrum of physiological activities but are also closely related chemically.

Our experiments measured the amount of steroid adsorbed from solution into the insoluble lipid films, using radiotracers, and the changes in surface pressure ($\Pi$) and surface potential ($\Delta\psi$) of the lipid films elicited by the steroids. It will be shown from these studies that low concentrations of
steroids influence the structure of insoluble lipid films. In addition, we will identify some general characteristics of the steroid-monolayer interactions. The details of these experiments form the main body of this paper. Some of the physiological implications of the results are also considered.

![Figure 1. Structures of the four soluble steroids used in this study. The dotted lines in androsterone and etiocholanolone indicate that these bonds recede behind the plane of the paper.](image)

**EXPERIMENTAL**

**A. Materials**

The following pure compounds (listed with melting points) were spread as monolayers from benzene-methanol (20:1) solutions: octadecylamine, Aldrich Chemical Co., Milwaukee, Wis. (76°C); methyl stearate, Calbiochem, Los Angeles, Calif. (38°C); stearic acid, Applied Science Laboratories Inc., State College, Pa. (69.5°C); octadecanol, Mann Research Labs. Inc., New York (58°C); cholesterol, Mann Research Labs. Inc. (149°C); mono-octadecyl phosphate (85.5°-86°C) (17). Octadecylacetamide was prepared from octadecylamine by treatment with acetic anhydride followed by recrystallization from ether (78.5°C).

The soluble steroids used in this study were androsterone (185°-185.5°C), etiocholanolone (152°-153°C), progesterone (128°-129°C); these were obtained from Southeastern Biochemicals, Augusta, Ga. Testosterone (155°C) was a gift from Dr. D. Johnson, National Institutes of Health. All steroids were tested for purity by thin layer chromatography; etiocholanolone showed one minor impurity which was removed. The purity of etiocholanolone was verified by mass spectrometry.

Progesterone-4-14C, testosterone-4-14C, stearic-1-14C acid, and glycine-1-14C were obtained from New England Nuclear Corp., Boston, Mass., and were isotopically diluted, when necessary to, give specific activities of 11.15, 16.75, 6.71, and 8.10 mCi/mnmole, respectively.

Solutions of the steroids in water were made by first dissolving the steroid in 0.3
ml methanol and then adding the concentrated solution to the desired volume of water. Controls using this concentration of methanol alone indicated that the alcohol does not influence the properties of the monolayers. Buffered solutions were prepared by titration of 2 mM sodium phosphate with NaOH.

B. Apparatus and Methods

The general experimental procedure followed throughout was to spread the insoluble lipid film on the aqueous steroid solutions. A Langmuir-type horizontal float film balance was used for measuring surface pressures and areas (18). This method measures directly the difference in surface tension between the steroid solution and the film-covered solution. However, surface pressures are generally defined as the difference between the surface tension of steroid-free solution and the surface tension of the solution covered by the film. Therefore, the surface tension lowering caused by the steroids alone must be added to the surface tension difference measured by the film balance to obtain the conventional value of surface pressure. Surface tensions of the steroid solutions were measured by the drop weight method (12).

Surface potentials, $\Delta V$, were measured with a $^{220}$Ra electrode and electrometer (18) with a reproducibility of $\pm 10$ mv.

Surface excess concentrations, $\Gamma_s$ (moles/cm$^2$), of $^{14}$C-labeled steroid solutions with and without the insoluble monolayers, were obtained by measuring the surface radioactivity with a thin window gas-flow G. M. Tube (Nuclear-Chicago, Des Plaines, Ill.) which was suspended at a fixed distance of 3 mm over the solution surface. The surface pressure $\Pi$ was monitored simultaneously with the surface radioactivity. The principle of the method for determining $\Gamma_s$ with labeled compounds has been described (19, 20). To separate experimentally the radioactivity of the solution interior from that due only to the adsorbed surface film, glycine-1-14C (which is not surface active) solutions were measured under the same counting geometry as the steroid solutions. The difference between the radioactivities of the glycine and steroid solutions represents the amount of radioactivity due to adsorbed steroid. To convert this difference into steroid excess surface concentrations, stearic-1-14C acid monolayers were spread on water under the same counting geometry, and the proportionality constant for the acid radioactivity to its surface concentration was obtained. All the radioactivities were corrected for the differences in their specific activities which were determined separately under the same conditions. All experiments were performed at 23°C.

