[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Interactions between Polycylic Hydrocarbons and Sterols in Mixed Surface Films at the Air–Water Surface

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Preliminary observations on the interactions of carcinogenic and related hydrocarbons with various sterols in mixed surface films of the two classes of substances on water have been reported in two previous papers from this Laboratory.[1,2]

It was found, as anticipated, that the hydrocarbons, owing to their lack of polar groups, displayed no ability to form monomolecular films when used alone. However, when a benzene solution containing any one of a considerable series of four or five ring hydrocarbons and a sterol was deposited on a water surface, the area of the resulting film exceeded that of a control film containing the same number of sterol molecules; this indicated that certain hydrocarbon molecules, in spite of their lack of polar groups, could be held at the water surface by virtue of their attraction for the sterol molecules adjacent to them in the mixed film. An estimate of the magnitude of these attractive forces was obtained by measuring the pressure which had to be applied to the film before the hydrocarbon molecules were squeezed out of their area determining positions.

The tendency toward such interaction between hydrocarbons and sterols was found to vary with the hydrocarbon used, the sterol used, and the hydrocarbon–sterol ratio initially introduced into a given mixed film. Certain hydrocarbons, such as 10-methyl-1,2-benzanthracene, appeared to dissolve in cholesterol or cholestanol films to form simple two-dimensional solutions. Other hydrocarbons, such as 3,4-benzpyrene, exhibited a more complex behavior, suggesting that under certain conditions each molecule of this class becomes associated with a definite number of cholesterol or cholestanol molecules at the moment when the film is formed, this degree of association being maintained as long as the hydrocarbon molecule remains in an area determining position in the film. Still other hydrocarbons, such as phenanthrene, were unable to form mixed films with the sterols.

Although these attractive forces between hydrocarbon molecules and sterol molecules in surface films were relatively small as compared to those involved in maintaining chemical bonds in simple organic molecules, they nevertheless appeared large enough to be of some potential biological significance in the transport of hydrocarbons by body fluids and, by their presence at the time of formation of essential cellular structural components, in causing abnormalities in such membrane and other cell structures as may depend on sterols.

Accordingly, a more intensive investigation of the properties of hydrocarbon–sterol mixed films was undertaken with a view to providing a broader chemical basis for eventually assessing the possible role of hydrocarbon–sterol solutions and of hydrocarbon–sterol molecular associations in the biological effects of carcinogenic hydrocarbons. The present paper presents surface film data obtained from 37 hydrocarbons and 7 sterols used in appropriate combinations. The preliminary interpretation of the structure of such mixed films is confirmed in all essential respects and the conditions leading to the various types of interaction between hydrocarbons and sterols have now been worked out in some detail. A preliminary survey of the effect of a fatty acid, a fat, or a phosphatide as a third component in the hydrocarbon–sterol mixed films has also been made.

Experimental Methods and Materials

Experimental Methods.—The apparatus and procedures for making force–area curves, except for the extensions of the methods reported below, are the same as those used in previous studies.[1]

One method of making force–area curves, referred to here as the P-A method, consists in altering the torsion on the pressure balance by regular small increments and then, at each pressure, finding the area of the film at which the balance pointer gives zero deflection at the end of a one-minute equilibration period. A second method of making force–area curves, referred to as the A-P method, consists in altering the area of the film by regular predetermined increments and then, at each area, finding the pressure at which the balance pointer gives zero deflection at the end of a one-minute equilibration period. A kymographic method of recording force–area curves, equivalent in result to the manual A-P method, also has been developed. The movable barrier is set at successively smaller areas and kept at each area for twenty seconds. The deflection of the film balance pointer is continuously registered on the kymograph (an example is given in Fig. 9). The degree of sta-

(1) G. H. A. Clowes, W. W. Davis and M. E. Krahl, *Am. J. Cancer*, **36**, 98 (1939).

(2) G. H. A. Clowes, W. W. Davis and M. E. Krahl, *ibid.*, **37**, 483 (1939).

bility of the film at each stage in the compression may be qualitatively determined from the constancy of the pressure during the twenty second interval at each area. A decreasing pressure indicates instability of the film.

Most of the experiments were carried out at room temperature within the range 29 ± 4°, the average being 27°. In the experiments concerned directly with the effect of temperature on the films, the temperature was held to within ±1° at 8°, 25° and 40°, the experiments at 8° being carried out in a cold room and those at 25° and 40° with the film tray partially submerged in a bath of water.

Source and Purity of Substances Used.—The sources and melting points of the sterols used are given in Table I; similar data for the hydrocarbons are given in Table II. The melting points were taken in duplicate in this laboratory with Anschütz thermometers. These determinations were made after the film experiments had been carried out, thus eliminating in the film studies any error due to decomposition beyond that indicated by the melting points.

Many of the hydrocarbons and sterols are highly sensitive to the action of light. To protect them, the method used by Dr. Fieser for shipment and storage was adopted. Wooden blocks 5 × 2 × 2 and 5 × 2 × 1 inches were bored to accommodate vials or bottles of hydrocarbons with as little free space as possible. These were made deep enough so that a wad of cotton could be placed in the bottom, the vial or bottle inserted, and the hole closed with a tight-fitting cork stopper. Under these conditions of storage the substances were not subject to photodecomposition except as a result of the infrequent short exposures when they had to be removed for sampling. The purification of ergosterol, as well as all film experiments with it, was carried out at night in artificial light, previously shown to cause no photodecomposition of the ergosterol or the hydrocarbon used with it.

Calculation of Molecular Areas for Hydrocarbons.—The A segment (Figs. 2, 10 and 2, 19) of each mixed film curve was extrapolated to zero pressure. This gave the area of a mixed film containing a known number of hydrocarbon molecules mixed with a known number of sterol molecules. The area of the control film containing the same number of sterol molecules was then subtracted from the total area. The area value obtained as remainder, when divided by the number of hydrocarbon molecules in the film, yielded the area per hydrocarbon molecule. This calculation, for reasons set forth later, was performed only for films having an A segment in the P-A curve. Here the additional area due to the hydrocarbon molecules was found to be proportional to the amount of hydrocarbon incorporated with the sterol in the film.

Experimental Results and Interpretations

Structure of Pure Sterol Films.—Previous studies[3] of surface films of sterols have indicated that each molecule of sterol in a monolayer on water is oriented with its hydroxyl group (in the 3 position) in contact with the water, the remainder of the molecule projecting upward with the side chains forming an upper layer immediately above the lower area determining layer of closely packed ring systems.

The areas occupied on the water surface at zero pressure by each of 13 sterols and sterol esters are given in Table I.

From these molecular areas it is evident that the configuration and degree of saturation of the sterol ring systems and the *cis* or *trans* arrangement of the OH at C_3 with respect to the CH_3 at C_{10} are more important than the configuration of the side chain at C_{17} in determining the area occupied by each sterol molecule at the water surface. The flexible alkyl side chains of the sterol molecules at C_{17} apparently can be readily accommodated in the space available above the area determining ring systems. Each of the sterols listed in Table I has two methyl groups which project nearly at right angles, at the C_{10} and C_{13} positions, from the same side of the virtually plane ring systems of the sterol molecules,[4] with the exception of neoergosterol which has only one located at C_{13}. The two sides of the ring system may be distinguished as methylated and non-methylated faces. The side chain at C_{17} emerges nearly at right angles from the non-methylated side. It is evident that the van der Waals attraction between adjacent sterol molecules in the film may vary according to whether the sterol molecules in the film are regularly oriented with like faces of adjacent molecules in contact, are regularly oriented with unlike faces in contact or have a random orientation in this respect. No evidence is at present available for deciding between these possibilities. For the present it may be assumed that all possible mutual arrangements occur in a proportion which can be predicted from the Maxwell-Boltzmann distribution law when and if the proper energetic factors become known.

From the data of Table I it may be noted that the area per sterol molecule in the crystal, as calculated from X-ray data, is smaller than that in the films, indicating that the perfection of packing of the sterol molecules in the film is not as great as in the crystal.

(3) N. K. Adam, "The Physics and Chemistry of Surfaces," second edition, Clarendon Press, Oxford, 1938, pp. 79–85.

(4) H. Sobotka, "The Chemistry of the Sterids," Williams and Wilkins Co., Baltimore, Md., 1938, pp. 48–68.

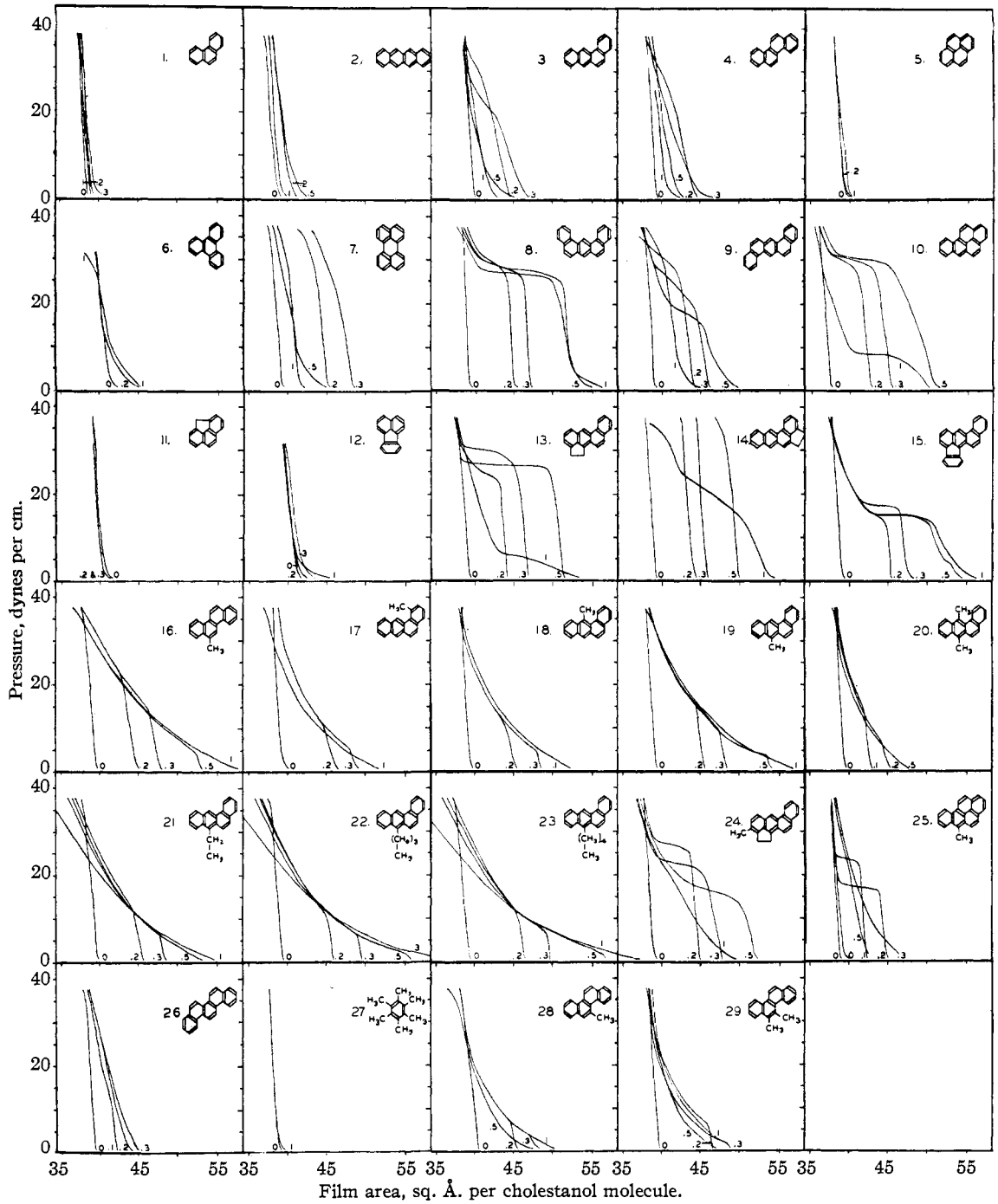

Fig. 1.—P-A (pressure–area) curves for mixed films of various hydrocarbons with cholestanol. The areas are given as sq. Å. per cholestanol molecule. The decimals accompanying the individual curves give the number of hydrocarbon molecules per cholestanol molecule initially spread in the film. The names of the compounds are found by reference to the key numbers of Table II.

