Reactions in Monolayers.

THE LIVERSIDGE LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY IN LONDON ON MARCH 15TH, 1945, AND IN MANCHESTER ON MARCH 22ND, 1945.

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IT is scarcely necessary to dilate on the significance of the contributions which Devaux, Sir W. Hardy, and Irving Langmuir have made to our knowledge of the structure of matter in the boundary state. The concept of the orientated monolayer has proved most suggestive and stimulating. As a result a vast corpus of information is now available as to the molecular orientation, the physical properties of the two-dimensional phases, and phase changes in such systems. We must, however, observe that the accumulation of knowledge on the chemical behaviour of matter in this state of molecular orientation has not proceeded so rapidly. Yet it is one of peculiar interest not only because it provides a method of examining how far co-operative processes can play a part in affecting the relatively short-range forces associated with chemical action, but also because it seems highly probable that reactions at and with such interfaces play an important part in biological processes, since not only can the monolayer be regarded as a definite level albeit perhaps a low one of organisation, but it is equally certain that interphases play a large if not a predominant rôle in living matter. It would thus appear to be a matter of more than transitory interest to examine such reactions in some detail.

FIG. 1.

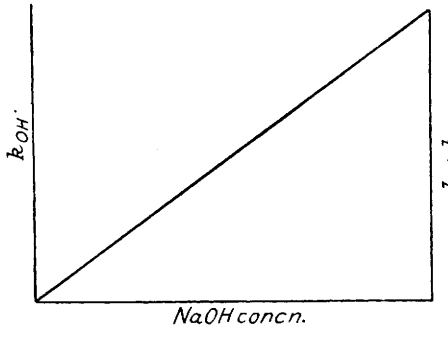
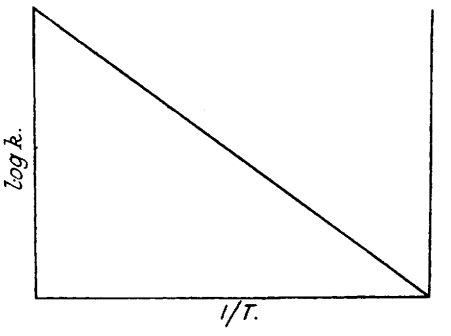


FIG. 2.



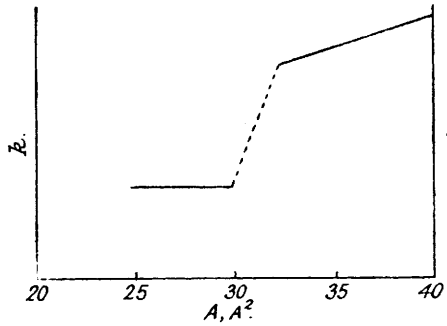


FIG. 3.

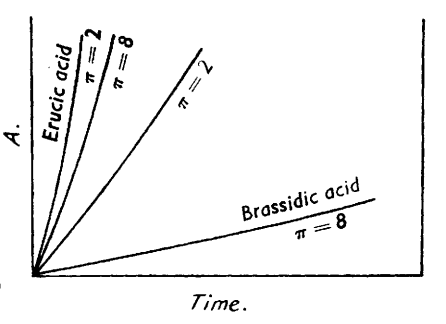


FIG. 4.

Although the quantities of matter involved in these surface reactions are relatively small, being of the order of only one mg. per square metre, yet the methods of following the chemical change are very sensitive. In general, we may utilise the change in the two-dimensional or surface pressure π as measured in a Langmuir trough, or preferably, since it is the polar portions of the molecules which are involved in chemical action, we can utilise the change in phase boundary potential ΔV caused by the reaction. The π–A and ΔV–n characteristics of a monolayer provide us with a great deal of information respecting the non-polar and polar portions of the molecules, respectively.

In studying such reactions our interest is naturally focused on examining what differences are to be observed between reactions proceeding in the bulk phase and those taking place in an orientated monolayer. Again, the monolayer is capable of being expanded or compressed and thus the molecular orientation, and in many cases, the physical state, is capable of being altered at will. How do these factors affect the reaction velocity? And if the reaction velocity is affected, we must enquire how far this is due to an alteration in the energy of activation or the entropy and the steric factor in the reaction. During the last fifteen years we have had occasion to examine a number of these surface reactions, and it has been found that both the energy term and the steric factor can be controlled. This is not the time or place to give a detailed account of these investigations, but we will examine a few typical ones.

