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# Interaction of Hydrocortisone with Model Membranes Containing Phospholipid and Cholesterol

**GARY W. CLEARY * and JOEL L. ZATZ ˣ**

**Abstract** □ Pure and mixed monolayers of lecithin and cholesterol were spread on substrates of dissolved hydrocortisone at 25 and 37°. The presence of hydrocortisone increased the surface pressure of dipalmitoyl and egg lecithin films that were in head contact. The increase in surface pressure was dependent on steroid concentration. There were no significant interactions with coherent cholesterol monolayers. Penetration of hydrocortisone was decreased by the addition of cholesterol to the monolayer system. These model membrane systems indicate that hydrocortisone interacts with the hydrated polar head group of the phospholipid and not with films whose molecules are in hydrocarbon tail contact.

**Keyphrases** □ Hydrocortisone—interaction with pure and mixed monolayers of lecithin and cholesterol □ Monolayers—lecithin and cholesterol, pure and mixed, interaction with hydrocortisone □ Lecithin—monolayers, pure and mixed with cholesterol, interaction with hydrocortisone □ Cholesterol—monolayers, pure and mixed with lecithin, interaction with hydrocortisone □ Membranes, model—pure and mixed monolayers of lecithin and cholesterol, interaction with hydrocortisone

Several literature reports have drawn attention to the possibility that the biological activity of certain steroids is due to an interaction with biological membranes (1–5). In a review of steroids and cell surfaces, Willmer (1) proposed a mechanism of steroid activity based on the penetration of steroid molecules between the hydrocarbon tails of membrane phospholipids. Erythrocytes were more resistant to lysis in hypotonic solution when steroids were present in low concentration (2). In high concentration, the steroids themselves caused lysis or precipitation. Another study (3) showed that the corticosteroids tended to stabilize lysosomes at pharmacological concentration ($10^{-4}$–$10^{-6}$ *M*) but to lyse them at higher concentrations. Cortisone and hydrocortisone exerted a protective effect on the membranes of erythrocytes (4) and rat liver cells (5).

## BACKGROUND

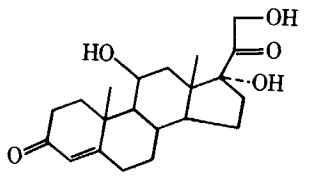
One approach to an understanding of steroid–membrane interactions is through model membrane systems. Monomolecular films provide an organized interfacial structure believed to be similar to that found in biological membranes. Studies of drug penetration into monolayers containing components of natural membranes have been useful in explaining the mode of action of many drugs (6, 7).

Current concepts of cellular membranes suggest the existence of a fluid mosaic structure of globular proteins embedded in, and partially protruding from, an organized but discontinuous lipid layer (8). The site of attachment for membrane-active steroids might be protein or lipid. But membrane proteins have not been well characterized. Because of the availability of pure lipid substances known to be components of biological membranes and in view of Willmer's hypothesis (1), it was decided to use monolayers containing lecithins and cholesterol as membrane models.

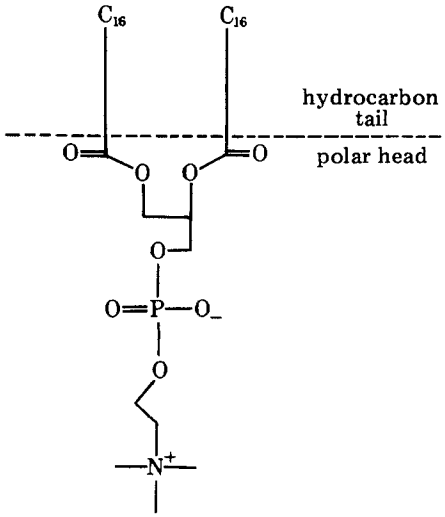
Few studies on the effect of steroids on monolayers have been reported. Progesterone penetrated monolayers of cholesterol and dipalmitoyl lecithin to some extent (9). Cortisone had little effect on monolayers of stearic acid or cholesterol (10), and the properties of stearyl alcohol monolayers were not affected by the presence of dissolved steroids (11).

Progesterone, testosterone, etiocholanolone, and androsterone had little effect on the surface pressure of lipid monolayers in the condensed state (12), but the surface potential of every monolayer was lowered by the same amount in the presence of a given steroid regardless of the nature of the polar group. This finding was attributed to alteration of the structure of water beneath the monolayers by the steroids. Similar changes in surface potential were caused by hydrocortisone in the presence of lecithin and cholesterol monolayers (13). However, the observed changes were an artifact of the experimental procedure and not a result of changes in water structure.

