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Summary

1. The heat capacity of methylamine has been determined from 11.5°K. to the boiling point. In the range 95 to 175°K. the heat capacity of solid methylamine exhibits hysteresis effects.

2. The melting point of methylamine is 179.70° (−93.46°C.) and the boiling point is 266.84°K. (−6.32°C.).

3. The heats of fusion and vaporization of methylamine are 1465.8 and 6169 cal./mole, respectively.

4. The vapor pressure of liquid methylamine has been determined over the range 190°K. to the boiling point and is represented by the equation:

$$\text{Log}_{10} P_{\text{mm.}} = -(2089.100/T) - 6.05920 \log_{10} T + 2.61668 \times 10^{-4} T - 5.47880 \times 10^{-7} T^2 + 25.44187$$

5. The "spectroscopic" entropy of methylamine calculated from the Raman spectrum and moments of inertia, is 58.06 e. u. per mole at one atmosphere and 266.84°K.

6. The molal entropy of the ideal gas at the boiling point, calculated from the experimental data, is 56.42 ± 0.3 E. u. This value is 1.6 E. u. less than the corresponding "spectroscopic" value.

7. The molal entropies of the superheated liquid and ideal gas at 298.16°K. and one atmosphere are 35.90 and 57.73 E. u. respectively.

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE GENERAL ELECTRIC COMPANY AND FROM THE LABORATORIES OF THE MOUNT SINAI HOSPITAL]

Multilayers of Sterols and the Adsorption of Digitonin by Deposited Monolayers

BY IRVING LANGMUIR, V. J. SCHAEFER AND HARRY SOBOTKA

In a recent note[1] it was shown that digitonin is adsorbed as a visible film on a properly oriented monolayer of cholesterol deposited upon a substrate of barium stearate multilayers of critical thickness. To test the specificity of this reaction we have studied the adsorption of digitonin on some other sterols. We have also built up multilayers of these sterols to determine the thickness of each monolayer and other properties.

Multilayers of Sterols.—The technique for making built-up films was similar to that which has been developed by Dr. Blodgett for barium stearate films.[2,3]

The plates upon which the films were deposited were rectangular pieces of polished chromium-plated brass 1″ × 3″ (2.5 × 7.6 cm.) The surface of the metal was covered with a thin layer of molten ferric stearate which was rubbed vigorously with a clean cloth while cooling. This gives a very uniform monolayer which we shall call the initial layer.

We now select two areas on the plate, which we designate as *blocks*, to be used for two separate purposes. One of these, the *sample block*, is to contain one or more monolayers of the sterol as a "sample" for investigation. The monolayers are deposited upon a *base* consisting of a barium–copper stearate multilayer. The other block, which we shall call the *reference block*, is a barium–copper stearate multilayer to be used as a standard (24.2 Å. per layer) for measuring the thickness of the sample layers. Each block is built up until its thickness lies within a *critical range* giving a minimum in the intensity of reflected polarized light from a sodium vapor lamp at some angle of incidence i, lying between 67–83°, at which angles the intensity of the reflected light is a very sensitive indicator of the thickness of the film.

The *critical thickness* corresponding to the minimum at an observed angle of incidence can be determined, with a sensitivity of about 1.5 Å., by making the film in steps (of about 2 monolayers) and finding the angle i at which adjacent steps match in intensity. The thickness which corresponds to the minimum is the average of the thicknesses of the two steps.

The stearate multilayers which we use for both the reference block and the base layers of the

(1) I. Langmuir and V. J. Schaefer, THIS JOURNAL, **59**, 1406 (1937).

(2) K. B. Blodgett, *ibid.*, **57**, 1007 (1935).

(3) K. B. Blodgett and I. Langmuir, *Phys. Rev.*, **51**, 964 (1937).

TABLE I

OPTICAL MEASUREMENTS OF MULTILAYERS OF STEROLS

Oleic acid piston oil $F = 30$; $N_R = 48$

Substance	i_R, deg.	N_B	N_S	i_S, deg.	t_S, Å.	a_{30}/a_2	R σ_S/σ_W	a_W, sq. Å.	a A. A. D., (sq. Å.)
Cholesterol with $10^{-3}M$ Cu	74	0	66	74	17.35	0.94	(0.99)	35.9	38.3
epi-Cholesterol	74.3	11	49	71.3	17.72	.92	0.98	34.8	
Cholestanol	74.7	11	49	73.0	17.86	.95	1.00	35.3	36.0
epi-Cholestanol	74.5	11	51	79.3	17.65	.93	0.99	35.3	38.3
Ergosterol	74.0	39	13 (X)	78.45	17.85	.86		35.5	36.0
Calciferol	78.3	11	53	77.2	16.58	.78		39.4	41.5

