# FILM REACTIONS AS A NEW APPROACH TO BIOLOGY*

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TOWARDS the end of the last century the biologist and physiologist were agreed that the biological entity was the whole living unit. This century has seen an attack on biological problems by the physical and organic chemist. The study of the living unit has been dropped, and in its place we find investigations on specialized processes such as oxidation and reduction or catalytic reactions. It is an unfortunate fact, as the late Sir William Hardy clearly pointed out, that in this method of approach the mechanism of the co-ordination or the integration of the activities of an assemblage of cells must remain insoluble. It is this very point which I think deserves some consideration. We must conclude that the mechanism of integration is at any rate dependent on a pre-existing organization of at least the major operative portions of the assemblage of cells. This raises a number of important problems such as: what types of organization are to be found in living material; how far control over chemical reactions can be effected by modification of the type or extent of such organization; and again how far different types of organization can modify such important factors as the chemical or physical state of a material or chemical equilibria in reacting systems; and lastly, what new properties or reactions make their appearance as a direct result of organization.

Whilst it has been frequently stated that one of the chief characteristics of living matter is that it contains a relatively large proportion of matter in what we designate the colloidal state, a closer analysis indicates that in fact the colloidal properties of living matter are due to the fact that an exceptionally large fraction both of material and of energy is present in films, membranes, fibres, fine capillaries and the like. It thus seems pertinent to inquire a little into the properties of surfaces of separation between bulk phases or of matter in the boundary state. These surfaces of separation can be considered as a new phase—the interphase—and for our discussion we must examine this phase and find in what respect it differs from the enclosing bulk phases.

While we must pay attention to the static properties such as composition, form and orientation, we must not forget that it is the dynamic properties of ingress and egress, of flow and chemical action in and with the two-dimensional contents of the phase, that we are particularly interested in, but any integrating features of the former are of great importance if it can be shown that they produce effects in the dynamics of the system which are not to be found in non-structural liquid or vaporous phases.

We already know that the composition of the interphase differs from that of either of the bulk phases in contact with it, and the general principles governing relationship between its composition and its three-dimensional partners were clearly enumerated by Willard Gibbs and Sir J. J. Thomson. Equally important are the considerations of Sir William Hardy and Irving Langmuir, who showed that in many cases when dealing with an interphase we were actually examining a monolayer—a hypothesis suggested by Lord Rayleigh. Finally, we know the molecules contained in the monolayer are orientated with respect to one another and to the plane of the interphase.

We have referred to the fact that molecules in a monolayer are orientated relative to one another and to the substrate and that this orientation can be altered by extension or compression. If the molecules in the monolayer undergo reaction with a reactant dissolved in the substrate, the rate of reaction may be modified by the charge in molecular orientation of the former. This is equivalent to a control of the steric factor and determining the path of approach of a reacting molecule or ion to the reactive portion of the other reactant. In this way both the reaction velocity and the height of the energy barrier or apparent energy of activation may be altered.

In the following table is given an example of such a variation in reaction effected by change in compression of a monolayer.

Attack on Lecithin Monolayers by 0·001 per cent black tiger snake venom at 20° and *p*H 7·2.

| No. of lecithin molecules per sq. cm. × $10^{-14}$ | Half-life in minutes. |
|---|---|
| 1·04 | 0·5 |
| 1·27 | 4 |
| 1·57 | 32 |
| 2·11 | 90 |

It is interesting to observe that these film reactions can be carried out with minute concentrations of strongly adsorbed reactants. Thus in the case of the attack of lecithin by snake venom to form lysolecithin, a half-life of about one hour is obtained with a concentration of venom as low

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as $2{\cdot}5\times 10^{-6}$ per cent. When cobra venom is examined by this method, it is found that only in extreme dilutions does any reaction occur. This inhibition at higher concentrations is due to proteins present in the cobra venom which are absorbed in preference to the enzyme by the lecithin monolayer. Egg albumin, although not so effective when added to black tiger venom, will produce a similar result. In addition to lecithinase present in snake venoms, other enzymes have been studied, and among them crystalline trypsin and crystalline pepsin, which rapidly digest monolayers of caseinogen, the former at *p*H 8 and the latter at *p*H 2. When the purified and crystalline enzyme preparations are employed, these enzyme actions on the protein monolayers behave exactly as in bulk phase, although the protein has undergone a process akin to denaturation. With unpurified proteolytic ferments, on the other hand, fatty acid protein complexes are invariably present which give rise to other phenomena.