This report describes the effect of hydrocortisone (I) on model membranes composed of monolayers containing dipalmitoyl (II) and egg (III) lecithins and cholesterol (IV). The molecular weights (in daltons) used



I

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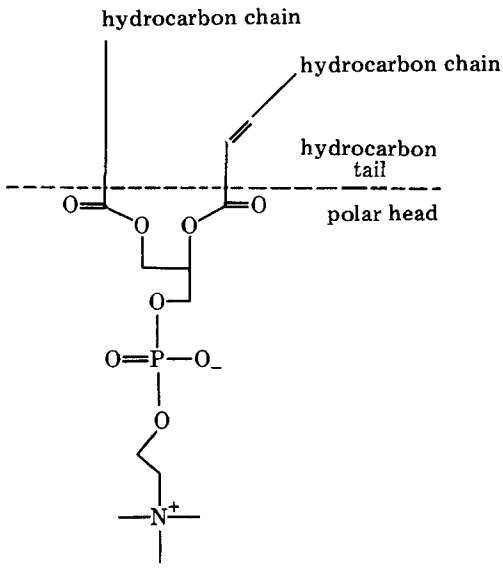


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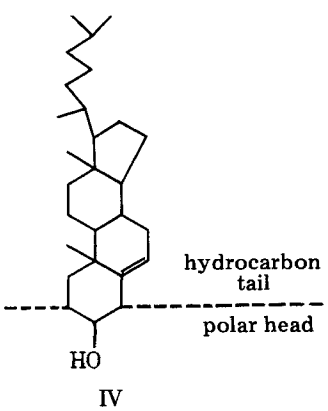


IV

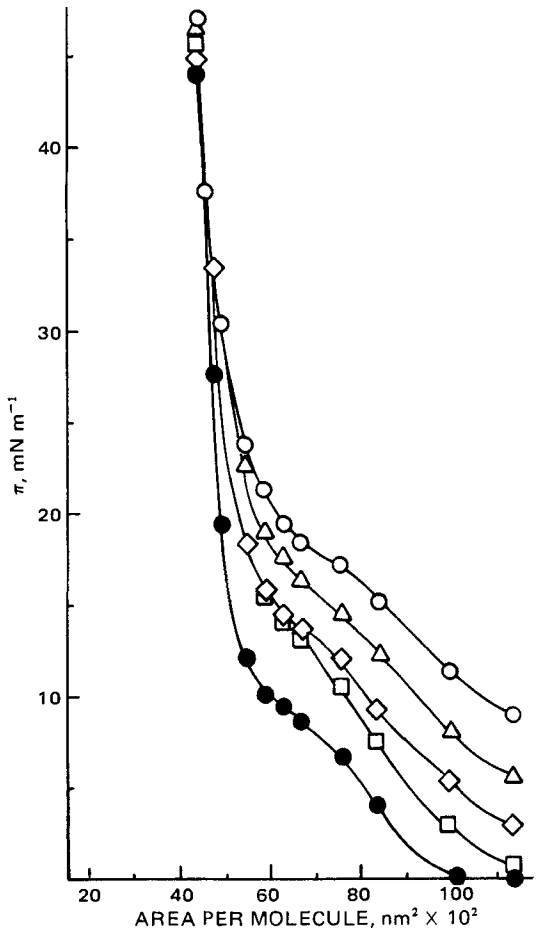


**Figure 1**—*The π–A curves of dipalmitoyl lecithin monolayers on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◊, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

in the calculation of surface area per molecule of the monolayer-forming substances were: I, 362; II, 752; III, 790; and IV, 387.

## EXPERIMENTAL

Hydrocortisone[1] was used as received. Its identity and purity were checked by melting point, optical rotation, paper chromatography, TLC, and IR spectroscopy. Cholesterol[2], dipalmitoyl lecithin[2], and egg lecithin[3] were reported as 99% pure by the suppliers. Organic solvents, spectrograde, were free of surface-active impurities (14). Sodium chloride was reagent grade. Water was double distilled with the final distillation in an all-glass still.

Surface pressure, $\pi$, measurements were made by the Wilhelmy plate method using a thin platinum plate suspended from a torsion balance[4] (15). The polytef[5] surface balance was similar to one described previously (16).