To test the validity of the radiotracer method, values of $\Gamma_s$ obtained directly with $^{14}$C-labeled steroids were compared with those calculated from surface tension data of steroid solutions (in the absence of insoluble monolayer) using the Gibbs adsorption isotherm

$$
\Gamma_s = -\frac{1}{RT} \int d\gamma \ln c_s \approx \frac{\Delta \gamma}{RT}.
$$

These values for testosterone and progesterone at a bulk solution concentration at $2 \times 10^{-4}$ M are given in Table I. Agreement between the two methods for $\Gamma_s$ is good. Included in Table I are the surface tensions of $2 \times 10^{-4}$ M solutions of all four
### Table I

**Properties of Steroid Solutions**

\( C_s = 2 \times 10^{-5} \text{ M}, T = 23^\circ \text{C}, \gamma_w = 72.7 \text{ dynes cm} \)

| Steroid              | \( \gamma \)  | \( \Gamma_s \) (radioisotope) | \( \Gamma_s^* \) |
|----------------------|----------------|-------------------------------|------------------|
| Progesterone         | 67.8           | \( 1.88 \pm 0.1 \times 10^{-10} \) | \( 1.85 \times 10^{-10} \) |
| Testosterone         | 71.5           | \( 0.34 \pm 0.1 \times 10^{-10} \) | \( 0.40 \times 10^{-10} \) |
| Etiocholanolone      | 67.3           | —                             | \( 2.1 \times 10^{-10} \) |
| Androsterone         | 67.3           | —                             | \( 2.1 \times 10^{-10} \) |

* Calculated with the Gibbs equation: \( \Gamma_s = \frac{-C_s(\delta\gamma)}{RT \delta C_s} \approx \frac{(\gamma_w - \gamma)}{RT} \).

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**RESULTS**

**A. \( \Pi-\Lambda \) Isotherms**

The \( \Pi-\Lambda \) isotherms for the insoluble lipid molecules used in this study, without steroid in the aqueous subphase, are given in Fig. 2. In general, the normal aliphatic compounds form liquid-condensed films (12). The limiting high

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**Figure 2.** \( \Pi-\Lambda \) isotherms of the insoluble monolayers in the absence of steroid.
surface pressures occur at about 20 ± 1 Å²/molecule, representing the area at which the oriented lipid molecules are tightly packed in the film. The Π-Å isotherm for cholesterol is also shown in Fig. 2, and its high pressure region is located at about 37 Å²/molecule. This area corresponds to the molecule close-packed and oriented with the —OH group immersed in the
aqueous phase, and with the fused-ring structure directed normal to the water surface.  

The \( \Pi - A \) isotherms of the insoluble monolayers on solutions containing steroid are shown in Fig. 3 for testosterone, Fig. 4 for progesterone, and Fig. 5 for etiocholanolone at a steroid concentration of \( 2 \times 10^{-5} \) m. The curves for androsterone are not shown because they are essentially the same as for etiocholanolone, but with the former 0.5–1.0 dynes/cm lower at each value of \( A \). In general, the steroids increase \( \Pi \) at every point on the isotherms of the insoluble lipid films.

