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FILM REACTIONS AS A NEW APPROACH TO BIOLOGY[1]

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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 coordination 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 know for example that at death the catalysts escape from control since the molecular structure of the moving parts gets disorganized. Again, Loeb showed that unfertilized sea urchin's eggs could be made to develop by immersion in salt solutions of sufficient concentration. In development a whole series of complicated chemical reactions are set in operation and it is clear that in the quiescent unfertilized egg all the chemical ingredients for the reactions are present but await some change in organization before reaction sets in. 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 finally how far different types of organization can modify such important factors as the chemical or physical state of

[1] Address of the president of the Section of Chemistry, British Association for the Advancement of Science, Dundee, August 31, 1939.

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, fibers, 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.

Whilst 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. I need not enlarge at this point on the structure and different physical states as well as the effects of variation of the external variables on the equilibria of the phases of monolayers of simple molecules such as derivatives of both paraffinic and cyclic hydrocarbon, since these have been exhaustively examined during the last twenty years, but monolayers both of macromolecules as well as those composed of binary and components of a higher order posseses a number of interesting and somewhat unexpected properties.

We find, for example, that macromolecules such as the methylated or acetylated starches and celluloses or the native proteins can be spread as monolayers. The chains are extended at the interface and in general the non-polar side chains penetrate into one (the non-polar) and the polar side chains into the other (the aqueous) phase. This separation of the side chains by the solvent action of the homogeneous phases can only be effected by suitable partial rotation along the chain involving the usual cis-trans motion. Thus no single protein chain can acquire along its entire length either the α or β keratin configuration unless the side chains alternate in polarity in suitable fashion. Monolayers of both the proteins and of the derivatives of starches and celluloses when suitably compressed acquire rigidity and interesting elastic properties; we are forming in fact a two-dimensional gel, the prototype of a membrane. We shall return to some of the reactions which are observed with such monolayers, but may observe in passing that these macromolecules in a monolayer are in part crossing one another by the accident of distribution, in part associate with one another through three separate factors: (*a*) the non-polar side chains forming a hydrophobic surface to a triplex sheet; (*b*) association through the –CO–NH linkages in the chains; (*c*) association between some of the polar heads in the substrate. It appears that at extremely great surface dilutions of many proteins actual molecular separation occurs, and we are thus presented with a simple method of determining the molecular weight from the relation $FA = RT$ of these complex bodies.

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 Tables 1 and 2 and Fig. 1 are given three different examples of such a variation in reaction effected by change in compression of a monolayer.

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 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 amongst them crystalline trypsin and crystalline pepsin which rapidly digest monolayers of caseinogen, the former at pH 8 and the latter at pH 2. When the purified and crystalline enzyme preparations are employed, these enzyme actions on the protein monolayers behave exactly as

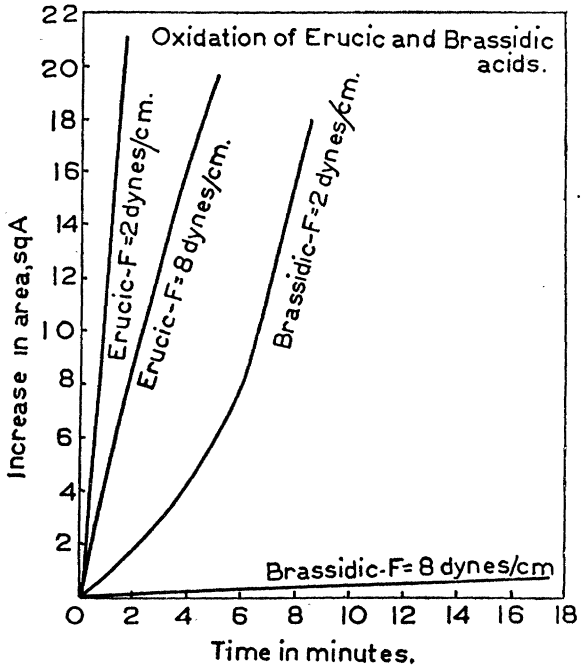

FIG. 1. Oxidation of Erucic and Brassidic acids by 0.005 per cent. $KMnO_4$ and N/100 H_2SO_4.

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.

TABLE 1

HYDROLYSIS OF TRILAURIN ON N/5 NaOH. T = 20°

F dynes/cm	E cals./gm mol.
5·4	10,000
10·8	13,200
16·2	16,100

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 oriented 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.