The hydrocortisone was dissolved in the subphase consisting of 0.9% sodium chloride solution prior to spreading the monolayer. Hexane was the spreading solvent for cholesterol. The lecithins and all mixed lipid systems were spread from solution in hexane–ethanol (4:1). The lipid solutions were spread on the subphase with a micrometer syringe[6]. Subphase temperature was controlled to within 0.1° with a thermostat[7]. The reproducibility of each $\pi$ reading in replicate experiments at the 95% confidence interval was ±0.5 mN m$^{-1}$ at all areas.

[1] Merck Sharp and Dohme Research Laboratories, West Point, Pa.
[2] Schwarz/Mann, New York, N.Y.
[3] Sylvana Co., Milburn, N.J.
[4] Rosano surface tensiometer, Newark, N.J.
[5] Teflon (du Pont).
[6] Agla, Burroughs-Wellcome, Sheffield, England.
[7] Lauda K-2/R circulator, Westbury, N.Y.

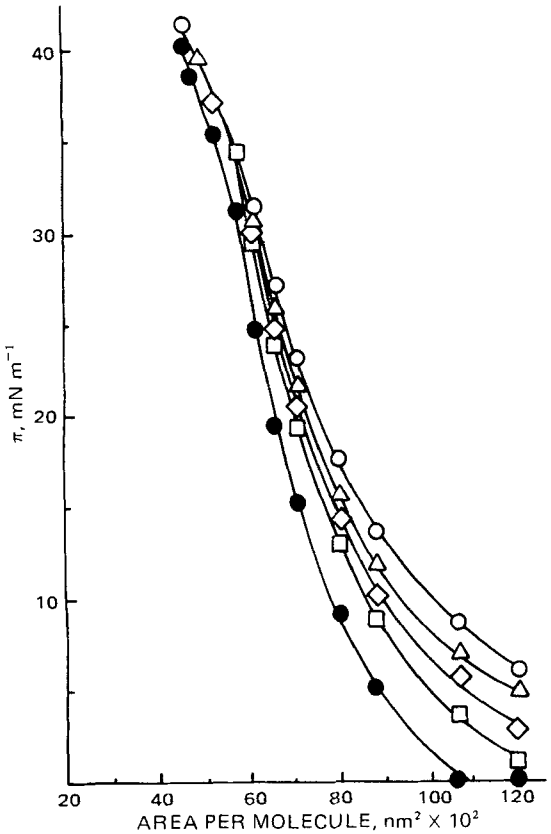


**Figure 2**—*The π–A curves of dipalmitoyl lecithin monolayers spread on a subphase containing dissolved hydrocortisone at 37°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◊, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

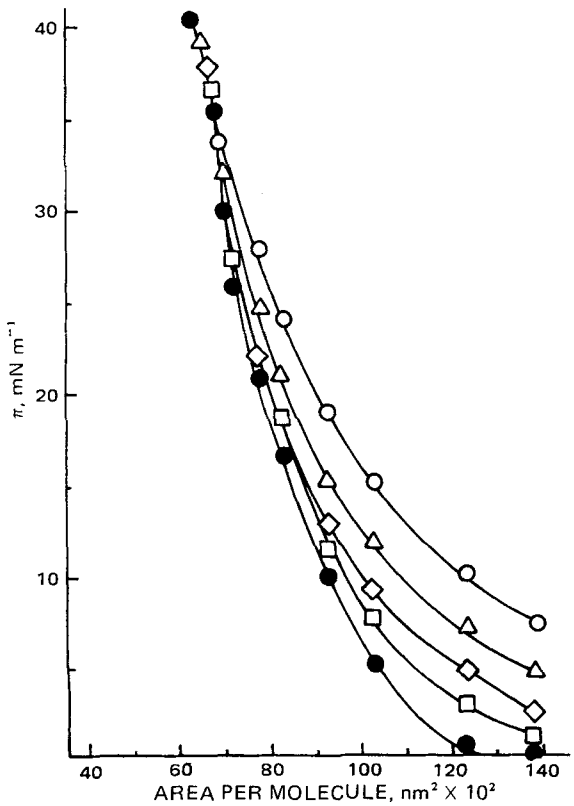


**Figure 3**—*The π–A curves of egg lecithin monolayers on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◊, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