sample block are deposited on the plate as Y-films (one layer on the down trip and one on the up trip, the plate emerging dry, hydrophobic) from monolayers of stearic acid spread on distilled water (from a quartz still) to which 10^{-4} M barium acetate, 2×10^{-4} M potassium bicarbonate, and 2×10^{-6} M cupric acetate have been added (pH 6.9). A surface pressure of $F = 30$ dynes cm.$^{-1}$ is applied to the monolayer by a piston oil consisting of purified oleic acid. The sterol multilayers are built up on the base of the sample block from monolayers spread upon distilled water in equilibrium with the carbon dioxide of the air (pH 5.8) at $F = 30$. Other experiments have shown that the sterol monolayers are not sensitive to changes in pH. Using sodium light polarized with its electric vector perpendicular to the plane of incidence (R_s ray), we find the critical thickness for the reference block to be $N_R = 48$ stearate layers (obtained by steps of 47 and 49 layers, including the initial layer) at the angle i_R given in Table I. This angle varies a few degrees for different plates because of differences in the polishing of the metal or the thickness of the rubbed-down initial layer of ferric stearate.

The sample block was usually made with a base of 11 stearate layers and the sample layers were built in steps (2-layer step interval) of 48 to 54 layers. The angle i_S as given in Table I is the angle at which two adjacent steps match in intensity.

The thickness t_S of the sterol monolayers (in Å.) was then calculated by the equation[4]

$$N_S t_S = 23.86\,(N_R - N_B) + 2.92 \times 10^{-3} N_R\,(\Theta_R^2 - \Theta_S^2) \quad (1)$$

where N_S is the number of sample monolayers in the sample block (average of the two steps which match at angle i_S); N_R and N_B are the number of monolayers in the reference block and in the base layers of the sample block, respectively; Θ_R and Θ_S are respectively the complements of the angles i_R and i_S (expressed in degrees).

This equation is based on a thickness $t_1 = 24.20$ Å., and an index, $n_1 = 1.498$ for the stearate. These are values that Dr. Blodgett has determined recently for the type of reference film we have been using. It also assumes that the refractive index n_S of the sterols (for sodium light) is 1.510, which is the value that Dr. Blodgett has recently found from careful measurements of cholestanol multilayers.

All of the sterols for which data are given in Table I, except cholesterol and ergosterol, can be built up rapidly and without difficulty as Y-multilayers upon a base of barium stearate. There is also no difficulty in depositing barium stearate Y-layers upon multilayers of these sterols.

Monolayers of cholesterol and ergosterol, under the same conditions, do not give Y-multilayers. They can, however, be deposited, slowly and with some difficulty, as X-multilayers: a monolayer being deposited on each down trip, but none on the up trip. These layers (like Y-layers) are dry on emergence from the solution (hydrophobic). We were only able to build 14 layers of ergosterol or 26 layers of cholesterol (as X-layers) before deposition became irregular, as indicated by variations of contact angle of the water solution against the plate during the up trips. With ergosterol we were able to add a sufficient number of barium stearate layers to bring the sample block to the critical thickness.

Cholesterol gave much more trouble, for it proved difficult to start building uniform layers on top of barium stearate or to deposit barium stearate on top of cholesterol layers. Rough preliminary measurements with 15 to 26 deposited X-layers, comparing with a barium stearate color gage by white light, showed that 16 layers of cholesterol produced a color change equal to

(4) The derivation of this equation together with a description of methods of studying monolayers and multilayers will soon be published in THIS JOURNAL.

that given by 12 layers of barium stearate. Such measurements gave $t_S = 17.4 \pm 0.4$ Å.

The data for cholesterol in Table I were obtained by measurement of Y-multilayers of cholesterol that were built up after adding 10^{-3} M cupric chloride to the water. With this amount of copper there is no tendency to form X-layers. We were not able to get uniform deposition of these cholesterol Y-layers on barium stearate nor to deposit barium stearate on Y-multilayers of cholesterol. By using as an initial layer a rubbed-down film of cholesterol we were able to build up 66 very uniform layers.

Although adsorbed copper atoms evidently serve as a cement to hold the A- and B-layers of cholesterol together,[5] it is remarkable that these monolayers contain no measurable amount of adsorbed copper. The test for copper was made by skimming off the monolayer between barriers, transferring it to a plate of white porcelain, squeezing out the water, and melting the compacted skim at 150°. The cholesterol skimmed off a 10^{-3} M cupric chloride solution after melting, was colorless and under a polarizing microscope showed the same type of doubly refracting, needle-like crystals as were given by cholesterol skims from pure water. The cholesterol was burned off at a red heat and left no ash visible under a microscope.