If a monolayer of a long-chain lactone, ester or glyceride is thrown upon the surface of a solution of sodium

hydroxide, the film undergoes hydrolysis and the rate of transformation into the acid ion can be followed conveniently. In Fig. 1 is shown the reaction velocity constant for the hydrolysis of γ-stearolactone at a constant lactone concentration of 4×10^{14} mols./cm.^{2} at 25° as a function of the concentration of sodium hydroxide in the substrate; in Fig. 2 the log of the velocity coefficient against the reciprocal of the temperature for $n = 3{\cdot}35 \times 10^{14}$ mols./cm.^{2}, yielding an apparent energy of activation of 12,000 cals./g.-mol. or a true energy of activation, when the viscosity of the constant substrate is considered, of 17,100 cals./g.-mol.; and in Fig. 3 the rate as a function of the molecular area.

It will be noted that, in agreement with bulk reaction, the reaction velocity is proportional to the concentration of alkali, and that the energy of activation is similar to that obtaining in bulk solution. We note, however, that over a 50% increase in the velocity constant is obtained if we allow the condensed film to expand. The reverse reaction, *viz.*, the lactonisation of monolayers of hydroxy-fatty acids on acid substrates has been followed more recently by this monolayer technique by Kögl and Howinja, and similar alterations in speed obtained.

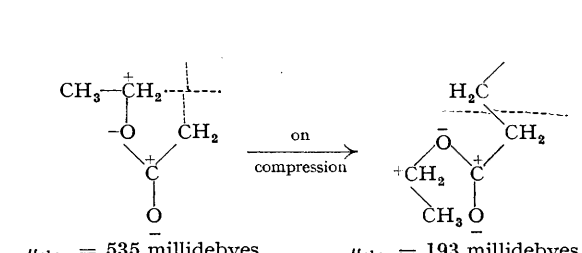
Further examination has shown that the alteration in rate of reaction produced by compression of the monolayer can affect both the steric term and the energy of activation. In the following table are the data for the hydrolysis of trilaurin on N/5-sodium hydroxide at 20°.

Surface pressure. dynes/cm.	E, cals./g.-mol.	k, sec.$^{-1} \times 10^3$.	Steric factor, P, calculated from Herz–Knudsen expression.
5·4	10,000	0·745	$1{\cdot}1 \times 10^{-6}$
10·8	13,200	0·787	$3{\cdot}1 \times 10^{-4}$
16·2	15,100	0·671	$4{\cdot}1 \times 10^{-2}$

Likewise, if we examine the rates of hydrolysis of a series of esters some remarkable differences are found in the velocity constants. Some of these are given in the following table.

Ester.	Type of film.	π, dynes/cm.	k, min.$^{-1}$, at 21°.
Ethyl palmitate	Liquid expanded	3	0·040
	Condensed	10·8	0·005
Methyl stearate	Condensed	3	0·021
Ethyl stearate	Liquid expanded	3	0·006
	Condensed	10·8	0·005
n-Butyl palmitate	Liquid expanded	3	0·042
	Condensed	10·8	0·023
Cetyl palmitate	Condensed	0·4	*ca.* 0·18
Octadecyl acetate	Condensed	3	0·15
Cetyl propionate	Liquid expanded	3	0·084
Methyl propionate	Condensed	9	0·021

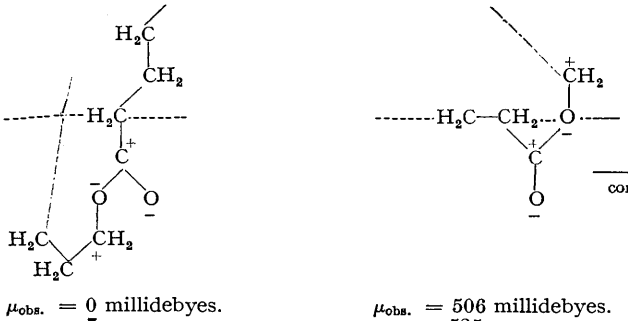
These relatively large changes in velocity of hydrolysis of the ester linkage can be associated with changes in the orientation of this group in the substrate surface. The path by which the hydroxyl ion in the solution



$\mu_{obs.}$ = 535 millidebyes.
$\mu_{calc.}$ = 525 ,,
A = 72 A.^{2}.
k = 0·04 min.$^{-1}$.