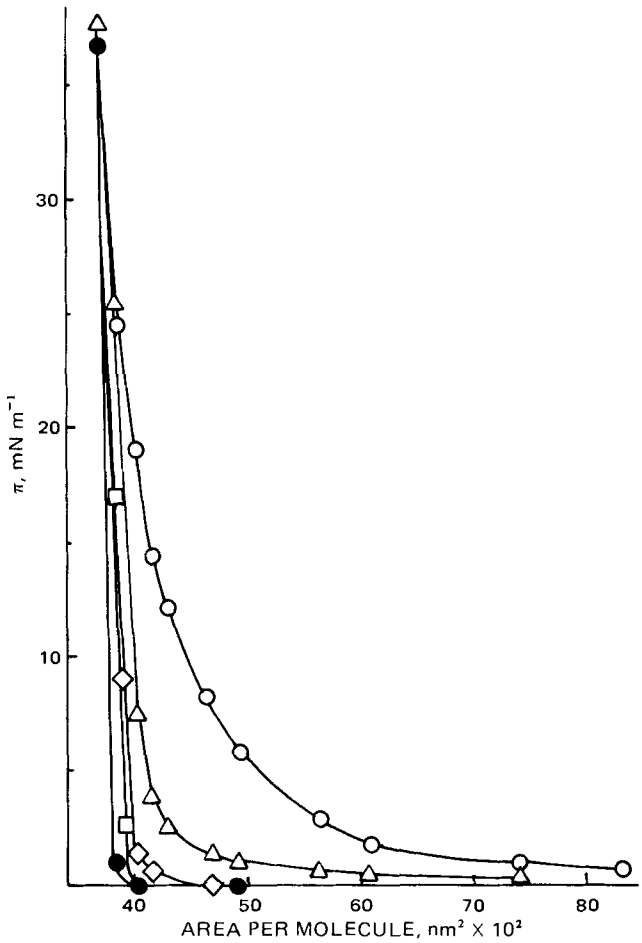


**Figure 4**—*The π–A curves of cholesterol monolayers on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◊, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

## RESULTS AND DISCUSSION

**Single-Component Monolayers**—The properties of dipalmitoyl lecithin monolayers were described previously (17). Surface pressure–surface area ($\pi$–$A$) curves for dipalmitoyl lecithin monolayers on a subphase containing dissolved hydrocortisone at 25° are shown in Fig. 1. The surface pressure of the monolayer was increased in the presence of hydrocortisone if the area per molecule of dipalmitoyl lecithin was greater than about 0.5 nm$^2$. Under these conditions, the lecithin molecules had their hydrated polar groups in close contact while the hydrocarbon "tails" were not tightly packed (17).

The increase in surface pressure that occurred when hydrocortisone was in the subphase was indicative of surface penetration by hydrocortisone molecules. The magnitude of the change in surface pressure was a function of hydrocortisone concentration. As the monolayers were compressed to surface areas smaller than 0.50 nm$^2$/molecule of dipalmitoyl lecithin, the $\pi$–$A$ curves for the systems containing hydrocortisone approached the curve obtained in the absence of the steroid. This finding was interpreted as an expulsion of steroid molecules from the monolayers. The removal of hydrocortisone from the surface coincided with a change in monolayer structure in which the lecithin polar groups underwent a change in hydration and the vertically oriented hydrocarbon tails became closely associated (17). The presence of hydrocortisone did not prevent this transition or alter the surface area at which it took place.

At a subphase temperature of 37°, the transition from an arrangement involving head contact to one of tail contact in dipalmitoyl lecithin monolayers (Fig. 2) took place at a much higher surface pressure than at 25° (17). Penetration of the monolayers by hydrocortisone was, again, a function of subphase concentration of the steroid. As the monolayers were compressed to smaller values of surface area available to the lecithin, the $\pi$–$A$ curves obtained with hydrocortisone present (Fig. 2) were shifted closer to the curve for lecithin alone, suggesting that hydrocortisone molecules were being forced from the surface in response to the change to a state of tail contact in the monolayer.