Force–Area Data on Hydrocarbon–Sterol Mixed Films.—The force area characteristics of the mixed films were determined principally by the P-A method which measures the film area required to bring the film pressure to a predetermined value.

P-A curves for each of 29 hydrocarbons with cholestanol (β-dihydrocholesterol) are shown in Fig. 1. Data for naphthalene and anthracene are

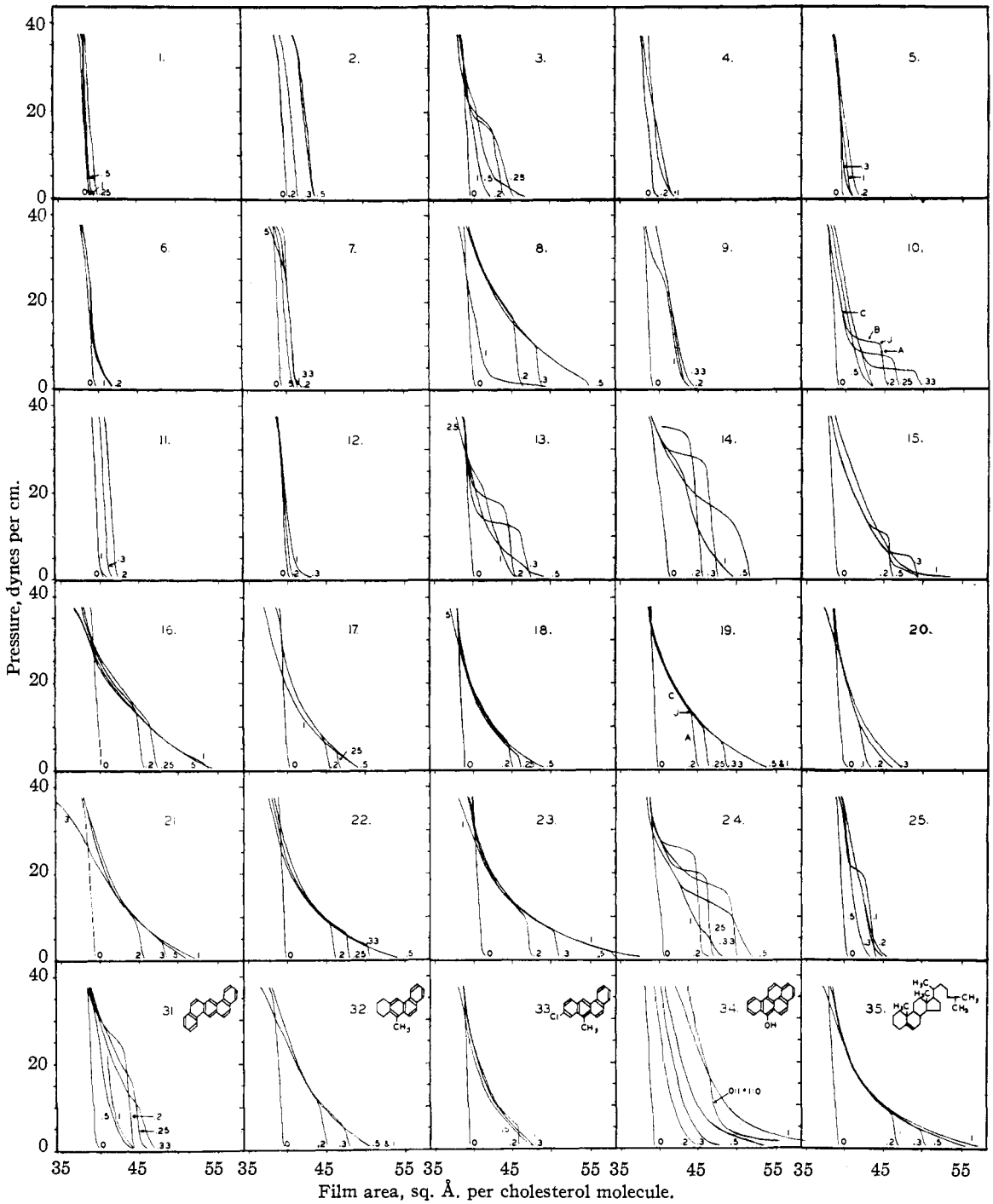

Fig. 2.—P-A (pressure–area) curves for mixed films of various hydrocarbons with cholesterol. The areas are given as sq. Å. per cholesterol molecule. The decimals accompanying the individual curves give the number of hydrocarbon molecules per cholesterol molecule initially spread in the film. The names of the compounds are found by reference to the key numbers of Table II.

not included because they, like phenanthrene, do not assume area determining positions in the cholestanol film.

P-A curves for each of thirty hydrocarbons with cholesterol are shown in Fig. 2. Data for naphthalene and anthracene are again omitted because they do not assume area determining positions in the cholesterol film.

TABLE I

CROSS SECTIONAL AREAS OF STEROLS AND STEROL ESTERS AS DETERMINED BY SURFACE FILM MEASUREMENTS, WITH X-RAY DATA FOR COMPARISON

Sterol or ester	Source[a]	M. p., °C.	Area, sq. Å. — Surface film data — Present work		Other sources	X-Ray data
Cholesterol	W-P	144.9–145.5	39.0	(31)[b]	40.5[d]	36.1[f]
Cholesterol acetate	EL-K	133.0–133.7	39.7	(3)		
Neoergosterol	RM-P	152.5–153.3	40.7 & 35.9[e]	(6)		
Cholestanol	EL-P	140.3–141.1	39.0 at 8°	(10)	38.8[d]	
			38.7 at 25°	(24)		
			40.3 at 40°	(10)		
Cholestanol acetate	IP	105.4–107.3	40.8	(2)		
epi-Cholestanol	RS	183.8–184.5	40.1	(6)	40.8[d]	
Ergosterol	PF-F	155.4–157.1	38.3	(6)	37.5[d]	36.1[f]
					37.5[e]	
Lumisterol					41.0[e]	37.0[f]
Calciferol					46.0[e]	37.2[f]
Stigmasterol	HLR	168.4–169.5	37.8	(2)		
Sitosterol (Cottonseed)	HK-T	136.6–137.1	38.2	(4)		
Coprostanol	EL-K	98.7– 99.9			41.8[d]	
epi-Coprostanol					43.8[d]	

[a] Sources of these substances are designated as follows: W-P, the Wilson Co., recrystallized from 95% alcohol; EL-K Dr. E. C. Kleiderer, Eli Lilly and Co.; RM-P, Dr. Russell E. Marker, Penn. State College, recrystallized from 95% alcohol; EL-P, prepared and purified in this laboratory; IP, Dr. Irvine H. Page, Indianapolis City Hospital; RS, Dr. R. Schoenheimer, Columbia University; PF-P, The Pfanstiehl Co., recrystallized at night from 95% alcohol; HLR, Hoffman LaRoche, Inc.; HK-T, Dr. Thornton, supplied by Dr. H. R. Kraybill, Purdue University. [b] The figure in parentheses gives the number of determinations of molecular area. The average value and the maximum observed deviation are given. In the previous preliminary publication the areas given for sterols and hydrocarbons were too large due to two causes: (1) an error made in calibrating the constant volume pipet which was used to deliver benzene solutions of film forming substances onto the surface; and (2) a further small error due to traces of adherent moisture in the sterol, which has been corrected by drying the sterols thoroughly. [c] The two values are obtained by extrapolating each of the two nearly perpendicular segments of the neoergosterol curve (Fig. 3, 10 and 19) to zero pressure. [d] Values on 0.02 *N* HCl from N. K. Adam, F. A. Askew and J. F. Danielli, *Biochem. J.*, **29**, 1786 (1935). [e] Values on 0.01 *N* HCl from J. F. Danielli and N. K. Adam, *Biochem. J.*, **28**, 1583 (1934). [f] J. D. Bernal and D. Crowfoot, *Chemistry and Industry*, **54**, 701 (1935). These are calculated as ab/n giving the area per molecule in the ab plane. These areas may differ from the minimum cross-section if the molecule is not perpendicular to the ab plane in the crystal.

P-A curves for representative hydrocarbons with ergosterol, neoergosterol, sitosterol, *epi*-cholestanol, coprostanol and stearic acid are shown in Fig. 3.

Data obtained by the manual A-P method were less significant than those obtained by the kymographic A-P method. Kymographic A-P families of curves for two systems are reproduced in Fig. 9.

Most of the hydrocarbons contribute, at zero pressure, to the area of the mixed films. Since the hydrocarbons contain no hydrophilic groups and have no tendency, when alone, to spread on the water surface, hydrocarbon molecules can be held in area determining positions only by attraction for the sterol molecules of the film.

Dr. I. Langmuir has suggested that the inability of naphthalene, phenanthrene, and anthracene to enter the films may be due to evaporation of these substances before the P-A measurements can be made. To test the possibility that the failure of these three substances to occupy area determining positions might be due to escape of the hydrocarbon from the air–water surface by evaporation or by solution, mixtures of naphthalene or phenanthrene with cholestanol were spread on water saturated with the hydrocarbon and in contact with air which was also saturated with the hydrocarbon. Even under such conditions, these two hydrocarbons failed to assume area determining positions in the cholestanol film.