**B. Surface Excess Concentrations, \( \Gamma_s \)**

The surface excess concentration \( \Gamma_s \) of progesterone and testosterone was measured with radiotracers over the same range of film areas as the \( \Pi - A \) isotherms of Figs. 3 and 4 (the \(^{14}\)C-labeled androsterone and etiocholanolone were not available). For both steroids, \( \Gamma_s \) decreased monotonically and approached zero as the insoluble lipid was compressed to its most compact

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1 It should be noted that for the normal aliphatic films at areas greater than 25 \( \text{Å}^2/\text{molecule} \) two discrete monolayer phases coexist: islands of liquid-condensed film in equilibrium with discrete film molecules which behave formally as a nonideal two-dimensional gas (21). Cholesterol monolayers behave similarly at areas greater than \(~50 \text{Å}^2/\text{molecule} \). The value of \( \Pi \) in this two-phase region of the isotherm is generally very low (<0.1 dynes/cm). We have not included these points in Fig. 2, but merely here state that at large film areas each of the systems has a small but finite value of \( \Pi \).
**TABLE II**
SURFACE CONCENTRATION $\Gamma_s$ OF PROGESTERONE AND TESTOSTERONE IN LIPID MONOLAYERS

$C_s = 2 \times 10^{-5}$ M, pH 7.4, $T = 23^\circ$C.

| Monolayer | Progesterone | Testosterone |
|-----------|--------------|--------------|
|           | $\Gamma_s \times 10^{10}$ (±0.1) | $\Gamma_s \times 10^{10}$ (±0.1) |
|           | moles/cm²   | moles/cm²   |
| **Area**  |             |             |
| A. Octadecanol | 0.2 1.8 0 0.7 | 0.1 1.9 0 0.8 |
| Methyl stearate | 0 1.8 0 0.7 | 0 1.8 0 0.7 |
| Stearic acid | 0.1 2.0 0.1 0.6 | 0.55 2.0 0.33 1.0 |
| Octadecylacetamide | 0 2.0 0.1 0.6 | 0 2.0 0.1 0.6 |
| Octadecylamine | 0 2.0 0.1 0.6 | 0 2.0 0.1 0.6 |
| m-Octadecyl phosphate | 0 2.0 0.1 0.6 | 0 2.0 0.1 0.6 |
| **Area**  | 38 A² 80 A² 38 A² 45 A² |            |
| B. Cholesterol | 0 1.9 0.1 0.5 |            |

*Area per insoluble lipid molecule.

area. Table II lists the values of $\Gamma_s$ which were obtained for the extreme limits of the experimental range of film areas: at the largest and at the most condensed areas studied.

**C. Surface Potentials, $\Delta V$**

The effects of the steroids on the surface potentials, $\Delta V$, of the monolayers as a function of film area from 20 to 40 A²/insoluble lipid molecule are shown in Fig. 6 for solutions of the various steroids and in the absence of steroid ($C_s = 0$). For the steroid-free studies ($C_s = 0$), $\Delta V$ was obtained only for the 20–25 A²/molecule range because at larger film areas the two-phase system of the condensed film in equilibrium with its surface vapor gives very erratic values of $\Delta V$ (12). In general, the addition of steroid decreases $\Delta V$. Despite the decreases in $\Delta V$, the steroids do not change significantly the slope of the $\Delta V$-$A$ curves in the range of 20–25 A²/insoluble lipid molecule.

**DISCUSSION**

**A. Characteristics of Steroid-Lipid Monolayer Interactions**

To establish whether specific interactions occur between the steroids and the insoluble lipids, it is useful to treat the surface mixtures as two-dimensional solutions (22, 23). Recent studies (24) have shown that mixtures of two lipid components at the air-water interface may be treated as "regular" solutions (25). Thus, we have applied the formal treatment of regular solutions of
surface films developed by Defay and Prigogine (26) to the \( \Pi - A \) data given in Figs. 3 and 4; the calculations for this analysis are presented elsewhere;\(^2\) to summarize these results, the data conforms to the regular solution treatment of Defay and Prigogine. We conclude therefore that the steroids and lipids in the present study also mix to form regular surface solutions.