Similar tests with stearic acid showed that with more than 10^{-6} M cupric chloride in the water the skims were distinctly green and left a black lava-like ash of copper oxide which gave the ferrocyanide test for copper after dissolving the ash in hydrochloric acid. All these tests for copper gave negative results with the cholesterol skim.

We were not able to build satisfactory multilayers of cholestene-4,5-one. A monolayer on water would not withstand a pressure of $F = 30$. Using castor oil as piston oil ($F = 15$) multilayers could be built up, but these showed a great deal of light scattering and formed milky films that did not show sharp interference colors, but gave an irregular mother-of-pearl appearance. Evidently the monolayers fold and crumple during deposition. Even with tricresyl phosphate as piston oil ($F = 9$) the same difficulty occurs.

The thickness t_S for a built-up film is not necessarily the same as that of the monolayer from which it is built. It is possible that in the process of transferring the monolayer from the water to the plate the surface density σ of the molecules undergoes a change. To detect such an effect we have made measurements of the area of the monolayer which is used up on the water when a plate is dipped a given number of times. A floating barrier, consisting of a circular waxed paper disk having a diameter only a few mm. less than the width of the trough, was used to separate the sterol monolayer and the oleic acid piston oil. As a "plate" on which to deposit the multilayer we used a chromium-plated cylinder (1" (2.5 cm.) diam.), with vertical axis, which was moved up and down through a definite distance. The displacement of the disk multiplied by the width of the trough gave the area of monolayer consumed. The vertical height of the portion of the cylinder coated by the deposited monolayer was measured after enough layers had been built up to be visible. This height multiplied by the perimeter of the cylinder and the number of one-way trips gave the area built up. The ratio of the area consumed to the area on the plate is called the deposition ratio R and may also be defined by

$$R = \sigma_S/\sigma_W \qquad (2)$$

where σ_S is the number of molecules per sq. cm. in each monolayer on the solid and σ_W is the number per sq. cm. in the monolayer on the water. The eighth column of Table I gives the values of R obtained in three cases. Similar experiments with barium stearate films gave $R = 0.99$.

For the deposited monolayers we also have the relation

$$\sigma_S V_S = t_S \qquad (3)$$

where V_S is the volume per molecule in the dedeposited film. Furthermore, on the water surface we have

$$\sigma_W = 1/a_W \qquad (4)$$

where a_W is the area per molecule at the pressure ($F = 30$) used during the deposition. Thus we obtain

$$a_W = RV_S/t_S \qquad (5)$$

The area a for "cholesterol and other natural sterols" was found by Adam and Rosenheim[6] to be 40.8 sq. Å. at $F = 0$ with a decrease to 40.2 at $F = 20$. We have made determinations (with the circular floating barrier) of the compressibility of the sterol monolayers by apply-

(5) A-Monolayers are those deposited on down trips and B-monolayers those on up trips. This nomenclature was introduced in our study of protein multilayers, I. Langmuir, V. J. Schaefer and D. Wrinch, *Science*, **85**, 76 (1937).

(6) N. K. Adam and O. Rosenheim, *Proc. Roy. Soc.* (London), **A126**, 25 (1930), and **B105**, 422 (1930).

ing a series of piston oils giving $F = 2$, 15 and 30. We find a linear decrease in area as F increases in this range but the compressibility is about three times that given by Adam and Rosenheim. The data in the seventh column of Table I give the ratio of the area at 30 and at 2 dynes cm.$^{-1}$.

X-Ray data for the molecular dimensions of some sterols are given by Bernal and Crowfoot.[7]

For cholesterol, V_S = 630 cu. Å.; c = 19.6 Å.; α = 117°; $c \sin \alpha$ = 17.46 Å. This value of V_S corresponds to a density of 1.011.

For ergosterol, V_S = 640 cu. Å.; c = 19.6 Å.; α = 115°; $c \sin \alpha$ = 17.76 Å.

For calciferol, V_S = 659 cu. Å.; c = 17.8 Å.; α = 95°; $c \sin \alpha$ = 17.76 Å.

It will be noticed that for cholesterol and ergosterol the basal plane spacings ($c \sin \alpha$) are very close to the values of t_S we have found for these substances. This would indicate that the angle of tilt is the same in the built-up films as in the ordinary crystals. In the case of calciferol the value of $c \sin \alpha$ is 7.1% greater than t_S. To bring them into agreement the angle α would need to be 111° which is about the same as in the other two sterols.