In the reactions which we have discussed, the chemical processes involved do not differ from those which would occur in similar systems in the disorganized state and the only effects of molecular organization into orientated monolayers are noted in the alterations produced in accessibility of the groups as revealed by the rapidity of the reactions and in the apparent energies of activation.

A further consequence of molecular orientation at interphases is found in those cases where radiation incident on the surface produces photochemical action after absorption of quanta by chromophoric groups in the monolayer. If, as is the case in ring compounds, the extinction coefficients are different along the three molecular or group axes, the photochemical reaction rate can be varied by alteration of the orientation by compression. Thus the rate of photochemical hydrolytic fission followed by oxidation in protein monolayers at those points along the chain where the chromophoric groups are situate can be varied within wide limits by simple expansion or contraction.

There are several processes in which an alteration in the properties of an interphase bring about a number of varied biological processes of great importance. I may mention the phenomena of lysis, agglutination, sensitization and the lethal activities of certain substances on various types of cells and micro-organisms.

Whilst the extent of mutual miscibility of two liquid phases is usually interpreted in terms of the relative internal pressures of the two liquids, we note from the molecular point of view, especially in the case of the large complex and the biologically important material, that we are really concerned with specific molecular interactions which may be identified as being due to those forces operative between the non-polar and the polar portions of the molecules respectively. In two-component monolayers the two molecular species are adlineated in respect to one another, and we should thus anticipate that it might be possible to form relatively stable two-component complexes which in three dimensions would only be detectable in terms of mutual solubility, and when a mutual solvent was present as a third component might not be observable at all. These conclusions are indeed fully borne out by investigations on two-component monolayers. It is found, for example, that strong complexes are formed in mixed monolayers of a variety of substances such as saponin with cholesterol or digitonin, or cetyl amine or sulphate with cholesterol.

Examination of a great variety of these systems has demonstrated that the free energy of formation of the complex is constitutive in the sense that its magnitude is dependent on the extent of interaction between the polar reactive groups and also that of the van der Waals interaction between the non-polar portions of the reacting species. The difference in properties of mixed films containing cholesterol on one hand and those containing, for example, epi-cholesterol is most marked, but when models are made of the two molecular systems, it becomes quite evident that the ease of adlineation of the hydrophobic portions of the molecule and the relative orientation of the polar group with respect to the axis of the molecule are the determining factors. The free energy changes involved in formation of these two-dimensional complexes are of the order of some 10,000 cal. per gm. mol. Complexes containing the constituents in ratios other than one to one can be prepared; thus cetyl alcohol and cetyl sulphate can form both a 1 : 1 and a somewhat unstable complex in the ratio of 1 : 3, whereas elaidyl alcohol produces an unstable 1 : 2 but no 1 : 3 complex. It is probable that with a more extended investigation of these interesting systems the basis for the most elementary form, that is, a two-dimensional crystallography of the type envisaged by Patterson, may be laid down.

I might mention in passing that the effects of cis-transisomerism on the free energies of the complexes are very characteristic and fully confirm the hypothesis we have advanced as to the importance of molecular adlineation; thus saturated aliphatic hydrocarbon chains with different reacting polar groups will form stable systems, likewise trans-olefinic chains can penetrate and pack both with one another and with saturated chains, but the cis- form is not capable of such adlineation. From the biological point of view, I think that the most interesting property of these systems lies in the mechanism of their formation, for on injection of one of the reactants beneath a monolayer