For regular solutions in general, the forces between components in the solution are nonspecific (25). With lipid mixtures in surface films the surface solutions have been shown to be dominated by van der Waals or dispersion forces between the lipid moieties, and dipole-dipole interactions do not make a significant contribution (24). The same general results have been obtained with the steroid hormone–insoluble lipid film mixtures of the present study. In agreement with regular surface solution theory (26), \( \Gamma_s \) decreases and approaches zero as \( \Pi \) increases, or as film area \( A \) decreases (Table II). If specific interactions between the steroid and lipid molecules exist such that compound formation resulted, one would expect the value of \( \Gamma_s \) to be significantly greater than zero at the low area range of the isotherm. With the possible exception of the amine films, \( \Gamma_s = 0 \) within the experimental error (see Table II, columns 1 and 3). Therefore, except for the amine films,

\(^2\) Muramatsu, M., and N. L. Gershfeld. A regular solution treatment for interaction between insoluble monolayers and soluble steroids on water surfaces. Manuscript in preparation.

Figure 6. Surface potential, \( \Delta V \), of insoluble films in the presence of \( 2 \times 10^{-6} M \) steroid as a function of insoluble film area \( A \). \( \Delta V \) for the films alone, \( C_s = 0 \), are also shown. \( T = 23^\circ C, \) pH 7.4. ■, octadecylamine; ▲, octadecylacetamide; ●, methyl stearate; △, octadecanol; ○, stearic acid; ◆, monooctadecyl phosphate.
there is no evidence for specific interactions between the soluble steroids and the polar groups of the insoluble lipid films in our study.

In this regard it has been suggested (5) that cholesterol in surfaces might enhance the adsorption of steroid hormones whose fused ring structures exhibit the same planarity as cholesterol. Testosterone and progesterone have the same basic fused ring structure as cholesterol but the mixing behavior of these hormones with cholesterol films was similar to the other insoluble aliphatic lipid films in that \( \Gamma_o \approx 0 \) when cholesterol is compressed to its smallest area, \( A \approx 38 \text{ A}^2 \) (Table II); the surface mixtures also followed regular solution theory. Thus, there does not appear to be any specific interaction of these hormones with oriented condensed films of cholesterol.

Despite the general lack of specificity of the interactions between steroid and insoluble lipid, the \( \Pi - A \) isotherms of the insoluble monolayers are selectively altered in the presence of steroids as seen in Figs. 3–5. Thus, for a given steroid at a constant area per molecule, \( \Pi \) generally increases in the order: phosphate > amine > carboxyl \( \approx \) alcohol \( \approx \) ester \( \approx \) amide \( \approx \) cholesterol. The results for each of the four steroids are summarized in Table III where values of \( \Delta \Pi \), the increase in \( \Pi \) caused by these steroids for a fixed value of the area of the insoluble lipid component, are listed.

To analyze rigorously the factors which cause the marked differences in \( \Delta \Pi \), the Gibbs adsorption isotherm must be used. The complete integrated

| Lipid monolayer       | Progesterone, \( \Delta \Pi \) | Testosterone, \( \Delta \Pi \) | Etiocholanolone, \( \Delta \Pi \) |
|-----------------------|-------------------------------|-------------------------------|---------------------------------|
| A. Octadecanol        | 4.5                           | 1.0                           | 5.5                             |
| Methyl stearate       | 5.5                           | 1.0                           | 5.5                             |
| Stearic acid          | 5.2                           | 2.0                           | 7.0                             |
| Octadecylacetamide    | 5.5                           | 1.0                           | 7.5                             |
| Octadeylamine         | 6.9                           | 5.6                           | 8.6                             |
| n-Octadecyl phosphate | 13.3                          | 8.3                           | 13.5                            |
| B. Cholesterol        | 6.3                           | 1.0\( \dagger \)             | -                               |

* Androsterone values are about 0.5 dynes/cm lower than for etiocholanolone; therefore, only values for the latter are presented.