Two-Dimensional Hydrocarbon–Sterol Solutions (Type S Films).—The first, and most general, type of P-A curve is illustrated by the data for 10-methyl-1,2-benzanthracene and cholesterol (Fig. 2, 19). This case includes those families previously[1] referred to as class 2 and some of those previously referred to as class 3. At zero pressure the area of the mixed film exceeds that of

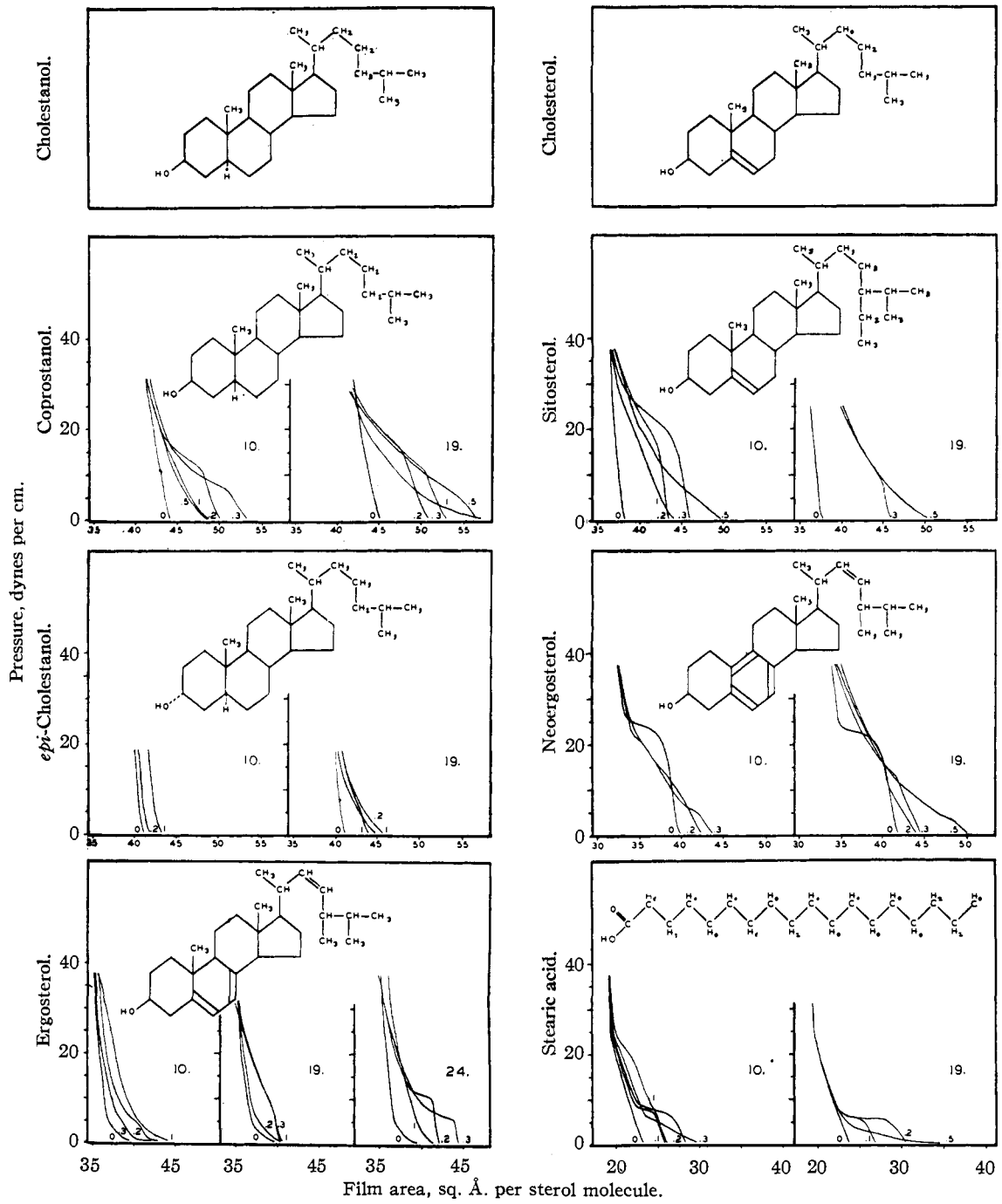

Fig. 3.—P-A (pressure–area) curves for mixed films of representative hydrocarbons with *epi*-cholestanol, coprostanol, ergosterol, neoergosterol, sitosterol and stearic acid. The areas are expressed as in Figs. 1 and 2. The names of the hydrocarbons are found by reference to the key numbers of Table II. The structural formulas for the sterols correspond to the names along the ordinates of the individual figures.

the pure sterol film by an amount which is proportional to the hydrocarbon content of the film, showing that all, or a constant fraction, of the hydrocarbon molecules originally spread with the sterol have taken area determining positions in the film. If all of the hydrocarbon is in such area determining positions, the area occupied by each hydrocarbon molecule can be calculated by dividing the increase in area of the film upon introduction of hydrocarbon by the number of hydrocar-

TABLE II

MOLECULAR AREAS OCCUPIED BY HYDROCARBONS IN MIXED FILMS WITH STEROLS AT ROOM TEMPERATURE

Key no.	Hydrocarbon	Source[a]	M. p., °C.	In mixed films with cholesterol: Interaction type[b]	In mixed films with cholesterol: Area, sq. Å.	In mixed films with cholestanol: Interaction type	In mixed films with cholestanol: Area, sq. Å.
36	Naphthalene	EK-168	80.3–81.3	N	...	N	...
37	Anthracene	EK-450x	215.6–216.5	N	...	N	...
1	Phenanthrene	EK-599	100.0–100.9	N	...	N	...
2	Naphthacene	LF-N	341.5–343.0	N	...	N	...
3	1,2-Benzanthracene	LF-H	161.4–161.8	A	22.7 ± 1.7 (3)[c]	A	24.2 ± 1.1 (4)
4	Chrysene	LF-N	253.2–253.8	?	20.6 ± 0.2 (2)	A	25.4 ± 0.6 (3)
5	Pyrene	LF-H	149.6–150.5	N	...	N	...
6	Triphenylene	LF-H	197.5–197.9	N	...	?	...
11	4,5-Methylenephenanthrene	LF-H	114.6–115.3	N	...	N	...
12	Fluoranthene	LF-H	110.0–110.7	N	...	N	...
7	Perylene	LF-N	277.9–279.1	N	...	A	28.3 ± 1.4 (4)
8	1,2,7,8-Dibenzanthracene	MSN	198.0–198.4	S	30.6 ± 1.4 (6)	A	27.5 ± 1.2 (6)
9	1,2,5,6-Dibenzanthracene	EK-P	266.6–266.9	?	29.2 (1)	A	25.3 ± 3.4 (4)
9a	1,2,5,6-Dibenzanthracene	HLR	262.7–264.0	?	27.5 ± 1.7 (2)		...
31	1,2,5,6-Dibenzanthracene	EK-3272	255.6–258.0	A	24.0 ± 0.6 (4)		...
10	3,4-Benzpyrene	HLR	176.3–177.0	A	30.8 ± 1.6 (6)	A	25.8 ± 1.0 (6)
26	Picene	MSN	363.5–364.5		...	?	...
13	Cholanthrene	LF-H	170.1–170.6	A	26.6 ± 1.3 (2)	A	24.4 ± 0.6 (3)
14	3,4′-Ace-1,2-benzanthracene	LF-H	233.1–234.3	A	21.4 ± 0.5 (3)	A	23.3 ± 2.4 (3)
15	15,16-Benzdehydrocholanthrene	LF-H	180.2–180.6	A, S	35.4 ± 0.6 (2)	A	30.1 ± 1.8 (3)
28	5-Methylchrysene	MSN	117.3–117.7		...	S	29.6 ± 0.4 (2)
16	6-Methylchrysene	LF-N	160.4–160.9	S	28.6 ± 1.2 (2)	S	27.6 ± 1.9 (6)
29	5,6-Dimethylchrysene	MSN	128.5–129.2		...	S	31.5 ± 3.6 (4)
17	1′-Methyl-1,2-benzanthracene	LF-H	138.5–139.0	S	26.6 ± 0.1 (2)	S	...
18	9-Methyl-1,2-benzanthracene	LF-N	138.0–138.8	S	29.6 ± 1.3 (4)	S	29.4 ± 1.4 (4)
19	10-Methyl-1,2-benzanthracene	LF-H	140.0–140.5	S	26.0 ± 2.0 (4)	S	27.5 ± 4.2 (17)
21	10-Ethyl-1,2-benzanthracene	LF-H	112.4–112.8	S	31.5 ± 0.2 (2)	S	29.1 ± 0.4 (2)
22	10-Butyl-1,2-benzanthracene	LF-H	96.4–96.7	S	36.4 ± 0.9 (4)	S	33.7 ± 1.1 (3)
23	10-Amyl-1,2-benzanthracene	LF-H	82.6–83.3	S	36.6 ± 0.4 (3)	S	36.2 ± 1.2 (5)
32	Tetrahydro-10-methyl-1,2-benzanthracene	LF-H	73.7–74.5	S	28.8 ± 1.2 (3)	S	29.2 ± 1.0 (2)
33	6-Chloro-10-methyl-1,2-benzanthracene	LF-N	157.4–157.7	S	31.7 (1)	S	31.3 ± 1.3 (4)
20	9,10-Dimethyl-1,2-benzanthracene	LF-W	122.6–122.9	S	31.4 ± 1.6 (2)	S	32.0 ± 2.8 (3)
24	20-Methylcholanthrene	HLR	175.3–177.1	A	28.5 ± 1.9 (6)	A	27.9 ± 2.6 (7)
25	5-Methyl-3,4-benzpyrene	LF-H	216.6–217.3	A	36.1 ± 0.0 (2)	A	33.1 ± 2.9 (3)
35	Cholestene	EL-P	88.4–89.1	S	37.1 ± 0.4 (6)		...
27	Hexamethylbenzene	EK-2294	159.0–162.0	N	...	N	...
	In mixed films with				Sitosterol		Coprostanol
10	3,4-Benzpyrene			A	27.3 ± 0.2 (2)	A	31.4 ± 0.2 (8)
14	3,4′-Ace-1,2-benzanthracene			A	26.5 (1)		...
19	10-Methyl-1,2-benzanthracene			S	27.3 (1)	S	27.9 ± 3.1 (3)
	In mixed films with				*epi*-Cholestanol		Stearic acid
10	3,4-Benzpyrene			N	...	A ?	27.5 ± 0.8 (2)
19	10-Methyl-1,2-benzanthracene			S	28.2 ± 0.9 (2)	A ?	...
	In mixed films with				Ergosterol		Neoergosterol
10	3,4-Benzpyrene			N	...	A	31.9 ± 3.3 (2)
24	20-Methylcholanthrene			A	29.3 ± 1.9 (2)		...
18	9-Methyl-1,2-benzanthracene					S	...
19	10-Methyl-1,2-benzanthracene			N	...	S	27.9 ± 1.9 (3)

[a] The sources of substances are designated as follows: EK, Eastman Kodak Company, the catalog number being given EK-P, Eastman Kodak Company, further purified in this Laboratory; LF-N, Dr. M. S. Newman, from laboratory of Dr. L. F. Fieser, Harvard University; LF-H, Dr. E. B. Hershberg, from laboratory of Dr. L. F. Fieser; LF-W, Dr. T. L. Webber, from laboratory of Dr. L. F. Fieser; MSN, Dr. M. S. Newman, Ohio State University; HLR, Hoffman La-Roche; EL-P, prepared and purified in this Laboratory. [b] A indicates that the interaction is predominantly of the as-

sociation type. S indicates that the interaction is predominantly of the solution type. A, S indicates that both types of interaction are exhibited with association type predominating. ? indicates that classification of interaction type cannot be determined from present data. N indicates that the expansion due to the presence of hydrocarbon is zero or very small and not proportional to the amount of hydrocarbon present, even at the lowest concentration of hydrocarbon used. [c] The figure in parentheses gives the number of determinations of molecular area. The average value and the maximum observed deviation are given. As noted earlier (footnote *b*, Table I) these values are lower than previously published values which were in error.