$\mu_{obs.}$ = 193 millidebyes.
$\mu_{calc.}$ = 198 ,,
A = 20 A.^{2}.
k = 0·005 min.$^{-1}$.

$\mu_{obs.}$ = 502 millidebyes.
$\mu_{calc.}$ = 525 ,,
A = 23 A.^{2}.
k = 0·15 min.$^{-1}$.



$\mu_{obs.}$ = 0 millidebyes.
$\mu_{calc.}$ = 7 ,,
A = 41 A.^{2}.
k = 0·18 min.$^{-1}$.

$\mu_{obs.}$ = 506 millidebyes.
$\mu_{calc.}$ = 525 ,,
A = 65 A.^{2}.
k = 0·084 min.$^{-1}$.

$\mu_{obs.}$ = 334 millidebyes.
$\mu_{calc.}$ = 329 ,,
A = 21 A.^{2}.
k = 0·021 min.$^{-1}$.

can approach and react with the group is thus the determining factor in affecting the velocity and, as we have seen, is revealed in both the energy term and the steric factor.

In the foregoing table are given diagrammatic representations of these orientations for various esters, together with the velocity constants of hydrolysis and the apparent dipole moment observed and that calculated on the basis of J. J. Thomson and Eucken's principle of vectorial summation.

We can see how the shielding of the ester group can be brought about by a short hydrocarbon chain when immersed below the surface. Similar orientation can of course take place not only at an air–water interface, but also at an oil–water interface, and since the esters are relatively insoluble in water, we might anticipate that the velocities of lipoclastic action of pancreatin on emulsions of the esters would show similar differences in velocity. This is actually found to be the case. For instance, ethyl butyrate and amyl propionate undergo hydrolysis respectively some 9 and 14 times more rapidly than the valerate. Complete protection is afforded by the benzoate group. The velocity of lipoclastic hydrolysis with pancreatin of a series of emulsified esters is given in the following table.

Ester.	*k*.	Ester.	*k*.
Ethyl butyrate	275	Benzyl propionate.....	225
Propyl butyrate	250	Ethyl benzoate	0
Amyl butyrate	80	Amyl benzoate	0
Butyl propionate	140	Amyl acetate	75
Amyl propionate	140	Amyl hexoate	20

The oxidation of monolayers of unsaturated acids likewise reveals several interesting results. The bulk oxidation of erucic and brassidic acids yields two dihydroxy-isomers. If these acids are spread upon dilute acid permanganate (*ca.* 0·005%), the films are observed to expand as the double bond is converted into the dihydroxy-derivative. It is evident that on compression of the films the accessibility of the double bond in the chain to the underlying oxidising substrate is diminished, and it is also clear that as far as this accessibility is reduced as the chains are brought into closer contact and adlineation by lateral compression, the *trans*-acid, *i.e.*, brassidic acid, will suffer the greater diminution in reaction velocity. This is clearly evident from the curves in Fig. 4. The compressed solid brassidic acid film at a compression of 8 dynes/mm. hardly undergoes oxidation at all. It is interesting to note that apparently both these acids yield the same oxidation product, the *cis*-dihydroxy-acid, when oxidised in the form of monolayers, apparently a case for directive oxidation.

The method is likewise applicable to the study of relatively rapid reactions; for instance, Alexander examined the reaction times of a film of *p*-hexadecylphenol in contact with solutions containing either a hypohalous acid, free halogen, or trihalide ions. Reactions with a half-life of as little as 40 seconds could be followed satisfactorily. In a similar manner Nasini and Mattei have followed the halogenation of long-chain unsaturated compounds by iodine chloride.

