Interaction of Soluble Proteins with Protein Monolayers

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Abstract

The direction and strength of intermolecular forces at an air-water or oil-water interface is such that many proteins in the interface are distorted in structure. This involves substantial changes in solubility and cross-sectional area. Many of the changes can be accounted for by rupture of the secondary and tertiary bonds and are often irreversible. The hydrophilic groups of the protein will be concentrated in the aqueous phase and participate in interactions with normal proteins in the supporting solution. It can be shown that certain types of interaction between these hydrophilic groups of a protein monofilm and a soluble protein are dependent on the interfacial pressure, that they are sensitive to a small (one or more amino acid) change in structure of the protein. Evidence is given that they are related to certain antigen-antibody type reactions between molecules in three-dimensional systems. Since many proteins in vivo are exposed to oil-water and air-water interfaces, this laboratory model may have physiologic as well as chemical significance.

Introduction

THE BEHAVIOR OF PROTEINS at an interface between a polar liquid and a nonpolar liquid appears to have many biological applications. In vivo, one of the most obvious examples of such an interface is the lipid chylomicrons circulating in plasma. For many years it has been recognized that a number of pathological circumstances lead to the cytoplasmic accumulation of lipid droplets (1). Another example of lipid droplet accumulation is the so-called fluorescent cells of normal organs (1a,2,3). These are presumed to be fluorescent lipid droplets and have been associated with cell growth and immunity. Both the immune process and the lipid droplet formation are associated with rapid protein synthesis. It is possible that protein is associated in an important way with the lipid-water interface.

At another order of magnitude there are an almost incalculable number of lipid protein interfaces in cell membranes and cell organelle membranes. This relationship of lipid and protein has been extensively studied by Stoeckenius (4).

Unfortunately water-lipid interfaces in vitro have presented formidable technical difficulties and have been little studied. The interfacial pressures of the air-water interface are such that the protein in the interface resembles in part the oil-water interface. There are some differences between the two systems however. Proteins spread more easily and completely at oil-water interface than at the air-water (5). Nevertheless the air-water interface can be an important preamble to the study of proteins at a lipid-water interface.

Even though the protein monolayer at a water-air interface has long been an attractive model for biological systems, there are still major problems associated with it. One of the major problems in protein monolayer work has been the problem of molecular structure. Until recent years the polypeptide sequence of protein has been unknown, and even today the secondary and tertiary structure of proteins is largely a matter of speculation. As a consequence, the principles of protein behavior at these model interfaces have been difficult to establish.

Since proteins may be classified in many ways, it may be useful to identify three types. One is the globular protein, of which plasma proteins are a good prototype. These are soluble in aqueous solution and circulate in the plasma of higher invertebrates and vertebrates. Another is the structural proteins which are not in solution in vivo. These often appear to be more unfolded than the globular proteins. Some appear to have many cross-links between polypeptide chains and to form helical structures. Collagen and its derivative, gelatine, would be a good example of this type. The third type is proteins which are mixed with other molecular species, such as the lipoproteins and the nucleoproteins. This type is of some value in interface chemistry because some of the principles of protein behavior of the interface are dependent on the degree of unfolding and on the nature of the nonprotein moiety.

Something is now known of the cellular mechanisms of synthesis for soluble proteins of the globular type. In Fig. 1 the polysome with messenger RNA is shown in a diagrammatic way as a synthesizing apparatus for a long chain of polypeptides. The important point is that the first steps of protein synthesis by current speculation are the formation of a linear polypeptide. This would necessarily have regions of relative aqueous insolubility. In one sense this original protein is in a denatured state. The steps that lead to the folding of this polypeptide in the cell have not yet been elucidated, but the consequences of the folding are the formation of a compact, globular-shaped molecule (Fig. 2).

This natural protein is now fully soluble. If a chemist were to come across the original unfolded precursor, it would probably be called a native or denatured protein. This molecule has the hydrophobic centers on the inside and is presumably surrounded by a cage of ice-like water molecules. The forces leading

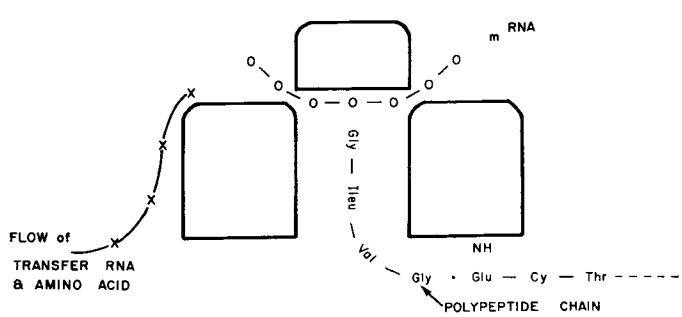

FIG. 1. A schematic of the polysome with a hollow central core. The polypeptide chain flows out of the polysome and is pictured as a linear molecule.

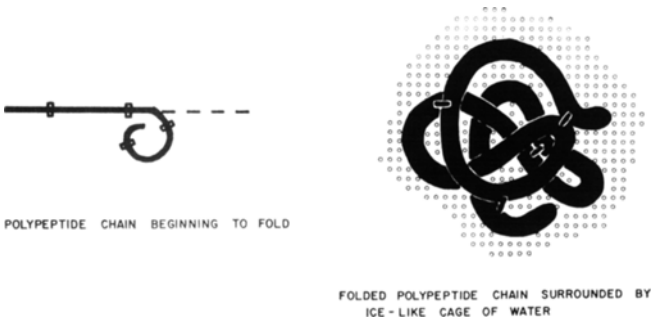

FIG. 2. The linear polypeptide from Fig. 1 somehow folds on itself and establishes cross-links, hydrophilic attractive forces, and an ice-like cage of water around the outside. The spreading of this folded protein at an interface is essentially the reverse of this process.

to the formation and perpetuation of this configuration are presumably hydrogen bonds, the attraction betweeen the nonpolar units on the inside of the molecule and the ice-like structure of water around it (6). In addition, there is a set of specific disulfide bonds which adds strength to this configuration.

This globular structure is in contrast to the sheet-like or pleated structure given for one of the major structural proteins such as collagen, and it is in contrast to the long α helix seen in the nucleic acids and some other molecules.

The importance of these considerations to the interface problem is that each form of protein in its own way suffers substantial distoration in its native configuration when it enters the air-water interface or a water-lipid interface. Many, if not all, of the forces leading to the globular forms of protein are disrupted by the interface forces. The molecule, if it can be successfully spread at the interface, will usually develop a configuration which behaves grossly as a large sheet of polypeptide chains (7). The long polypeptide chains appear to move freely, though probably not at random in the interface, and segments of these chains often behave physically as if they were an independent molecular species. This is similar to the behavior of other polymers in an interface (8). Even though portions of the spread polypeptide chain have colligative properties as if they were independent of each other, it is obvious that amino acid groups along the chain have certain required spatial relations to each other if for no other reason than that they are limited in their range of motion by the polypeptide chain.

As a result of these changes, protein monofilms of some of the plasma proteins will behave on compression at the interface as a gas first and then as a liquid.

Another predictable configuration of the protein in the interface has to do with the position of hydrophilic groups. The water-soluble side-chains are pointing down into the subsolution, and the lipid side-chains are held above the interface.

When one investigates the reaction of these side-chains, it is apparent that several principles should be considered. The active sites probably would be water-soluble. The active sites may be displaced in relationship to each other by virtue of the expansibility or contractibility of the film. In a three-dimensional solution, this phenomenon would require a distortion of the internal structure of the molecule. It is probable that the active sites of the monofilm have some ordered arrangement. They are not randomly arranged though they may have a large number of possible configurations.

In the monofilm the spatial arrangement of the active sites is under partial control of the investigator because he can contract or expand the area covered by a "set of active sites" simply by compressing or expanding the film.