\( \dagger A = 45 \text{ A}^2/\text{cholesterol molecule.} \)
form of the Gibbs equation for the conditions of our experiment may be written as

\[
\Delta II = \int_0^\infty \left[ \Gamma_f \left( \frac{\partial \mu_f}{\partial \mu_s} \right) + \Gamma_s + \Gamma_f \left( \frac{\partial \mu_f}{\partial \mu_s} \right) + \Gamma_w \left( \frac{\partial \mu_s}{\partial \mu_s} \right) \right] \, \mathrm{d}\mu_s \tag{2}
\]

where \( \mu \) is the chemical potential, \( \Delta II \) is the increase in surface pressure listed in Table III, and where the subscripts \( i, s, f, \) and \( w \) refer to electrolyte, steroid, insoluble film, and water, respectively. Several of the parameters in equation 2 may be immediately eliminated as not contributing to \( \Delta II \): \( \Gamma_f \) is held constant; \( \partial \mu_i / \partial \mu_s \) and \( \partial \mu_w / \partial \mu_s \) are the same for each monolayer since the same solution is used in each steroid series, and both electrolyte and water are equilibrated throughout the system, i.e., in the bulk as well as in the surface. Moreover, since the differences in \( \Gamma_s \) shown in Table II, columns 2 and 4, are small, they too cannot account for the differences in \( \Delta II \) found among the various insoluble monolayers. Therefore, we conclude that the observed differences in \( \Delta II \) must be due to one or more of the following, \( \partial \mu_f / \partial \mu_s \) (which contains all the configurational and interaction elements of the film structure), \( \Gamma_f \), and \( \Gamma_w \), the excess surface concentration of electrolyte and water, respectively. At present these parameters cannot be evaluated independently from the \( \Pi-A \) isotherms. However, we shall now demonstrate from the surface potential measurements that \( \Gamma_f \) also does not contribute to the differences in \( \Delta II \) of Table II.

The surface potential \( \Delta V \) will be influenced by the nature of the permanent dipoles, the presence of ionogenic groups, and the structure of the aqueous phase and the attendant water dipoles in the region of the surface film. These contributions to \( \Delta V \) may be expressed formally by treating the oriented array of dipoles in the film as a parallel plate condenser (27). In the absence of steroid

\[
\Delta V = 4\pi n_f \mu_f^+ + 4\pi n_w \mu_w^+ + \Psi_0 \tag{3}
\]

where \( n \) is the number of dipoles per square centimeter of surface, \( \mu^+ \) is the vertical component of the dipole moment in the surface with \( f \) and \( w \) referring each of the parameters to the lipid and water molecules in the surface film, respectively; \( \Psi_0 \) is the electrical double layer potential which arises from the presence of ionogenic groups in the film. In the presence of steroid at constant \( \Gamma_f \) (or \( n_f \))

\[
\Delta V_s = 4\pi n_f \mu_f^+ + 4\pi n_w \mu_w^+ + \Psi_0 + 4\pi n_s \mu_s^+ \tag{4}
\]

where the prime notation indicates possible new values for these parameters, and \( s \) refers to the steroid contribution. It should be noted that for the ionogenic amine, phosphate, and carboxyl films \( \Psi_0 \), and hence \( \Delta V_s \), is a func-
tion of $\Gamma_i$, while for nonionic ester and alcohol films $\Psi_0$ is zero, and hence $\Gamma_i$ is also zero.

While equations 3 and 4 cannot be evaluated absolutely because the appropriate values of the surface dipole moments are not known, the difference between the two equations represents the influence of the steroids on $\Delta V$ and provides a useful test for contributions of $\Psi_0$ and $\Gamma_i$. The difference between equations 4 and 3 will be signified by the notation $\Delta(\Delta V)$. Values of $\Delta(\Delta V)$ were calculated from the data given in Fig. 6, for the range of insoluble film areas between 20 and 25 $\text{Å}^2$/lipid molecule. As noted earlier, the steroids generally lower $\Delta V$ for the steroid-free system, but $\Delta(\Delta V)$ is virtually independent of the film area between 20 and 25 $\text{Å}^2$. Thus, only the value of $\Delta(\Delta V)$ calculated at 24 $\text{Å}^2$/lipid molecule is presented in Table IV.