I have mentioned cases of ester hydrolysis and oxidation as typical of a great variety of surface chemical reactions. All exhibit similar characteristics in that, when a surface film undergoes reaction with a reactant in the substrate, we have some measure of control, by orientation of the surface molecules, of the path of approach of the diffusible reactant. Alteration of this path of approach implies an alteration in the energy of activation as well as in the steric factor. These alterations are directly reflected in a change in reaction velocity. Another field of enquiry has led to interesting results in connection with these surface reactions. It is well known that ultra-violet erythema is due to the photochemical liberation after localised ultra-violet irradiation of the skin of a colloid, the H colloid of low diffusibility. The diffusion rate of the H colloid and the photochemical threshold of erythemic response suggest that the H colloid is derived from the photochemical decomposition of a protein. Further, we may observe that in the natural proteins only certain amino-acid groups, *viz.*, those containing the chromophoric aromatic groups derived from tyrosine, phenylalanine, and tryptophan, are responsible for light absorption (threshold $\lambda = 2400$ A.). In order to gain some insight into the photochemical reaction involved, monolayers of stearylanilide containing the –CO·NH linkage and the chromophoric benzene ring were illuminated with light of the wave-band 2350—2400 A. It was found that a hydrolytic reaction occurred resulting in the formation of a monolayer of stearic acid and aniline dissolving in the substrate. The quantum efficiency of this photochemical surface reaction is greatly dependent on the orientation of the benzene ring with respect to the incident light, as is revealed in the following table.

Molecular area, A.^{2}	27·6	28	28·5	30	31	32	33
Apparent quantum efficiency θ	0·5	0·4	0·2	0·16	0·10	0·04	0

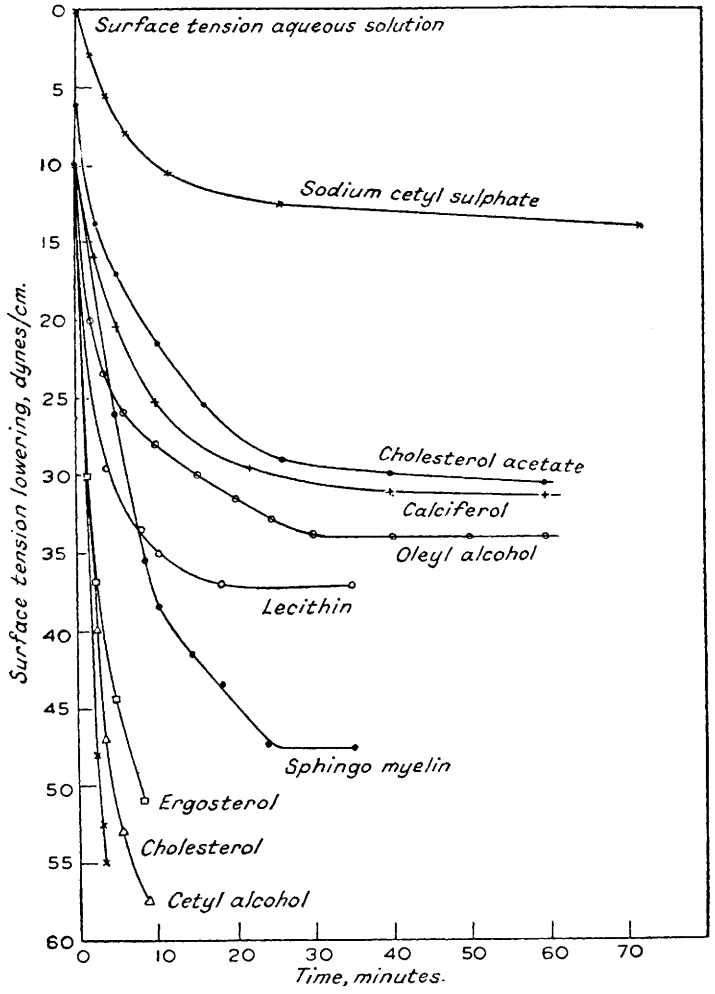
By examination of the change in phase boundary potential on illumination of monolayers of egg albumen, it was concluded that some 8·2% of the total number of –CO·NH groups in the monolayer were split by hydrolysis. This is indeed very close to the numerical proportion of aromatic amino-acid residues present in this protein (7%). The molecular lengths of the proteoses formed by this process of photochemical fragmentation which would locate the position of these chromophoric groups in the main chain awaits fuller investigation. The preliminary results of an essentially similar investigation have been published by Carpenter in the U.S.A. Part of the residues liberated in this process undergo oxidative changes; thus the glycyltyrosine fraction is eventually converted into a melanin-like pigment. We thus observe that a whole series of hydrolytic and oxidative changes, including indole ring closure, can be brought about by suitable radiation. In this case we are dealing with the excitation of chromophores which are part of the natural struc-

ture of the proteins. It is possible to incorporate alien chromophores into the protein and bring about similar photochemical reactions.