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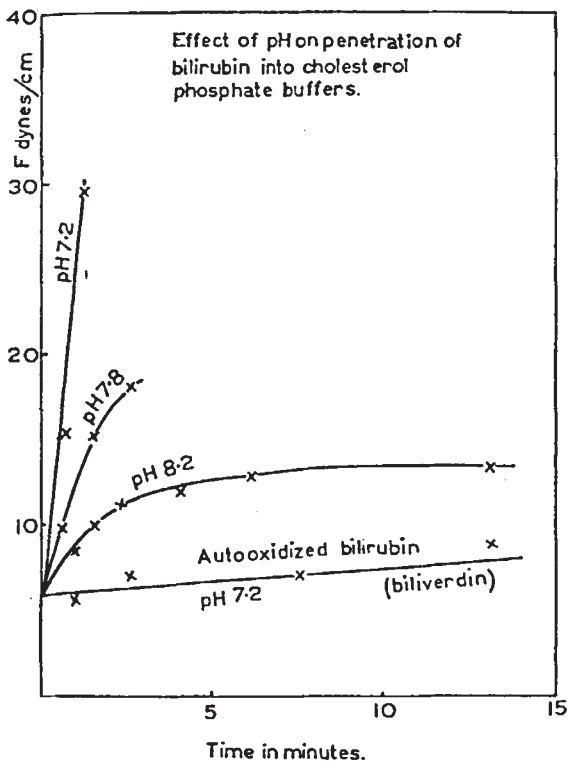
of the other, it is found that penetration of the latter by the former will take place to form the complex monolayer. This penetration, if carried out at constant area, naturally involves a rise in the two-dimensional pressure, or if at constant pressure a rise in area is involved. We have indeed examined the formation of complexes under both these conditions, and the changes involved are frequently remarkable. Thus the injection of a few mgm. of saponin under a film of cholesterol compressed to a pressure of 10 dynes/cm. will cause an increase of pressure of more than 50 dynes/cm.; whilst a film of cetyl alcohol at 20 A.$^2$ per molecule




expands to no less than 78 A.$^2$, even when the pressure is maintained at 23 dynes/cm. on the injection of only 1 mgm. in 300 c.c. of cetyl sulphate.

In some cases a small alteration in the *p*H of the substrate may affect the ease of penetration of a reactant to a marked extent. In the accompanying diagram is shown the effect of such a variation on the rate and extent of penetration of bilirubin into cholesterol as a function of the *p*H.

It is possible to examine the reactivity of various substances in respect to penetration of monolayers. I have referred to the penetration of monolayers of cholesterol, and we note that some substances such as digitonin or cetyl sulphate or amine possess this property to a remarkable extent. Of the other important cell wall constituents we include phospholipins and the proteins. Little information as yet is available on phospholipins, but our knowledge of the reactions of this type in the case of the proteins, especially the alcohol-soluble and thus readily dispersible protein gliadin, has been greatly extended in recent years.

The stability of the protein monolayers is, as we have seen, due partly to their mutual association; if these are broken down by stronger associating reactants we might anticipate a dispersion of the monolayer resulting in a solution of the protein in the form of a protein-reactant complex. This phenomenon is readily observed on injection of even minute quantities of such substances as sodium oleate, cetyl sulphate, or psychosin beneath a protein monolayer.

Other substances may react by penetration into the protein layer but not effect dispersion. By spreading monolayers with various head groups and examining the reactions caused on injection, it is possible to identify the reacting group in the protein monolayer. A characteristic group of protein complexes formed in monolayers is the lipo proteins; thus gliadin forms a remarkable complex with cholesterol in the ratio 4 : 1 by weight. Here the cholesterol is anchored to specific groups in the gliadin, in particular the amino and carboxylic groups. At high pressures (20 dynes) the cholesterol is forced up above the protein monolayer and the surface becomes one essentially of cholesterol. Nevertheless the cholesterol is still anchored to specific portions of the protein, for on release of the pressure the lipo-protein film is re-formed. This extrusion and re-forming process can be repeated several times before the complex structure breaks down. It is interesting to note that saponin, which penetrates cholesterol with extreme ease, but proteins only slightly, will penetrate these lipo-protein films except at those pressures where the cholesterol is separated from the substrate by the protein monolayer to which the cholesterol is anchored.

