CLI. MONOLAYERS OF PROTEOLYTIC ENZYMES AND PROTEINS.

IV. MIXED UNIMOLECULAR FILMS.

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WHILST a considerable knowledge exists as to the properties and reactions of unimolecular films of pure substances at an air/liquid interface, comparatively little is known of the behaviour of two or more different substances present together as a mixed film. Such information is of importance in connection with reaction kinetics in unimolecular films and also in regard to the properties of naturally occurring membranes, which cannot be treated in terms of one substance or even of one class of substance.

The most important observations on mixed films are those of Adam [1928] on the so-called "condensing" effect of large molecules on smaller molecules when both are present in a mixed unimolecular film. Thus addition of cholesterol or of tripalmitin to myristic acid, which itself forms an expanded type of film with a limiting area at zero compression of about 50 sq. Å., causes the myristic acid to assume the liquid condensed state with a much smaller limiting area per molecule. According to Adam the vibratory movements of the hydrocarbon chains, which are of large amplitude in the expanded state, are greatly hindered by the large and physically inert cholesterol or tripalmitin molecules. Adam further emphasised that there was no evidence of direct compound formation in the type of mixed films examined by him, since the two-dimensional vapour-pressure is not reduced more than would be expected from the proportion of cholesterol molecules present in the film. In a less simple case Leathes [1925] has observed a similar effect of cholesterol in condensing an expanded film of lecithin, a point of some interest in connection with the stability of living cell membranes. By the method of surface potentials Hughes [1935] showed that the condensation in this case was not accompanied by any appreciable change in the dipole moments of the two components, but that the molecular orientation was retained to a higher compression in an equimolecular mixture than in any other mixture.

In the present work a further study has been made of more varied types of mixed films and it is shown that the condensing effect is but one of several distinct phenomena observable in these systems. Thus it was found that the stability of mixed films was closely related to an effect sometimes encountered in studying chemical reactions in unimolecular films. This effect, which may be termed "film penetration", is met with in certain cases when a capillary active substance (*B*) is introduced into the solution beneath a pre-existent film of another substance (*A*). There are three possibilities. First, the film *A* may be completely replaced by a unimolecular film of *B*; secondly, *B* may penetrate the

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film of *A* and produce a stable mixed film of *A* and *B*; or finally neither replacement nor penetration occurs, *i.e.* no change can be detected in the original film. Examples of each behaviour have been observed and the relation of each to the physical and chemical properties of the system is discussed.

EXPERIMENTAL.

Equimolecular mixtures were prepared in light petroleum solution of the following pairs of substances: oleic acid and hexadecyl alcohol; sphingosine and hexadecyl alcohol; oleic acid and tripalmitin; and mixtures in the molecular proportions of 3 : 1 respectively were prepared of oleic acid and triolein; oleic acid and tripalmitin; hexadecyl alcohol and triolein.

The mixed films obtained by spreading from these solutions were examined by the methods of surface potentials and of surface pressures. The dark-field microscope technique of Zocher and Stiebel [1930] was applied to examine cases of doubtful homogeneity of the surface film. The results for the surface pressures and surface potentials are shown in the accompanying figures, where *n* as abscissa represents the total number of molecules per square centimetre of both kinds.

(i) *Oleic acid and tripalmitin* (Figs. 1 and 2).

At p_H 7.4 (*M*/25 phosphate buffer) oleic acid forms a liquid expanded film of the soap with a limiting area per molecule at zero compression of 53 sq. Å. At the same p_H the mixture gives a limiting area of 55 sq. Å. per molecule and on compression the surface pressure and potential rise uniformly till $n = 3.1 \times 10^{14}$ mols./sq. cm. Beyond this point the surface pressure remains constant at 21–22 dynes/cm., while the surface potential falls rapidly to a constant value of 175 mv. at $n = 5.35 \times 10^{14}$. The surface pressure level corresponds to the equilibrium breaking pressure of a tripalmitin film and up to this point the observed surface potential curve is that corresponding to a simple mixed film of the two constituents. On further compression it must be supposed that the tripalmitin is collapsing under the pressure of the oleate, which remains as a unimolecular film. The final surface potential is the maximum obtainable with sodium oleate alone at this p_H . Optical examination of the mixed film reveals numerous minute crystallites in the broken film. These are not observed with a pure tripalmitin film, which shows long parallel strain lines on fracture and has no tendency to break up into these highly characteristic crystallites noticed in the mixed film. In other words the oleic acid may be said to "peptise" the tripalmitin.