We may now calculate a_W (at F = 30) as given in the ninth column of Table I, by equation (5) from the values of V_S and R (we assume that the first four sterols have the same value of V_S).

The last column gives values of a (in sq. Å.), extrapolated to F = 30, from the data of Adam, Askew and Danielli.[8]

A comparison shows that our values of a_W vary much less than the A. A. D. values and average 5% lower. This may indicate that the molecular volume in the deposited films is higher than in crystals, perhaps because of hydration.

Contact Angles of Liquids on Sterol Multilayers.—Drops of water, glycerol and petrolatum were placed on the horizontal plates covered by multilayers of sterols, and the contact angles were observed.

Calciferol gave an angle of 41° with water and 70° with glycerol, but all the other sterols gave over 80° with both liquids. Petrolatum gave 21° with calciferol and angles from 49 to 60° with the others. The plates were then inclined until the drops moved across the plate. It was found that the calciferol under the drop had become hydrophilic (remained wet with either water or glycerol). The other sterols showed large differences between the advancing and receding angles with water, but much less with glycerol. The petrolatum dissolved the monolayers under the drops.

Other portions of the monolayers were then dipped repeatedly into a clean water surface (10 round trips). A stearic acid film (but not a barium–copper stearate film) lost the equivalent of several stearate layers by such treatment. The *epi*-cholesterol and calciferol films lost the equivalent of about 4 stearate layers, but the other sterols showed only slight decreases in thickness.

Adsorption of Digitonin upon a Deposited Monolayer of Sterol.—A plate is prepared having three steps of 45, 47, and 49 layers of barium copper stearate. The angle i_R is measured at which the match between the 47 and 49 layer is obtained. The sterol to be tested is spread on water and subjected to a pressure of 30 dynes cm.$^{-1}$. The prepared plate is then lowered into water through the monolayer and then, while still immersed in water, it is lifted out from the tray by means of a dipper which consists of a small Pyrex beaker provided with a long glass handle. A stream of distilled water pours into the dipper and is allowed to overflow and so remove the monolayer from the surface of the solution. An amount of digitonin is then added to the water in the dipper to give a 0.15% solution. This is stirred for one minute and the plate is withdrawn through a gentle stream of distilled water. The plate is dried, and then the angle i_S is determined at which two steps match. Table II gives the results of the determination of the thickness t_S of the A-layer of sterol with the adsorbed film of digitonin. Calculation was made using equation (1) which assumes that n for the digitonin is the same as for the sterol. It is probable that n does not differ sufficiently from 1.51 to cause serious error.

Blank experiments made without digitonin in the water showed that the A-layers of sterols which were deposited on the down trip did not generally come off during the up trip through a cleaned water surface. In this respect the sterols behave differently from stearic acid, for an A-film of stearic acid deposited upon a barium stearate film escapes completely onto the water surface when the plate is withdrawn from the water. The calciferol A-monolayer, however, was very

(7) J. D. Bernal and D. Crowfoot, *Chem. and Ind.*, **54**, 701 (1935).
(8) N. K. Adam, F. A. Askew and J. F. Danielli, *Biochem. J.*, **29**, 1780 (1935).

TABLE II

OPTICAL MEASUREMENTS OF DEPOSITED A-MONOLAYERS OF STEROL WITH ADSORBED DIGITONIN

$N_R = 48.$; $N_S = 1$

Substance	i_R deg.	N_B	i_S deg.	t_S, Å.	Character of surface	σ_D/σ_S
Cholesterol	72.5	46	72.3	47	Hydrophilic	0.73
epi-Cholesterol	70.25	48	76.3	28	Hydrophobic	.25
Cholestanol	70.6	46	70.8	49	Hydrophilic	.78
epi-Cholestanol	70.7	48	78.2	33	Hydrophobic	.37
Ergosterol	72.3	46	71.2	42	Hydrophilic	.60
Calciferol	70.65	48	72.2	8	Hydrophobic	..

largely lost on the up trip and the others showed (by monochromatic light at the critical angle) a somewhat streaked appearance and a loss of 2 or 3 Å. from the expected thickness of 18 Å. for the monolayer.

The data of Table II show that the *epi*-compounds adsorb very little digitonin as compared with the natural forms of the sterols.