A simple experiment can be carried out to demonstrate certain changes in a protein monofilm by changes in surface area. Fig. 3 illustrates a water-

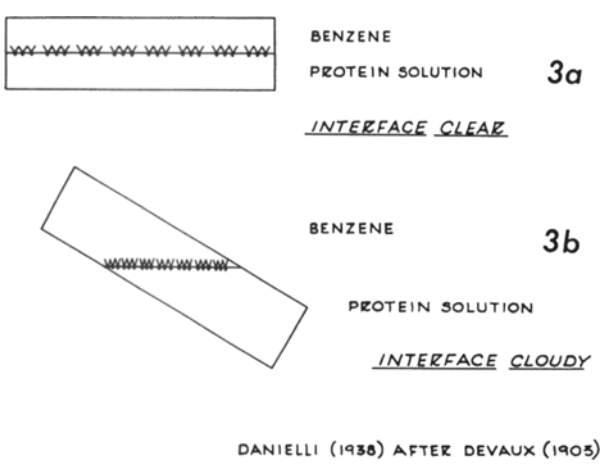

FIG. 3. A schematic of a closed vessel with benzene above and water below with a protein monofilm between. Changing the area of the interface by tipping the vessel leads to compression of the protein monofilm. This is observed by the change in optical density.

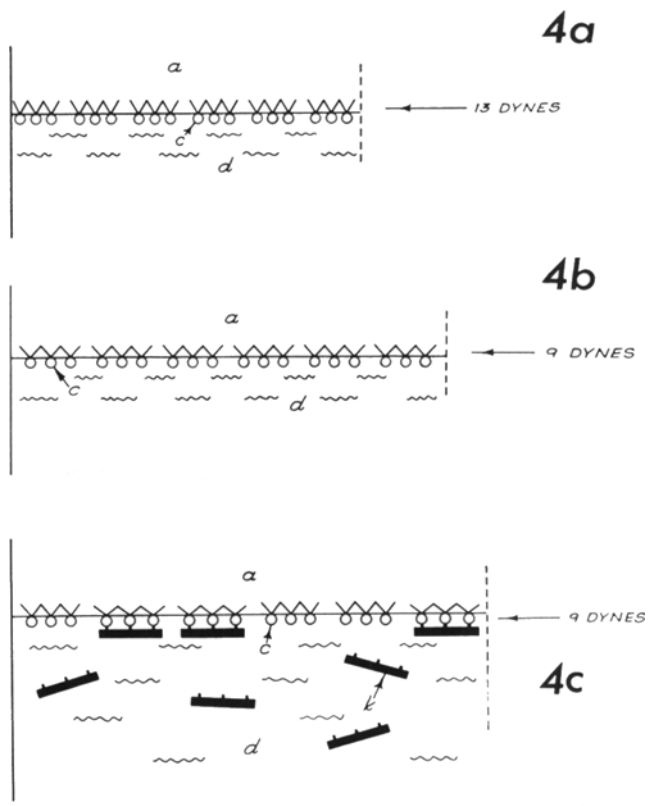

FIG. 4. A schematic of a protein monofilm at an air-water interface subjected to two different lateral pressures. With compression (4a), the hydrophilic reactive sites are crowded together 13 dynes. When the reactive sites bind to a protein in the subsolution (4a) so that they cannot be crowded together, the 13-dyne pressure may not reduce the surface area to the same extent as 9 dynes (4b) without this restraint.

benzene system in a closed vessel. This shows a maximum interface between the benzene and water. If the protein is introduced into the aqueous phase, movement of protein to the interface apparently occurs as part of the random diffusion of the protein molecule. Once contact is made with the interface, the protein enters this region selectively.

As it does this, the protein solubility in water diminishes. This change in solubility apparently occurs in sequential steps; each step alters the water solubility of the molecule so that the hydrophobic elements of the protein are now exposed to the solution and preferentially they enter the benzene phase. The protein is able to enter this interface over a period of time until a certain critical surface pressure is established. At this point there appears to be no further entry of the molecules into the benzene-water interface. If at any time following this process, this vessel is tilted (Fig. 3) so that the benzene-water interface is reduced in area, the protein molecules are crowded one against another with a sharp increase in pressure and an increase in optical density of the monofilm. At a critical pressure known as the collapse point, protein monofilms are forced from the interface into the subsolution. It is not known what happens to the molecular figurations of protein monofilms as they collapse. Most proteins appear not to recover their original configuration when the film collapses.

The protein monofilm is shown in Fig. 4 as if it were in a typical Langmuir balance. The interface at the top is under 13 dynes pressure. The protein monofilm is relatively close-packed. The reactive sites of the molecule, which are available to the subsolution, are relatively close to one another. At a lower pressure of 9 dynes the molecules are less closely packed, reactive sites are presumably at a greater distance from each other. If a new protein species is introduced into the solution, as illustrated by black bars at the bottom of the figure, then a match of reactive sites between the two molecular species may occur. This match will be dependent upon the relative spacing between specific reactive sites. If the match in the distribution of reactive sites is exact, there would presumably be no change in surface pressure or in the molecular configuration of either protein. If the match in the distribution of reactive sites is discordant, then presumably no reaction is possible. If however a match of reactive sites can occur but only with a relative change in the distribution of reactive sites on the molecules in the monofilm, then a change in surface area at constant pressure should be observed. This configuration of reactive sites on the monofilm is to a certain degree under the investigator's control.

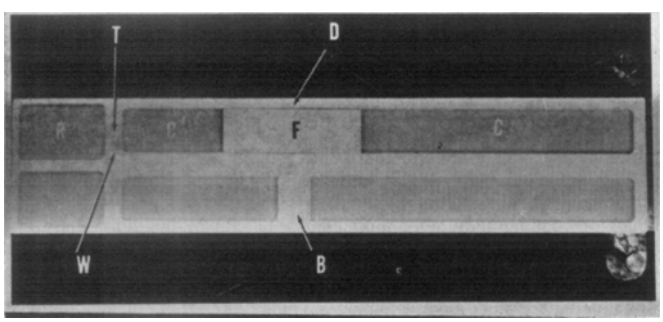

FIG. 5. A Teflon tray made to hold solutions on which protein monofilms may be formed. R and C^1 to be compressed by a float F backed by a piston oil C, which acts as a servomechanism to maintain constant pressure on F. A mercury seal D prevents leaks; a glass-filled Teflon block keeps solutions on both sides separated.

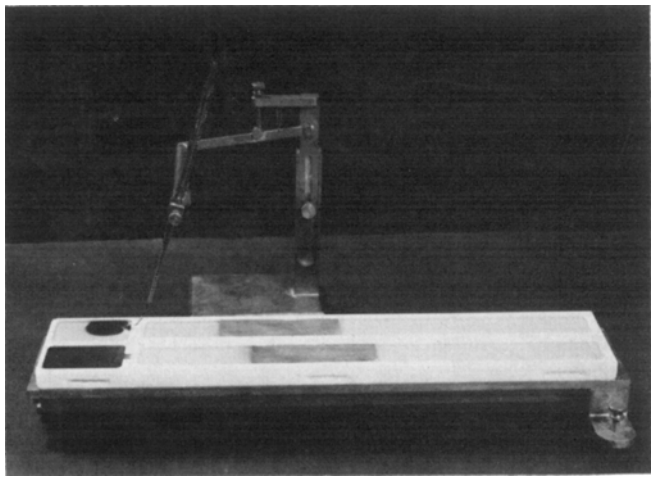

FIG. 6. The technique of injecting soluble proteins in the subsolution. India ink for greater contrast was used in place of protein.

Schulman and Rideal (9) discussed specific molecular interactions at an interface in terms of simple molecules and concluded that this system could be used to determine some degrees of specific molecular interaction. Their molecules were not flexible and would not have the large number of different reactive sites that one finds in the protein monolayer.

In order to investigate the protein monolayer and its molecular interactions with the molecules in the subsolution, a special apparatus was designed and utilized (10–11). Such an apparatus is illustrated in Fig. 5. This is a Teflon block about one foot in length, in which two compartments have been milled. Since both compartments are alike, only one will be described. A reaction area R is constructed so that a protein monofilm may be formed on its surface. Under this test area of the film, a suitable reacting molecule can be injected. The monofilm in R is in direct contact with a floating plastic film, which moves freely back and forth as if it were a two-dimensional piston in the larger compartment. This piston or Float F is held at constant surface pressure by a piston oil, as described originally by Cary and Rideal (12). This functions to permit changes in area of the compartment R and therefore all the other compartments while the entire system is held at constant surface pressure. This is a kind of molecular servomechanism and permits experiments to be carried out with the monofilm always at standard pressure. Special details of the construction and operation of these units are given elsewhere (10).

