

Surface Balance Studies of Bile Acid Monolayers

II. Monolayers of Lithocholic and Glycolithocholic Acids

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Lithocholic and glycolithocholic acid monolayers have been studied with the same experimental technique as described in Part I². The pressure-area curves for both the lithocholic acids differ appreciably from the curves for the cholanic acids, evidently due to the fact that there are two hydrophilic groups in the former and only one in the latter molecules. The molecules lie flat on the substrate surface and on compression the layer is first composed of densely packed lying molecules. When this monolayer collapses, pairs of lithocholic acid molecules, probably with their hydrophilic groups opposite each other, slide up on the monolayer and form a termolecular layer of lying molecules. This triple layer collapses in turn at a somewhat higher pressure. The transformation of the monolayer to a triple layer is accompanied by an increase in internal energy amounting to about 3 000 cal/mole.

Conclusions about the probable arrangement of the bile acid ions in the micelles present in bile salt solutions are drawn from the results of the monolayer studies.

The samples of lithocholic and glycolithocholic acids were synthesized as previously described by one of us (A.N.)¹. The methods used in the investigation of the monolayers have been described in Part I of this series². Chloroform was employed as solvent for the acids. The solution of lithocholic acid contained 2×10^{18} molecules per ml and that of glycolithocholic acid 0.67×10^{18} molecules per ml. Moderate variations in the rate with which the monolayers were compressed was not found to have any influence on the shape of the surface pressure-area curves.

EXPERIMENTAL RESULTS

Figs. 1 and 2 show typical pressure-area curves for lithocholic and glycolithocholic acid monolayers recorded on salt-free and salt-containing substrates of pH 2. In some cases also a 3 M sodium chloride solution saturated with lito-

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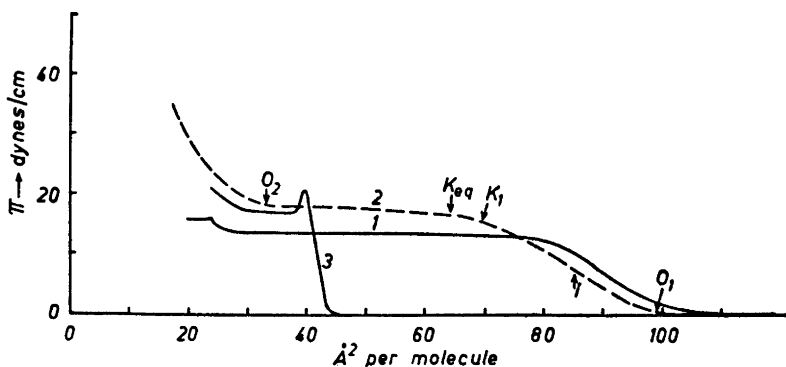


Fig. 1. Typical monolayer pressure-area curves of bile acids. Substrate: water of pH 2. 20°C.

1. Litocholic acid.
2. Glycolitocholic acid.
3. Cholanic acid.

cholic acid was used as substrate; the solubility of the acid is so low that no transfer of the acid from the bulk of the solution to the surface was detected with the surface balance during 3 h; the pressure-area curves for litocholic acid recorded on this substrate were both qualitatively and quantitatively identical with the curves recorded on 3 M sodium chloride without prior saturation of the latter with the acid.

The pressure-area curves of litocholic acid monolayers on 3 M sodium chloride were recorded at various temperatures between 10° and 40°C. Temperature had no effect on the shape of the curve; the area values were almost unchanged, but the surface pressure decreased linearly with increasing temperature (Fig. 3).