It thus appears not unlikely that the materials such as cytoplasm, and especially in the more stratified chloroplasts, must be regarded as a protein gel framework to which is attached the enzymes, the phosphatides and lipoids, and the means of attachment is as we have seen due to the interaction both of the non-polar as well as of the polar portions of the molecules concerned. Another important conclusion to be drawn from monolayer experiments is that these penetrative reactions involve not only a new head group interaction, but in many cases also the breaking of such a head group interaction already existing in the monolayer prior to penetration. Several biological analogies may be mentioned. Thus, since lysis of blood cells can be brought about both by protein and cholesterol penetrants, we must conclude that

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it has lipo-protein surface. Several micro-organisms can be sensitized for lysis by cholesterol penetrants by a prior treatment with cholesterol. Again, cilia of Mytilus appear to be mainly lipoidal, those of Paramecia chiefly protein, as judged by the criterion of penetration.

The carrier action of desoxycholic acid on fatty acids can readily be demonstrated in monolayers, as desoxycholic acid does not interact with other lipoids, nor to any great extent with proteins. We find also that the hæmolytic activity of a long-chain alcohol is negligibly small owing to the fact that it is practically insoluble in water; but it readily forms a soluble complex with a long-chain sulphate and can be transported to the cell wall in this form. There both the sulphate and the alcohol can penetrate separately, the former acting both on the protein and on the lipoid, the latter only on the protein, and produce lysis.

Yet another reaction of this type has been described by Peters and Wakelin, who found that the complex ovoverdin containing protein and astacin could be split to form a lipo-protein containing soap by the addition of small amounts of saturated long-chain fatty acids setting free the astacin. On the addition of calcium ions the process is reversed. They likewise direct attention to the fact that it seems probable that the co-enzyme in an oxidase system may be separated from the enzyme by the formation of such a lipo-protein complex.

Somewhat more complex in behaviour are the blood coagulants, heparin and the synthetic sulphate celluloses. It is found that their biological activities run parallel to the ease with which they penetrate films of cholesterol. It is not unlikely that they operate by breaking down a cholesterol cephalin complex, setting the latter free.

We have referred to the fact that, for the penetration of a monolayer by a substance injected into the substrate, primary interaction between the reactive head groups occurs, followed by solution, that is, penetration and adlineation, of the tail. In the case of reactants containing two or more reactive head groups, it is found that these can associate with head groups in the monolayer and thus form a series of links. Here another important factor is found operative. If the injected bipolar molecule possess a hydrophobic portion of such a structure that it can pack or adlineate with its neighbours beneath the monolayer, the resultant composite film is remarkably stable. Thus the long-chain dibasic acids are adsorbed on to, but do not penetrate, monolayers of amines, whilst the diamidines are adsorbed by, but do not penetrate, monolayers of cholesterol. Substances containing the phenolic group are of particular interest in this respect, as they include a number of biologically important substances. They react with amine groups quite readily, and to a less extent, with the imido group in a polypeptide chain. Gallic and tannic acids react with great ease with monolayers both of amines and with proteins. It is interesting to note that the reactivity of tannic acid with the spaced amine groups of the protein is high, and that subsequent injection of fatty acids beneath such treated monolayers results in the dispersion of the galloylamine or galloyl-protein complex film, but not in the tanned one—an indication of the effectiveness of the interlinkage produced in the non-dispersible network by the multiple point contact of the large tannic acid molecules.

Another significant biological similarity has been noted when we measure the extent of penetration of a series of substances containing identical hydrophobic 'tails,' for example a $C_{12}$ chain, but with different head groups, into a monolayer of a typical lipoid such as cholesterol. In all cases the extent of interaction as measured by the increase in surface pressure caused by the injection of 0·33 mgm./100 c.c. under a film of cholesterol originally extended to 40 A.$^2$ per molecule is found to be closely parallel to the hæmolytic activities and lethal activities on Paramecia of these substances.

These latter can be placed in order both of monolayer penetration and biological activ ty :

$$RNH_3^+ > RSO_4' > RSO_3' > RCOO' > RN(\overset{+}{C}H_3)_3 > RNH(\overset{+}{C}H_3)_2 >$$ bile acids.