One would presume that the reactive sites of a monofilm maintained under constant pressure should bear the same relationships to each other unless, of course, some new molecular configuration is established by mixing with the molecule in the subsolution. Fig. 6 shows the apparatus after the introduction of India ink into the subsolution as if it were a reacting protein. This technique can be used with rapid mixing so that contact of the monofilm with the soluble protein then occurs as a process of simple diffusion. This means that time is a major determinant in the number of contacts of the soluble protein with the monofilm and therefore with the behavior of the monofilm.

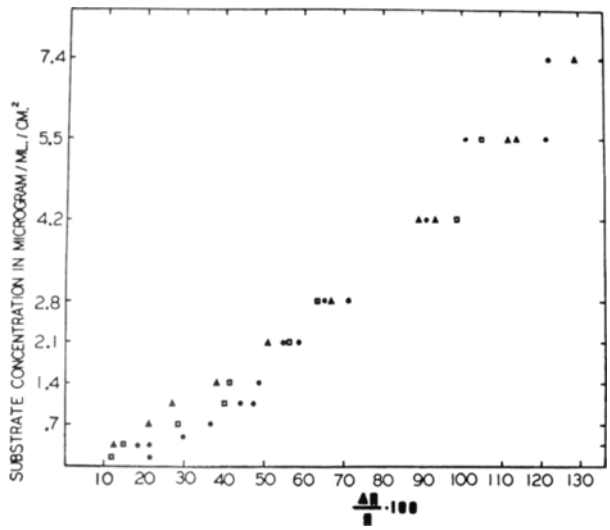

FIG. 7. Effect of concentration of soluble molecules in subsolution on film expansion. Pork insulin monofilms were reacted with protamine sulfate (Lilly, NF) to demonstrate reproducibility and linearity of reaction. Different symbols represent three different months in which the experiments were performed (0.8-lambda mercury droplets; 30 min of reaction).

Reproducibility of Isobaric Changes

Insulin forms a stable monofilm at an air-water interface, and it can be acquired in highly homogenous crystalline preparations. It can be recovered from monofilms with its original hormonal activity relatively intact. The amino acid sequence is also known. For these several reasons, insulin provides an ideal model of a monofilm made from globular protein. In Fig. 7 repeated determinations of the proportionality between the isobaric area changes of insulin monofilm and the concentration of protein in the subsolution have been plotted during the course of a year. These indicate a high degree of reproducibility and proportionality of reaction at a standard time.

As another example of the proportionality of the isobaric area changes, a thymus nucleohistone monofilm reacted with serum is shown in Fig. 8. These indicate that, over a 25-fold range of concentration of the protein on the subsolution, the area changes are proportional to the protein concentration in the subsolution.

Specificity of Isobaric Changes

The molecular structure of the protein monofilm, as well as the molecular characteristics of the reacting soluble protein, can be shown to determine the extent

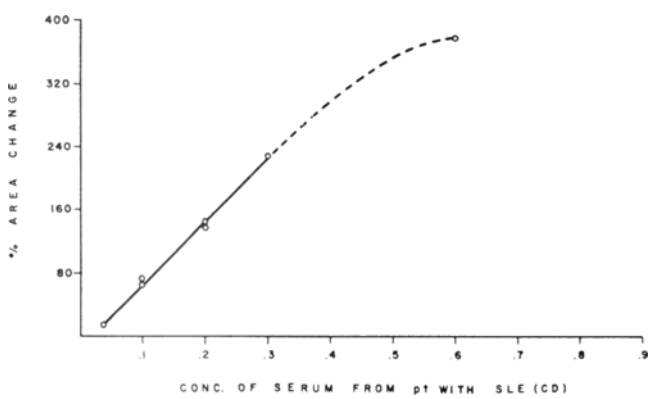

FIG. 8. Proportionality of isobaric area change of a calf thymus nucleohistone monofilm over 0.15 M phosphate buffer at pH 7. SLE (systemic lupus erythematosus).

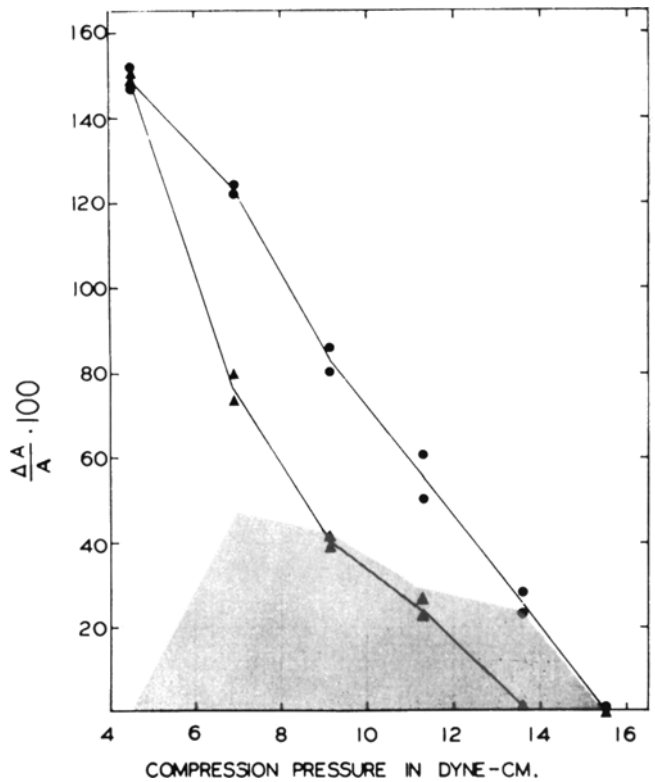

FIG. 9. Comparison of isobaric area change of an insulin monofilm and a γ-globulin in subsolution. The difference of the two curves is plotted as a shaded area (▲ with a γ-globulin monofilm and a γ-globulin in subsolution ●). This area represents the "specific interaction."

of isobaric area changes. In Fig. 9 the area change of an insulin monofilm reacted with serum γ-globulin

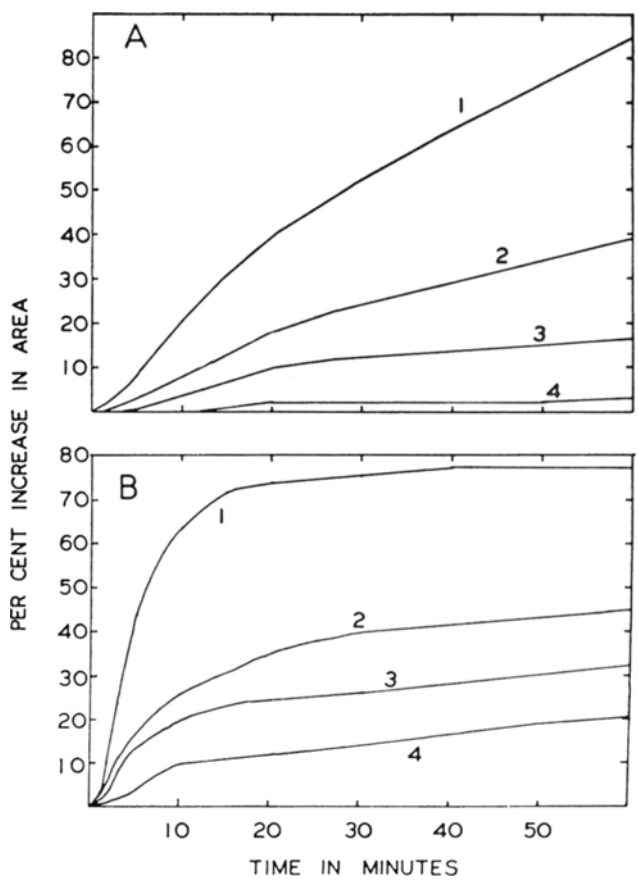

FIG. 10. Percentage increase in area.