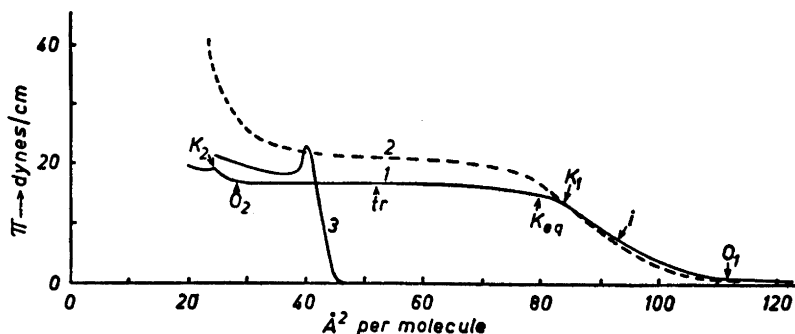


Fig. 2. Typical monolayer pressure-area curves of bile acids. Substrate: 3 M sodium chloride of pH 2. 20°C.

1. Litocholic acid.
2. Glycolitocholic acid.
3. Cholanic acid.

Table 1. Characteristic data for lithocholic acid and glycolithocholic acid monolayers.

Substance	Substrate moles NaCl per litre	Points on the π -A — curve							Physical property
		0	0_1	i	K_1	tr	0_2	K_2	
Lithocholic acid	0	119	107.8	91.3	81.0	51.3	28.7	23.6	Area values, \AA^2 per molecule
› ›	3	121	112.0	93.4	84.0	52.0	29.8	24.5	
Glycolithocholic acid	0	105.2	98.7	86.9	69.6	—	33.8	—	
› ›	3	113.7	106	88.4	76.4	—	43.6	—	
Lithocholic acid	0	0.4	0.6	6.3	12.3	13.8	14.2	16.5	Surface pressure values, dynes per cm
› ›	3	0.5	0.8	8.4	14.0	16.9	17.1	19.8	
Glycolithocholic acid	0	0.4	0.8	6.5	16.6	—	18.1	—	
› ›	3	0.5	0.9	9.7	18.9	—	21.3	—	
Lithocholic acid	0			-0.016					compressibility values $-\frac{\partial A}{A \partial \pi} = -\kappa$ cm per dyne
› ›	3			-0.013					
Glycolithocholic acid	0			-0.018					
› ›	3			-0.014					

The notations used in Fig. 1 to designate various points on the pressure-area curves will be employed in the following discussion. For comparison, the pressure-area curve for cholanic acid has also been drawn in the figures.

Observations made with talcum powder indicated that the viscosities of the surface films of lito- and glycolithocholic acids undergo marked changes at two stages during the compression; at the first stage the film becomes much more viscous and in the second stage it becomes solid.

Characteristic monolayer data for the two bile acids at 20 °C are given in Table 1.

DISCUSSION

The pressure-area curves for the two lithocholic acids differ appreciably from the curves for cholanic acids. The differences are evidently due to the fact that whereas the cholanic acid molecule contains only one hydrophilic group, *viz.* a carboxyl group at the end of the alkyl chain, the lithocholic acids contain an additional hydroxyl group on carbon atom 3 at the opposite end of the molecule. Molecules with two hydrophilic groups separated a longer distance from each other, exhibit a tendency to lie more or less parallel to the substrate surface and generally form monolayers of the gaseous type. The pressure-area curves in Figs. 1 and 2, however, seem to suggest that the lithocholic acids form monolayers of the condensed type on compression.

We have calculated with the aid of Stuart's molecule models the dimensions of the lithocholic acid molecules and the areas the molecules take up when they lie in different positions in densely packed monolayers (Table 2). The largest values in the table give the areas taken up by the molecules when they lie flat on the surface with their longitudinal axes parallel to the surface. The ring skeleton of the molecule is rigid but the alkyl chain can be folded; consequently

Table 2. Molecular dimensions and area requirements calculated from molecular models.

	Length of molecule Å	Area requirement in monolayer					Area requirement with molecules lying on edge in termolecular layer Å ² per molecule
		molecules lying flat Å ² per molecule		molecules on edge Å ² per molecule		molecules vertical, minimum projection area Å ² per molecule	
		max.	min.	max.	min.		
Litocholic acid	18.5	107	85	79	76	44.0	28—25
Glycolitocholic acid	22.0	122	85	93	76	44.0	28—25

it is possible for the area taken up by the molecule to decrease when the monolayer is compressed without the hydrophilic groups losing contact with the substrate surface. This minimum area requirement is also given in Table 2.