The condensing action of tripalmitin on the oleate molecules is not great in the 1 : 3 ratio. The area per molecule at zero compression for the oleate alone is 53 sq. Å. at this p_H of 7.4, while in the mixture it is 50 sq. Å. In the 1 : 1 ratio the area per molecule is reduced to 40 sq. Å. at zero compression, assuming that the tripalmitin molecules have a constant area per molecule of 60 sq. Å.

Compression of the 1 : 1 mixed film gives the same collapse point on the surface pressure curve and a similar peak in the potential curve at a higher surface potential corresponding to the greater number of molecules with the higher electric moment (μ for tripalmitin $= 10.0 \times 10^{-19}$ e.s.u. and for oleic acid 2.1×10^{-19} at p_H 2, 1.0×10^{-19} at p_H 7.0). On further compression the potential falls to that of sodium oleate alone. The calculation of the condensing effect of the tripalmitin on the oleate molecules is made as follows.

Let n = total number of molecules/sq. cm. of surface. In the 1 : 1 ratio let *A* be area/mol. in sq. Å. of oleate molecules, assuming tripalmitin to have an area/mol.

of 60 sq. Å. The observed value of n at zero compression of mixed film $= 2.0 \times 10^{14}$, $\frac{1}{2}n \times 60 \times 10^{-16} = \text{area occupied by tripalmitin in 1 sq. cm. of film}$, therefore $(1 - 30 \times 10^{-16} n) = \text{area occupied by oleate, i.e.}$

$$A = \frac{(1 - 30 \times 10^{-16} n)}{\frac{n}{2}} \times 10^{16} = 40 \text{ sq. Å.}$$

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 4.

Figs. 1 and 2. A, Tripalmitin; B, oleic acid; C, 1 tripalmitin : 3 oleic acid; D, 1 tripalmitin : 1 oleic acid.

Figs. 3 and 4. A, Triolein; B, oleic acid; C, 1 triolein : 3 oleic acid.

(ii) *Oleic acid and triolein* (Figs. 3 and 4).

The 3 : 1 mixed film is homogeneous and liquid expanded from zero compression at $n = 1.4 \times 10^{14}$ mol./sq. cm. up to $n = 2.5 \times 10^{14}$ mol./sq. cm. At higher values of n the surface potential decreases while the surface pressure curve rises much less steeply than over the initial portion of the curve, indicating partial collapse of the triolein. The final collapse pressure and potential correspond to a mixture of triolein and oleic acid richer in oleic acid than the original 3 : 1 oleic acid : triolein.

The mutual effect of the two substances is negligible in that the observed limiting area per molecule in the mixed film (at zero compression) is within experimental error equal to that calculated for the simple mixture.

(iii) *Hexadecyl alcohol and oleic acid* (Figs. 5 and 6, 7 and 8).

This mixed film was examined at p_H 2 ($N/100$ HCl) and at p_H 7.4.

At p_H 2 the mixed film collapses at the same pressure as oleic acid alone, indicating that the hexadecyl alcohol is displacing the oleic acid. The surface

potential of the mixed film shows that in the early stages of compression a uniform mixed film is obtained, but that ultimately the potential rises to 410 mv. corresponding to a film of hexadecyl alcohol alone. The observed value of n at the final collapse point further shows that about half of the original total number of molecules in the film have been expelled, presumably as minute droplets of oleic acid. The condensing action of hexadecyl alcohol on oleic acid is negligible at this p_H (cf. Table I).

Fig. 7.

Fig. 5.

Fig. 8.

Fig. 6.

Figs. 5-8. A, Hexadecyl alcohol; B, oleic acid; C, 1 mol. alcohol : 1 mol. acid.

At $p_H 7.4$ the behaviour is different in that the surface potential never rises to that of hexadecyl alcohol alone. The surface pressure does not show a sharp collapse point corresponding to incipient collapse of the oleic acid but continues rising though less steeply beyond this point—as in the case of triolein and oleic acid. This difference in behaviour must be attributed to the formation of a relatively stable complex between the ionised oleate molecule and the hexadecyl alcohol, which increases their mutual adhesion at $p_H 7.4$ but does not exist at $p_H 2$.

Again, the limiting area at zero compression in the mixed film at $p_H 7.4$ is greater than that calculated assuming the areas of the components to be unchanged. Thus if we assume the hexadecyl alcohol to be unaltered, with an area per molecule of 20 sq. Å. the calculated area per molecule of oleic acid in the mixed film is 75 sq. Å. instead of 53 sq. Å. when alone. This must mean an expansion of the hexadecyl alcohol by the oleic acid molecules at this p_H (cf. Table I).