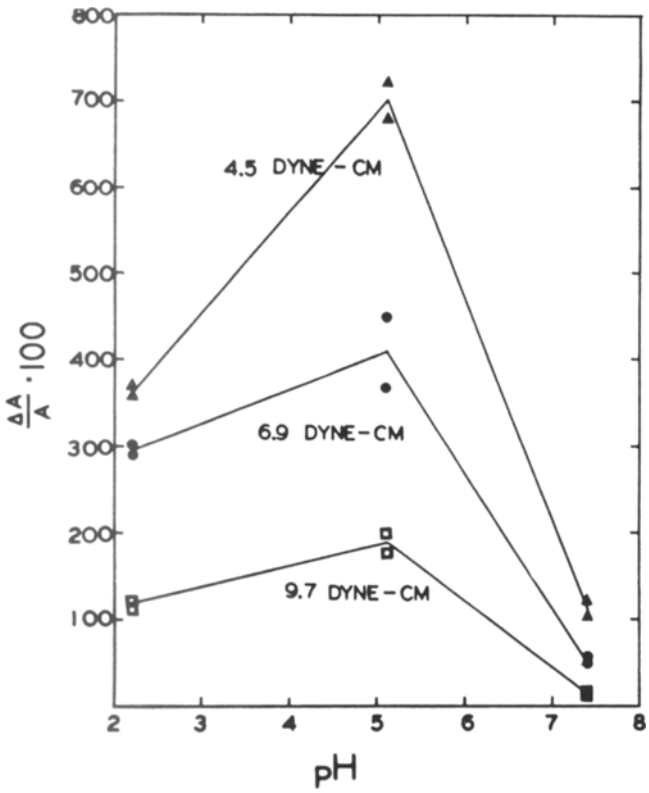

FIG. 11. The effect of pH on the interaction between an insulin monofilm and γ-globulin in subsolution at different surface pressures.

is compared under similar conditions with the isobaric changes of a γ-globulin monofilm with the γ-globulin also in the subsolution. In the latter instance, one may view the isobaric area changes as a consequence of the spontaneous nonspecific entry of γ-globulin into the interface. The fact that area changes are different

	8	9	10
Cattle	**Ala**	**Ser**	**Val**
Pig, Sperm whale	**Thr**	**Ser**	**Ileu**
Sheep	**Ala**	Gly	**Val**

FIG. 12. The amino acids at position 8, 9, 10 of the insulin molecular.

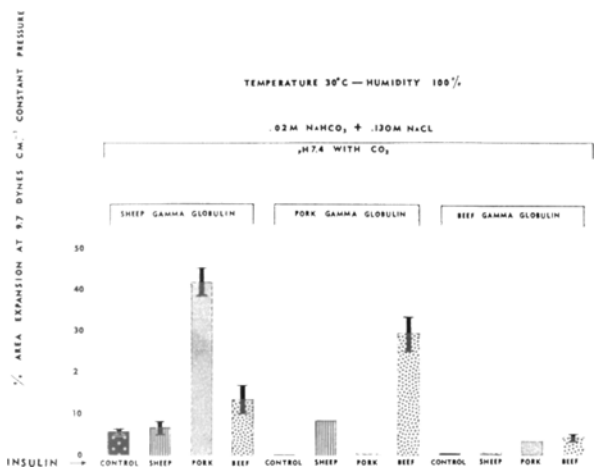

FIG. 13. Expansion of three species of insulin monofilm with 0.1 mg γ-globulin (Cohn Fr. 11).

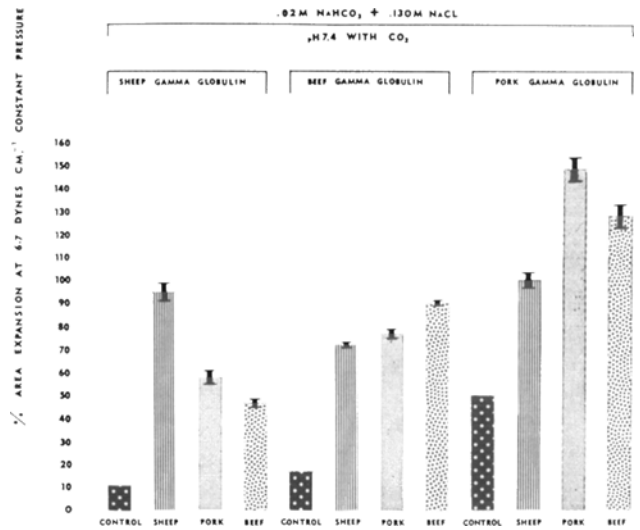

FIG. 14. Expansion of three species of insulin monofilm with .075 mg (three species of) γ-globulin (Cohn Fr. 11).

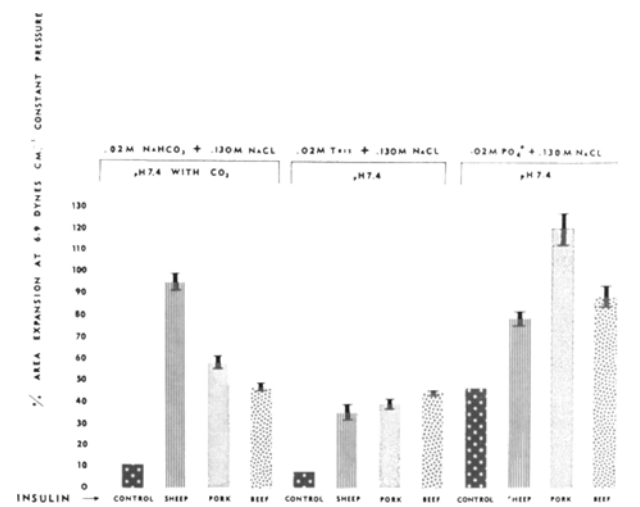

FIG. 15. Expansion of three species of insulin monofilm with 0.1 mg sheep γ-globulin (Cohn Fr. 11).

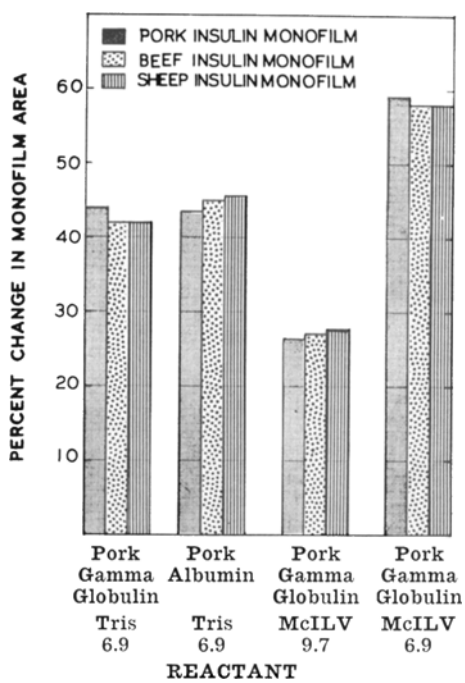

FIG. 16. Percentage change in monofilm area.

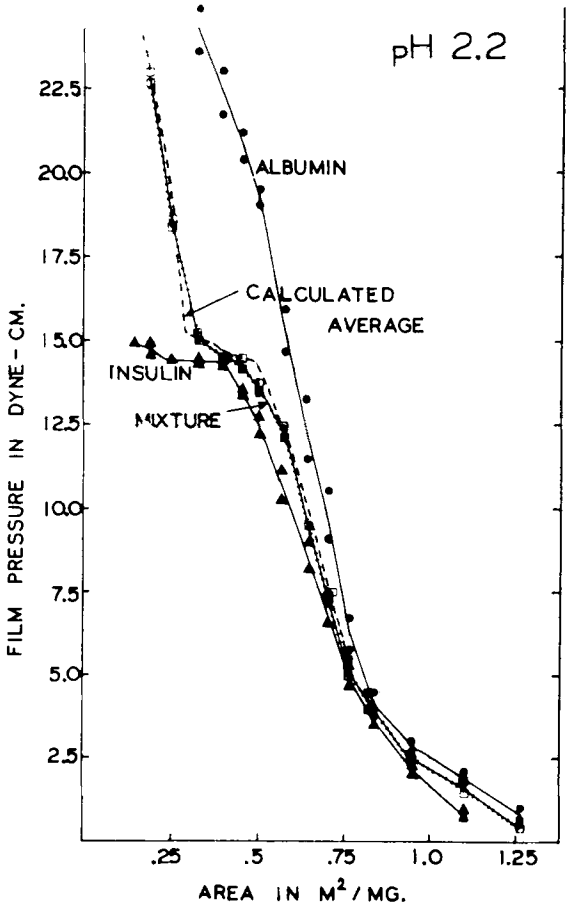

FIG. 17. Comparison of force area curves.

