``Surface Elasticity'' of Protein Films. I. Egg Albumin

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under the conditions of these experiments, photosynthesis and respiration do not follow the simple course represented by the solid line in Fig. 1.* It is hoped that experiments now being undertaken will make clearer the cause of the discrepancy.

The experiments reported above were performed in the Physics Laboratory of Johns Hopkins University, and are a part of a larger program under the direction of Professor J. Franck. The writer wishes to express his appreciation to Professor Franck and to the others who have contributed materially to the research. Professor B. Livingston very kindly made available the facilities of the Plant Physiology Department. Professor H. Pfund contributed valuable advice concerning the energy measurements and provided a thermocouple of his own construction. Dr. Dean Burk of the Fixed Nitrogen Laboratory has been a most helpful consultant on the biological aspects of the problem.

* The type of induction period which has been observed for many plants would lead to an effect opposite to that described here. According to Warburg's experiments (reference 3), chlorella does not exhibit an induction period at low light intensities. However, in higher plants, induction periods have recently been shown to occur at light intensities which are low, although not so low as those used in experiments on quantum efficiencies (references 4 and 5).

[3] O. Warburg, Biochem. Zeits. **103**, 188 (1920).

[4] E. D. McAllister, Smithsonian Miscellaneous Collections, **95**, No. 24 (1937).

[5] E. Smith, J. Gen. Physiology **21**, 151 (1937).

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"Surface Elasticity" of Protein Films. I. Egg Albumin*

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The limiting area is the quantity by which protein films are usually described. This may give some indication of the "spreading tendency" of the protein, but it is not necessarily related to other mechanical properties of the film, since an unknown proportion of the protein may remain unspread, or may pass into solution in the substrate. This is demonstrated by an experimental study of the limiting areas and the surface elasticities, $M_S = -A \cdot dF/dA$, of egg albumin films as a function of *p*H. It is shown that the $M_S - F$ curves for this protein are of characteristic form, with a single well-defined maximum, $(M_S)_{max}$. It is shown further that the characteristic large variations of limiting area with *p*H are not reflected in the variation of $(M_S)_{max}$ and it is concluded that throughout the range of H ion concentrations studied the observed elasticities are due to a monomolecular film of true limiting area ~1.0 m²/mg. The lower values of the apparent limiting area obtained at certain hydrogen ion concentrations are due principally to unspread material which does not contribute significantly to the mechanical properties of the film. It is suggested that the $M_S - F$ curves should be employed in place of the apparent limiting areas in the description of protein films and that the values of $(M_S)_{max}$ may provide a convenient means of showing specific differences between films of different proteins.

INTRODUCTION

IT is well known that an organic substance insoluble in water but containing a suitable proportion of polar groups can be caused to form a thin layer at an air-water interface. The effect of such layers upon the interfacial tension has been extensively studied in relation to the area covered by a given quantity of the substance, and convergent lines of evidence suggest that over a wide range of interfacial tensions and areas the films may be homogeneous and one molecule in thickness, varying only in the mode of packing of the nonpolar heads and in the orientation of the polar groups with respect to the surface.

In the correlation of force-area (force = lowering of interfacial tension) relations with the structure and orientation of organic molecules it has been customary to make considerable use of "limiting areas" obtained by extrapolating por-

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† Commonwealth Fund Fellow.

tions of the force-area curves to zero pressure, a procedure that is fully justified by the evident relationship of such a quantity to the molecular dimensions of the substance constituting the film. Such limiting areas have indeed proved of value in elucidating the structure of substances of unknown constitution. There are, however, certain obvious circumstances under which the use of limiting areas in the characterization of films could be misleading. Such, for example, would be partial solubility of the substance in the substrate, failure of an undetermined fraction to spread to form an homogeneous film, dilution of the substance added with unknown amounts of other materials, or indefiniteness of molecular weight or particle size. Naturally "limiting areas" obtained from the study of such films will be of little value for descriptive purposes and of less value for the estimation of molecular quantities.

All of these considerations apply to the proteins. These substances may, as a rule, be spread either from the solid or from solution. The film appears to be insoluble in water, but a certain variable fraction of the material added probably enters the substrate during the spreading process and may or may not be adsorbed subsequently at the surface. A further portion may remain unspread, either lying in discrete masses upon the surface of the homogeneous film, or, possibly, forming some sort of micellar network which might influence the mechanical properties of the film. In either case, the computed "limiting area" would have little meaning and might be expected to depend on the particular method of spreading employed. Again, under some conditions the process of spreading may be so slow that there is no certainty that the "limiting area" finally attained relates to the material originally spread rather than to a product of some slow chemical transformation. Thus the limiting area of a protein film is an arbitrary quantity which under the most favorable circumstances may be connected with the so-called "spreading tendency" of the material but which has no necessary connection with the properties of the film itself. It is unfortunate that protein films are usually described solely by their limiting areas, while the force-area curves from which these values are derived are not recorded.