TABLE III

MOLECULAR DIMENSIONS, MOLECULAR AREAS IN THE *ab* CRYSTAL PLANE, AND MOLECULAR CROSS-SECTIONS IN THE PLANE PERPENDICULAR TO THE γ OPTICAL DIRECTION, (THE PROBABLE LONG AXIS OF THE MOLECULES), CALCULATED FROM X-RAY AND OPTICAL DATA OF BERNAL AND CROWFOOT[5] AND IBALL[6]

Hydrocarbon	Ref.	Molecular dimensions, Å. Length	Width $= b$	Thickness $a \sin \gamma/n$	Molecular area in *ab* crystal plane ab/n, sq. Å.	Molecular cross-section in plane perpendicular to γ axis $ab \sin \gamma/n$, sq. Å.
Phenanthrene	5	9.62	6.11	4.28	26.5	26.2
Fluorene	6	9.43	5.70	4.23	24.2	24.2
1,2-Dimethylphenanthrene	5	10.9	6.35	4.14	26.3	26.3
1,2,7-Trimethylphenanthrene	5	12.2	6.4	3.95	25.3	25.3
Retene	5	11.7	6.25	4.27	26.7	26.7
1,2-Cyclopentenophenanthrene (Stable)	5	11.6	6.05	3.73	27.9	22.8
1,2-Cyclopentenophenanthrene (Metastable)	5	11.4	6.4	4.05	26.0	26.0
Diels' hydrocarbon, $C_{18}H_{16}$, (γ-methylcyclopentenophenanthrene)	5	12.1	6.25	4.25	26.6	26.6
Chrysene	5	11.4	6.18	4.02	25.8	24.0 (25.4)[b]
3,4-Benzpyrene (orthorhombic)	5	11.19	..	..[a]	29.1	29.1 (25.8)
Methylcholanthrene	5	13.7	..	..[a]	27.7	24.7 (27.9)
Methylcholanthrene	6	13.85	..	..[a]	27.5	24.5
1,2,5,6-Dibenzanthracene	5	13.75	6.59	3.92	25.8	25.8 (25.3)
5,6-Cyclopenteno-1,2-benzanthracene	6	13.80	..	..[a]	25.8	25.0
Picene	5	14.4	6.16	4.10	25.3	25.3
Diels' 2nd hydrocarbon, $C_{25}H_{24}$	5	17.2	6.16	4.28	33.9	26.3
Cholesterylene	5	20.5	..	..[a]	30.4	30.4
Cholestane	5	20.5	..	..[a]	30.8	30.7

[a] In these cases the b and $a \sin \beta/n$ dimensions do not represent molecular width and thickness, respectively. [b] Area in mixed films of hydrocarbon and cholestanol (see Table II).

bon molecules introduced. Molecular areas calculated in this way are given in Table II. These molecular areas of the hydrocarbons are, in general, somewhat higher than the cross-section areas, calculated from X-ray data on such hydrocarbons in the crystalline state,[5,6] presented for comparison in Table III.[7]

The area increase per hydrocarbon molecule is, with the S type of mixed films under consideration, essentially constant with increasing hydrocarbon content.

As the pressure is increased, the film area remains virtually unchanged along segment A of each curve, showing only the compression characteristic of the pure sterol film. Beyond the point J indicated in Figure 2, 19, further increase in pressure along segment C produces a gradual decrease in total film area. For films of increasing hydrocarbon content the C segments of the individual curves overlap exactly to form a common envelope for the family made up of curves of constant sterol and varying hydrocarbon content.

This decrease in film area with increasing pressure apparently indicates removal of hydrocarbon molecules from positions between the sterol molecules to non-area determining positions in an excess phase outside (but capable of very close contact with) the film.

At any given pressure value along the common envelope of the family of P-A curves for 10-methyl-1,2-benzanthracene and cholesterol, the number of 10-methyl-1,2-benzanthracene molecules retaining area determining positions in the cholesterol film is fixed and is independent of the initial concentration of the hydrocarbon in the film. As shown by the curves in Fig. 4, which are essentially rectilinear at low pressures, the logarithm of the mole fraction of hydrocarbon in the

(5) J. D. Bernal and D. Crowfoot, *J. Chem. Soc.*, **138**, 93 (1935).
(6) John Iball, *Z. Krist.*, **94**, 7 (1936); **95**, 397 (1936).
(7) Column 6 of Table III presents molecular cross-section values without correction for tilt of the molecules from the vertical; Column 7 presents molecular cross-section values corrected for tilt on the basis of the data of references 5 and 6.

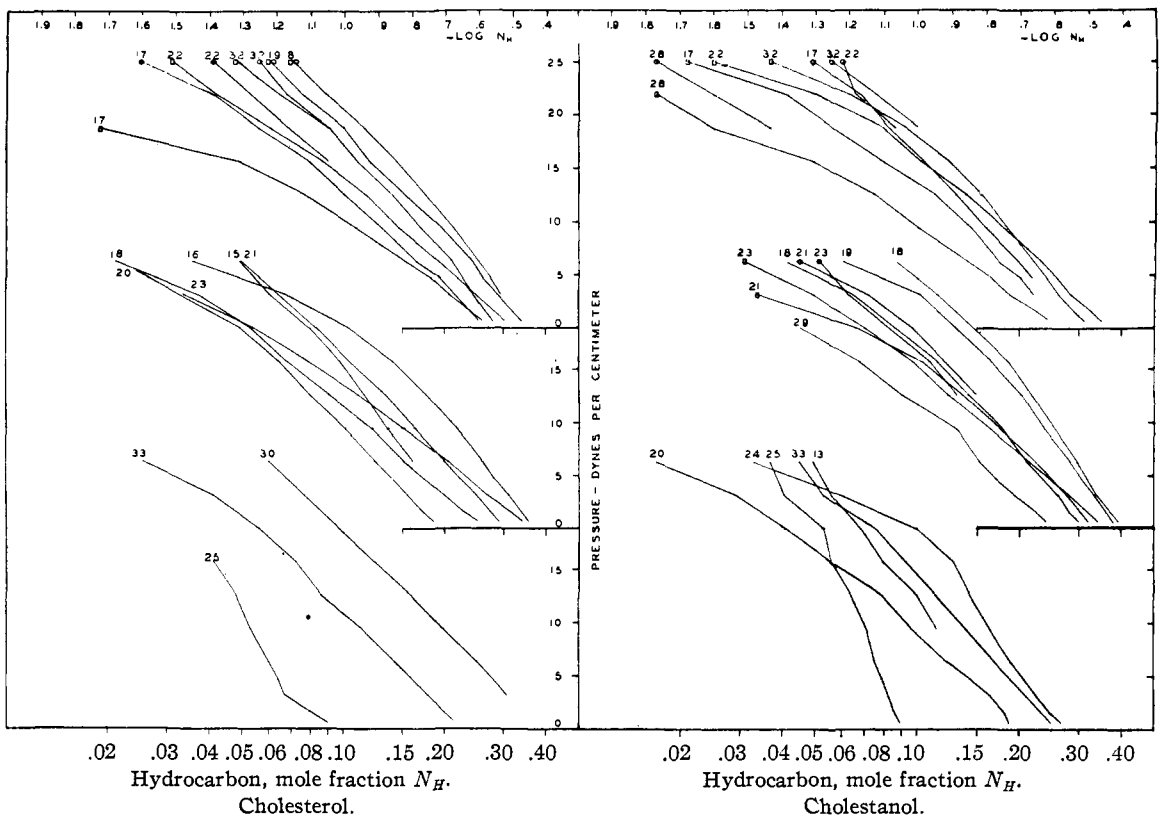

Fig. 4.—Relation of logarithm of hydrocarbon mole fraction (N_H) in area determining layer of mixed films to film pressure (P). Curves marked O and □ are for films having an initial hydrocarbon–sterol ratio of 0.2 to 1 and 1 to 1, respectively. See text for detailed explanation. The curves are designated as in Figs. 1 and 2.

film, log N_H, varies linearly with the pressure applied to the mixed film. Taken in conjunction with the reversible passage of such hydrocarbon molecules into and out of the area determining positions in mixed films, to be discussed in detail below, these log N_H–P curves indicate that 10-methyl-1,2-benzanthracene, and other hydrocarbons in mixed films having P-A curves of like form, behave as if in two-dimensional solution in the area determining layer of the sterol film, existing there in equilibrium with hydrocarbon in an excess phase of constant effective concentration or thermodynamic activity.

In the data plotted in Fig. 4 it will be noted that the best straight line relationship between log N_H and P is found in those mixed films where the amount of hydrocarbon initially spread is lowest. The curves of Figs. 1–2 show that some mixed films which had a high hydrocarbon content at zero pressure occupy at high pressures an area smaller than that of a pure sterol film containing the same number of sterol molecules. These effects apparently are due to the fact that a large amount of hydrocarbon present in the excess phase can serve as a solvent phase which dissolves a fraction of the sterol molecules and holds them away from area determining positions, the activity of the hydrocarbon in this hydrocarbon–sterol excess phase being lower than in an excess phase of pure hydrocarbon. It is possible to extend this treatment for 10-methyl-1,2-benzanthracene and cholesterol to cover all mixed films which show a straight line relationship between P and log N_H. This includes, at high hydrocarbon–sterol ratios, some of the films in the systems previously[1] designated as Class 1.