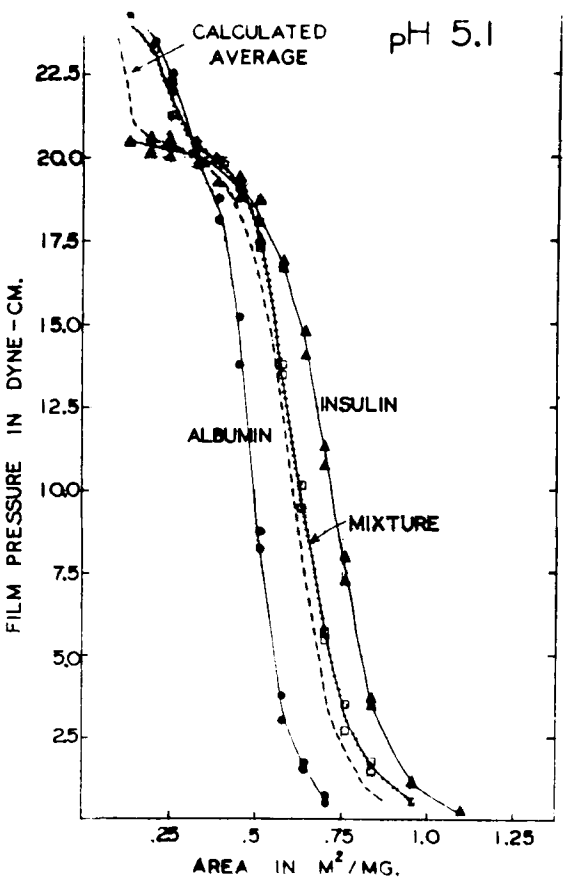

FIG. 18. Effect of change in pH on force area curve.

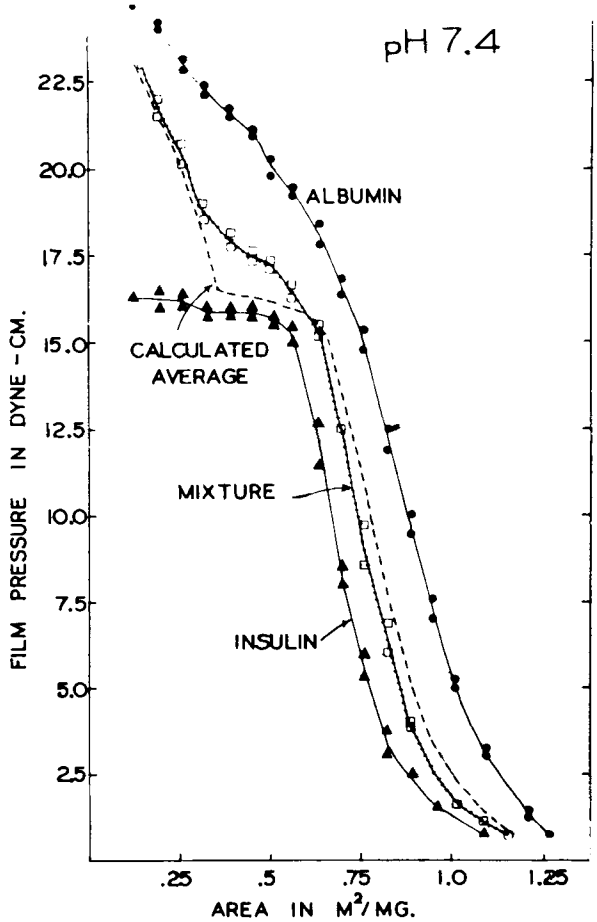

FIG. 19. Another effect of change in pH.

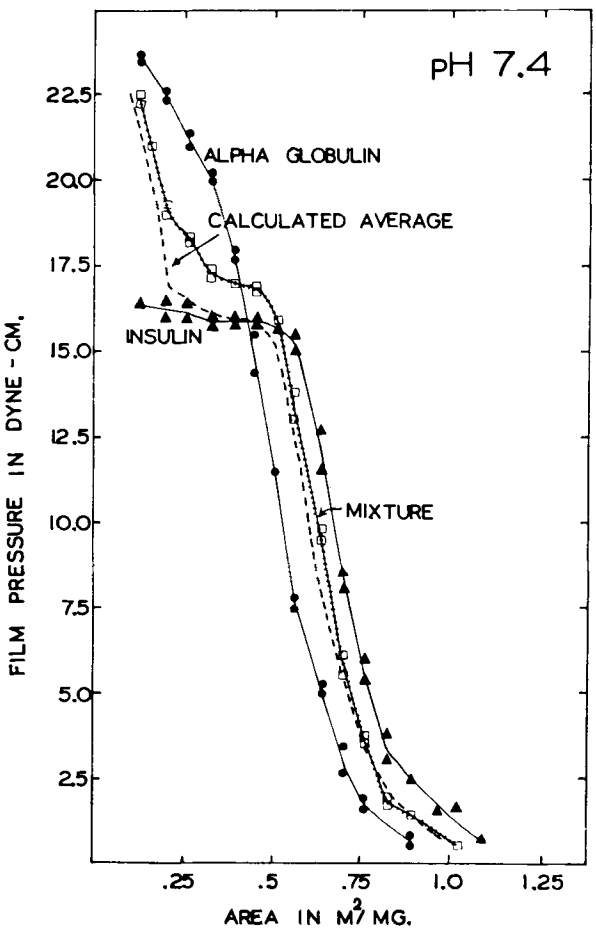

FIG. 20. Indication of lateral interaction owing to pH.

for different protein monofilms is evidence for a specific kind of molecular interaction with insulin and γ-globulin. The role of the surface pressure in revealing this specific interaction is indicated by the difference, which is plotted as the shaded area in Fig. 9. In this instance, the most specific interaction appears at a compression pressure of about 7 dynes. The isobaric area change is a time-dependent phenomenon.

The kinetics of the reaction shows a time dependence for both the specific and the nonspecific isobaric area change. In Fig. 10 the time course of several reactions is plotted. It is of some interest that the shape of the curves for each type of reaction is different.

In the systems studied so far, the maximum reaction between a protein monofilm and a soluble protein has usually been in the pH range between the isoelectric point of the two proteins. In Fig. 11 the reaction between an insulin monofilm and serum γ-globulin is expressed over the pH range. It is apparent that, at several different pressures, the maximum change of area occurs about 5.2 pH. It is also apparent that the greatest reaction occurs at the smallest pressure.

Dependence on Molecular Structure

A set of three insulin monofilms from three animal species was reacted with the γ-globulin from each animal species. The monofilm polypeptides differed from each other at Position 8, 9, and 10 (Fig. 12).

The insulin monofilms had similar force area curves, but the isobaric area changes, when reacted with globulins from each species, show a quite different pattern, than that of the indicated γ-globulin monofilm with the same γ-globulin in the subsolution.

In Fig. 13 the behavior of each monofilm with each of three γ-globulins is shown at pressure of 9.7 dynes cm^{-1}. The subsolution was an isotonic bicarbonate buffer. It is apparent that each γ-globulin has a specific kind of interaction and each monofilm also has a specific interaction. If a generalization can be made from these data, it should be to the effect that interspecies interaction is more pronounced than intraspecies interaction.

In Fig. 14 this same type of experiment is performed at a much lower pressure, 6.7 dynes cm^{-1}. The interrelationships are markedly changed. In this experiment the reactive sites of the monofilm are modified by the simple technique of altering the surface pressure. This presumably has its greatest effect in changing the spatial distances between reactive sites in the monofilm. The pattern of interaction is now markedly different from Fig. 13.

A similar set of experiments has been carried out with different buffers all at the same pH (Fig. 15). The several buffers make sheep γ-globulin behave in a manner similar to beef γ-globulin (Tris. + NaCl) or pork γ-globulin (PO_4 + NaCl). The data show the great dependence upon ion species in the set of variables determining the interaction.

It is shown that the differences in behavior of the three monofilms can be eliminated by changing the pH (Fig. 16). The efiect of amino acid substitutions at positions 8, 9, and 10 is now eliminated.

