catalytic thermal decomposition of (a) ethylene for the hydrogenation of acetone at 110°, (b) methane for the hydrogenation of carbon dioxide at 315°, and of nitrous oxide at 73.5°

2. The experimental evidence indicates that the surface of the nickel catalysts studied was non-uniform because (a) catalysts which no longer catalyzed the decomposition reactions were still active at lower temperatures in the hydrogenation reactions, (b) certain parts of the surface were more active than others in the hydrogenation of acetone, (c) heating in hydrogen a surface upon which the hydrogenation of nitrous oxide had occurred caused selective activation or poisoning according to conditions, (d) the nickel surface responsible for about two-thirds of the hydrogenation of carbon dioxide could be poisoned without affecting the nickel surface which catalyzed the hydrogenation of nitrous oxide.

PROVIDENCE, R. I. RECEIVED JULY 16, 1940

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND THE CHEMISTRY DEPARTMENT OF THE NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

An Electrophoretic Study of the Proteins of Egg White

BY LEWIS G. LONGSWORTH, R. KEITH CANNAN AND D. A. MACINNES

Due to its availability, its importance in nutrition, and as a source of proteins for biological investigations, egg white has been the subject of many researches.[1] As the result of much painstaking work, the number and relative amounts of the major components of that material have been ascertained. With the advent of the electrophoretic method, as improved by Tiselius,[2] it has, however, been possible to obtain much additional quantitative information regarding this very complex protein system. Up to the present time the only electrophoretic study of egg white that has been published is that of Young,[3] who used the original Tiselius apparatus which yielded simple schlieren bands. He reported "five or six separate migrating boundaries" at *p*H 7.2. With the addition of the scale method of Lamm,[4] the diagonal schlieren method of Philpot,[5] and Svensson,[6] or the schlieren scanning method developed in this Laboratory,[7] and described in detail in a recent article,[8] it is possible to obtain, in addition to the number and mobilities, data concerning the concentrations of the constituents present. In the research to be described below the schlieren scanning method was used. In all, 134 separate electrophoretic experiments were made on egg white and its constituents and considerably more than that number of electrophoretic patterns were obtained. In the following only representative or typical patterns can be shown, but all of the results are consistent with the interpretation we have given.

With the exception of two modifications: *i. e.*, a double length section of the electrophoresis cell, and a cell support, to be described below, the electrophoresis apparatus used has remained as outlined in recent articles from this Laboratory.[8,9,10] The double length section, II, is shown in position in the support in Figs. 1-a and 1-b. This section fills most of the field of the schlieren lens, without the disturbing horizontal glass plates obscuring the middle of the field, as in the more usual four section apparatus. With this arrangement it is possible to spread the boundaries over more than twice the distance, with the possibility of finding more detail in the electrophoretic patterns.[11] The filling of the apparatus, however, involves a somewhat different procedure than with the older type of cell and is as follows. As before, boundaries are initially formed between a buffer solution in which a protein is dissolved, and the buffer itself. The protein bearing solution is placed in the bottom section, III, the filling extending somewhat into section II. The bottom section is isolated by pushing it to the left. The

(1) The early researches on egg white have been excellently summarized by Hektoen and Cole, *J. Infectious Diseases*, **42**, 1 (1928).

(2) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(3) Young, *J. Biol. Chem.*, **128**, cxiv (1939).

(4) Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, Ser. IV, **10**, No. 6 (1937).

(5) Philpot, *Nature*, **141**, 283 (1938).

(6) Svensson, *Kolloid-Z.*, **87**, 181 (1939).

(7) Longsworth, THIS JOURNAL, **61**, 529 (1939).