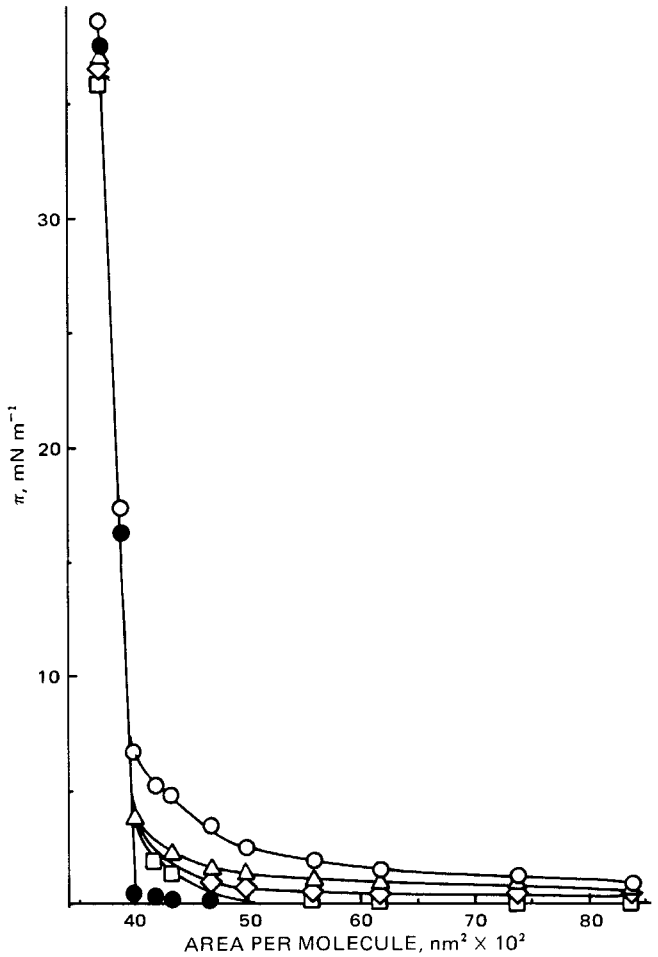


**Figure 5**—*The π–A curves of cholesterol monolayers spread on a subphase containing dissolved hydrocortisone at 37°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◇, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

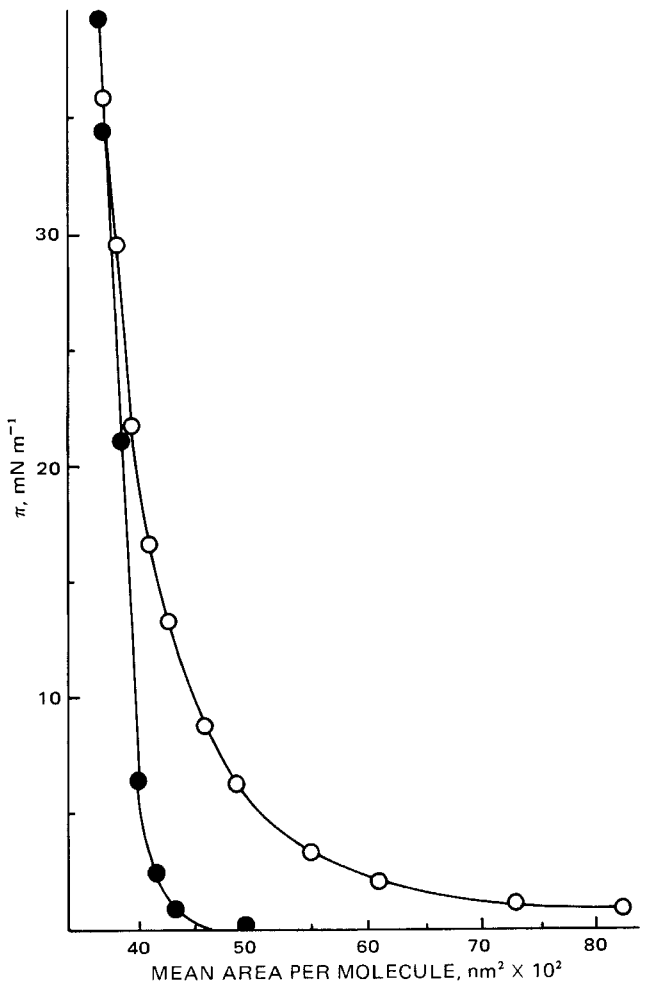


**Figure 6**—*The π–A curves of monolayers of dipalmitoyl lecithin and cholesterol in a 1:2 molar ratio on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

Egg lecithin contains a mixture of phosphatidyl cholines. Approximately 53% of the fatty tails are unsaturated (18). The *π–A* curve (Fig. 3) was of the expanded type, and compression of the monolayer did not force the hydrocarbon groups together until near the collapse point (19). As with dipalmitoyl lecithin, the extent of monolayer penetration by hydrocortisone depended on subphase concentration. Compression caused the *π–A* curves for the systems containing steroid to approach the curve for egg lecithin alone, and all curves merged just before the collapse pressure was reached.