Cholesterol, cholestanol, and ergosterol give hydrophilic films which are completely wet when withdrawn from the water and as the water evaporates interference colors are shown, indicating that there is no finite contact angle between the water and the film. After drying they can be wetted again readily. *epi*-Cholesterol, *epi*-cholestanol, and calciferol, on the other hand, when withdrawn from water are only momentarily covered with a film of water which immediately peels away, showing a definite angle, soon leaving the slide dry without requiring evaporation of the water film. Drops of water placed on these films gave contact angles over 80°. The films are thus hydrophobic.

Each of the hydrophilic films in Table II is much thicker than a sterol monolayer (18 Å.) proving a marked adsorption of digitonin. If we take V_D (assuming a density 1.4 and a molecular weight of 1229), the volume per molecule, to be 1450 cu. Å. for digitonin, we find by equation (3) $\sigma_D = 2.0 \times 10^{14}$ molecules cm.$^{-2}$ for the digitonin adsorbed on the cholesterol. For the sterols $\sigma_S = 2.8 \times 10^{14}$ so that for each cholesterol molecule there is 0.72 molecule of digitonin. The last column of Table II contains these ratios for the other sterols. In the case of calciferol the monolayer was largely lost onto the water surface while withdrawing the plate from the water.

We have also studied the adsorption of digitonin upon B-monolayers of cholestanol and *epi*-cholestanol. In this case we deposited two successive AB layers (two round trips) of the sterol upon a stepped base of barium stearate (43 to 49 layers) and then lowered the plate into water in a small beaker through a cleaned water surface. Digitonin was then added and after one minute was washed out. The results were not appreciably different from those obtained by adsorption on an A-monolayer of sterol.

In the sterol monolayer on water the molecules must be oriented with their OH radicals (hydrophilic ends) in contact with the water. We should therefore expect that the A-monolayer under water has its OH radicals outward—in a position to react with digitonin; but the OH of the B-monolayer is turned inward and should not react with digitonin.

The fact that the A- and B-monolayers behave alike therefore indicates that the individual sterol molecules are being turned over continually (end for end) by thermal agitation. The relative members oriented in one or the other of two directions depend upon the difference of potential energy between the two positions in accord with the Boltzmann equation. The energy of adsorption of the digitonin may thus cause nearly all the sterol molecules in the outside layer to become oriented with the OH radicals outward.[9]

Another indication of this ready overturning of sterol films is the fact that the contact angles of water placed upon A- or B-monolayers are not noticeably different.

We see by Table II that the sterol monolayers which become hydrophilic after immersion in a digitonin solution are those that show the largest thickness of adsorbed digitonin. These data, as well as those obtained with B-monolayers, show, however, that the *epi*-sterols, although they give hydrophobic films, adsorb almost half as much digitonin as the natural sterols.

It is possible that a thorough washing of the film before drying would give a more striking contrast between the adsorption on the hydro-

(9) We have recently found that aluminum or thorium salts can overturn the outer B-layer of barium stearate Y-films and make the surface hydrophilic.

philic and on the hydrophobic surfaces. We have observed, however, that too vigorous washing can cause loss of part of the sterol monolayer. It is desirable to make a much more thorough study than we have yet made of the best technique for washing films on which there are adsorbed layers.

The present evidence is that the *epi*-sterol monolayers do take up an appreciable amount of digitonin which probably results from a penetration of digitonin molecules between and even under the sterol molecules.

Adsorption of Digitonin by Sterol Monolayers on Water.—Schulman and Rideal[10] have shown that when digitonin is injected under a monolayer of cholesterol on water at an initial pressure of $F = 10$ and the area is then kept constant, F increases to over 60, the film becomes solid and there is a change in surface potential.

We have spread monolayers of several sterols on much more dilute solutions of digitonin and have noted the changes in area and surface rigidity. We have deposited the composite films on prepared plates and have measured the thickness and other properties.

Cholesterol, cholestanol, and ergosterol (see Table III) when spread on digitonin solutions of 5×10^{-5} M form such solid films that spreading occurs slowly and with difficulty. A small drop of "indicator oil" (oxidized petroleum oil that spreads to give visible films) applied to the surface tears the monolayer in jagged cracks, indicating rigidity and tensile strength. With the *epi*-compounds and with calciferol the monolayers form rapidly and without difficulty, giving liquid films of low viscosity.