We shall have occasion to discuss the mechanism of this process of incorporation in more detail later, but for the present we observe, for example, that the injection of traces of a copper salt or of hæmatoporphyrin under a protein monolayer will sensitise the monolayer to reaction in visible light, and phenols will sensitise the monolayer to the near ultra-violet radiation. It is of interest to note in this connection that a variety of phenols are readily adsorbed by the proteins in the cell walls of paramœcia, which are thus rendered sensitive to ultra-violet radiation and rapidly destroyed.

This process of incorporation of alien chromophores in the macromolecular protein monolayer is only one example of a reaction very common in orientated monolayers. If a dilute solution of a reactant is introduced

FIG. 5.



Sodium cetyl sulphate $3{\cdot}3 \times 10^{-4}$ *per cent. injected at* pH 7·2 *under various films, at* 10 *dynes surface pressure.*

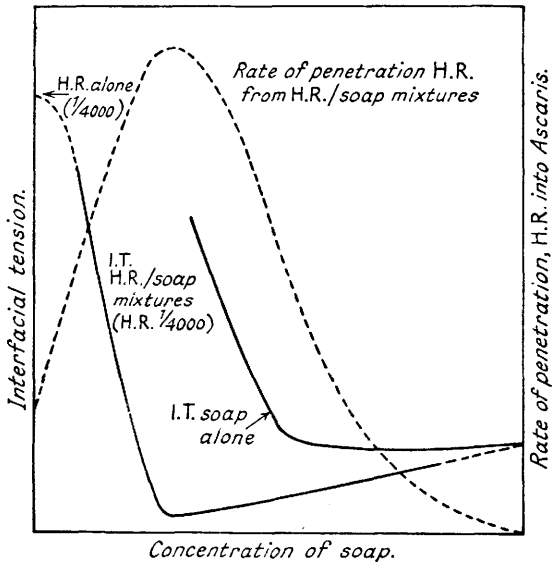
beneath a monolayer, reactions can be detected by a change in the phase boundary potential. The reaction need not necessarily be one such as hydrolysis in which a chemical change in the nature of the polar group is involved, but may consist in molecular association between the molecules of the monolayer and those injected. Where these molecular associations or complexes are formed by interaction of polar groups, we find that this associating mechanism can be interpreted in general as a hydrogen bonding.

If the injected molecules possess non-polar portions, these may penetrate up into the monolayer and interact with its non-polar portions. Here the van der Waals forces of two-dimensional solution are involved, but for non-polar portions of symmetrical form, two-dimensional cybotaxis or molecular adlineation likewise occurs, The result of this process of penetration is an expansion or an increase in pressure of the two-dimensional film. and since the polar group interaction is stoicheiometric, we obtain a two-dimensional pattern or ordered system. These two-dimensional complexes are only stable below certain limiting pressures, and in general one component can be ejected by increasing the pressure on the film. In Fig. 5 is shown the effect of injecting a

$3{\cdot}3 \times 10^{-4}\%$ sodium cetyl sulphate solution at pH 7·2 beneath various monolayers maintained at constant area and originally at 10 dynes/cm. pressure. The specific nature of the complex formation will be noted. Several biologically active substances exhibit remarkable power in penetration; *e.g.*, saponins and digitonin when present to the extent of only a few parts per million penetrate films of cholesterol with great rapidity. The specificity is most marked, since with cholesterol the surface pressure rises by some 50 dynes/cm., but scarcely any effect is noted with cholesterol acetate or with calciferol. We may note that sodium cetyl sulphate hæmolyses blood cells with great ease, but its hæmolytic activity is removed by the addition of cholesterol.