Figure 4 demonstrates the effect of hydrocortisone on cholesterol monolayers at 25°. At the lower concentrations, hydrocortisone caused little change in the *π–A* curve; but at a subphase concentration of 19.7 × $10^{-5}$ *M* hydrocortisone, the surface pressure of the cholesterol monolayer increased significantly. However, this effect may have been due to physical entrapment of hydrocortisone molecules during compression of the cholesterol surface films. Surface potential work showed that some adsorption of hydrocortisone takes place at the air–water interface (13). If desorption were slow relative to compression, some hydrocortisone might remain at the surface even in the absence of an appreciable interaction with the monolayer. The cholesterol monolayers were unstable in the sense that when the surface area per cholesterol molecule was held constant, surface pressure readings dropped with time. In contrast, the surface pressure measurements made on the lecithin monolayers remained stable for at least 20 min whether or not hydrocortisone was present.

At a subphase temperature of 37°, hydrocortisone caused a small increase in surface pressure when the area per cholesterol molecule exceeded 0.40 $nm^2$ (Fig. 5). The condensed portion of the *π–A* curve was unaffected, indicating that hydrocortisone was not retained in the monolayer. These results are in agreement with the report of Gershfeld and Heftmann (10). When the molecular area of cholesterol is greater than 0.40 $nm^2$, the cholesterol molecules exist as individual units or clusters, leaving some aqueous surface available for adsorption of hydrocortisone. Compression forces the cholesterol molecules together (the monolayer becomes coherent) and causes desorption of hydrocortisone.

The incorporation of hydrocortisone into monolayers can be thought of as a distribution phenomenon in which the surface concentration depends on the characteristics of the surface film as well as the bulk concentration of steroid. With a monolayer in a condensed state (with close packed vertical hydrophobic groups), hydrocortisone cannot penetrate between the fatty tails. Hydrophobic interactions between hydrocortisone and the film molecules cannot compete successfully with the interactions between film molecules.

In earlier work on monolayers in which no penetration by steroids was observed (10, 11), the surface films studied were condensed. With expanded monolayers, hydrocortisone can enter the surface under two sets of conditions. First, adsorption occurs at monolayer-free aqueous interfaces so it should also take place when monolayer molecules are widely separated at the surface. Such simple adsorption does not require any interaction with film molecules but can occur only when the monolayer is gaseous, *i.e.*, at surface areas where the monolayer without hydrocortisone is less than 1–2 mN $m^{-1}$. This mechanism appears to be operative in cholesterol monolayers.

The second condition involves penetration of a monolayer in which the film molecules are in contact without tight packing of hydrocarbon tails. The effective molecular area of film molecules is determined by the size of the hydrated polar group. With this arrangement, steroid molecules might displace water of hydration and associate with film molecules. This mechanism appears to be operative in the penetration of lecithin monolayers by hydrocortisone.

**Mixed Monolayers**—Cholesterol causes changes in the molecular organization of dipalmitoyl lecithin monolayers (17). Since the pene-

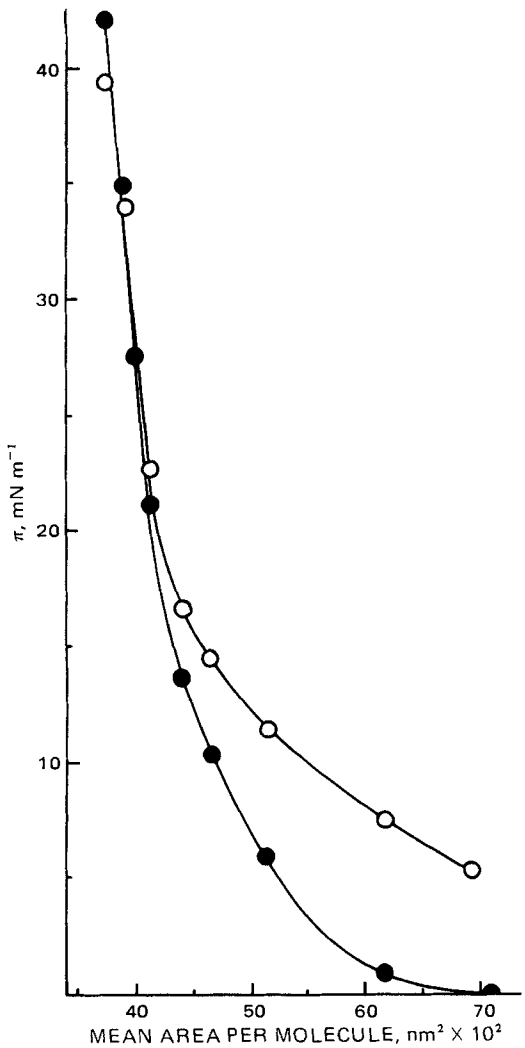


**Figure 7**—*The π–A curves of monolayers of dipalmitoyl lecithin and cholesterol in a 2:1 molar ratio on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; and ○, 19.7 × $10^{-5}$ M hydrocortisone.*