A comparison of the experimental data for the litocholic acids and the calculated area requirements shows that the observed value $A_0 = 100\text{--}110$ Å² per molecule closely corresponds to that of a molecule lying flat on the surface. Accordingly, already at the point 0 the monolayer is composed of densely packed molecules lying on the surface. The pressure-area curves exhibit no sharp break point in the range $120\text{--}70$ Å²; the pressure increases slowly and at the inflexion points the monolayers have a compressibility, $-\kappa$, equal to $0.013\text{--}0.018 \times 10^{-3}$ cm/dyne.

The monolayer can be compressed to an area of $85\text{--}65$ Å² per molecule before the curve bends at the break point $K_1\text{--}K_{eq}$ and becomes almost horizontal. The latter area values approximately correspond to the smallest area requirements we have calculated for densely packed molecules which still have both hydrophilic groups in contact with the substrate surface. The high compressibility of the monolayer thus appears to be due to the fact that the molecules, while remaining in the horizontal position, rearrange themselves with increasing lateral pressure so that they first take up a smaller area and are then forced to fold to some extent.

In the relatively wide range of more or less constant surface pressure which follows after the break point at $K_1\text{--}K_{eq}$, the surface film is liquid but highly viscous. In the case of litocholic acid this state of the film prevails until it has been compressed to an area of about 29 Å² per molecule, after which the pressure begins to increase. When the area is decreased to 24 Å² per molecule, the curve shows a sharp break point at K_2 which resembles a normal collapse point, and after this point has been passed the film solidifies. When trying to interpret the pressure-area curve in this range the following facts may be considered. The fact that the area values for litocholic acid did not differ when the substrate was a pure 3 M sodium chloride solution and a similar solution saturated with the acid shows that the form of the pressure-area curve between the points K_1 and K_2 is not due to the transfer of litocholic acid from the monolayer into the substrate. If, after the break point $K_1\text{--}K_{eq}$,

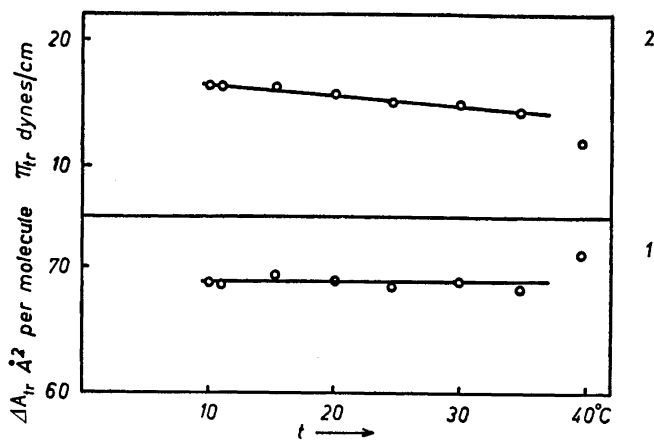


Fig. 3. 1. The change in the molecular area at the transition point, ΔA_{tr} , in the transformation of litocholic acid monolayer into a triple layer. 2. Variation of the surface pressure at the transition point.

larger and larger numbers of the monolayer molecules were forced to assume a vertical position in such a way that one of the hydrophilic groups (*e. g.* the hydroxyl group) is forced away from the substrate surface, this would finally lead to a monolayer composed of molecules in a more or less vertical position. Such a monolayer must, however, collapse after it has been compressed to an area of 44 \AA^2 per molecule (*cf.* Part I²). The experimentally observed value of 24 \AA^2 per molecule is, however, so low that it is impossible that such a monolayer of vertically oriented molecules can exist down to this point. The observed area value is on the other hand in fairly good agreement with the area requirement per molecule in a termolecular layer of molecules lying densely packed on their edges. From experiments performed using the built-up technique it is known that an arrangement of molecules such as that shown in Fig. 4 is very stable. In our case, conditions are furthermore suitable for the formation of hydrogen bonds between the hydrophilic groups when the molecules are arranged in this way. It therefore seems probable that the monomolecular layer "collapses" already at the break point K_1 — K_{eq} , but when doing this pairs of litocholic acid molecules with their hydrophilic groups opposite each other slide up on the monolayer and form a termolecular layer of the type shown in Fig. 4. This transition of the monolayer into a triple layer seems to be reversible. The termolecular film collapses at K_2 ; here we have the real collapse point of the surface layer system. The termolecular film is liquid, but