The solubility of a given hydrocarbon in the mixed film, as measured by the mole fraction of the hydrocarbon in the film at any given pressure in the range corresponding to section C of the curve, is nearly the same in cholesterol, cholestanol, coprostanol, sitosterol, and neoergosterol; the solubilities in *epi*-cholestanol and ergosterol are much smaller. With a given sterol there is a considerable variation in the solubility of the various hydrocarbons. In both the cholesterol

TABLE IV

MOLAR FREE ENERGY CHANGE, ΔF, REQUIRED TO REMOVE VARIOUS HYDROCARBONS FROM SOLUTION TYPE HYDROCARBON–STEROL MIXED FILMS (N_H = 0.2) TO THE EXCESS PHASE AT 25°

Hydrocarbon	Collapse press., dynes per cm.	Area per mole sq. cm. × 10^{-9}	ΔF Ergs × 10^{-9}	ΔF Kcal.
In Mixed Films with Cholesterol				
6-Methylchrysene	11.3	1.73	19.6	0.47
1'-Methyl-1,2-benzanthracene	3.9	1.51	5.9	.14
9-Methyl-1,2-benzanthracene	3.1	1.79	5.5	.13
10-Methyl-1,2-benzanthracene	8.5	1.58	13.4	.32
10-Ethyl-1,2-benzanthracene	6.3	1.91	12.1	.29
10-Butyl-1,2-benzanthracene	5.6	2.20	12.3	.29
10-Amyl-1,2-benzanthracene	6.4	2.22	14.2	.34
Tetrahydro-10-methyl-1,2-benzanthracene	7.1	1.74	12.4	.30
6-Chloro-10-methyl-1,2-benzanthracene	1.6	1.92	3.1	.07
9,10-Dimethyl-1,2-benzanthracene	− 0.6	1.90	− 1.1	− .03[a]
Cholestene	9.3	2.23	17.3	.41
1,2,7,8-Dibenzanthracene	10.4	1.86	19.4	.46
In Mixed Films with Cholestanol				
5-Methylchrysene	2.8	1.79	5.0	.12
6-Methylchrysene	14.3	1.67	23.9	.57
5,6-Dimethylchrysene	2.8	1.91	5.3	.13
1'-Methyl-1,2-benzanthracene	5.0	2.0	10.0	.24
9-Methyl-1,2-benzanthracene	7.4	1.78	13.2	.31
10-Methyl-1,2-benzanthracene	12.9	1.66	21.4	.51
10-Ethyl-1,2-benzanthracene	8.2	1.76	14.5	.35
10-Butyl-1,2-benzanthracene	8.2	1.97	16.2	.39
10-Amyl-1,2-benzanthracene	7.4	2.18	16.2	.39
Tetrahydro-10-methyl-1,2-benzanthracene	7.8	1.76	13.7	.33
6-Chloro-10-methyl-1,2-benzanthracene	3.9	1.90	7.4	.18
9,10-Dimethyl-1,2-benzanthracene	0.0	1.94	0.0	.00[a]

[a] The solubility of 9,10-dimethyl-1,2-benzanthracene is so low as to make the ΔF value zero or negative at N_H = 0.2. The negative value is obtained by extrapolation of curve 20 in Fig. 4.

and the cholestanol films, for example, the two dimensional solubility of 10-methyl-1,2-benzanthracene tends to be relatively high and that for 9,10-dimethyl-1,2-benzanthracene relatively low, that of 9-methyl-1,2-benzanthracene being intermediate.

A direct measure of the free energy of mixed film formation from a pure sterol film and hydrocarbon as it exists in the excess phase can be obtained as follows. The free energy change, ΔF (ergs), for the removal of one mole of hydrocarbon, at mole fraction of hydrocarbon, N_H, to the excess phase, the reverse of the process just mentioned, is approximately

$$\Delta F = KAP_{N_H}$$

where K is Avogadro's number, P_{N_H} is the collapse pressure (dynes/cm.), at the mole fraction N_H, and A is the area (sq. cm.) per molecule of hydrocarbon. From the data listed in Table IV, it is evident that the free energy change, at N_H = 0.2, for removal of a given hydrocarbon from a mixed film with cholestanol is about the same as for removal of the same hydrocarbon from a mixed film with cholesterol. If, as appears likely, the thermodynamic activity of hydrocarbon in the excess phase is about the same as that in the crystalline state, these figures represent free energies of solution in ideal two dimensional solutions.

An appraisal of the effect of structure of the hydrocarbon on the binding energies in sterol films would give important information on the nature of the forces involved in the interaction. Such an analysis would involve consideration of the relative forces binding the different hydrocarbons in their crystals. The physical data necessary to calculate the free energy of sublimation of these hydrocarbons are not available.

It was stated above that the movement of 10-methyl-1,2-benzanthracene molecules between the cholesterol film and the excess phase is reversible. Comparison of the re-expansion data of Fig. 5 with the compression data of Figs. 1 and 2 shows that when such mixed films, after compression up

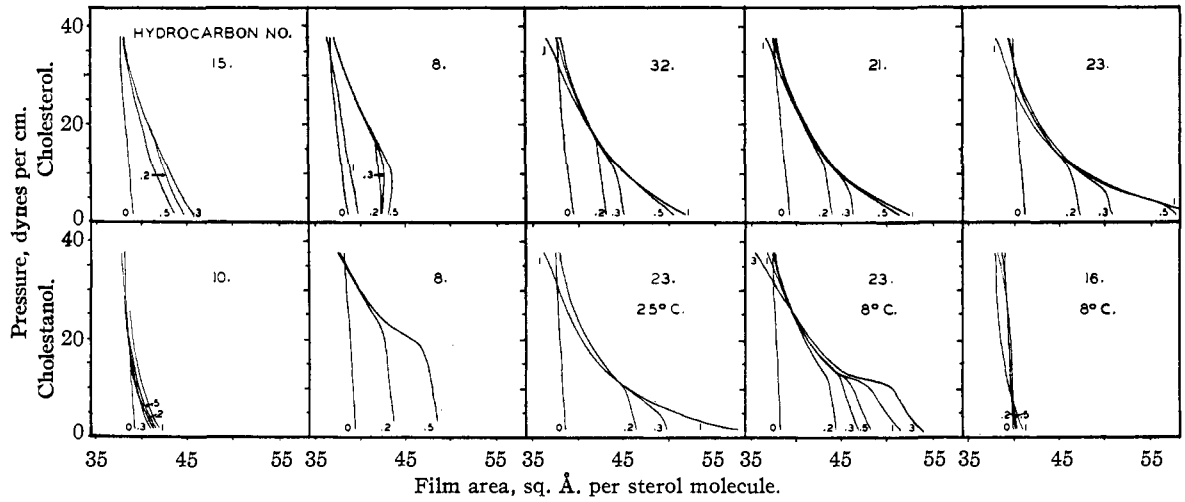

Fig. 5.—Curves obtained by allowing mixed films of various hydrocarbons with cholesterol or cholestanol to re-expand, after carrying each mixed film to the point of collapse, with successive pressure decrements of 3.3 dynes. The curves are designated as in Figs. 1 and 2. See text for relation of data to those of Figs. 1 and 2.

to a pressure of 40 dynes, are allowed to re-expand under successive pressure decreases of 3.3 dynes, the hydrocarbon molecules re-enter the film to re-establish the same relation of N_H and P as holds during compression. The identical forms of A-P and P-A curves for these systems indicate that the hydrocarbon positions in the mixed films can be reversibly re-occupied at all stages of the removal of hydrocarbon.

There is, however, a slow irreversible loss, from the excess phase, of 10-methyl-1,2-benzanthracene and other hydrocarbons which give mixed films showing rectilinear log N_H–P curves. There is thus a decrease in the limiting area at zero pressure after the film has been slowly compressed and re-expanded. The rate of this loss has been best measured by subjecting the mixed film to a given pressure for definite times during which a definite part of the hydrocarbon is in the excess phase, then reducing the pressure to 1.7 dynes, measuring the amount of hydrocarbon which returns to the film, and determining the irreversible loss of hydrocarbon from the film by difference. Data for the irreversible loss of 9-methyl-1,2-benzanthracene from mixed films with cholestanol at 8°, 25° and 40°, presented in Fig. 6, show that the rate of this irreversible loss tends to increase with temperature and with the amount of hydrocarbon in the excess phase.

The average rate of this slow irreversible loss during the determination of a force–area curve has been measured by finding the difference between the limiting area of the mixed film when compression is begun and the area after re-expansion has been completed. Comparison of these data for the 10-alkyl-1,2-benzanthracenes shows that the rate of this irreversible loss from the excess phase decreases with increase in the length of the aliphatic side chain attached to the ring system and as shown in Table V, increases with temperature.

The location of the excess phase with respect to the area determining layer of the film and the nature of the irreversible loss from this phase remains, for the moment, an open question. In a previous paper,[1] to which reference must be made for the detailed statement, it was suggested that the excess hydrocarbon phase formed a layer above an area determining hydrocarbon–sterol layer. A suggestion, made by Dr. I. Langmuir, that the slow, irreversible loss of hydrocarbon may be due to evaporation of hydrocarbon from such an excess hydrocarbon phase above the film is being investigated.

An alternative mechanism for the irreversible loss of hydrocarbon from the film which merits consideration is suggested by experiments, to be reported in detail at a later date, in which the water solubilities of the 10-alkyl-1,2-benzanthracenes have been found to increase with decrease in length of the alkyl side chain, that is, parallel to the irreversible loss of hydrocarbon from the film. For example, the solubility of 10-amyl-1,2-benzanthracene in water is approximately 1 microgram per liter as compared with approximately 70 micrograms per liter for 10-methyl-1,2-benzanthracene. According to this second proposed

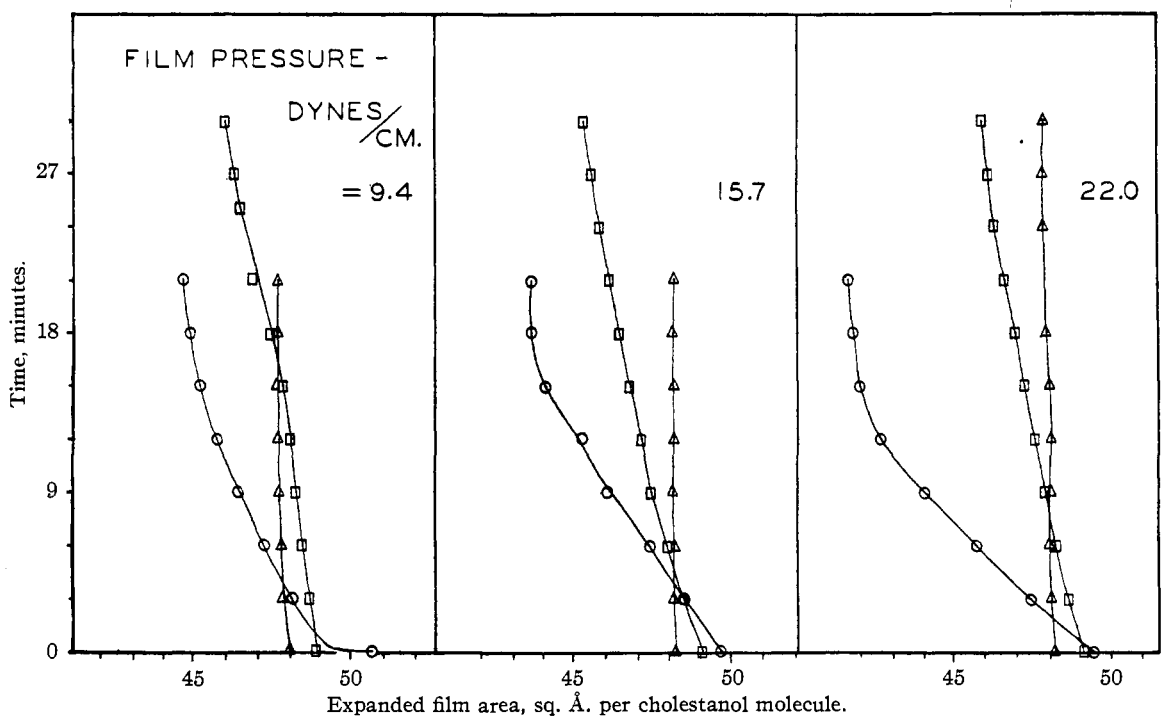

Fig. 6.—Areas occupied by mixed films of 9-methyl-1,2-benzanthracene with cholestanol (0.3 molecule hydrocarbon per 1 molecule sterol) when re-expanded to a pressure of 1.6 dynes after being held for the times indicated at the pressures indicated. Curves marked △, □ and O were made at 8°, 25° and 40°, respectively. The times do not include that required for the process of re-expansion and for area measurement. The slopes of the curves indicate the relative rates of irreversible loss of hydrocarbon at the three temperatures.

mechanism, application of pressure to the mixed film forces hydrocarbon molecules from their area determining positions in the film into an excess phase, possibly as microcrystals in contact with the water and in immediate proximity to the film.