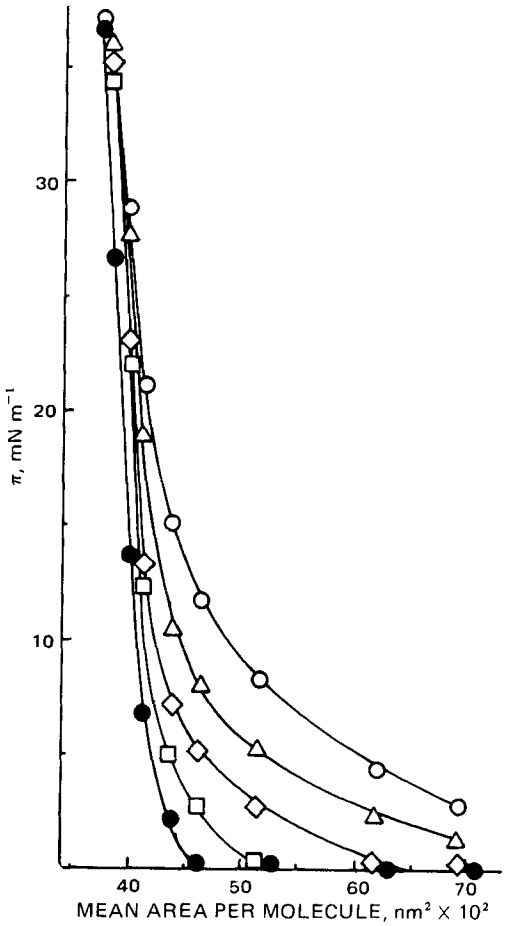


**Figure 8**—*The π–A curves of monolayers of dipalmitoyl lecithin and cholesterol in a 1:1 molar ratio on a subphase containing dissolved hydrocortisone at 25°. Key: ●, no hydrocortisone; □, 2.98 × $10^{-5}$ M hydrocortisone; ◇, 8.20 × $10^{-5}$ M hydrocortisone; △, 13.7 × $10^{-5}$ M hydrocortisone; and ○, 19.7 × $10^{-5}$ M hydrocortisone.*

tration of monolayers by hydrocortisone depends on the monolayer state, it was of interest to see whether the inclusion of cholesterol in surface films of lecithin influences interactions of the model system with hydrocortisone. Accordingly, some experiments were conducted in which mixed dipalmitoyl lecithin–cholesterol monolayers were spread on subphases containing hydrocortisone (Figs. 6–9). Only one subphase concentration was used with the dipalmitoyl lecithin–cholesterol mixed monolayer in a 1:2 molar ratio (Fig. 6). The *π–A* curve obtained with hydrocortisone present was the same as the one produced when the monolayer contained only cholesterol. The influence of the lecithin seemed to be completely swamped.

A single subphase concentration of hydrocortisone was used with the dipalmitoyl lecithin–cholesterol mixed monolayer in a 2:1 molar ratio (Fig. 7). The change in surface pressure due to hydrocortisone was smaller than with a monolayer of pure dipalmitoyl lecithin. The equimolar mixed systems at 25° (Fig. 8) and 37° (Fig. 9) were similar in structure (17). At 25° (Fig. 8), hydrocortisone increased the surface pressure only at mean molecular areas corresponding to a gaseous monolayer, one in which the film molecules are separated from one another. In a coherent system, such as a membrane, no space would be available to steroid molecules. At 37° (Fig. 9), the situation was quite similar, although there was a small region of head contact in the *π–A* curves. All curves met at about 15 mN $m^{-1}$.

Comparison of these results for mixed monolayers with the experiments on single-component lecithin monolayers shows that monolayer penetration by hydrocortisone was diminished effectively in the presence of cholesterol. Previously (20), the addition of cholesterol to lipid dispersions decreased the uptake of hydrocortisone and other steroids by the membranes.

Application of the heads-or-tails model (17, 19) provides an explanation for the effect of cholesterol. In mixed monolayers containing cholesterol and dipalmitoyl lecithin, cholesterol molecules change the packing in the monolayer under conditions where the cholesterol molecules become interspersed among the hydrocarbon tails of the dipalmitoyl lecithin molecules. The molecular assembly is more compact, and the molecular area is determined by the cross-sectional area of the hydrocarbon portion.