Several significant results are obtained on the injection of substances beneath monolayers of a protein. We may take as examples, saponin, long-chain fatty acids, and cholesterol. In the first case there is but little interaction; it might be suggested that, since saponin reacts readily with monolayers of long-chain alcohols and cholesterol but not with protein monolayers, its lytic properties are due to its interaction with the lipoid and especially the cholesterol portions of the cell wall. Long-chain fatty acids on injection under a protein form a lipo-protein complex by penetration of the acid into the protein monolayer. On addition of more of the soap (pH 7·2), the lipo-protein monolayer is displaced or is " deterged " from the surface by a soap monolayer and enters into the bulk phase as a lipo-protein complex. This effect is more emphasised the longer the chain of the acid. Thus a limit is set to the biological activity of a homologous series owing to the fact that the surface complex can be displaced or " deterged " from the surface if the capillary activity of the drug forming the penetration complex is great enough to displace the complex from the surface and bring it into

FIG. 6.



solution. This phenomenon, Schulman and I found to occur in the case of the 4 : 4′-dihydroxydipropylstilbene compound when injected under proteins. The injection of cholesterol beneath a protein monolayer results in the penetration of the protein by the cholesterol to make a lipo-protein complex. On compression of the film the cholesterol is pushed up and the protein pushed down into the aqueous phase. Eventually we obtain a film of cholesterol. The importance of these surface complexes may be illustrated from some experiments of Alexander and Trim on the penetration of hexylresorcinol into *Ascaris*, and the effect of adding a long-chain ion which forms a two-dimensional complex with the vermicide. In Fig. 6 (HR = hexylresorcinol; IT = interfacial tension) we note how the apparent toxicity increases as the surface concentration of the complex, as measured by the lowering of an oil–water interfacial tension, increases. On addition of excess of the carrier, the biological activity falls as micelle formation of the soap in the bulk phase commences; this micelle contains some hexylresorcinol and its toxic effect is consequently impaired.

A somewhat different aspect of this " complex " penetration mechanism is to be found in processes of sensitisation. For instance, several micro-organisms can be sensitised for lysis by cholesterol penetrants by a prior treatment of the bacteria with cholesterol which is adsorbed. Another reaction of this type is described by Peters and Wakelin, who found that the complex ovoverdin, which contains both a protein and astacin, could be split to form a lipo-protein containing soap by the addition of small amounts of long-chain fatty acids, the astacin being displaced and set free. On the addition of calcium ions the process is reversed. They likewise draw attention to the fact that it seems probable that the co-enzymes in an oxidase system may be separated from the enzyme by the formation of such a lipo-protein complex.

In a somewhat brief investigation of the blood coagulants heparin and the synthetic sulphate celluloses, we found that their biological activities ran parallel to the readiness with which these substances penetrated

films of cholesterol, suggesting that their mechanism of operation consists in forming a heparin–cholesterol complex from the cholesterol–cephalin complex present, setting the latter free. We have already referred to the fact that, in addition to the class of substances which react with and penetrate monolayers and possess a certain specificity in these reactions due to specific head-group interaction on the one hand, and also the adlineation in which the dispersive forces or van der Waals interactions are involved of the hydrophobic portions, there exists a class in which only the polar head groups interact and the molecules are anchored to the base of the monolayer but do not penetrate it. This adsorption is accompanied by a change in the phase boundary potential and in the rigidity or viscosity of the film. Examination of various films revealed interesting changes in properties when the free amino-groups in protein monolayers were brought into reaction with phenolic groups by injection of various types of phenols under the monolayer. Gallic acid reacts but slowly with a protein monolayer, but tannic acid, even in very dilute solutions, reacts rapidly. This tanned monolayer is no longer elastic and behaves more like a pellicle or skin. Injection of protein dispersing agents, such as sodium oleate to which we have referred above, no longer affects these skins. We must suppose that the tannic acid is attached by more than one hydroxyl group to the amino-groups of the protein and thus links the system together by multipoint contact. The 4 : 4′-dihydroxystilbenes behave in a similar manner, but with increasing length of aliphatic chain, protein dispersion can be obtained. Substances which are adsorbed on protein monolayers in this way are found to be those which agglutinate red blood cells and likewise kill paramœcia without cytolysis.

Certain dyes like Janus-green are found to be slowly adsorbed on protein monolayers when weak concentrations are employed. On increasing the dye concentration, the surface pressure of the monolayer commences to increase and the dye behaves like a weak penetrating agent. Here again the film behaviour is reflected in the transition from an agglutinating to a hæmolysing behaviour, and we note how sensitising agents can act.