TABLE V

IRREVERSIBLE LOSS OF HYDROCARBON DURING A SINGLE CYCLE OF COMPRESSION AND RE-EXPANSION

Hydrocarbon	Temp., °C.	Hydrocarbon molecules (per sterol molecule) lost from film at the following hydrocarbon/sterol ratios 0.2	0.3	0.5
1'-Methyl-1,2-benzanthracene	8	0.15	0.13	
	25	.07	.08	
	40	.16	.25	
9-Methyl-1,2-benzanthracene	8	.01	.01	
	25	.05	.04	
10-Methyl-1,2-benzanthracene	25[a]	.06	.12	0.15
	25	.07	.13	
	40	.12	.15	.27
10-Ethyl-1,2-benzanthracene	25	.06	.07	
10-Butyl-1,2-benzanthracene	25	.03	.03	.03
10-Amyl-1,2-benzanthracene	8	.01		
	25	.02	.01	.03
	25	.00	.00	
	40	.00	.00	
9,10-Dimethyl-1,2-benzanthracene	8	0.01		
	25	.02		
	40	.07		
Tetrahydro-10-methyl-1,2-benzanthracene	25	.03	0.04	
6-Chloro-10-methyl-1,2-benzanthracene	25	.00	.00	
5-Methylchrysene	25	.15	.21	
6-Methylchrysene	25	.07	.10	0.14
	25	.15	.19	
	25	.04	.10	
	40	.12	.15	
5,6-Dimethylchrysene	25	.12	.14	

[a] A twenty-minute cycle was used for this determination.

From such an excess phase hydrocarbon molecules may re-enter the film when the pressure is reduced or, having passed into water solution, be irreversibly lost to the film, the rate of this loss being presumably a function of the water solubility of the hydrocarbon in question. In agreement with this latter interpretation is the fact that these hydrocarbons, when present in colloidal suspensions in the water under pure sterol films, have been found to penetrate a given sterol film at rates which closely parallel both the water solubilities of the

hydrocarbon in question and the rates at which they are irreversibly lost from a mixed hydrocarbon–sterol film. While these parallels between rate of irreversible loss and solubility are suggestive, the evidence is insufficient to arrive at any final decision regarding the matter.

The implications of these two proposed alternatives are now being further examined experimentally in an effort to decide whether one or both of them will account satisfactorily for the properties of the excess phase and the rate of hydrocarbon loss from it.

Two Dimensional Hydrocarbon–Sterol Systems with Molecular Association (Type A Films).—A second type of P-A curve, to be contrasted with the first type which was illustrated by the 10-methyl-1,2-benzanthracene and cholesterol data, is illustrated by the 0.2, 0.25 and 0.33 curves in the family for 3,4-benzpyrene and cholesterol (Fig. 2, 10). At zero pressure the area of the mixed film exceeds that of the pure cholesterol film by an amount which is proportional to the hydrocarbon content of the film, showing, as was the case with 10-methyl-1,2-benzanthracene, that all or a constant fraction of the hydrocarbon molecules originally spread with the sterol have taken area determining positions between the cholesterol molecules in the film. As the pressure is increased, the film area remains essentially unchanged along segment A. At J the film collapses without further increase in pressure; the film areas for the 0.2, 0.25, and 0.33 curves drop to that for the 1 curve at the same pressure. Further increase in pressure then results, as in the case of mixed films similar to those of 10-methyl-1,2-benzanthracene and cholesterol, in a more gradual removal of the rest of the hydrocarbon molecules from the film.

When mixed films of 3,4-benzpyrene and cholesterol, after compression up to a pressure of 40 dynes along the curves of Fig. 2, 10, are allowed to re-expand under successive pressure decreases of 3.3 dynes, the films re-expand only along the common C portion of the curves and, in the A and B sections of the curves, in no case return to the area observed at the same pressure during the compression (Fig. 5). This indicates that, at areas beyond the area intercept of the extrapolated C portion of the curves, the area determining positions from which 3,4-benzpyrene molecules were removed by compression along the B segment are not re-occupied.

These mixed films, at areas along the C portion of the curve, are capable of sustaining a permanent constant film pressure. In many films of this type, at areas corresponding to points along the B segments of the curves, only a small or zero pressure can be permanently supported by the film, which collapses spontaneously to a lower pressure as soon as the collapse process has been initiated at the point J. This behavior indicates a decrease in stability of the hydrocarbon in the partially collapsed film; this instability is more clearly demonstrated by the kymographic A-P curves of Fig. 9.

From the data of Figs. 5 and 9 it appears that hydrocarbons which, like 3,4-benzpyrene, show this type of interaction, are held in stable two dimensional solution in the sterol film only along the C segments of the curves. The two-dimensional solubility of each such hydrocarbon in the film is relatively low in comparison to that of hydrocarbons behaving like 10-methyl-1,2-benzanthracene. At pressures along the A segments of the 3,4-benzpyrene curves the hydrocarbon molecules appear to be held in an association complex with the sterol molecules rather than in pure two dimensional solution. The relative stability of the complexes formed by the various individual hydrocarbons can be estimated only approximately from the collapse pressure at the point where the B segment of the curve sets in. The pressure required to continue the collapse usually decreases when the point J is passed, indicating that the films are in a metastable condition even before actual collapse begins. Examination of the characteristics of these mixed films showing metastable association leads to a possible explanation of the nature of the association complex.

The first outstanding characteristic of this type of mixed film systems is that the maximum number of hydrocarbon molecules which can assume area determining positions in films with this type of interaction is 1 hydrocarbon molecule for every 2 sterol molecules. From this limiting ratio it is postulated that, in this type of mixed film, each hydrocarbon molecule is held between two sterol molecules. It is further suggested that the hydrocarbon may be in contact with the non-methylated faces of the two adjacent sterol molecules, an arrangement which appears to provide optimum packing and hence optimum van der Waals attraction between the hydrocarbon and the sterol molecules.

This hypothesis as to film structure has been

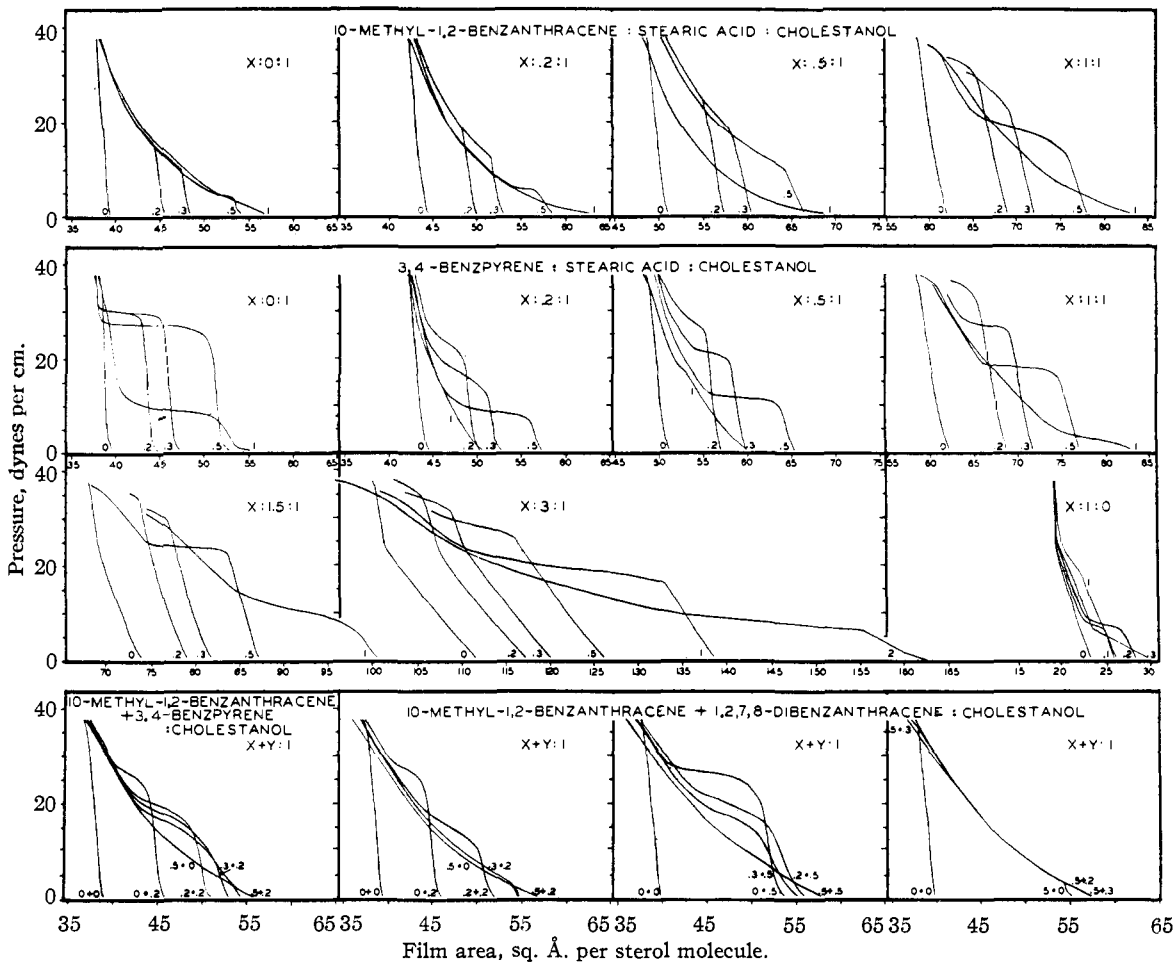

Fig. 7.—P-A (pressure–area) curves for three component mixed films. The numbers accompanying the individual curves give the values of x or, in the lower curves, x and y. The areas are given as sq. Å. per cholestanol molecule.

partially tested by testing the following prediction arising from the hypothesis: Any influence which tends to oppose the perfection of the face to face mutual orientation of sterol molecules in the film should decrease the possibilities for holding hydrocarbon molecules in the association complex and therefore the tendency toward the formation, and the stability, of these mixed films displaying two dimensional molecular association. This prediction is confirmed by four groups of experiments.

(a) The presence of one or more double bonds in the sterol ring system leads to a decrease in the collapse pressure required to remove the hydrocarbon molecules from area determining positions in the film (compare data for 3,4-benzpyrene and cholesterol with those for 3,4-benzpyrene and cholestanol, Figs. 1, 10 and 2, 10). The sterol double bonds must be expected to have at least a weak attraction for the water surface, thus disturbing the mutual packing of sterol and hydrocarbon molecules. Double bonds in the B ring also tend to prevent the A and C rings from lying in a common plane, a situation which also may reduce the potentialities for hydrocarbon–sterol packing.