The extent of this change in packing depends on the cholesterol content. When cholesterol is the principal component of the mixed systems, all molecules are in tail contact. In the 1:1 mixed system, all molecules are in tail contact over most of the surface pressure range. When dipalmitoyl lecithin is the principal component, some lecithin molecules are in tail contact while a portion are in the same state as they would be in a monolayer of pure dipalmitoyl lecithin at the same surface pressure. Conversion of lecithin molecules from a head contact situation to one of tail contact blocks possible sites of hydrocortisone penetration, making it more difficult for hydrocortisone to enter the monolayer.

**Extrapolation to Biological Membranes**—Although extrapolation from a simple model system to a complex biological system is always hazardous, some general statements seem justified. The ability of hydrocortisone to penetrate lecithin monolayers at 37° that are coherent (but in head contact) suggests that hydrocortisone can associate with lecithin molecules in biological membranes. The uptake by membranes is expected to be dependent on the bulk concentration. Cholesterol monolayers must be in the gaseous state to interact with hydrocortisone. This is not likely in membranes. In mixed monolayers, cholesterol reduces monolayer penetration by hydrocortisone. Membranes containing cholesterol should admit fewer hydrocortisone molecules into the plane of the membrane than those that are free of cholesterol.

**Orientation of Hydrocortisone in Penetrated Monolayers**—The inability of hydrocortisone to be accommodated between hydrocarbon chains in lecithin monolayers whose molecules are in a state of tail contact

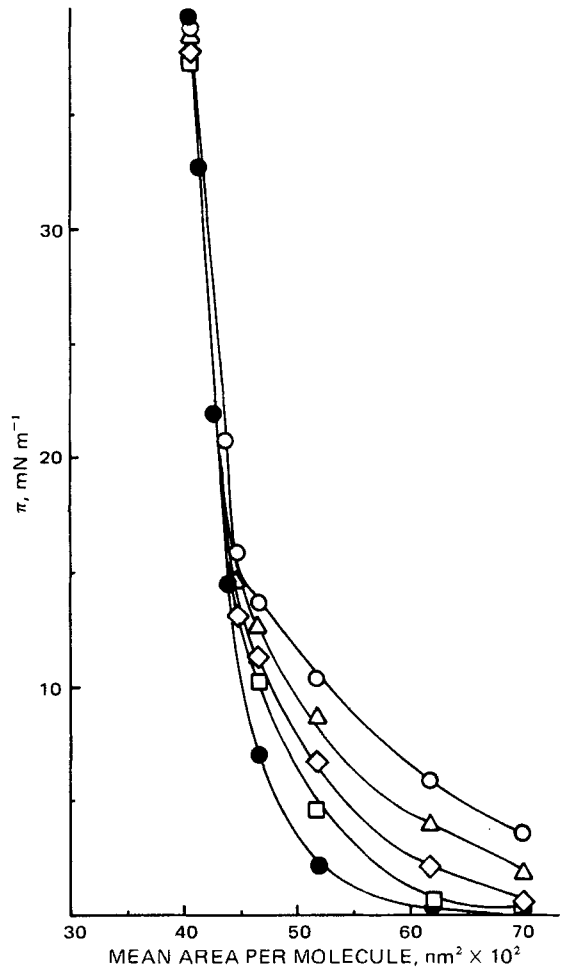


**Figure 9**—*The π–A curves of monolayers of dipalmitoyl lecithin and cholesterol in a 1:1 molar ratio on a subphase containing dissolved hydrocortisone at 37°. Key: ●, no hydrocortisone; □, 2.98 × 10⁻⁵ M hydrocortisone; ◇, 8.20 × 10⁻⁵ M hydrocortisone; △, 13.7 × 10⁻⁵ M hydrocortisone; and ○, 19.7 × 10⁻⁵ M hydrocortisone.*

is in marked contrast to the behavior of cholesterol, which is completely miscible (17). The difference is most likely due to the location of polar groups on the hydrocortisone molecule. Cholesterol has a single polar group at one end of the molecule, allowing orientation at the interface so that the polar group is in contact with water while the hydrophobic portion of the molecule protrudes from the surface. Hydrocortisone has polar groups at both ends of the molecule, thus favoring a horizontal orientation so that all polar groups can remain hydrated. Support for this orientation comes from spin label studies showing that corticosteroids do not orient in the hydrocarbon tail region of lipid bilayers (21). Willmer's theory of steroid hormone activity (1) was based on the notion that steroid molecules would be retained between the phospholipid fatty chains so that the long axis of the steroid molecule would be normal to the surface. The results of this study, therefore, contradict Willmer's hypothesis.

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