TABLE III

MONOLAYERS OF STEROLS ON DIGITONIN SOLUTIONS (5×10^{-5} M)

Substance	Character of film	$N_{S/S}$, Å.	Contact angle with water
Cholesterol	Solid	53	60
epi-Cholesterol	Liquid	54	>90
Cholestanol	Solid	60	0
epi-Cholestanol	Liquid	59	83
Ergosterol	Solid	54	31
Calciferol	Liquid	46	>90

With a surface viscosimeter[11] consisting of a floating disk one inch (2.54 cm.) in diameter suspended from a tungsten wire attached to a torsion head we have measured the absolute surface viscosities μ_S of monolayers of sterols on pure water and on a digitonin solution (5×10^{-6} M). On pure water all the sterols gave μ_S less than 0.002 g. sec.$^{-1}$, which was about the lower limit of sensitivity of the viscosimeter. On the digitonin solution viscosities of 0.010 and 0.006 g. sec.$^{-1}$ were obtained for monolayers of *epi*-cholesterol and *epi*-cholestanol under a pressure of $F = 15$. The monolayers of cholesterol and cholestanol on the solution gave a viscosity of more than 1.0 within one minute. After five minutes on the surface these films became so rigid that the deflection of the disk produced by one complete turn of the torsion head was less than 0.1°. The fact that the deflection did not increase perceptibly in one minute shows that the surface viscosity had risen to values greater than 2000 g. sec.$^{-1}$—an increase of a million-fold over that observed without digitonin.

The absolute surface elasticity E_S (for shearing stress) was greater than 500 g. sec.$^{-2}$.

The viscosity, elasticity, and maximum shearing stress of films should give quantitative measures of the number and strengths of the cross linkages between molecules in the monolayers. For example, if each digitonin molecule were to combine with a single sterol molecule, the digitonin would be strongly adsorbed; but we should expect no marked increase in viscosity, nor would the film show rigidity or elasticity. The rigid films of the digitonides of the normal sterols thus prove that each digitonin molecule can become firmly attached to at least two sterol molecules and that each sterol molecule can be attached to at least two digitonin molecules. A quantitative, experimental and theoretical study of the mechanical properties of such monolayers will probably give very detailed knowledge of the nature of the interaction between molecules.

A very sensitive test for the adsorption of digitonin by a sterol monolayer on a solution is to skim off the monolayer, transfer it to a glass slide, and observe it with a polarizing microscope after heating to various temperatures.

The skim from a sterol monolayer on pure water has a sharp melting point and shows characteristic doubly refracting crystals (except calciferol which shows no double refraction). The skims obtained from monolayers spread on 10^{-5} M digitonin solution show no double refraction and no definite melting point. Above 250° they show the charring characteristic of sugars. These

(10) J. H. Schulman and E. K. Rideal, *Proc. Roy. Soc.* (London), **122**, 29–45 (1935).

(11) I. Langmuir, *Science*, **84**, 379 (1936).

tests showed that the monolayers of both the natural and the *epi*-sterols adsorb digitonin.

We have found that it is possible to deposit the composite sterol digitonide films on prepared plates. For example, on a down trip an AD film is deposited giving PRAD, where D represents digitonin. To remove the plate from the solution without the deposition of another layer the monolayer on the water must be scraped off before the up trip. If the surface is not scraped, the up trip results in the formation of a hydrous film which may be represented by PRADWDB, W being a layer of water of a thickness of several microns (far too thick to give interference colors with white light).

If another down trip is made before the film is dried, the DB layer returns to the water surface. If, however, the film is dried (dehydrated) at room temperature so as to produce a "dehydrous film" (PRADDB) a second AD layer is deposited on top of the DB film during the next down trip. Repetition of this process, drying after each up trip, permits the building up of multilayers. In these respects the sterol-digitonide monolayers resemble monolayers of proteins.[5]

From the 5×10^{-5} M digitonin solution ADDB layers were deposited on prepared plates and gave for the total thickness the values in the third column of Table III. With cholesterol the digitonide was so rigid that it was only possible to deposit the ADDB layer by cutting the monolayer away from the sides of the trough. The thickness is about the same for the different sterols and is only about 50% greater than that of two sterol monolayers. Since the amount of expansion was not measured in these experiments, the digitonin–sterol ratio cannot be calculated.

The ADDB layers were markedly different in their contact angles and stability toward water. The sterols which gave solid monolayers had low contact angles with water, but those that gave liquid films showed large angles. Dipping the plates in water caused a loss of nearly all of the ADDB film of the *epi*-compounds, but produced no change in the other films.

It thus appears that the monolayers of all the compounds take up digitonin, largely between the sterol molecules, but those that form solid films hold the adsorbed digitonin tenaciously and are stabilized by it, while the others lose their digitonin and are thus easily disintegrated by being raised through a water surface.