Fig. 4. Schematic arrangement of molecules in a triple layer of litocholic acid.

after the collapse at K_2 , crystallization occurs, the earlier the lower the temperature.

As mentioned above, variation of the temperature between 10° and 40 °C does not effect any changes in the shape of the pressure-area curve of lithocholic acid (Fig. 3). We are inclined to consider the transition from the liquid monolayer to the liquid triple layer as a transformation of the first order and have calculated the changes in molar heat content, ΔH_{tr} , in the transformation on the basis of the two-dimensional analogue of the Clausius-Clapeyron equation. The values of the equilibrium transition pressure, π_{tr} , and of the net change in monomolecular area, ΔA_{tr} , at the transformation point at different temperatures are given in Fig. 3. For the molar heat content, ΔH_{tr} , we have calculated in this way the value 1 800 cal/mole, and for the net change in the molar internal energy, ΔE_{tr} , we have found values of the order of 3 100—2 900 cal/mole.

The glycolithocholic acid monolayers behave in a similar manner as the lithocholic acid monolayer. The phenomena are not, however, so distinct, primarily because the film becomes solid already before the triple layer collapses, *i. e.* when an area of 30 Å² per molecule is attained. The pressure rises but no collapse is observed to occur.

It may be mentioned that the "collapse" of the monolayers of both lithocholic acids at appreciably lower surface pressures than that where the corresponding cholanic acid monolayer collapses (Figs. 1 and 2) can be ascribed to the prone arrangement of the lithocholic acid molecules on the substrate surface. With this arrangement, the area of contact between the hydrocarbon parts of the molecules is only half that in a monolayer with the molecules in a vertical position. Accordingly the cohesion forces per unit area between the molecules in the monolayer must be weaker in the former case.

The results of our monolayer studies can be used to draw conclusions about the probable arrangement of bile acid ions in the micelles present in bile salt solutions. In cholocate solutions the conditions are probably such that micelles of essentially similar structure as those in the fatty acid salt solutions can be formed, *i. e.* micelles with the long anions arranged more or less parallel in double layers or in cylindrical or spheroidal aggregates in which all the hydrophilic groups face the surrounding water. In lithocholate solutions a different micellar structure is more probable. In the smallest aggregates, it can be expected that the most advantageous arrangement from the energetic viewpoint is one in which 2 to 4 ions lie back to back with the hydrocarbon parts touching each other and the hydrophilic groups projecting outwards. Even larger aggregates may be assumed to be formed in a similar manner, for example, a double layer of ions with contiguous hydrocarbon parts. The size that such an aggregate can attain must however be very limited. When the aggregation proceeds further, a new arrangement must sooner or later result in which a part of the ions turn so that their hydrophilic groups approach each other, in the same way as in the termolecular films (Fig. 4). Ion-dipole interaction may play a role in keeping these anions together. A similar arrangement is possible in the micelles of the desoxycholates and cholates which have, respectively, two and three hydroxyl groups in their molecules. Our discussion thus leads to the conclusion that the association in these bile salt solutions

should result in the formation of different types of aggregates with different properties. This conclusion is to some extent confirmed by experimental findings. Our earlier investigations have revealed that the association in solutions of bile salts (desoxycholates and cholates) proceeds stepwise and that the properties of the micellar substance deviate from each other in different concentration ranges³⁻⁴.

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