We may conclude that the most reactive group in the protein macromolecule is the amino group, since the —NH—CO— group is poorly reactive, a point of some interest when we examine the reactions of lecithin and of cephalin. This order of head group reactivity receives confirmation when penetration into monolayers containing these head groups is examined, that is, on inverting the system. When we compare the reactivities of a series of long-chain compounds with identical head groups, it is found that biological activity and film penetration commences with $C_9$ when attached to a very reactive head group, with $C_{12}$ when attached to a poorly reactive group, and reaches a maximum value at *c.* $C_{18}$. It is interesting to note that it is not necessary for all the carbon atoms to be in the form of a chain but that they may be enclosed in rings; thus activity commences with diphenyl derivatives and increases with addition of carbon atoms to an optimum, as in the bile acids, stearic acid, diethyl stilbene and benzpyrene. By examining the reactivity of substances containing two reactive groups at various spacings underneath protein monolayers, it is possible to obtain some idea as to the statistical distribution of the reactive groups in the monolayer. It would appear that

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some 12·5 A. is the mean distribution of the amine groups beneath a gliadin film. In the native protein such spacings are naturally different, and thus reactions involving two-point contact will not take place in bulk phase unless the spacing is unaffected by two-dimensional unrolling of the protein.

We have referred to the modification which must be introduced into either the Overton Meyer or Traube concepts of biological activity, that is, lipoid solubility or capillary activity necessitated by the concept of specific head group interaction. We see that a definite limit is also set to the hydrophobic portion of the molecule, not only on account of the decreasing solubility in the aqueous phase causing difficulty in transport, and on account of the ease of adlineation or packing having an optimum of $C_{18}$ for association with sterols or fats, but also because a new phenomenon sets in with long chains, namely, dispersion of the monolayer. It is possible that this phenomenon of film collapse and dispersion may be a generally important factor in setting the upper limit to the chain length, or more generally the capillary activity of homogeneous series of biologically important substances, for example, anæsthetics. This dispersion of protein films may have biological counterparts in adsorption on specific portions of the cell surface similar to the hæmolytic activity of long-chain compounds such as oleic acid, which readily disperses protein films.

This method of attack permits us to investigate the nature of the coatings of cells or unicellular animals and plants by examining the effects of lipoid or protein penetrating substances on them.

Thus both red cells and Paramecia are affected by both lipoid and protein monolayer penetrating (cytolysing) or adsorbing (agglutinating) agents, and we deduce that their surface structures must contain lipoproteins or consist of a lipoid protein mosaic; whereas certain other unicellular animals frequently found associated with Paramecia and in addition the cilia of Mytilus are not affected by protein dispersants but are readily influenced by lipoid penetrating agents, and their coatings in consequence must be chiefly lipoidal in nature.

Examination of the carcinogenic hydrocarbons by the monolayer technique reveals the interesting fact that, whilst they themselves are unreactive, they are readily converted into extremely reactive water-soluble photo-oxides. These substances are not only reactive to protein monolayers like the water-soluble dibenzanthracene endosuccinate, but also are paramecicidal, the parallelism between the biological activity and monolayer reaction being maintained.

It has been the purpose of this address to re-emphasize the importance of the fundamental concepts introduced by Sir William Hardy and Dr. I. Langmuir as to the structure of matter in the boundary state. I have attempted to show that there is implicitly contained in the concept of molecular orientation a whole series of properties and events for which there are no analogies in homogeneous bulk phase systems. We note that many of the modes and types of the reactions which can be effected in monolayers, and which can be defined with precision and their mechanism established with a considerable degree of assurance, are unique for such interphases, but are again observed in living and organized material. It is with this object of ultimate correlation with biological behaviour that we have taken up the detailed study of interfacial reactions at Cambridge, and I should like to express my indebtedness to Dr. J. Schulman, who has been associated with me in this object.

Many 'vitalistic' models have been proposed in the past, and whilst it might be correct, although unscientific, to suggest that the ultimate level of integration in living matter is incapable of examination and definition, yet I believe that one is justified in asserting that at least one of the important levels to which due attention must be given for a proper understanding of biological activities is that of the ordered interface.

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# CULTURAL SIGNIFICANCE OF ANTHROPOLOGICAL STUDIES*

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THE vision of a universe in evolution has shown men that what had been accepted as absolutes are in several cases by-products of evolution. Old beliefs are increasingly relegated to the domain of folklore survivals, and the effects of this upon motive power in personal and social conduct are major factors of the present crisis in world affairs. Having been so long accustomed to accept an absolute basis which has now been undermined, men have hastily sought a new

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