With a solution as concentrated as 5×10^{-5} M a very appreciable amount of digitonin may be held in solution in the water layer W of the hydrous film ADWDB and when this is dried it may leave a residue of a thickness of several Å. For this reason and also to avoid the difficulty due to the great rigidity of the films of the digitonides of the natural sterols, some experiments were made with a solution of 10^{-5} M. On this solution monolayers of cholestanol increased in area for about fifteen minutes, reaching a final area per cholestanol molecule of $a = 68$ sq. Å. at $F = 20$ (using a surface balance instead of piston oil). The *epi*-cholestanol spread about 6 times more rapidly and in four minutes reached a limiting value of 74 sq. Å. at $F = 20$.

We then deposited an AD layer which had been on a 10^{-5} M digitonin solution for twenty minutes on a prepared plate and obtained a total thickness of 40 Å. with cholestanol and 39 Å. with *epi*-cholestanol. From these data, by the equation

$$\sigma_S V_S + \sigma_D V_D = t$$

we calculated σ_D and got, for the ratio σ_D/σ_S, 1.5 for cholestanol and 1.6 for *epi*-cholestanol. The film of the latter was very unstable and in places had lost some of its thickness in being taken out of the solution.

In interpreting these high values of σ_D/σ_S it must be kept in mind that digitonin is strongly adsorbed on a water surface even when no sterol monolayer is present. Within a few minutes a 10^{-5} M solution becomes covered by a digitonin monolayer which does not easily go into solution when subjected to pressure. The attempt was made to deposit such films onto a prepared plate. Variable results were obtained (using castor oil as piston oil) giving thicknesses of 4 to 12 Å. for an AB layer. The castor oil piston oil, however, is not satisfactory as it is made solid by the digitonin.

With a still more dilute solution of digitonin, 2×10^{-6} M, the area of a cholestanol monolayer increased much more slowly so that after twenty-one minutes a was only 58 sq. Å. and was still increasing. An ADDB film was deposited and was found to have a total thickness of only 38 Å. which is less than that of the AD film of the previous experiment. The ratio σ_D/σ_S is only 0.62.

In order to obtain further insight into the nature of the forces between sterols and digitonin,

we have attempted to build other types of multilayers of digitonides. A PRAD film, prepared as previously described, was dried and was then lowered into a digitonin solution covered by a sterol monolayer to see if a PRADAD film could be formed. Because of the hydrophilic D-layer the solution ran up on the plate during the down trip, giving a PRADWDA from which the DA layer returned to the water surface against 30 dynes cm.$^{-1}$ pressure. The up trip followed by drying gave the usual PRADDB film.

We then lowered a PRAD film into pure water covered by a sterol film in an attempt to get a PRADA film. We found, however, that no film was deposited on the down trip. The following up trip gave PRADWB and after drying gave PRADB which showed an increase of thickness of 18 Å. over the original PRAD film. We then lowered the PRADB film into clean water with the intention of adding digitonin to the water and so to form a PRADBD film, but we were unable to do this since the B-layer escaped onto the water surface.

In these experiments we found no differences in behavior between the natural and the *epi*-sterols.

We may therefore conclude that the digitonin molecules, although they can form cross-links between natural sterol molecules in a monolayer, cannot serve as links between two separate sterol monolayers. This suggests that the points of attachment to sterol all lie on one side of the digitonin molecule. The digitonin molecules in an AD layer exert a far greater attraction for other oriented digitonin molecules (in an overlying DB layer) than they do for a sterol layer (as in an AD-B film). This is indicated by the fact that water breaks the linkages between AD and B but not between AD and DB.

The adsorption of digitonin on deposited monolayers of various sterols not only illustrates the specificity of the adsorptive forces but also confirms some of our views of the structure of sterols. The specificity of the adsorption of digitonin on deposited layers of natural sterols parallels the affinity between those sterols and digitonin as revealed by the formation of insoluble digitonides. Cholesterol, cholestanol and ergosterol are known to form digitonides in bulk in dilute alcohol; *i. e.*, by bringing together an aqueous solution of digitonin with an alcoholic solution of the sterol, whereas *epi*-cholesterol, *epi*-cholestanol, and calciferol do not. This characteristic property is duplicated in the adsorption experiments. The structural difference between the natural sterols and the *epi*-sterols is due to inversion of the substituents on carbon atom 3. For a number of reasons one assumes that the hydroxyl group on carbon atom 3 points above the median plane of the carbon skeleton in the natural sterols and below in the *epi*-sterols, under the assumption that the angular methyl group on carbon atom 10 is oriented upward.[12]

Although cases are known in which digitonin precipitability depends on other criteria, *e. g.*, on the steric constellation on carbon atom 17, the relative position of the hydroxyl on carbon atom 3 is a valuable guide in most instances and may be correlated to the shape of the molecule as a whole. According to the t_S values given above, the molecule of most sterols in the deposited monolayers is slanted at angles identical with those in the corresponding ordinary (three-dimensional) crystals.