It is possible to calculate the surface potential in the mixed film from the known electric moments (μ_1 , μ_2) of the two constituents, assuming that these

have not been altered in the mixed film from the original values. We may write $\Delta V = 4\pi \frac{n}{2} (\mu_1 + \mu_2) \times 300 \times 10^3$ mv. for the surface potential of the mixed film of two components in equimolecular properties, n being the total number of molecules per sq. cm.

At p_H 2 it so happens that the vertical components of the electric moment per molecule of oleic acid and hexadecyl alcohol are identical ($\mu = 2.1 \times 10^{-19}$ E.S.U.); and as seen in Fig. 6 the $\Delta V/n$ curve for the mixture is an extension of the $\Delta V/n$ curve for oleic acid or hexadecyl alcohol alone. At p_H 7.4 however, the value of μ for oleic acid has fallen to 1.0×10^{-19} E.S.U., owing to ionisation, and the mixed film of oleate and alcohol has a surface potential close to the value calculated by the above equation. At $n = 4.0 \times 10^{-14}$ mols./sq. cm. (25 sq. Å.) the observed value of ΔV is 200 mv. The calculated value is 230 mv.

(iv) *Sphingosine and hexadecyl alcohol* (Figs. 9, 10, 11 and 12).

A much clearer example of complex formation in mixed films is afforded by an equimolecular mixture of sphingosine and hexadecyl alcohol.

Fig. 11.

Fig. 9.

Fig. 12.

Fig. 10.

Figs. 9–12. A, Hexadecyl alcohol; B, sphingosine; C, 1 hex. alc. : 1 sphingosine.

Sphingosine alone on acid or neutral solutions forms unimolecular films which slowly dissolve in the underlying solution. The curves B for the surface pressures and potentials of sphingosine alone at p_H 2 and p_H 7.4 are obtained by compressing the films as rapidly as possible; the films are liquid expanded but the recorded areas per molecule are probably too small on account of the solubility effect. If now the equimolecular sphingosine-hexadecyl alcohol

mixture be examined a stable mixed film is found at p_H 2 or p_H 7.4. This film collapses at an area per molecule of 20 sq. Å. and has a surface potential much higher than that of hexadecyl alcohol. The sphingosine is clearly not displaced by the hexadecyl alcohol although the latter can exert a much higher two-dimensional surface pressure than sphingosine. The alcohol must be supposed to hold the sphingosine in the film in an oriented mixed monolayer, or in other words the solubility of the sphingosine film is greatly reduced by the presence of the hexadecyl alcohol. The rise of surface potential is due to the high vertical component of the electric moment of sphingosine. As in the case of oleic acid and hexadecyl alcohol we may calculate the surface potential in the mixed film. The value of μ for sphingosine at p_H 7.4 is 3.5×10^{-19} E.S.U. at $n = 3.0 \times 10^{14}$ mols./sq. cm., and for hexadecyl alcohol $\mu = 2.1 \times 10^{-19}$ E.S.U. ($n = 5.0 \times 10^{14}$ mols./sq. cm.).

The calculated value of ΔV in the mixed film at 25 sq. Å. ($n = 4.0 \times 10^{14}$) is 422 mv. The observed value is 420 mv. Compression to $n = 5 \times 10^{14}$ (20 sq. Å.) gives an increase of ΔV to 470 mv.; the calculated value is 518, but the discrepancy is ascribable to partial collapse of the mixed film. It is clear that the potential of a mixed film may be equal to, less than or greater than that of one component, depending on the actual dipole moment of the other component and the degree to which the orientation is retained on compression of the mixture.

The condensing effect.

In the case of the hexadecyl alcohol and oleic acid mixed film at p_H 7.4 it was noted that the oleic acid apparently causes an expansion of the hexadecyl alcohol film, and that the hexadecyl alcohol is thus not condensing the expanded film of oleic acid. It is useful to summarise these area changes in the following manner. If there is no mutual effect on the limiting area per molecule, and x is the mol-fraction in the mixture of component 1, we may write:

$$A = A_1x + A_2(1 - x),$$

where A is the calculated area per molecule at zero compression, A_1 and A_2 being the values for the two components.

In Table I these calculated values are compared with the observed values in the various mixed films.

Table I.