(b) Substitution of *epi*-cholestanol for cholestanol prevents the occurrence of the oriented association (compare data for 3,4-benzpyrene and *epi*-cholestanol, Fig. 3, 10, with that for 3,4-benzpyrene and cholestanol, Fig. 1, 10). This is apparently due to the projection of the hydroxyl normal to the methyl free face of the *epi*-cholestanol ring system which, either by its effect on the tilt or the mutual packing of the sterol molecules, prevents formation of the oriented molecular complex.

Coprostanol, which is identical with cholestanol except that its A ring is bent out of the plane of the B and C rings at the A-B juncture, displays a

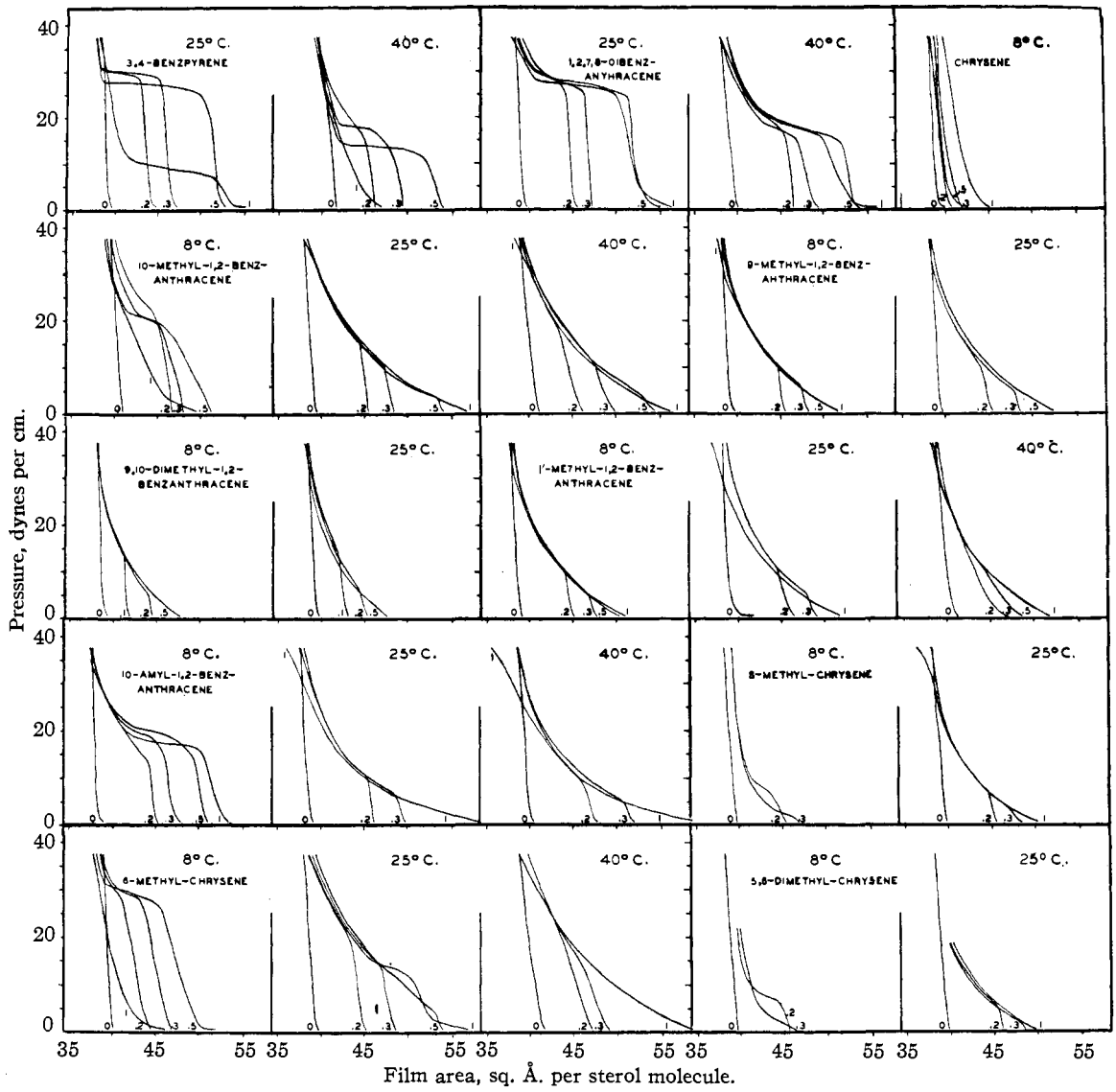

Fig. 8.—P-A (pressure–area) curves for mixed films of representative hydrocarbons with cholestanol at various temperatures. The areas are given as sq. Å. per cholestanol molecule.

weaker interaction with 3,4-benzpyrene than does cholestanol.

(c) Inclusion in the film of a third component which disturbs the packing in the film reduces or eliminates the tendency of certain hydrocarbons to display oriented molecular association with sterol molecules. A second hydrocarbon which in two component mixed films with sterol displays only solution type interaction, or stearic acid in small amounts, are examples of such third components (see effect of 10-methyl-1,2-benzanthracene or of stearic acid on films of 3,4-benzpyrene with cholestanol, Fig. 7).

(d) Increased temperature has the predicted effect of decreasing the stability of the association type mixed films (see Fig. 8).

From the above hypothesis as to film structure it may also be predicted that any influence which stabilizes the orientation of hydrocarbon and sterol molecules in the mixed film should improve the possibilities for the two-dimensional hydrocarbon–sterol molecular association. This is found to be achieved by decreasing the temperature to 8° (see Fig. 8).. At this low temperature a number of hydrocarbons show association with the sterol, although they are not able to do so at 25° or 40°.

Better orientation and stronger association are

also achieved by including a sufficiently large amount of stearic acid as a third component in the film. The data of Fig. 8, supported by further data from kymograph records, indicate that sufficiently large stearic acid concentrations in the film may cause the collapse pressure in cholestanol films containing small amounts of 3,4-benzpyrene to be raised to a point where the entire film collapses before any hydrocarbon is forced from the film.[8]

A second characteristic of mixed film systems displaying molecular association is the manner in which the pressure required to initiate collapse of hydrocarbon from these mixed films varies with the ratio of hydrocarbon to sterol. In Figs. 1, 2, 3 and 9 it will be noted that the pressure required to initiate collapse in films of this type tends to decrease as the hydrocarbon–sterol ratio increases. At some critical ratio (between 0.33:1 and 0.50:1 for 3,4-benzpyrene and cholesterol) the collapse pressure becomes zero and, at higher ratios, the association type interaction can no longer be detected.

A possible explanation for this dependence of collapse pressure on film composition arises from the conditions under which the film is initially laid down on the surface. As the benzene solution evaporates, hydrocarbon molecules may be (a) carried out into area determining positions between sterol molecules or (b) partially or wholly deposited from benzene solution in minute crystals of virtually pure hydrocarbon. The distribution of hydrocarbon molecules between these two positions will depend, first, on the capacity of the sterol film to accommodate and hold hydrocarbon molecules, and, secondly, on the tendency of the hydrocarbon to escape from the benzene spreading solution into a pure phase. The relative tendencies of the various hydrocarbons to escape, during the spreading, into a pure phase has been approximately determined by measuring their solubilities, and their tendencies to form saturated solutions, in benzene. In accord with expectation from the above explanation there was found to be a rough correlation between the critical hydrocarbon–sterol ratio at which complete collapse occurs and the tendency of the hydrocarbon in question to resist crystal formation from the benzene solution.

One further characteristic of those mixed films which display molecular association is their easily observable viscosity, indicating some more or less rigid cross linkage between the molecules of the film. No measurable viscosity is apparent in the solution type mixed films or in films of pure cholesterol or cholestanol. The viscosities of association type films cannot at present be expressed in quantitative units because, during the course of measurements by the rotating disk method, a decrease in the damping occurs.

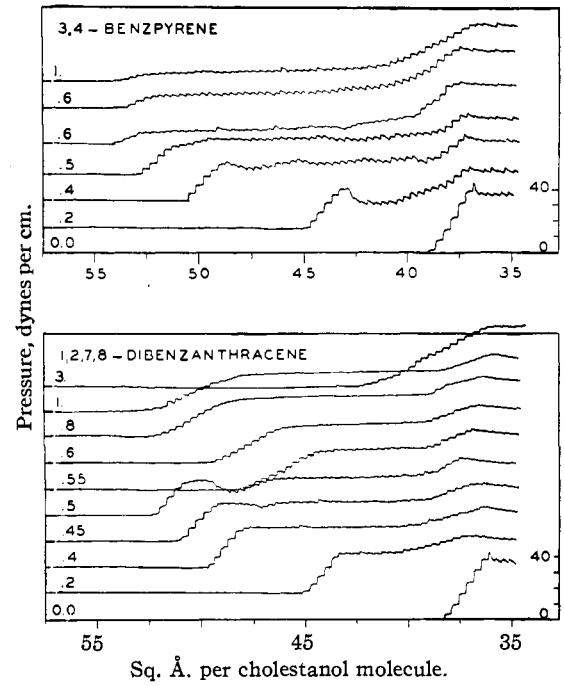

Fig. 9.—Kymographic A-P (area–pressure) curves for mixed films of 3,4-benzpyrene with cholestanol and 1,2,7,8-dibenzanthracene with cholestanol. The number of hydrocarbon molecules per sterol molecule is shown at the left above the curve to which it refers. Each curve starts at zero pressure and the pressure increase in each case is obtained by reference to the pressure scale in the lower right corner of each part of the figure.

Influence of Hydrocarbon Side Chains on the Type and Reversibility of Hydrocarbon–Sterol Interaction.—At 25° the area per hydrocarbon molecule for the 10-normal alkyl-1,2-benzanthracenes in solution type films increases with the length of the side chain (Table VI), indicating that each hydrocarbon molecule in the area determining layer of the film is oriented so that the side chain occupies space in this layer. At 8°, under conditions where they give association type films, the respective molecular areas for the 10-normal alkyl-1,2-benzanthracenes are smaller than in the solution type films and virtually independent of

(8) Preliminary experiments with systems of 3,4-benzpyrene and cholestanol or cholesterol containing lecithin, hydrolecithin, cephalin, or tristearin as a third component have yielded results similar to those for stearic acid here described.