We see that $\Delta(\Delta V)$ has a characteristic value for a given steroid which is independent of the chemical nature of the lipid film. Since $\Psi_0$ and $\Psi'_0$ are zero for the nonionic films (i.e., alcohol, ester, amide), the constant value of $\Delta(\Delta V)$ for these films must be characteristic of some nonionic process. For the case of the ionogenic films (i.e., carboxyl, phosphate, and amino), if $\Psi_0$, and hence the ionic distribution in the surface, is affected by the steroid, one would surely expect a different value for $\Delta(\Delta V)$ than that obtained with the nonionic films. The fact that $\Delta(\Delta V)$ is independent of the charge in the film indicates that $\Psi_0$ and concomitantly $\Gamma_i$ is not affected by the steroids.

To verify that the steroids do not influence $\Psi_0$, $\Delta(\Delta V)$ for $-\text{OH}$, $-\text{COOH}$, and $-\text{PO}_4$ films was measured at pH 2, where the dissociation of the acids is greatly repressed. The results with progesterone are shown in Table V where it is seen that even though $\Delta V$ (and hence $\Psi_0$) for $-\text{COOH}$ and phosphate is markedly affected by the change in pH, $\Delta(\Delta V)$ is still constant and independent of pH. As expected, with the nonionic $-\text{OH}$ film $\Delta V$ is not

### Table IV

SURFACE POTENTIALS, $\Delta V$, OF MONOLAYERS ON SOLUTIONS OF STEROIDS $C_s = 2 \times 10^{-5}$ m, pH 7.4. Area per insoluble lipid molecule = 24 $\text{Å}^2$.

| Monolayer                  | $\Delta V$ | Progesterone | Testosterone | Androstene | Etocho- lanolone |
|----------------------------|------------|--------------|--------------|-------------|-----------------|
|                            | $\text{ms} \pm 10 \text{ ms}$ | $\text{ms} \pm 10 \text{ ms}$ |               |             |                 |
| Octadecanol                | +425       | -250         | -40          | -285        | -345            |
| Methyl stearate            | +525       | -250         | -70          | -285        | -350            |
| Stearic acid               | +230       | -250         | -90          | -285        | -345            |
| Octadecylamine             | +750       | -230         | -40          | -280        | -350            |
| Octadecylacetamide         | +585       | -230         | -50          | -260        | -320            |
| $m$-Octadecyl phosphate    | +70        | -250         | -50          | -260        | -350            |
| Cholesterol (40 $\text{Å}^2$/molecule) | +370       | -235         | -60          | --          | --              |
influenced by pH, nor is $\Delta V$. Since $\Psi_0$ is a function of $\Gamma$, it follows that the steroids do not affect $\Gamma$.

It is apparent, therefore, that the variations in $\Delta \Pi$ listed in Table III can be due only to the contribution of $(\partial \mu / \partial \mu)$, the chemical potential of the insoluble lipid component of the film, and $\Gamma_w$, the excess concentration of water in the surface. It is important to recognize that this separation into lipid and water components is somewhat arbitrary and masks the physically more realistic picture of hydrated lipid films on water. The results of a variety of experiments (28) suggest that the polar regions of condensed lipid films are hydrated. Recent spectroscopic (29) and thermodynamic arguments suggest that the hydrated structure is characteristic of the polar group. Unfortunately, the film balance experiment itself cannot provide direct evidence for any specific model of film hydration (30), and, consequently, the extent to which the hydration contributes to the surface pressure of the lipid film is presently not known. However, closer examination of the surface potential data does give an indication of the influence exerted by the steroids on hydration.