Components		(Sq. Å.)						
1	2	p_H	x	A_1	A_2	A (calc.)	A (obs.)	Difference
Hexadecyl alcohol	Oleic acid	2	0.5	21	55	38	41	+3
		7.4	0.5	21	53	37	48	+11
Hexadecyl alcohol	Sphingosine	2	0.5	21	(67)	(44)	42	(-2)
		7.4	0.5	21	63	42	33	-9
Tripalmitin	Oleic acid	7.4	0.25	60	53	55	53	-2
		7.4	0.5	60	53	56.5	50	-6.5
Triolein	Oleic acid	7.4	0.25	132	53	73	72	-1
Triolein	Hexadecyl alcohol	2	0.25	132	21	49	57	+8

(The solubility of sphingosine at p_H 2 makes the estimated area per molecule in this case low.)

The cases of condensation, *i.e.* a negative result in the final column, are seen to occur with sphingosine and hexadecyl alcohol and with the equimolecular mixture of tripalmitin and oleic acid. In each case the expanded film must be

ERRATUM

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For $5 \cdot 10^{-14} \%$ *read* $5 \cdot 10^{-4} \%$

considerably reduced in its limiting area. But this effect is clearly not dependent merely on the physical nature of the films of the two components. If instead of sphingosine and hexadecyl alcohol we take oleic acid and hexadecyl alcohol we now observe an increase in total area per molecule, slight at p_H 2 but at p_H 7.4 amounting to a 30 % increase. This must mean that the expanded film of oleic acid is causing an expansion of the condensed film of hexadecyl alcohol.

A similar expansion is noticed in the mixed film of 3 mols. oleic acid to 1 mol. of hexadecyl alcohol, to the extent of a 16 % increase on the estimated value. The original view of Adam that the inert molecules simply hinder the vibrations of the expanded molecules must now be extended to cases such as these where expanded molecules may increase the thermal agitation of the condensed molecules. It is of interest that where both components are in the expanded state, as in the case of triolein and oleic acid, the mutual effect is negligible.

The case of tripalmitin and oleic acid appears to be of the type discussed by Adam, the effect being one of condensation and proportional to the amount of tripalmitin in the mixture. The diversity of the other cases indicates that the area changes in a mixed film may be to some extent specific to the chemical nature of the molecules concerned, in that a complex film may be produced whose characteristics are very different from those of either component.

FILM PENETRATION.

In the introduction mention has been made of the possible effects of introducing a capillary active substance (*B*) beneath a unimolecular film of a substance *A*. Three possibilities were noted, (i) displacement of *A* by *B*, (ii) penetration of *A* by *B* to give a mixed film of *A* and *B*, (iii) no change in the film of *A*.

Examples of the third and simplest case are found when *B* is a relatively soluble substance. Thus ethyl alcohol, ethylamine, benzenesulphonic acid or aniline hydrochloride produces no change in any unimolecular films of insoluble substances so far studied. Again, sodium acetate in concentrations up to 30 % causes no displacement of a unimolecular film of tripalmitin, but sodium oleate at a concentration of 0.0005 % completely displaces the tripalmitin film in 2 hours at p_H 7.4. This same concentration of sodium oleate will not displace a film of hexadecyl alcohol, but penetrates to form a mixed unimolecular film of oleate and hexadecyl alcohol in about 20 minutes. These preliminary results were extended.

The experimental procedure was to inject the constituent *B* beneath the film of *A* spread on a solution of appropriate p_H . The bulk concentration of *B* was $5 \cdot 10^{-14}$ %, unless otherwise stated; the temperature was 18–20°. The films were examined by the methods of surface potentials and surface pressures and by the ring surface tension apparatus. It was found that the ease of displacement or of penetration of *A* by *B* depended on several factors:

- (a) the physical nature of the film;
- (b) the nature of the penetrating molecules *B*;
- (c) the bulk concentration of *B*;
- (d) the degree of adsorption of *B* at the interface as dependent on the Gibbs adsorption relation.

These factors are exemplified in the following cases. The cases of displacement, as opposed to penetration alone, are few and will be considered first.

Displacement. A = tripalmitin or triolein; B = sodium oleate.

(a) *Physical nature of film.* At p_H 7.4 a tripalmitin film in the solid condensed state with an area of 58 sq. Å. per molecule at 15 dynes/cm., corresponding to close-packed vertical chains, is completely displaced in 2 hours by sodium oleate. A triolein film in the liquid expanded state at 100 sq. Å. per molecule and 14 dynes/cm. is displaced in about 30 minutes. This indicates that close packing of the film reduces the ease of penetration and thus also of displacement.