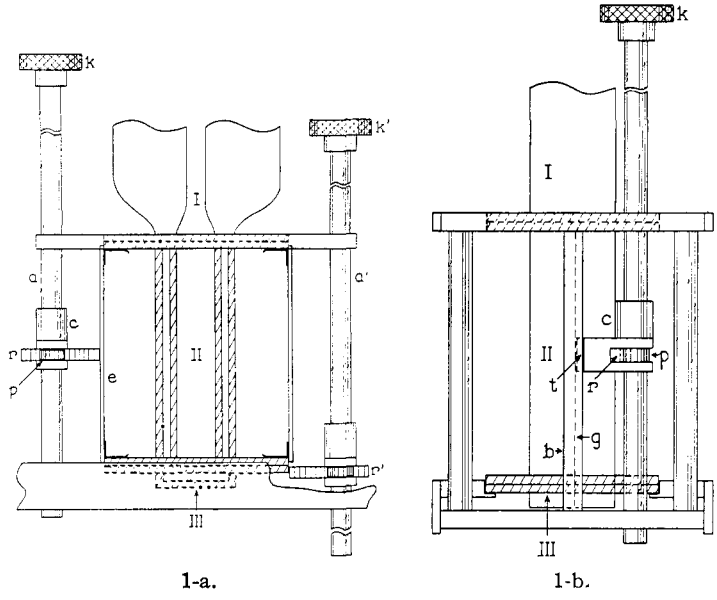
(8) Longsworth and MacInnes, *ibid.*, **62**, 705 (1940).

(9) Longsworth, *Ann. N. Y. Acad. Sci.*, **39**, 187 (1939).

(10) Longsworth and MacInnes, *Chem. Rev.*, **24**, 271 (1939).

(11) The double length section was suggested to us by Prof. Arne Tiselius before his return to Sweden. Although most of the results to be outlined in this paper were obtained before the new sections were available they have been used in all our recent work, and we regard them as a distinct contribution to the electrophoretic technique.

protein solution in one side of section II is rinsed out and then filled with buffer above the junction with section I. The other side is then filled with the protein solution, also above the junction with the top section. The center section, II, is then displaced to the right, after which the bottom section is returned to the center. The excess protein solution is next rinsed out of the top section I, which is then filled with the buffer solution. The electrode vessels are next attached to the electrophoresis cell and filled as described in previous articles.[12] Now by returning section II to the center position boundaries between the protein and buffer solutions are formed at the junctions of sections I and II in one side of the channel and between II and III in the other side. These boundaries may be brought out from behind the plates by injecting some buffer solution into the closed side of the apparatus in the manner described elsewhere.[10] By applying a potential in the appropriate direction, boundaries will rise in one side of section II of the cell, and descend on the other, provided, of course, that the components of the protein mixture carry like electrical charges.



1-a. 1-b.

Figs. 1-a and 1-b.—A modified Tiselius electrophoresis cell and support.

Because of the necessity of moving the bottom section III, a support for the electrophoresis cell that is both simpler and more flexible than the one used previously in this Laboratory was devised. It is also shown in Figs. 1-a and 1-b. The device for moving the sections of the cell to the right, for example, consists of a rod, a, of Fig. 1-a, carrying a pinion, p, which engages the rack, r, carried by the collar, c. The rod, a, slides vertically through holes in the supporting frame while a tongue, t, Fig. 1-b, of the collar moves in a groove, g, in the bar, b, and prevents the collar from rotating when the rod is turned. The rod, a′, Fig. 1-a, carries a similar rack and pinion arrangement for displacement of the cell sections to the left. The racks, r and r′, are provided with pins to limit their movement.

The operation is as follows. Suppose section II of the cell is to be displaced to the right. The rods a and a′ are shifted vertically until the racks are in the positions shown in Fig. 1-a. Turning the knurled knob, k, causes the rack, r, to press against the metal insert, e, which communicates the pressure to the edges of section II and displaces this section. At the same time the knob, k′, is held with the other hand to prevent movement of section III. As may be noted in Fig. 1-b, the pressure is applied somewhat away from the center of the edges of the plates but no difficulty has been experienced on this account. With this arrangement the bottom section III can be moved, which is necessary in the procedure described above. Furthermore, by extending the support vertically any number of center sections can be manipulated with one pair of sliding racks and pinions.