The values of $\Delta (\Delta V)$ listed in Table IV are practically independent of the film area between 20 and 25 $A^2$/ insoluble film molecule. If we now recall the fact that the surface excess concentration of steroid, $\Gamma_*$ (see Table II), is zero, with the exception of the amine films, when the insoluble film area equals 20 $A^2$ we can conclude that the large value of $\Delta (\Delta V)$ at the highly compressed area must be the result of the steroid in the bulk solution beneath the surface film. This conclusion follows by recognizing first that there is no steroid in the plane of the insoluble film. Secondly, at 20 $A^2$/molecule the insoluble lipid molecules are packed almost as closely as in the crystalline state ($\sim 18 A^2$); thus it seems unlikely that the lipid molecules will have sufficient freedom of rotation to change drastically the surface dipole moment contribution of the lipid molecules to the surface potential of the monolayer (equation 4).

| Film                  | $\Delta V$ | $\Delta (\Delta V)$ | $\Delta V$ | $\Delta (\Delta V)$ |
|-----------------------|------------|---------------------|------------|---------------------|
| Octadecanol           | $+400$     | $-260$              | $+425$     | $-250$              |
| Stearic acid          | $+375$     | $-265$              | $+230$     | $-250$              |
| $m$-Octadecyl phosphate | $+270$     | $-250$              | $+70$      | $-250$              |

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The values of $\Delta(\Delta V)$ of Table IV therefore reflect either the contribution of steroid dipoles or a perturbation of the water dipoles beneath the surface film. Two points would seem to argue against the former; one is that the values of $\Delta(\Delta V)$ should bear some relation to the vacuum dipole moments of the steroids. To test this point the vacuum dipole moments were compared with the average $\Delta(\Delta V)$ values of the steroids (Table IV); as seen in Table VI there is no correlation between the two. Secondly, a simple calculation of the maximum value of $\Delta(\Delta V)$, which can be obtained—allowing for the maximum value of the steroid vacuum dipole moment (see Table V), and using the relation $\Delta(\Delta V) = 4 \pi \eta \mu^2$, indicates that it is physically impossible for this

| Steroid          | $\Delta(\Delta V)^*$ | $\eta \mu^2$ |
|------------------|------------------------|--------------|
| Androsterone     | $-276\pm10$            | 3.7          |
| Etiocholanolone  | $-343\pm8$             | 3.6§         |
| Progesterone     | $-242\pm9$             | 2.7          |
| Testosterone     | $-58\pm14$             | 4.1          |

* Mean values and the mean deviations of the values given in Table IV.

§ Neudert, W., and H. Röpke, 1965. Atlas of Steroid Spectra. Springer-Verlag New York Inc., New York.

§ Estimate, obtained by comparing the dipole moments of the isomers: 5α-androstan-3α-ol-17-one, 5α-androstan-3β-ol-17-one, and 5β-androstan-3β-ol-17-one (see § above).

small a concentration of steroid ($\sim 10^{-11}$ moles/cm$^2$, i.e. the limit of the radioisotope detection method) to produce the values of $\Delta(\Delta V)$ listed in Table IV. Hence, we conclude that the $\Delta(\Delta V)$ results of Table IV principally reflect the influence of these steroids on the distribution and orientation of water dipoles at the film surface.

It is of interest to note that the influence of these steroids on $\Delta(\Delta V)$ appears to be unique, in that other compounds which have similar fused ring structures do not give the same type of results with insoluble lipid films. For example, cholesterol in mixed lipid films usually changes the surface potential approximately in proportion to its mole fraction in the surface film (31), while for the soluble steroids $\Delta(\Delta V)$ appears to be independent of the surface concentration of the steroid. Another compound for comparison is the steroid alkaloid veratrine; at a concentration of $2 \times 10^{-6}$ M, veratrine will produce values of $\Delta(\Delta V)$ which, unlike the steroid hormones, depend on the chemical nature of the surface film (N. L. Gershfeld, unpublished results).