Behavior of Mixed Films of Proteins

One explanation for the behavior of protein monofilms is that interactions develop between parts of the molecules of each species which are in the interface together. Such a situation can be studied by placing both the proteins in the interface simultaneously. This may be done by mixing the two proteins before spreading or by simultaneous spreading of the two proteins. In Fig. 17 force area curves at pH 2.2 of insulin and albumin, and a mixture of insulin and albumin are compared. The predicted behavior of the mixture is calculated by the method of Ries (12) and is also plotted on this figure. It is apparent that the predicted behavior would mean that each molecular species acts independently of the other in modifying the force area of the mixture. At this pH the collapse pressure of the mixture which occurs at about 15 dynes is identical to the calculated value and indicates that insulin is driven into the solution at this pressure.

In Fig. 18 it is apparent that the mixed film now behaves at pH 5.1 over the force area curve in an entirely different manner. The calculated behavior of the mixed film departs from the observed behavior, and this is most apparent near the collapse pressure of 14 dynes. Interaction between the insulin members of the monofilm and the albumin members occurs

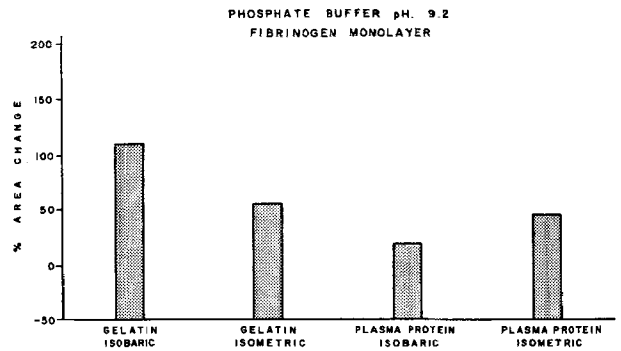

FIG. 21. Comparison between isobaric reaction and isometric reaction in terms of area occupied by complexed monolayer after reaction is completed.

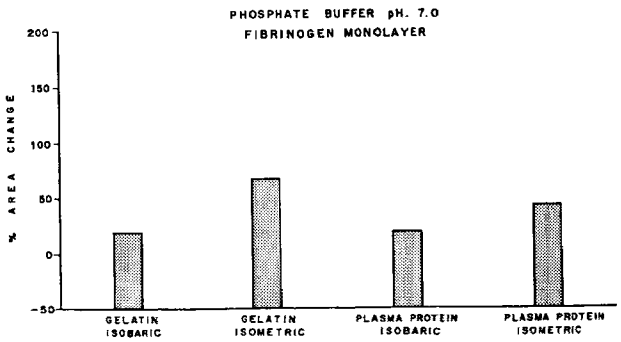

FIG. 22. Comparison between isobaric reaction and isometric reaction with change of pH from that in Fig. 21.

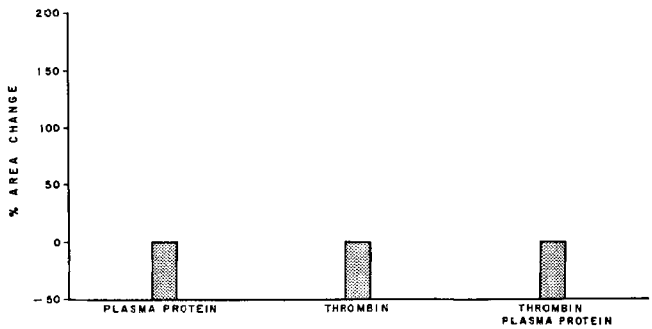

FIG. 23. Isobaric area changes of a fibrinogen monolayer at air-water interface. Reactant injected into 0.05 M phosphate buffer at pH 7.4.

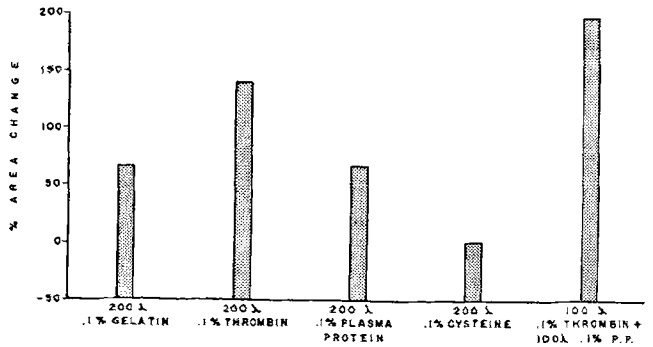

FIG. 24. Isobaric area changes of a fibrinogen monolayer at air-water interface. Reactant injected into 0.15 M phosphate buffer at pH 7.4.

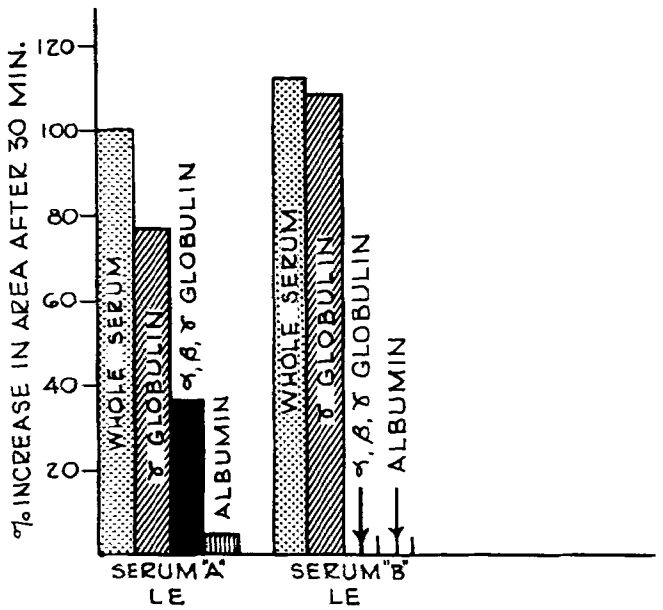

FIG. 25. Binding with human nucleohistone.

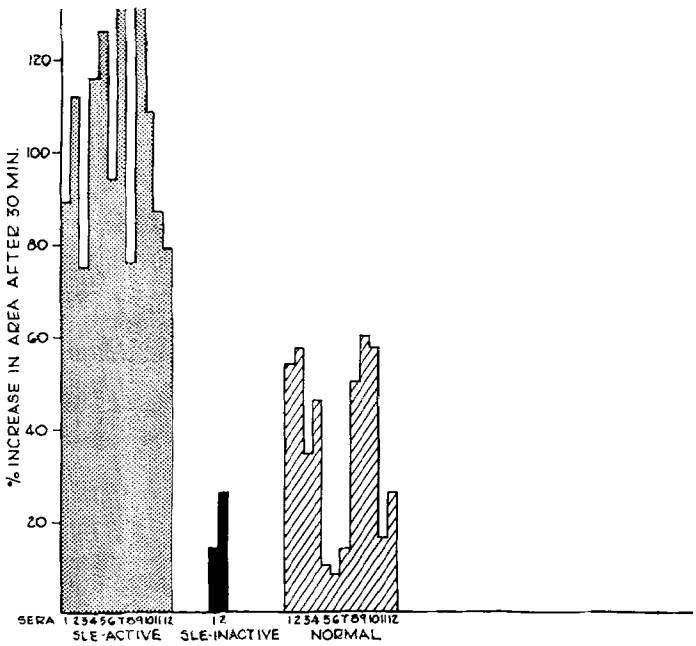

FIG. 26. Nucleohistone binding by human sera.

at this region of the curve. This phenomenon is also observed at a pH of 7.4 (Fig. 19), and it appears to be true also of the albumin *alpha* globulin mixture studied at pH 7.4 (Fig. 20). This may mean that there is lateral interaction between the insulin and the serum protein molecules at these particular pH values.

Use of Fibrinogen Monolayers

In order to demonstrate certain other principles about protein monolayer interaction, use is made of the fibrinogen film. A specific example of the variation in results between isobaric and isometric area changes is given in Fig. 21, showing data for the interaction of fibrinigen monolayers and several reactive proteins in the subsolution. This study was carried out to indicate that monofilms which are experiencing a continued change in surface pressure are different from the monofilms in which the pressure is held constant.