(b) *Nature of molecules of B .* At p_H 12 sodium oleate does not displace tripalmitin. At p_H 2 oleic acid takes from 3 to 4 hours to displace tripalmitin, but around neutrality the rate of displacement is a maximum. The fact that no displacement occurs at high p_H values is probably related to the much smaller surface tension lowering caused by the soap in this region as opposed to the very large lowering brought about in neutral solutions. The diminished rate of displacement in acid solutions is due to the formation of an emulsion of oleic acid and the resulting diminution in molecular concentration.

(c) *Bulk concentrations of B .* The rate of displacement of tripalmitin films by sodium oleate at the fixed p_H of 7.4 depends on the bulk concentration of oleate. At 5×10^{-3} % displacement is complete in about 30 minutes, at 5×10^{-4} % in about 2 hours, while at 5×10^{-5} % it is incomplete in 10 hours.

(d) *Surface adsorption of B .* As already mentioned, relatively soluble capillary active molecules such as ethyl alcohol even in high concentrations (up to 5 %) cause no displacement; presumably since the adsorption beneath the film is too small. Very long chain substances, stearic and higher fatty acids and their sodium salts are much less effective than sodium oleate (or myristate) since they are too insoluble in the bulk solution to give a high enough concentration in the Gibbs layer.

Penetration (without displacement).

In these cases the experimental observation is that a film A at an initial very high surface compression, say 20 dynes/cm., can admit molecules of a second substance B , from the underlying solution, although this substance itself may only be capable of exerting a much smaller surface pressure. The surface pressure rises rapidly to the highest value—up to 40 dynes/cm. in some cases—which can be exerted by A alone, while the surface potential may rise, fall or remain unchanged.

An example of this behaviour is afforded by the injection of sodium oleate beneath hexadecyl alcohol.

1 mg. of sodium oleate was injected beneath a film of hexadecyl alcohol at a compression of 12 dynes/cm. spread on 300 ml. of $M/25$ phosphate buffer at p_H 6.5. The initial value of the surface potential of the alcohol film, 410 mv., fell in 20 minutes to 290 mv. and reached a final value of 270 mv. The film changed from the viscous liquid condensed state to a very rigid mixed film. The value of the surface potential of the acid soap film on the free surface of the liquid was 132 mv. Simultaneously the surface pressure rose from 12 to 40 dynes/cm. as measured by the ring method and by the Langmuir trough. At p_H 2 no change in ΔV occurs, μ for oleic acid and hexadecyl alcohol being identical ($\mu = 2.1 \times 10^{-19}$), although a similar change in surface pressure takes place as at p_H 6.5. A more spectacular case is shown by the injection of 1 mg. of psychosine sulphate beneath a film of hexadecyl alcohol at 10 dynes/cm. compression and a surface potential of 420 mv. at p_H 2. The surface pressure measured by the ring method rose in 30 minutes to a constant maximum value of 33 dynes/cm., while the surface potential increased by 178 mv. to 598 mv.

Similar large rises in surface pressure and film potential are observed with both sphingosine and psychosine in solution beneath films of hexadecyl alcohol, cholesterol and palmitic acid, and to a lesser extent beneath films of triolein, lecithin and tripalmitin.

Another case is that of sodium hexadecyl sulphate in solution beneath hexadecyl alcohol. Using the sulphate at 5×10^{-4} % the surface pressure of the hexadecyl alcohol film increased from 16 to 40 dynes/cm. in 2 minutes at p_H 2. The corresponding change in surface potential was from 426 mv. to 336 mv. in 2 minutes, finally reaching a value of 291 mv. The remarkable feature to be noticed is that the surface pressure at the free surface of this solution of sodium hexadecyl sulphate was very small; between 5 and 6 dynes/cm. Reduction of the bulk concentration to 5×10^{-5} % merely increased the time of reaching the final equilibrium (which was unchanged), while the surface pressure at the free surface was now less than 1 dyne/cm.

The phenomenon is very general. The film (*A*) may be a long chain acid, alcohol, amine, ether, tripalmitin, triolein, lecithin or cholesterol. The substance in solution (*B*) may be a long chain amine, sulphate, sulphonate, psychosine or sphingosine. Mixed films result in all cases except for fatty acids beneath fats and ether, when displacement results.

The points requiring explanation are, first, the mechanism of penetration; secondly, the formation of a stable mixed film, although one constituent may possess a much higher solubility than the other; thirdly, the fact that the rise or fall in surface potential of the original film corresponds to the entrance of many more molecules of the second component than there was initially room for.