It should be noted that the behavior of androsterone and etiocholanolone
essentially parallels that of progesterone and testosterone with respect to their effects on the II-A isotherms (Fig. 5) and Δ(ΔV) (Table IV). While we cannot measure Γ, for these steroids, we believe the conclusions obtained for progesterone and testosterone apply as well to androsterone and etiocholanolone.

In summary, the amount of a given steroid adsorbed in the surface is largely independent of the chemical nature of the insoluble lipid film molecules. The steroid-lipid interactions in surface mixtures are nonspecific, and probably involve van der Waals forces between the hydrocarbon moieties of each species; with the possible exception of amino groups, interactions between the permanent dipoles of the steroids and the lipids do not occur. Film structure is selectively affected by steroid, and the structural changes are accompanied by a reorientation of water dipoles in the surface.

A striking characteristic of the steroid-lipid interaction is the fact that it is nonspecific, and yet the structural changes which occur depend on the chemical nature of the lipid film. As a preliminary attempt to explain this effect we propose that the marked differences of ΔII listed in Table IV arise primarily from changes in the polar region of the condensed lipid films. Furthermore, we believe that the action of the steroid is to alter the hydrated state of the polar moiety, perhaps by perturbing the hydration layers associated with the film. The structural changes which follow will depend on how extensive a contribution hydration makes toward the film structure; clearly it will be characteristic of the polar moiety.

More explicitly the steroid-monolayer interaction may be written as

\[
\text{film (hydrated)} \quad \xrightarrow{\text{steroid}} \quad \text{film (hydrated')}
\]

where the prime indicates a different hydrated state of the film, and where the steroid enters only indirectly into the transformation process. This mechanism is consistent with the lack of binding specificity, the dependence of Δ(ΔV) only on the steroid and not the polar group of the insoluble film, and the fact that ΔII is a characteristic of the polar group.

Evidence for support of this model must demonstrate that steroids can influence the aqueous region near the monolayer. While the film balance experiment cannot give direct evidence of the water contribution to film properties, some associated studies of surfaces do indicate that water is an integral component of the lipid films (28) and that steroids do alter the water region adjacent to the films (16).

B. Physiological Implications of Steroid-Lipid Interactions

The steroid-lipid monolayer interactions which have been discussed are sufficiently general that one may assume they will occur in biological systems wherever these components of the monolayers are known to exist. The significance of these interactions to the pharmacological properties of the
steroid hormones may best be discussed within the context of the two theories briefly alluded to in the Introduction. Implicit to both theories is the concept that a specific interaction between the "target site" (e.g., the enzyme or membrane) and the steroid hormone results in a structural or conformational change in the system and a concomitant new level of physiological activity. On the basis of our results the concept of a unique target site for each steroid hormone may be misleading. Rather, our results suggest that the hormones exert a general effect upon all cellular structures, or their components, which are hydrated and are accessible to the steroid. It is important to note that for many steroid hormones multiple physiological responses are usually observed upon administering a particular hormone (32). This nonspecific behavior is clearly inconsistent with a specific target-site model, but is compatible with the more general picture of steroid interactions developed from our monolayer studies.

The interaction of the steroids with amino groups cannot be ascribed any special significance at this time. However, the steroid-amino interaction may contribute to the formation of the complexes which form between steroids and proteins (9).

The four steroids used in this study gave qualitatively similar results; the differences in behavior must be ascribed to the chemical differences among the four. However, it is premature to speculate upon the cause of these differences until more steroids have been examined.

In conclusion, the steroid hormones are capable of altering structured lipid systems at physiological concentrations even as low as $10^{-7}$ M (13). A molecular mechanism has been proposed to account for the structural changes induced by low concentrations of steroids which is consistent with the ubiquitous behavior of the hormones.

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