The problem, then, is to find a new means of characterization of protein films in terms of some quantity which can be derived from the force-area diagrams, which is independent of the actual area per milligram or per molecule of protein spread, and which is sufficiently sensitive to be of value in detecting specific differences between proteins or between films of the same protein when spread upon different substrates. As a quantity satisfying the first criterion we have chosen $-A \cdot dF/dA$ which may be called the "surface elasticity," M_S, since the above definition is in conformity with that given by Quincke[1] (1870), who first used the term, and by Schütt,[2] who first investigated the elastic properties of protein films. Plotting M_S against F for several different proteins, we obtained curves which differed reproducibly from one another and which encouraged us to believe that the second criterion also might be satisfied by M_S. At this stage, however, it appeared necessary to make a careful preliminary study of these curves for a single well-defined protein, and of their variation with time of spreading, amount of material spread, and *p*H. For this purpose we chose crystalline egg albumin, the "spreadability" of which has been studied in some detail by Gorter,[3] ter Horst[4] and others. It is the purpose of this paper to present the results obtained.[4a]

Experimental

The surface balance, resembling in most particulars that described by Harkins and Myers,[5] was designed by Mr. A. J. Rawson of this Department. The float was of phosphorbronze coated with a thin layer of high melting paraffin wax; leakage past the float was prevented in some experiments by thin waxed-silk threads, in others by gold ribbons 0.00068 mm thick, rendered more flexible by vertical corrugations and fixed in position with Wood's metal. All other surfaces in contact with the film or the

[1] G. Quincke, Pogg. Ann. **105**, 1 (1858); **139**, 1 (1870). Quoted by Schütt, reference 2.

[2] K. Schütt, Ann. d. Physik (4) **13**, 712–746 (1904).

[3] E. Gorter, Am. J. Dis. Children **47**, 945–956 (1934); Article in C. L. A. Schmidt's *Chemistry of the Proteins and Amino-Acids* (Thomas, Springfield, Illinois, 1938), p. 442.

[4] M. G. ter Horst, Rec. trav. chim. Pays-Bas **55**, 33–42 (1936).

[4a] J. B. Bateman, Cold Spring Harbor Symposium on Quantitative Biology, p. 148 (1938).

[5] W. D. Harkins and R. J. Myers, J. Chem. Phys. **4**, 716–724 (1936).

underlying solution were of chromium-plated brass conditioned with a rubbed-down film of ferric stearate prepared as described by Langmuir and Schaefer.[6] The dimensions of the tray were 25.5×76.3×1 cm.

The water used was obtained from a tin-lined still and collected in a glass carboy. Chemicals used in making up buffer solutions were of reagent quality. With the exception of those of *p*H 1.0, buffers were made up to a total concentration of 0.01 *M* and their *p*H measured with a Beckmann glass electrode. The following solutions were used.

*p*H 1.0	0.1 *M* HCl
2	0.01 *M* HCl
4.7	acetic acid-sodium acetate
6–9	$Na_2HPO_4-KH_2PO_4$
11	Na_2HPO_4-KOH

The final experiments were made with a specimen of crystalline egg albumin prepared by Sörensen's method by Chambers and Flosdorf and stored in lyophil form since 1933. In other experiments a crude commercial preparation (Merck) was used. The solid protein was kept in a microweighing bottle (Pregl "pig") which was weighed on a Sartorius microbalance (for the loan of which we are indebted to the Department of Bacteriology) in order to determine the amount of material transferred to the surface. The latter operation was performed with a platinum loop, the protein being added in small amounts until a pressure of about 0.3 dyne/cm was obtained. The surface was then increased by one-third and left for 15–60 minutes, according to the rate of spreading at the particular *p*H used. The first compression curve was taken in all cases before spreading was complete, but at a time when it was sufficiently slow to be inappreciable over the duration of the measurement. The average time required for a single complete compression was 13 minutes. The curves obtained upon expansion were sometimes recorded, but since in all cases the film showed considerable hysteresis the presentation of these curves will be deferred. This hysteresis, although rarely mentioned in the literature, appears to be a fairly constant property of protein films which requires investigation. All measurements were made at room temperature (23–25°C).