In regard to this last point, it is clear from the experiments, already described in the first section, on those systems where the mixed films can be studied, that whether the surface potential rises or falls depends simply on the respective values of the dipole moments of the two components. In no case is there convincing evidence of any interaction of the dipoles consequent on mixed film formation. It is, however, remarkable that in the cases of film penetration the final value obtained for the surface potential of the mixed film is never far removed from the value corresponding to an equimolecular mixture of the two components—although in the penetration cases the constituent in the underlying solution is present in about 100 times the actual amount of the material in the original film.

In illustration we may refer to the cases of psychosine and sphingosine. In the form of its sulphate, in neutral and acid solutions, psychosine is very soluble in water and insoluble in light petroleum or benzene, thus excluding a study of its mixed films obtained by direct spreading. The related compound, sphingosine, is capable of study by spreading from benzene solution, and its mixed films have been described. Thus the equimolecular mixed film of hexadecyl alcohol and sphingosine is remarkably stable in spite of the much greater solubility of sphingosine in water. Injection of 10^{-3} g. of sphingosine beneath a film of hexadecyl alcohol containing 10^{-5} g. gives a rise of surface potential to a value approximately equal to that of the equimolecular mixture of sphingosine and hexadecyl alcohol at its maximum compression. While it is impossible to measure the dipole moment of psychosine owing to its extreme solubility, yet the similarity of its behaviour to that of sphingosine on injection beneath unimolecular films implies that its action is much the same.

In these cases of film penetration with formation of stable mixed films it is difficult to avoid the conclusion that the stability of the resultant film is due to the formation of a complex molecule of the two constituents whose stability

is due in part to the hydrocarbon chains, and whose formation is not attended by radical changes in the dipole moments of the polar portions of the molecule.

A tentative generalisation may now be made.

(1) If in a mixed film of *A* and *B*, compression results in expulsion of one component (*A*) from the oriented monolayer, then injection of *B* beneath a film of *A* will result in complete displacement of *A*. That is, the mixed film is unstable and the *AB* complex, if it exists at all, is unstable.

(2) If the mixed film is stable on compression, penetration alone will occur on injection of *B* beneath *A*, and will result in a stable (*AB*) mixed film.

The mechanism of penetration and of displacement is at present obscure, but it is highly probable that displacement is in the first stages due to the formation of an unstable complex of the two molecules which is itself rapidly displaced by excess of the second component beneath the film. In the case of penetration of a close-packed solid condensed film such as that of tripalmitin by sodium oleate it is possible that the soap molecules reach the surface through minute cracks in the film and then spread over and peptise the tripalmitin film. In cases of penetration of liquid films such a mechanism is improbable, since it is difficult to visualise cracks in a liquid film. A possible explanation may be found along the following lines. One must suppose that the molecules in the film (*A*) are in equilibrium with a definite, though small, bulk concentration of its molecules. The equilibrium $A_{\text{film}} \rightleftharpoons A_{\text{dissolved}}$ will be disturbed on the introduction of a second molecule *B*, which would alone set up its own equilibrium $B_{\text{film}} \rightleftharpoons B_{\text{dissolved}}$. When a molecule of *A* leaves the surface, either *A* or *B* may now return and an interchange of *A* and *B* will be effected to an extent dependent on the relative adhesional forces of *A* to *A*, *A* to *B*, and *B* to *B*. If the attractive forces between *A* and *B* are greater than the cohesive forces between *A* and *A* or *B* and *B* a mixed film of an *AB* complex will result, whose stability is related to the stability of this complex. Such molecular complexes are formed between hexadecyl alcohol (or cholesterol) and oleic acid, hexadecylsulphonic acid, hexadecyl sulphate, psychosine or sphingosine; and their stability is sufficient to prevent ultimate displacement by the more soluble constituent. In other cases, such as triolein and oleic acid, the complex must be regarded as being very loose, and complete displacement ensues.

SUMMARY.

A study of two-component unimolecular films has been made, the components being long chain alcohols, acids, glycerides, sphingosine and psychosine. On compression of a mixed film one of two phenomena was found to occur: one component may displace the other from the surface or both components may remain as a stable mixed film. In the latter case there is some evidence of the existence of a complex between the two components which does not involve measurable dipole interaction. In these cases also it is possible to obtain the same mixed film by injection of the one component into the solution beneath a film of the other component. Otherwise complete displacement of the film results.

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