The isometric reaction was done in the same way as the isobaric reaction except that the film was held to a constant area during the interaction. At the appropriate time the film was allowed to expand while a constant pressure of 9.7 dynes was maintained. An equilibrium was rapidly achieved but, as can be seen from the figure, this occurs at quite a different area from the one with the constant pressure.

The importance of these experiments, Fig. 21 and Fig. 22, lies in the fact that a difference in the interaction does occur when the pressure is constantly rising, as in the isometric interaction. It is presumed that a series of new interactions occurs because of the continuous reduction in area of each group of interacting sites.

Thus gelatin gives a larger isobaric area change than does gelatin with an isometric change. In contrast to this, plasma protein reacts with less increase in area under isobaric conditions than it does under isometric conditions. Both interactions are, of course, pH-dependent. (Compare Fig. 21 with Fig. 22.)

Interaction Without Changes

Fibrinogen in the presence of calcium and thrombin undergoes, in three-dimensional solutions, specific interaction to form fibrin. Under these same conditions fibrinogen, when spread as a monofilm, does not undergo an area change. Although it is not indicated in Fig. 23, evidence for specific interaction with the monofilm thrombin is apparent in changes in viscosity. This is not the case with the interaction with plasma protein. Fig. 24 indicates that the area change in the monofilm, in which an isobaric area change occurs, does occur when the buffer concentration is altered.

Interaction of Nucleohistone

In Fig. 25 a comparison between the several components of human serum and human nucleohistone indicates that the human serum components presumably participate in an interaction with the nuclear histone film. This may actually be related to the phenomenon of the L.E. cell phenomenon, which occurs as a diagnostic manifestation of the disease, systemic lupus. These sera have components which are highly reactive with the nuclear histone; therefore examination of some paired sera from patients with systemic lupus and normal individuals followed. Fig. 26 indicates that isobaric area changes will occur in the nuclear histone monofilm in the presence of sera with a large amount of these reactive components.

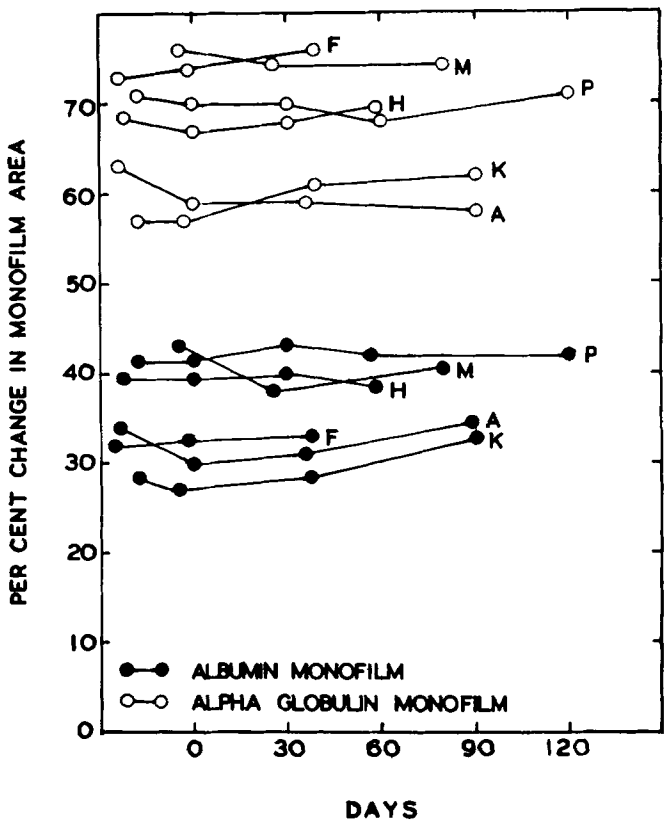

FIG. 27. Reactions of serum components in albumin and α-globulin.

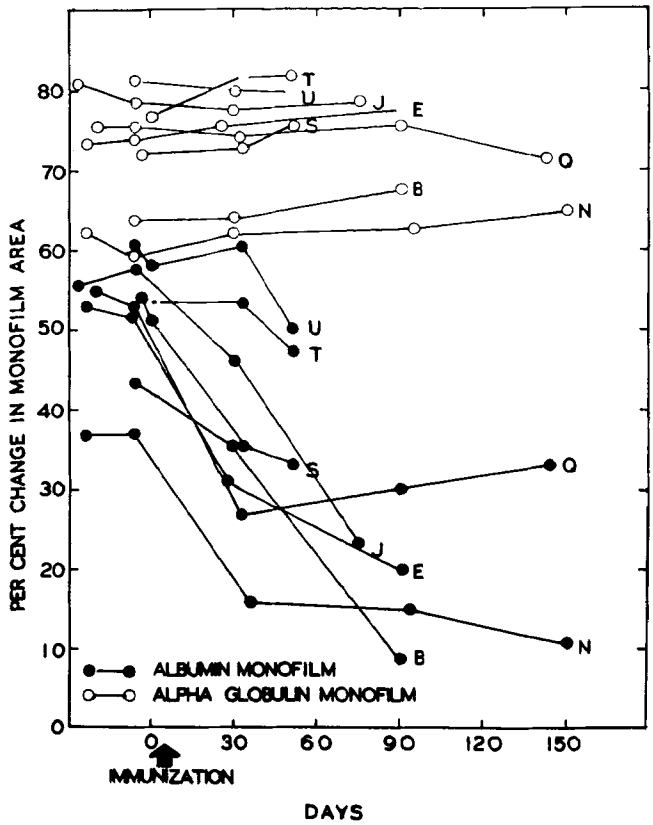

FIG. 28. Parallel with standard changes in immune body production.

Interaction of Immune Sera

In another study aimed at identifying specific molecular interactions, a group of rabbits was immunized with bovine serum albumin. These animals were compared with a controlled series in which the rabbits were not exposed to bovine serum albumin. As an additional control, the γ-globulin from both serum and animals was tested against an α-globulin monofilm as well as against the bovine serum albumin monofilm. The α-globulin fraction presumably does not participate in the immune phenomenon. It can be seen in Fig. 27 that, during the course of 100 days or more, the serum components have reactions in both albumin and α-globulin. The standard amount of γ-globulin from control animals was relatively constant for this entire period. The animals immunized against bovine serum albumin produced a γ-globulin which did not change in their reactivity to the α-globulin monofilm, but, in this instance, there was a substantial reduction in the percentage of expansion of the bovine serum albumin monolayer. This occurred parallel with the standard changes in immune body production, Fig. 28.

Discussion

A large number of proteins may be spread in a monolayer at the air-water interface. This can be done in a highly reproducible way even though the new configuration for the protein is in many respects uncertain. It is possible however to predict that the new configuration involves a selective repositioning

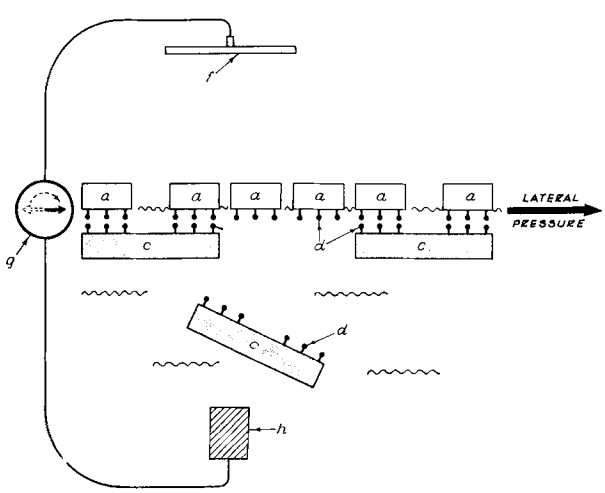

FIG. 29. Soluble molecules with separation of clusters.