TABLE VI

MOLECULAR AREAS OF HYDROCARBONS IN SOLUTION TYPE AND IN ASSOCIATION TYPE INTERACTION IN MIXED FILMS WITH CHOLESTANOL

Key No.	Hydrocarbon	Temp., °C.	Exptl. mol. areas, sq. Å., from individual films at following hydrocarbon–sterol ratios 0.2	0.3	0.5	Interaction type	Derived mol. areas for two interaction types, sq. Å. Solution	Association
17	1′-Methyl-1,2-benzanthracene	8	30.0	28.8		S	29.4	
		25	34.6	32.6		S	33.6	
18	9-Methyl-1,2-benzanthracene	8	30.8	29.1		S	29.9	
		25	30.1	30.9		S	30.5	
19	10-Methyl-1,2-benzanthracene	8	29.5	24.3	22.6	A, S	29.5	20.2
		25	30.0	29.5	28.6	S	29.3	
		40	25.7	29.1	26.7	S	27.2	
21	10-Ethyl-1,2-benzanthracene	25	28.7	29.5		S	29.1	
22	10-Butyl-1,2-benzanthracene	25	28.7	34.7	33.2	S	32.2	
23	10-Amyl-1,2-benzanthracene	8	33.5	29.2	25.1	A, S	33.5	19.2
		25	37.3	36.2		S	36.7	
		40	36.8	38.9		S	37.8	
20	9,10-Dimethyl-1,2-benzanthracene	8	31.2	31.2		S	31.2	
		25	38.6	32.2		S	35.4	
28	5-Methylchrysene	8	22.8	23.2		A, S		23.0[a]
		25	30.0	29.2		S	29.6	
16	6-Methylchrysene	8	20.6	22.3	21.1	A		21.3
		21.5	28.4	30.4	24.7	S, A	28.4	21.4
		25	27.9	27.2	29.5	S, A	28.2[b]	
		40	29.5	25.1		S	27.3	
29	5,6-Dimethylchrysene	8	24.0			A, S		24.0[a]
		25	29.5	30.3		S	29.9	
3	1,2-Benzanthracene	25	23.2	22.3		A		22.7
4	Chrysene	25	25.1			A		25.1
8	1,2,7,8-Dibenzanthracene	25	28.7	26.4	26.4	A, S	28.7[b]	25.7
		40	33.4	30.9	27.4	A, S	33.4	22.8
10	3,4-Benzpyrene	17	27.0	28.3	26.4	A		27.6
		25	24.9	24.9	25.9	A		25.2
		40	29.3	31.0	28.5	A, S	29.3[b]	24.3
14	3,4′-Ace-1,2-benzanthracene	25	25.7	22.6	21.6	A		23.3
		40	27.8	25.1	25.9	A		26.3

[a] Value is too high due to the presence of some solution type interaction which is not excluded here in the calculation.
[b] Value is too low due to the presence of some association type interaction which is not excluded here in the calculation.

the length of the side chain. This latter observation suggests that, in the association type films, each of the alkyl-1,2-benzanthracenes is oriented so that its side chain is accommodated in a non-area determining layer, leaving the 1,2-benzanthracene nucleus to be accommodated, as at 25°, between the sterol ring systems.

It also appears that, when excessive thermal agitation does not interfere, the presence of a hydrocarbon side chain which is so placed on the aromatic nucleus as to be readily accommodated in a non-area determining layer may help to stabilize the hydrocarbon in the association complex (compare data for chrysene and 6-methylchrysene, both with cholestanol, Fig. 8). The presence of a hydrocarbon side chain where it *must* be accommodated within the sterol ring system layer, when the hydrocarbon ring system is in that layer, may prevent the packing required for formation of the association complex. This conclusion is supported by the fact that, at 8°, 10-methyl-1,2-benzanthracene forms an association type film, whereas the 1′-methyl-, 9-methyl-, and 9,10-dimethyl-1,2-benzanthracenes do not. Similarly, 6-methylchrysene forms a very stable association type film while 5-methylchrysene and 5,6-dimethylchrysene display little or no ability to associate with sterol molecules.

To summarize the side chain effects, it may be said that the presence of an alkyl side chain at-

tached to the hydrocarbon increases, as a rule, the reversibility of the passage of hydrocarbon between the area determining layer of the film and the excess phase of hydrocarbon, and, simultaneously, decreases or abolishes the tendency of the hydrocarbon molecule to enter the oriented association with the sterol molecules of the film.[9] The side chain frequently increases the two dimensional solubility of the hydrocarbon in the sterol film. This may possibly be due to a great decrease in the forces holding the hydrocarbon molecules in the crystal.

Discussion

The experimental data here presented emphasize two possible ways in which polycyclic hydrocarbons react with sterols. The simplest reaction consists in a solution of hydrocarbon in sterol films. A reaction of the same or an analogous type may be presumed to occur, whether in purely chemical or in biological systems, wherever the concentration of sterol molecules is sufficient. In biological systems such an accumulation of sterol molecules may well occur at the surface of protein molecules or other relatively large aggregates. The greatest potential biological importance of this type of reaction may lie in its availability as a mechanism of transport of polycyclic hydrocarbons from the point of administration to the site of action in the animal organism.

The other reaction is of a more specific type and apparently requires each hydrocarbon molecule to be held between two closely adjacent and properly oriented sterol molecules. For this type of reaction to occur requires (a) an outside influence, such as orientation at a water–air or other appropriate interface such as that mentioned above, to impose a suitable orderly arrangement upon the sterol molecules; and (b) a proper configuration of the hydrocarbon, both as to ring system and side chains, to permit it to pack between adjacent sterol molecules sufficiently well to allow van der Waals forces to hold it there. The greatest potential biological significance for this second type of reaction appears to be in its availability as a means of binding hydrocarbon molecules into the structure of living cells. This possibility gains in probability from the fact that this second type of reaction is greatly augmented by the presence, in the films as third components, of such normal cellular components as fats, long chain fatty acids, and phosphatides. Further considerations relating to the biological implications of the present experiments will be published in appropriate biological journals at a later date.

(9) In rare cases, where the hydrocarbon side chain can be accommodated by the sterol film without spoiling the packing, both oriented association and a certain degree of reversibility may be displayed by a single hydrocarbon, as in the case of 10-amyl-1,2-benzanthracene with cholestanol at 8° (Fig. 8).

In three-component films, such as that of cholestanol, 3,4-benzpyrene, and 10-methyl-1,2-benzanthracene the presence of a hydrocarbon capable of reversible re-entry into the area determining layer of the film confers a limited reversibility on the hydrocarbon capable of association in the film.

The authors wish to acknowledge their great indebtedness to the various persons who have furnished materials for this investigation. They are especially grateful for the coöperation and friendly advice of Dr. L. F. Fieser and his past and present associates, Dr. M. S. Newman and Dr. E. B. Hershberg, who have furnished the many polycyclic hydrocarbons without which this investigation could not have been carried out. The authors also wish to thank Mr. T. V. Parke for performing a number of the experiments here reported.

Summary

1. From measurements on surface films at the air–water interface, the molecular areas of nine sterols have been determined. Other data regarding areas of sterols in surface films have been assembled and compared with previously published X-ray measurements on the dimensions of sterol molecules (Table I).

2. P-A (pressure–area) and A-P (area–pressure) measurements have been made for a series of mixed films, each containing a sterol and a hydrocarbon in known ratio. Experiments of this type were performed at a number of hydrocarbon–sterol ratios with each of 35 hydrocarbons (chiefly polycyclic compounds including or related to the carcinogenic hydrocarbons) in combination with cholestanol or with cholesterol (Figs. 1 and 2). Certain representative hydrocarbons were also used with each of the following: *epi*-cholestanol, coprostanol, ergosterol, neoergosterol, sitosterol and stearic acid (Fig. 3). These studies were carried out both by the usual manual techniques and by a new method which employs a kymograph to make an automatic permanent record of the force–area relationships.

3. From analysis of data on mixed films obtained in this way it has been found that most of the hydrocarbons studied display sufficient reactivity toward sterol molecules to be held between the sterol ring systems at the air–water

surface. The hydrocarbon molecules are apparently held in the mixed films in two principal ways:

(a) In a two dimensional solution which the hydrocarbon molecules can enter or leave reversibly; the logarithm of the mole fraction of hydrocarbon held in such solution, at film pressures below 15 dynes, varies linearly with the pressure applied to the film by the movable barrier (Fig. 4 and Table IV). At any given pressure the extent of this solubility depends on the structure of both the hydrocarbon and the sterol.

(b) In association complexes with sterol molecules; one hydrocarbon molecule apparently is held between two appropriately oriented sterol molecules. For such association to occur the hydrocarbon must have a favorable molecular configuration with respect to ring system and side chain. Any influence which favors a high degree of orientation among the solvent sterol molecules, such as low temperature or the presence of a third film component which leads to advantageous packing, favors this association. Any influence which disrupts the sterol orientation, such as high temperature, unfavorable sterol substituents or double bonds, or presence of an improperly packing third film component, detracts from the possibilities for formation of such hydrocarbon–sterol complexes (Figs. 5, 7, 8).

4. The molecular areas have been determined for most of the hydrocarbons studied (Table II) and compared with the dimensions of the same hydrocarbon molecules in the crystal, as obtained from previously published X-ray data (Table III).

Molecular areas for certain representative hydrocarbons have also been determined at temperatures from 8° to 40° (Table VI).

5. It is suggested that the solution type of interaction may possibly have some significance in the transport of polycyclic hydrocarbons in the animal organism. The association type of interaction, which is especially pronounced when the hydrocarbon–sterol films contain a fat, stearic acid, or a phosphatide as a third component, may possibly provide a mechanism whereby the polycyclic hydrocarbons may be bound into, and act as a modifying influence in biological structures.

INDIANAPOLIS, INDIANA RECEIVED SEPTEMBER 3, 1940

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Synthesis of 9,10-Dimethyl-1,2-benzanthracene and of a Thiophene Isolog

BY REUBEN B. SANDIN[1] AND LOUIS F. FIESER

Among the numerous homologs and dimethylene derivatives of 1,2-benzanthracene which have been tested for carcinogenic activity, those exhibiting the highest potency are cholanthrene, 20-methylcholanthrene, and 9,10-dimethyl-1,2-benzanthracene. The 10-methyl derivative falls somewhat below these hydrocarbons in general ability to evoke tumors rapidly in various tissues, and among the other isomers 9-methyl-1,2-benzanthracene comes next in the order of potency.[2] It would be interesting to know whether the structural specificity discernible in the hydrocarbon series is manifested among compounds of analogous structure in which one of the four benzenoid nuclei is replaced by a heterocyclic ring having aromatic characteristics. The synthesis of a thiophene isolog of one of the above potently carcinogenic hydrocarbons was undertaken both from this point of view and in order to provide a basis for interpreting such biological results as may be obtained with the recently synthesized pyridine isolog of 20-methylcholanthrene.[3] Since this substance differs from the hydrocarbon in being basic as well as in possessing a heterocyclic ring, a control substance was desired representing a variation of only one of these two features.

As a likely starting point for the synthesis of a compound of the desired type having a thiophene ring in place of the terminal linear ring of a *meso*-alkyl-1,2-benzanthracene, the condensation of α-thienylmagnesium iodide with 1,2-naphthalic anhydride was investigated. A keto acid mixture was produced in moderately good yield, and it was found possible to separate the higher melting isomer (II) readily as the crystalline sodium salt and to isolate the isomer I from the mother liquor.

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(2) For a summary of most of the pertinent data, see Fieser, *Am. J. Cancer*, **34**, 37 (1938).

(3) Fieser and Hershberg, THIS JOURNAL, **62**, 1640 (1940).