TABLE 2

ATTACK ON LECITHIN MONOLAYERS BY 0·001 PER CENT. BLACK TIGER SNAKE VENOM AT 20° AND PH 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

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 photochemcial 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. It is true that we are not yet certain in any one case as to the exact composition, structure or thickness of the cell membrane, but we are certain that the surface structure must be organized in the sense that forces of molecular orientation must be operative in the membrane. Whilst, as we have seen, a monolayer membrane of a protein may be destroyed by suitable enzymes, yet the phenomena which I am now referring to do not appear to be the result of chemical action in the usual sense of the word. The reactions themselves do not appear to possess large temperature coefficients indicative of sensible energies of activation, although it must be admitted this fact is frequently obscured by other processes operative at the same time. We may mention in passing the thermal denaturation of proteins is a reaction in which the apparent and true energies of activation are markedly different, a fact emphasized by the investigations of Steinhardt and La Mer. A second criterion is to be found in the fact that these processes are nearly all catastrophic in character, *i.e.*, the process under investigation being recorded as a hit or miss. As far as quantitative results are possible in such systems it appears that a definite quantity of reactant related naturally to the extent of all surface is required to bring about the reaction, and further that this quantity is removed from the environment on to or into the cell wall. One further point of interest is that the relation between the quantity on or in the cell wall and the concentration in the environment can be expressed in terms of an adsorption isotherm. Not too much stress may be

laid on this last point, because the adsorption isotherm may equally well be replaced for existing experimental data by a partition function between two phases or by the mass law operative between easily dissociable salts.

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 sulfate 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 the 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 is of the order of some 10,000 cals. per gm. mol. Complexes containing the constituents in ratios other than one to one can be prepared; thus cetyl alcohol and cetyl sulfate 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, *i.e.*, 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 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 over 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 cc of cetyl sulfate.

If sodium cetyl sulfate be injected beneath a monolayer of cholesterol this substance will penetrate to form, as we have seen, a complex. By suitable adjustment of the pressure this complex can be maintained at the definite composition of one to one, excess of the sodium salt being ejected as the pressure is raised. It is found that the pressure on the film has to be raised as the concentration of sodium salt in the substrate is raised; some of the values obtained are given in Table 3.

TABLE 3

Conc. sodium cetyl sulfate gms/cc $\times 10^{-7}$	Equilibrium pressure in dynes/cm for the 1·1 complex formation
1·0	17
2·0	32
3·0	38
4·0	45
5·0	47
6·0	48

We conclude that there must be a more or less complete layer of the sodium sulfate adsorbed beneath the surface of the complex. We are thus led to the view that in many oil-in-water emulsions as well as in the micellar aggregates found in soap solutions the gegen ion atmosphere around the emulsion particle or micelle must contain a number of molecules of the emulsifying soap, a somewhat novel conclusion as to their structure. Further, that emulsions formed from such complexes should be remarkably stable with a low interfacial energy. In some cases a small alteration in the pH of the substrate may affect the ease of penetration of

a reactant to a marked extent. In the 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 pH. 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 sulfate 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,

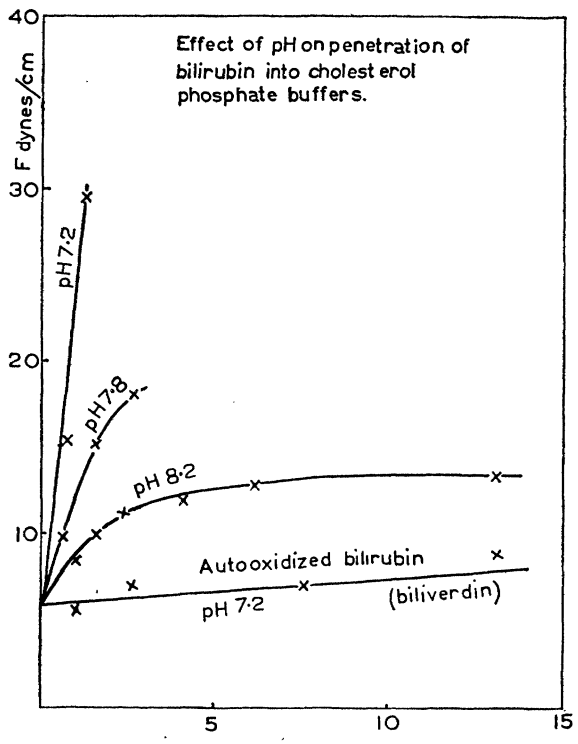

FIG. 2

especially the alcohol soluble and thus readily dispersible protein gliadin, has been greatly extended in recent years.