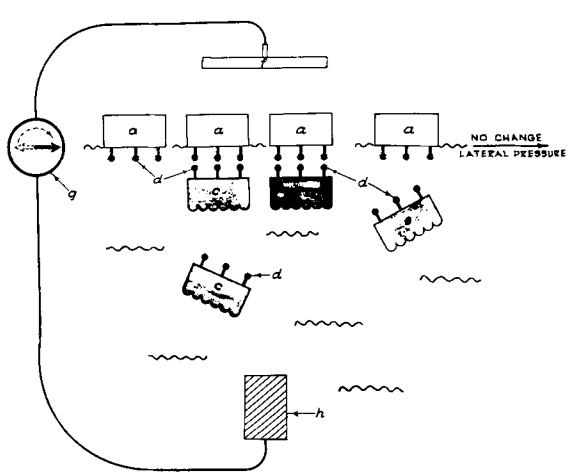

FIG. 30. Interactions with no area change.

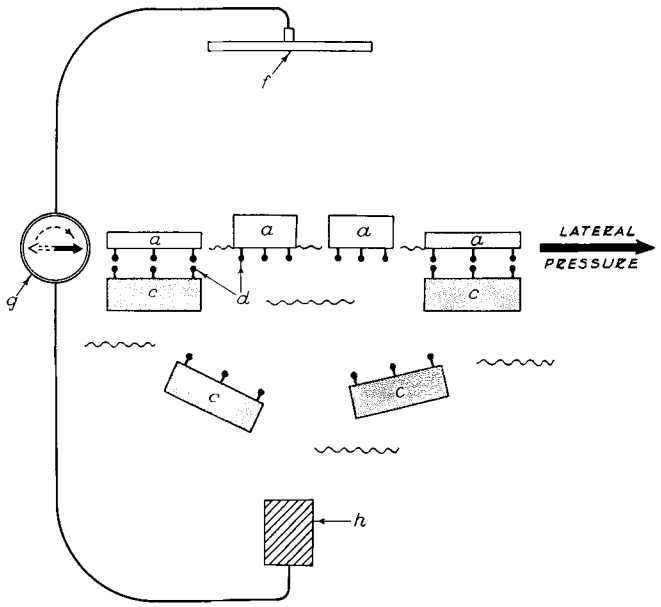

FIG. 31. Placement of soluble protein in interface.

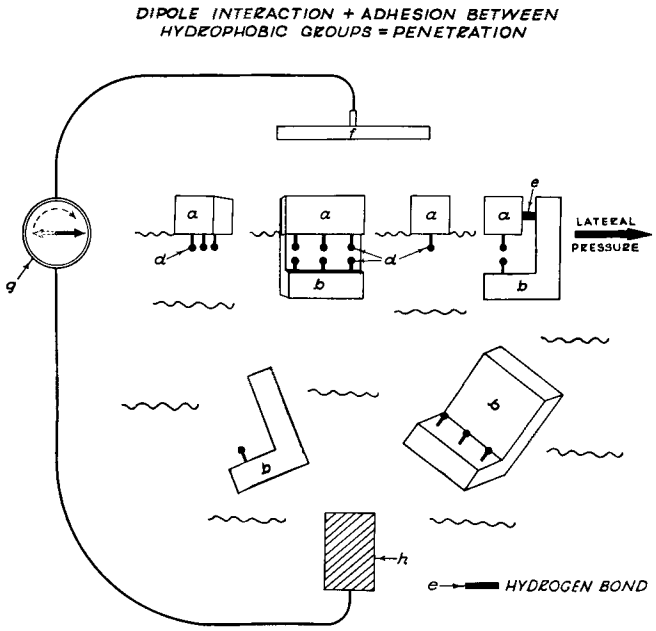

FIG. 32. Force area curve effects of insulin and γ-globulin.

of the hydrophilic groups so that they point down into the water phase.

For operational convenience, the protein monofilm may be looked upon as a kind of elastic sheet with reactive sites dangling from one surface. These reactive sites must form clusters which are tied together by the polypeptide chains. If the elastic sheet is stretched (the film expanded), the individual components of each cluster will separate from each other. It is probable that the location of any one reactive site is really a statistical expression to describe the probability of any one position for the site at any given time. There are also many configurations which are highly improbable.

For these reasons it has seemed useful to keep the distribution statistics as simple as possible. To do this, it is necessary that expansion or contraction of each cluster, as a result of pressure changes transmitted through the film, be minimized. Findings demonstrate that there is a substantial difference between isobaric and isometric conditions in the monofilm.

Several kinds of molecular interaction with the monofilm may be visualized. There may be soluble molecules in which the clusters are separated from each other (Fig. 29). There may be interactions in which the fit of each cluster to the soluble protein is so close that no area change occurs, as with the reaction of some fibrinogen molecules (Fig. 30) with thrombin. There may be interactions in which each cluster comes close enough to the fit on the soluble protein but in which the difference in fit does require an expansion of the area subtended by the cluster. The film will expand against a constant pressure as a consequence. There may be (Fig. 31) a placement of the soluble protein in the interface. Such a phenomenon is the most likely explanation for the force area curve effects of insulin and γ-globulin (Fig. 32). In these cases the collapse pressure of mixed films may be altered.

A kind of specificity in the interaction of molecules in an interface with molecules in a subsolution has been reported by others (14–16). We have previously reported on the specificity of the interaction of protein monofilms and soluble protein in the aqueous phase (10,11). This report extends these observations.

It can be shown that positions 8, 9, and 10 on the insulin molecule modifies the interaction. It can be shown that the production of immune γ-globulin alters the γ-globulin complex of a series of animals so that the isobaric interaction with a protein monofilm is substantially altered. In the case of γ-globulin produced as a result of immune stimulation, one may observe that Freund's adjuvant, an important technique to augment immune response, has an enormous oil-water interface. From what is known of protein behavior at an interface, the protein antigen must be collected in large part at these interfaces in the Freund's emulsion. It can be shown that the existence of a group of natural antibodies to nucleoprotein is associated with a change in the isobaric behavior of the sera to nucleoprotein monofilms.

All of these interactions depend on the pH and the nature of buffer ions. One can only speculate on the molecular configurations involved in these interactions, but there are a number of lines of evidence which partly identify the nature of the "specific" interaction.

ACKNOWLEDGMENT

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Discussion

DR. HAYDON: Thank you very much, Dr. Arnold, for that persuasive account of protein interactions at interfaces—it is obviously a very complicated question.

DR. SMALL: Concerning your first model of the expansion of the protein monolayer, cannot penetration of the monolayer explain the data? Have you measured changes in surface potential or surface viscosity? These techniques might give some clue as to the type of interaction going on in the monolayer.

DR. ARNOLD: Well, we have not, as you have noticed, indicated what kind of molecular association occurs here. The specificity, I thought, was demonstrated by stability of mixed films. The specificity will not show the dipole-dipole interactions, but there is facilitated entry if nothing more if one film enters another. The evidence I showed you has to be sufficient otherwise I cannot prove to you that these are specific interactions.

DR. HAYDON: I should like to point out that there is a problem in distinguishing between effects due to protein-protein interaction and effects due merely to the interaction of the second protein with the air/water interface.

DR. ARNOLD: I think that is quite true. It seems to me we did present evidence on this point which is that we studied the effect of the protein entering its own monofilm. A protein entering its own monofilm gives us quite a different kind of result than proteins which can interact. You are quite correct in stating that any protein will enter a monofilm up to a certain pressure, which is probably near or at the collapse pressure of its own monofilm. If you have a second monofilm with different characteristics, the behavior is different, and the interaction is quite different.

DR. SMALL: Certainly, apparently conflicting results may arise from differences of method. Dr. Arnold is using the injection technique, that is, injecting a substance under a monolayer spread at a given pressure and measuring area change at that constant pressure. The way one measures the condensation effect of the cholesterol is to add a given mixture to the surface and study the isotherm. No injection technique is involved. Condensation is present if the area of the mixed film is less than the sum of the areas of the two separate species. The two techniques give different kinds of data.

DR. CHARLES Y. C. PAK (National Institutes of Health, Bethesda, Md.): I would like to make the following comments regarding the specific monofilm expansion, which might answer some of the questions raised so far. First, the specific monofilm expansion is a calculated value, unlike the total and nonspecific expansions which were directly determined experimentally. Thus the contention that it represents the difference between the total and nonspecific expansions cannot be tested directly. Second, the specific monofilm expansion was shown to be first-order, whereas the other two types of expansions were not. Further, in most of the experiments reported here, the specific monofilm expansion reaches the maximum value at about 30 min, whereas the other expansions show a continued rise. Thus, most of our experiments were performed for 30 min.