[6] I. Langmuir and V. J. Schaefer, J. Am. Chem. Soc. **59**, 2400–2414 (1937).

General Character of Surface Elasticity Curves

The force-area curves which were used as a starting point in the determination of M_S were of the general character of those described by Gorter (1934). These consist of a low pressure region, in which the curve is convex towards the A axis, followed by a transition, usually around $F=6$ dynes/cm, to a high pressure region in which F increases more or less linearly, tending to flatten off gradually above $F=19$. Above $F\sim25$ this decrease in slope becomes much more marked and is accompanied by irreversible changes in the film. It is thus obvious that the curve differs in the high pressure region from the curve of constant surface elasticity, $F=-\ln A$, and measurement shows that this is also the case in the low pressure region. Within the limits of experimental error, $M_S=0$ when $F=0$.* The low pressure portion is represented by a rapid increase of M_S with increasing pressure, reaching a maximum in the neighborhood of the transition to the high pressure region. The maximum is followed by an almost linear decrease, the extent of which depends upon the extent of the linear portion of the $F-A$ curve, and this by a more rapid decrease which continues beyond the limit of reversible compression ($F\sim25$ dynes/cm) (see

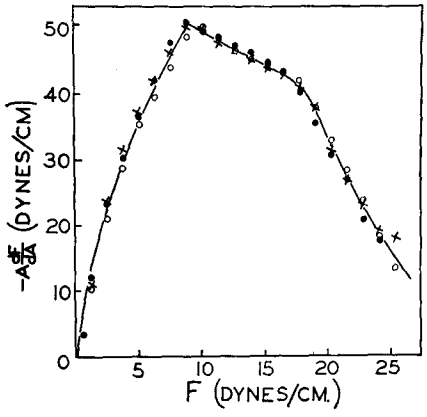


FIG. 1. Force-elasticity curves for egg albumin on 0.01 *M* phosphate buffer, *p*H 7. (● 60 minutes, ○ 120 minutes, × 180 minutes after spreading.) Averaged data from three experiments. Force in dynes cm^{-1}, elasticity in dynes cm^{-1}.

* Since egg albumin forms a coherent film, this statement is untrue without the qualification concerning experimental error, which is too great to permit an exact determination of M_S when $F=0$.

Fig. 1). In the experiments described here, while recording the complete curves whenever necessary, we have given most attention to variations in the maximum value of M, $(M_S)_{max}$, and the corresponding values of F, F_{max}, since these data are convenient for descriptive purposes and must also be of some physical significance.

Relation of M_S to Other Moduli of Elasticity

M_S is an empirical modulus suitable for describing the behavior of a two-dimensional body to which a constraint is applied to prevent any change of dimensions normal to the applied force. It is related to the two-dimensional bulk modulus, K_S, and the two-dimensional modulus of rigidity, N_S, by the equation $M_S = K_S + N_S$. Its relation to the three-dimensional moduli can only be estimated by assuming the film to be isotropic and of mechanically definable thickness z. In this case, $M = zY/(1-\sigma^2)$, where Y is Young's modulus and σ is Poisson's ratio. If $M_S = 50$, $z = 10$A, and $\sigma = 0.5$, then $Y = 3.75 \cdot 10^8$ dynes/cm^2, which is about 100 times greater than the value for 45 percent gelatin estimated from the data of Leick[7] and Sheppard and Sweet.[8]

Effect of Time on M_S

In a number of experiments at different pH values the first measurements were made as soon as further increases in force had become negligible over a period of about five minutes, and other measurements were made at intervals of about one hour, the film being left in the expanded state in the intervening period. For three hours after spreading, there is usually a tendency for $(M_S)_{max}$ to decrease by two to five percent. This decrease, however, cannot be attributed wholly to the direct effect of prolonged contact with the substrate, since it is dependent rather upon the change in limiting area (A_0) than upon time. In those occasional cases where the increase of A_0 during the three-hour period was large, the decrease in $(M_S)_{max}$ was also larger than usual, and there was a consistent tendency for the decrease in $(M_S)_{max}$ to increase with the percentage change in A_0. The same tendency appeared when A_0 was increased by removing half of the film from the surface and allowing the remaining portion to expand. In one such case this caused A_0 to increase by 40 percent, while $(M_S)_{max}$ decreased by 7.7 percent. The data show that when A_0 is constant there is an average decrease in $(M_S)_{max}$ of about three percent per hour during the first three hours, while an additional decrease of about 3.5 percent is caused by a ten percent increase in A_0. In a single film which was left for six hours A_0 increased by 18 percent and $(M_S)_{max}$ decreased by only nine percent. The comparatively slight importance of these variations is illustrated in Fig. 1, which shows the curves obtained at 60, 120 and 180 minutes after spreading a film at pH 7.0. Nevertheless we considered it advisable when recording the effects of other factors to make measurement within one hour of spreading.