The stability of the protein monolayer 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 sulfate 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 are 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 nonpolar 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 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 paramoecia 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 hemolytic 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 sulfate and can be transported to the cell wall in this form. There both the sulfate 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 draw 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 behavior are the blood coagulants heparin and the synthetic sulfate 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, *i.e.*, 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 neighbors 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 in the dispersion of the galloyl-amine 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

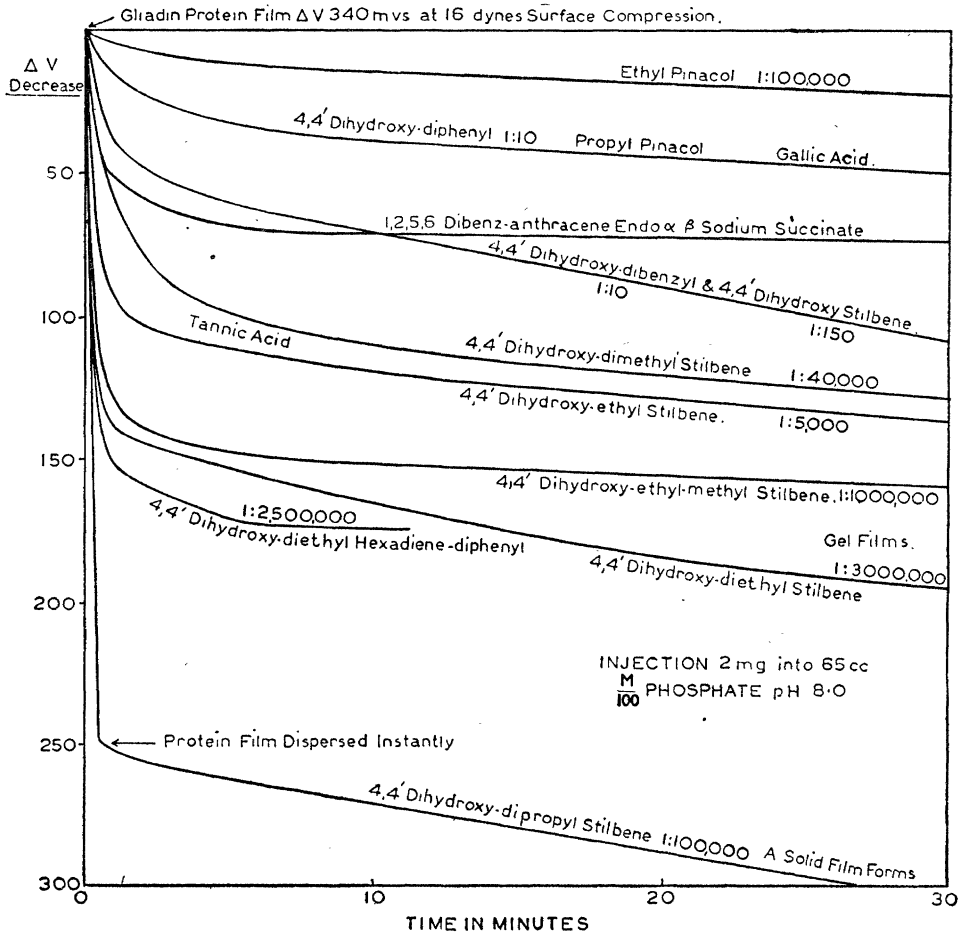

FIG. 3

by the multiple point contact of the large tannic acid molecules.

The extremely reactive oestrogenic compounds of Dodds and Lawson, of which the *pp'* dihydroxy diphenyl hexadiene and stilbene derivatives form a definite series in which the ratio of hydrophobic to hydrophilic portion can be varied, present an interesting series, the results of injection of which under a protein monolayer are shown in the attached curve.

It will be noted that reaction sets in rapidly, but equilibrium is only finally attained after some 15 to 20 minutes, and that there is a parallelism between the estrogenic activity and the protein adsorption except in the first and the last members, points we shall refer to later.

In apparent conformity with Traube's view, there is a marked increase in adsorption with increase in the number of CH_2 groups in the molecule, and this adsorption is cut down by the insertion of polar groups. This effect is clearly exemplified in comparing the diethyl stilbene or dibenzyl compounds with the corresponding pinacols. If, however, reactive polar groups, *e.g.,* the phenolic hydroxyl, had been inserted instead of the primary alcoholic groups in the pinacols, a marked increase rather than a decrease in adsorption would have been observed, for the following compounds react in order of increasing adsorption on both amine and protein monolayers—cresol, gallic acid, digallic acid, purpurogallin and tannic acid. This order is, however, reversed to give a normal Traube series when these substances are injected under a relatively non-reactive monolayer such as a long chain acid.