The hydroxyl group at the end of an *epi*-sterol molecule forms an angle with the interface entirely different from the angle in a natural sterol, because of the difference in position in reference to the median plane of the molecule. Let us assume[8] that the natural sterols carry the hydroxyl group "approximately in line with the axis of the molecule," whereas in the *epi*-derivatives it is "almost at right angles to the molecule." The directions of the two valences in reference to the interface differ from each other approximately by the "tetrahedral" angle of 109°, under the assumption that the tilt of the molecule remains essentially the same.

Although the nature of the valence which joins digitonin and sterols is not well known, the above results indicate that digitonin is readily adsorbed onto a sterol monolayer from which hydroxyl groups protrude at a steep angle into the water phase. The number of the adsorbed molecules seems to approach the molecular ratio 1:1, but the observations on mechanical properties suggest that the adsorbed molecules are not definitely correlated or assigned to individual molecules of sterol in the deposited monolayer.

Much less digitonin is adsorbed on deposited A-layers of *epi*-sterols in which the hydroxyl group is assumed in a position about parallel to the interface.

(12) H. Sobotka, "Chemistry of Sterids," Chapter IV, Williams and Wilkins, Baltimore, in press.

The experiments on adsorption of digitonin by sterol monolayers on water are complicated by the "penetration" effect. As the amount of expansion, indicative of this effect, was not measured in those cases in which the thickness could be subsequently evaluated, one cannot yet appraise the possible change of orientation of the sterol due to interspersion of digitonin. However, the results indicate that the high specificity resulting in striking differences between deposited monolayers of digitonide forming sterols and *epi*-sterols is obscured, in the case of monolayers on water, by penetration. Yet the affinity of the natural sterols for digitonin, the greater insolubility of their digitonides (and the resulting tendency toward regular alignment) is reflected by the solid character of the film and by the observed stability on passing through a clean water surface. The slower course of penetration by digitonin and the observation of smaller contact angles with water seem to indicate that the affinity of digitonin for the natural sterols even checks the penetration of the highly surface active saponin toward the surface and perhaps keeps the carbohydrate portion of the molecule on the digitonide–water interface. The adsorption of molecules with such extremely hydrophilic groupings as the numerous hydroxyls of hexose residues onto deposited films by the present method offers new and interesting aspects. The possible "intertwining" action of these hydrophilic portions may account for the unexpectedly low t_S of ADDB layers and for the great tensile strength of such layers.

Calciferol, in which ring II of the cyclopentanophenanthrene skeleton is disrupted, forms monolayers slightly thinner than the isomeric ergosterol. The failure of calciferol to combine with digitonin or to adsorb it on deposited layers may be due either to modification of the nucleus, or, as in the *epi*-sterol, to a suspected epimerization on carbon atom 3 during the first stages of the irradiation of ergosterol.

We are indebted for various specimens to Dr. Erwin Schwenk, Schering Corporation of America, Dr. R. E. Marker of Pennsylvania State College, and Dr. Arthur Knudson of the Albany Medical College. The sterols gave satisfactory tests for purity; only the sample of *epi*-cholesterol contained traces of cholesterol. Calciferol, not a sterol in the strict sense, but used as a control substance, was a crystalline fresh sample of commercial origin.

Summary

Monolayers of several sterols, cholesterol, cholestanol and the corresponding *epi*-sterols ergosterol and calciferol can be built up as multilayers on chromium plates. The thickness per monolayer (16.6 to 17.9 Å.) is about the same as is found by X-ray analysis for 3-dimensional crystals.

Digitonin is adsorbed as a visible film (up to 30 Å. thickness) from a 10^{-3} M solution upon monolayers of cholesterol, cholestanol and ergosterol, but only slightly upon monolayers of the *epi*-sterols or calciferol. The specificity of this adsorption parallels the digitonin precipitability of these sterols.

Digitonin from a substrate solution penetrates between sterol molecules in a monolayer on the surface and causes an expansion to about double area even against a pressure of 30 dynes cm.$^{-1}$. The *epi*-sterols give liquid films which expand rapidly, while the normal sterols give very rigid films which expand slowly. These composite (digitonide) monolayers can be transferred as double layers to a prepared plate giving a total thickness of about 60 Å. The films from the *epi*-sterols are far more unstable than those of the normal sterols and are hydrophobic, while the latter are hydrophilic.

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