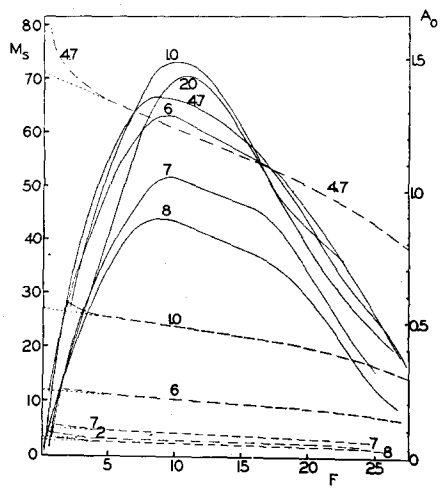


FIG. 2. Force-area (broken lines) and force-elasticity (continuous lines) curves for egg albumin. Numbers attached to each curve give pH of substrate; total concentration 0.01 M except at pH 1.0, for which substrate was 0.1 M HCl. Force in dynes cm^{-1}, elasticity in dynes cm^{-1}, area in apparent m^2 mg^{-1}, determined from weight of solid protein transferred to surface.

F_{max} is usually 8–9 dynes/cm, but the values in individual experiments are scattered over a range of about ± 1.5 dynes/cm, without any consistent trend in relation to time or limiting area. The wide scatter may be attributed largely to variations in the manner of drawing $F-A$ curves through the experimental points.

[7] A. Leick, Ann. d. Physik (4) **14**, 1939–1952 (1904).
[8] S. E. Sheppard and S. S. Sweet, J. Am. Chem. Soc. **43**, 539–547 (1931).

Effect of *p*H on A_0 and on $(M_S)_{max}$

The present measurements of the limiting area are in broad agreement with those of other workers (Gorter and Philippi,[9] ter Horst[4] and Seastone[10]), showing a sharp maximum in the neighborhood of the isoelectric point of about 1.45 m²/mg, and falling off on both the alkaline and the acid side (Fig. 3, curve 3) to about one-tenth of this value, again rising rather sharply below *p*H 2 and also showing an appreciable rise at *p*H 11.7, the highest used in these experiments. Over the same range F_{max} shows small irregular variations (Fig. 3, curve 2), while $(M_S)_{max}$ (Fig. 2; Fig. 3, curve 1) shows a slow rise on the acid side and a steep decline on the alkaline side of the isoelectric point. The position of the isoelectric point is not made apparent by any uniqueness in the value of $(M_S)_{max}$ at this point, nor does this curve resemble in any way the curve of limiting areas. In Fig. 2 this contrast between the regular variation of $(M_S)_{max}$ and the disproportionate changes in the weight of protein required to form a film of given area at different *p*H values is clearly illustrated by the presentation in the same diagram of the M_S-F curves and the $F-A$ curves from which they were derived. In this diagram changes in the character of the curves with *p*H also become apparent, the "plateau" corresponding to the linear portion of the $F-A$ curves being practically absent at *p*H 1 and 2, and becoming more pronounced with more alkaline substrates.

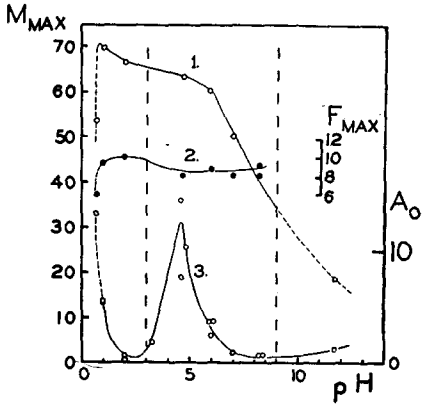


FIG. 3. $(M_S)_{max}$ (top curve), $(F)_{max}$, (middle curve) and apparent limiting areas (bottom curve) as function of *p*H. Units as previously. Vertical broken lines mark boundaries of *p*H stability region as given by Svedberg (J. Am. Chem. Soc. **52**, 2855–2863 (1930)).