A wide variety of substances have been examined from this point of view, namely, their extent of interaction with protein monolayers, and it has been found that there is a direct parallelism between their extent of interaction and their lethal action on paramecia. Another significant biological similarity has been noted when we measure the extent of penetration of a series of substances containing identical hydrophobic "tails," *e.g.,* 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 cc under a film of cholesterol originally extended to 40 A^2 per molecule is found to be closely parallel to the hemolytic activities and lethal activities on paramecia of these substances.

These latter can be placed in order both of monolayer penetration and biological activity as follows:

$$RNH^+_3 > RSO'_4 > RSO'_3 > RCOO' > RN(CH^+_3)_3 > RNH(C^+H_3)_2 > \text{Bile acids.}$$

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, *i.e.,* 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 *ca.* 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 may be enclosed in ring systems; 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 some $12 \cdot 5$ 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, *i.e.,* 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, as exemplified in the figure, sets in with long chains, *viz.,* dispersion of the monolayer, most marked in the case of 4 4′ dihydroxy dipropyl stilbene. 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, *e.g.,* anesthetics. This dispersion of protein films may have biological counterparts in adsorption on specific portions of the cell surface similar to the hemolytic activity of long chain compounds such as oleic acid, which readily disperses protein films. Another interesting parallelism has been observed in the surface reactivities and estrogenic powers of two isomeric compounds (*pp'* dihydroxy diethyl dibenzyl), one being markedly differentiated from the other in both protein adsorption and in estrogenic activity. Here models indicate that the trans arranged

rings can pack laterally with one another in sheet form much more readily and with a greater degree of adlineation than the cis structure, imparting stability to the adsorption complex formed with the former substance.

In advancing these somewhat novel principles based upon the hypothesis of a parallelism of film reactions and biological activity, it is desirable to point out exceptions. It is found, for example, that 4 4′ dihydroxy diethyl pinacol is a much more effective estrogenic agent than either its parameciicidal activity or adsorption on protein monolayers would suggest. The view might be advanced that on certain living tissues it can be partly converted by enzymitic dehydration to the extremely active 4 4′ dihydroxy diphenyl hexadiene. Another interesting exception is to be found in desoxycholic acid, which is the only hemolytic agent in the bile acid (*ca.* 1:550) series and is likewise lethal on paramecia. It is as we have seen unreactive to films of protein, cholesterol and glycerides, and in fact a specific interaction with fatty acids is involved.

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 (cytolyzing) 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 endosuccinnate, but also are parameciicidal, the parallelism between the biological activity and monolayer reaction being maintained.

Many attempts have been made to construct model systems to yield potential differences analogous to the bioelectric potentials observed in tissues. The work of Beutner, Bauer, Cremer and others suggests that potential differences of magnitude corresponding to those found in living systems can be obtained by interposing suitable oil phases between electrolytes of different composition, and the fact that the penetration of large molecules into living cells frequently follows their lipoid solubility has given support to the theory that the seat of the bioelectric potential lies in the lipoid-like cell wall. The order of thickness of such cell walls can not exceed a few molecular layers, and we must take this fact into consideration. We have noted that at the lipoid-water interface there will exist an orientated layer of dipoles, and on placing a monolayer at the interface, the original array of solvent dipoles will be replaced by one consisting of the material of the monolayer. If an electrolyte be brought to equilibrium in both the homogeneous phases, it is clear that opposite the monolayer in both the aqueous phase and in the lipoid phase adsorption and electrokinetic potentials[2] will be built up of such magnitudes that the total potential fall across the interface, which may be written $\xi\text{oil} + \Delta V + \xi\text{water}$, must be zero. If the lipoid phase be replaced by air no diffuse double layer can be built up, since the gaseous ions produced by the usual radioactive source are continually drawn into the liquid phase; there is in consequence a permanent potential fall equal to $\Delta V + \xi\text{water}$, which is the one customarily measured.

It is evidence that a bioelectric potential difference may be caused by a sudden alteration in ΔV, for the compensating potential differences ξoil and ξwater must take time to readjust themselves by diffusion to the new equilibrium values. Since in general the electrolyte concentration in the aqueous phase is high, it seems probable that ξwater will adjust itself to the new value acquired by ΔV as rapidly as ΔV can be caused to change either by mechanical, electrical or chemical means. Thus surges in potential difference across the interface due to a periodic alteration in ΔV may be caused by the slow readjustment of ξoil, for it is in this phase that the ionic concentration is low.