Effect of Varying the *p*H of the Solution Beneath a Preformed Film

The foregoing results show that the films formed at various hydrogen ion concentrations are closely similar in their mechanical properties, differing only in the proportion of the protein added that is able to spread in a monolayer; it is almost inconceivable that, if the actual quantities of protein in a homogeneous film showed tenfold variation at different hydrogen ion concentrations, the surface elasticities should vary by only a few percent. A critical test of this conclusion was made in the following manner: a film was spread at the isoelectric point (4.55), a compression curve taken, and the *p*H of the underlying solution then changed to about 2.0. This was accomplished by means of narrow Pyrex tubes running the length of the Langmuir trough and provided with small holes through which the necessary volume of 0.4 *M* HCl could be passed. The solution could then be stirred by gentle lateral movements of the tubes, and the completeness of mixing checked by *p*H measurements. Three curves were made at *p*H 2.1, the last one after one-half of the film had been scraped from the surface. The *p*H of the substrate was then changed to 0.65 by a further addition of HCl, and the measurement repeated. The results are summarized in Table I, together with the results of a second experiment

TABLE I. *Effect of p*H *change on preformed films of egg albumin.*

Expt.	*p*H	Weight of protein in surface (mg)	A_0 (m²/mg)	$(M_S)_{max}$ (dynes/cm)	Time after spreading (minutes)
1.	4.74	0.150	1.07	62.1	13
	2.1	0.150	1.13	62.1	43
	2.1	0.150	1.11	66.9	54
	2.1	0.075	1.15	62.8	64
	0.65	0.075	1.51	39.0	114
2.	4.68	0.122	1.08	60.5	14
	2.05	0.122	1.07	64.0	39
3.	4.83	0.172	0.796	66.6	15
	10	0.172	0.895	56.3	40

[9] E. Gorter and G. T. Philippi, Proc. Kon. Akad. Wet. Amsterdam **37**, 788–793 (1934).
[10] C. V. Seastone, J. Gen. Physiol. **21**, 621–629 (1938).

of the same kind. In a third experiment the substrate was made more alkaline by the addition of a solution containing KOH and Na_2HPO_4; the results of this change are likewise given in Table I.

Discussion

It follows from a comparison of the data given in Fig. 3, with those in Table I that, to a first approximation at least, there is a 1−1 correspondence between the elasticities of an egg albumin film and the *p*H of the substrate at the time of measurement, irrespective of the conditions under which the film was originally formed. Approximately the same variations of $(M_S)_{max}$ with *p*H is apparent whether the measurement is made upon a film spread at the *p*H in question or at some other *p*H. The resemblance extends even to other details in the shape of the M_S-F curves, such as the length of the linear portion. It is evident that throughout the *p*H range the mechanical properties are those of films of which the limiting area is about 1.0 m^2/mg, with comparatively insignificant variations. From this point of view smaller apparent values of A_0 are to be regarded as experimental artifacts, referable to the failure of a certain proportion of the added protein to form a monolayer. We shall not discuss at length the reason for this inability to spread; it may be due to inactivation of the unspread portion, as is suggested by the failure of a significant increase in A_0 to occur even when such films are left in the expanded state or when the *p*H is changed to a value more favorable to the spreading of the original protein (Seastone[10]). It may, on the other hand, be caused by the presence of discrete clumps of unspread material lying on the upper surface of the monolayer, as the observed optical (Zocher and Stiebel[11]) and electrical (Gorter and Philippi[9]) heterogeneity of the film indicates. The surface elasticities also show clearly that the unspread fraction does not contribute markedly to the mechanical behavior of the film, although some slight effect may be discerned in the tendency of $(M_S)_{max}$ to decrease as spreading becomes more complete.

In discussing the form of the elasticity curves and the physical significance of $(M_S)_{max}$, it is convenient to refer to the view, supported by several workers and put forward in its most recent form by Mitchell,[12] that compression of the most highly expanded film is accompanied by a rotation of the polypeptide chain in such a manner that the polar side chains, initially lying

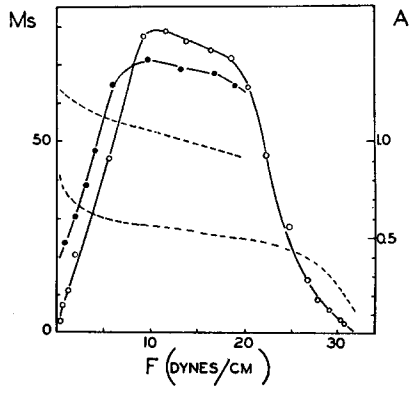


FIG. 4. Force-area (broken lines) and force-elasticity (continuous lines) curves for α-aminostearic acid at *p*H 9.0 (circles and lower force-area curve) and the tripeptide of α-aminocaprylic acid (solid points; *p*H not specified). Elasticities calculated from force-area curves of Porter (1937) and Gorter (1938), respectively. Units as before.

in the plane of the water surface, approach a limiting position in which they are normal both to the axis of the main chain and to the surface, while the nonpolar side chains are in a similar manner removed from the surface, forming a hydrophobic upper surface to the film (Hughes and Rideal[13]). Mitchell identifies the completion of this process with the transition between the low pressure and high pressure regions, and it is reasonable to assume that the value of $(M_S)_{max}$ provides a more exact indication of the transition point. X-ray studies and observations of the birefringence of multilayers of egg albumin deposited from compressed monolayers (Astbury, Bell, Gorter and van Ormondt[14]) confirm the belief implicit in the hypothesis outlined that the polypeptide chains are arranged in parallel rows transverse to the direction of compression. Accordingly the observed compressibilities in the

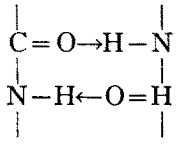
[11] H. Zocher and F. Stiebel, Zeits f. physik Chemie **A147**, 401–435 (1930).

[12] J. S. Mitchell, Trans. Faraday Soc. **33**, 1129–1139 (1937).

[13] A. H. Hughes and E. K. Rideal, Proc. Roy. Soc. **A137**, 62–77 (1932).

[14] W. T. Astbury, F. O. Bell, E. Gorter and J. van Ormondt, Nature **142**, 33 (1938).

high pressure region must be the resultant of the several forces involved in closer packing of polypeptide chains and their accompanying side chains—the attraction or repulsion of polar groups, interaction of the main chains and of the nonpolar side chains, and possibly the dehydration and plication of the main chains. That these forces result in a rapid reversible decrease in elasticity indicates that with the completion of reorientation of side chains cohesive forces between adjoining groups become predominant and may lead upon further compression to the thread formation observed by Devaux[15] (see also Mathieu[16]). Mitchell considers that coordinated hydrogen linkages between the main chains



may occur, and some support for this view may be drawn from the infra-red absorption spectra of dry and hydrated gelatin (Ellis and Bath[17]). However, the dependence of $(M_S)_{max}$ on pH might suggest rather that the ionizable groups are chiefly responsible for the increase in compressibility, although it is then difficult to account for the absence of a minimum value of $(M_S)_{max}$ at the isoelectric point, where the lateral adhesion should be much increased by the equal distribution of positive and negative charges.

Both α-aminostearic acid on the alkaline side of the isoelectric point (Porter[18]) and the tripeptide of α-aminocaprylic acid (Gorter, 1938; pH unspecified) give surface elasticity curves resembling those of egg albumin (Fig. 4). In these cases also the detailed interpretation of the results is obscure, but they at least make it evident that simpler substances of known constitution can show the same general behavior as the proteins. We consider it undesirable therefore at this stage to discuss the elasticity of protein films in terms of particular hypotheses as to protein structure or in relation to particular phenomena, such as hydration and gelation, which are more or less specific to the proteins.

The discussion of the facts presented in this paper in connection with the study of biological phenomena is of interest in view of the current belief that the properties of living systems are very largely the properties of interfaces. The observed effects of pH change on surface elasticity suggest that small environmental pH changes are unlikely to produce such radical changes in the properties of membranes as a consideration of the curves of "spreading tendency" might have suggested. To the extent that biological membranes are fixed or "preformed" structures, their stability over a wide range of pH can be readily understood; to the extent, on the contrary, that biochemical processes are dependent upon a constant interchange of labile or surface-active materials between plasma and interface, or between homogeneous solution and micelle, we may expect relatively violent effects of environmental change, analogous to the striking variations of spreading tendency which are observed when proteins are brought into contact with different aqueous solutions.

[15] H. Devaux, Bull. soc. francaise de phys. No. 406, 84–85 (1937).
[16] M. Mathieu, Cold Spring Harbor Symposium on Quantitative Biology, p. 186–7 (1938).
[17] J. W. Ellis and J. Bath, J. Chem. Phys. **6**, 723–729 (1938).
[18] E. F. Porter, J. Am. Chem. Soc. **59**, 1883–1888 (1937).