Another source of biological potentials is to be found in the case where the chemical potential of the electrolyte is not the same in the two phases, bringing into existence a diffusion potential across the interface from source to sink. It is clear that a monolayer can only affect the diffusion potential provided that its permeability to the ions is not only comparable to that of the homogeneous phases on each side, but that it also is not equally permeable to both ions. Experiments have shown that monolayers and even built-up multilayers of considerable thickness of proteins are surprisingly permeable to ions and we must presume that the bioelectric potentials do not involve only a protein membrane between the ionic source and sink.

[2] Whether adsorption or electrokinetic potentials will be built up in any specific case will depend on whether short or long range forces are involved *i.e.*, on the magnitude and spacing of the dipoles. If the dipole system occurs across a relatively thick multilayer the potentials will be purely electrokinetic.

It thus appears that there is some justification for the assumption that it must be a lipoid or a lipo-protein membrane.

It has been the purpose of this address to re-emphasize the importance of the fundamental concepts introduced by Sir W. 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 behavior that we have taken up the detailed study of interfacial reactions at Cambridge, and I should like to express my deep indebtedness to my colleague, 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.

OBITUARY

THE MAYO BROTHERS AND THEIR CLINIC

THERE was nothing mysterious or supernatural about this twentieth century Lourdes at whose doors incredible numbers of the lame, halt and blind have for years been daily delivered from the ends of the earth. Nothing supernatural—unless possibly the flawless, life-long devotion of two brothers for one another be so regarded. Not since the somewhat mythical attachment of those fifth-century physicians, Cosmos and Damian, both of whom in due time came to be sanctified, has there been anything quite like it.

Rochester, Minnesota, fifty years ago, then scarcely on the map, was a prairie town near the headwaters of the Mississippi where in a humble way, at St. Mary's Hospital, the Clinic had its beginning. It was, to be sure, a Catholic foundation in which Sisters of Mercy doubtless prayed for the recovery of their patients. But it was not primarily for prayer, however efficacious, that the afflicted as by a magnet came to be drawn to that particular shrine.

It was rather the world-wide reputation of two forward-looking men whom I like to remember as they were thirty years and more ago, young and vigorous; each blessed with rare surgical judgment, each with hands which seemed possessed, in an emergency, with an uncanny ability to do, unflustered, just the right thing at the right moment.

At this shrine there was plenty of ritual, to be sure, but it was the ritual of the well-drilled, silent, operating room where for every movement there is a reason; where the incense in the air is not to conceal corruption but to produce painless sleep; where the water in which gloved fingers are dipped is holy only because it is sterile.

Their father, the senior Dr. Mayo, pioneer and Indian fighter, was still alive when I first came to know the place in its early simplicity. There were then but two operating tables, at one of which "Dr. Will" officiated, at the other in an adjoining room "Dr. Charlie." They were thus affectionately differentiated by every one—staff, patients, employees and fellow townspeople —not to mention the countless visiting doctors who even then were wearing a path to their door.

For these also soon came from all parts of the world, often by special trains, to see for themselves what modern miracles were being performed daily in this once obscure country town. To what they could learn and carry away for their own use they were more than welcome, for our profession has no trade secrets. The more widely knowledge can be disseminated, the better for every one.

And so, as the years slipped rapidly by, a great tower of healing, known everywhere as the Mayo Clinic, was finally erected—a living memorial to a great idea, not a mere place of worship for tradition dead and gone, like the Basilica of SS. Cosmos and Damian built some fifteen centuries ago in Rome by Pope Felix IV.

Another contemporary pair of no less self-effacing brothers—Wilbur and Orville Wright of Dayton, Ohio —were also at about the same time dreaming dreams of a different sort that in no less spectacular fashion came likewise to be fulfilled. Like the Mayos, they seem to have imbibed in their youth the flavor of the old Northwest Territory where the offspring of the early settlers were reared to think more highly of serving mankind than of helping themselves.

One is led to wonder whether imaginative visions of such kind are not more likely to occur and be more possible of realization for those who live where horizons are broad than for those cooped up in metropolitan centers where, even could the rising or the setting sun ever be seen, there would be no time to stop and commune with it.

Different as W. J. and C. H. Mayo were from each other, I have always felt that there was something Lincolnesque about them both. It was shown not only