Typical electrophoretic patterns of egg white (diluted eight times) are shown in Fig. 2 for the *p*H values 4.45, 6.12 and 8.0, all at an ionic strength of 0.1. The patterns are given for both legs of the electrophoresis channel. It will be recalled from the discussions in the earlier papers that: (a) an ordinate in these patterns is proportional to the gradients of refractive index in a given layer of the solution and the corresponding abscissa indicates the position of the layer in the electrophoresis cell; (b) assuming a constant coefficient of refractive index, the area under each peak is proportional to the concentration of the corresponding component (or components) having a given electrophoretic mobility;

(12) Neoprene sleeves are now available from the Pioneer Rubber Co., Willard, O., for making the junction between the cell and electrode vessels. This material lasts much longer than rubber in spite of the fact that it must be greased to make a water-tight and insulating joint. It becomes hard, temporarily at least, if kept at zero degrees for an extended period.

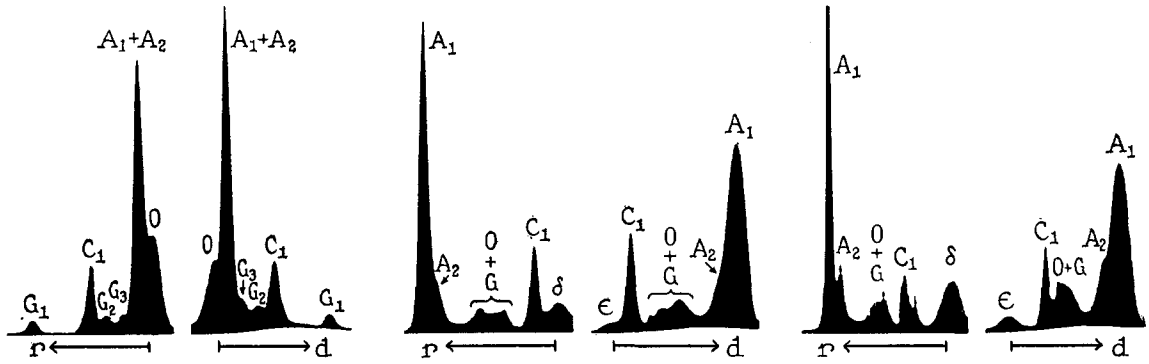


Fig. 2.—Electrophoretic patterns of egg white, diluted 8×, in buffers of 0.1 ionic strength.

and (c) that the mobility may be deduced from the observed displacement of the boundary from its initial position. Of these patterns that shown in Fig. 2(a) has been most readily interpreted. To anticipate the conclusions of this research, comparison with patterns obtained with the separate (or partially separated) components has made possible the identification of the sources of various peaks in Fig. 2(a), as follows: O, ovomucoid (including the δ and ϵ effects); $A_1 + A_2$, ovalbumin; C_1, conalbumin, and G_1, G_2, G_3, globulins. (In addition there is an insoluble fraction, which precipitates on dilution and dialysis against the buffers used. This is the material known as mucin and, due to its insolubility, has not yet been studied electrophoretically.) In the *p*H range studied, 3.9 to 8, the pattern shown in Fig. 2(a) is the only one to show peaks corresponding to all of the constituents. The omission of peaks from the other patterns may be due to the fact that two or more constituents may have the same mobility at a given *p*H value, and, also, complexes may exist between the proteins. The patterns shown in (b) and (c) of Fig. 2 illustrate the second phenomenon, as will be more fully shown below. In the following discussion the methods for obtaining and testing the purity of the separate components will also be considered, data will be given as to the variation of the mobilities of the components with the *p*H, and evidence will be furnished as to the complexity of several of the proteins. A quantitative analysis of egg white obtained from the electrophoretic patterns will also be given.

An Electrophoretic Study of a Method for Preparing Ovalbumin.—Although the components of a protein mixture, such as egg white, may be separated electrophoretically the quantities so far obtainable in this manner are limited. It has, in general, been found more efficient to use electrophoretic analysis to study and control the more usual methods of separation and purification. As an example, electrophoretic patterns were obtained at each stage of the separation and purification of ovalbumin following the procedures recommended by La Rosa.[13] These patterns are given in Fig. 3 of which (a) is that for untreated egg white at *p*H 3.92. It will be seen that this is similar to that for *p*H 4.45, shown in Fig. 2(a), except that the separate peak for the globulin G_3 is missing, since, as will be shown below, this component has, at this *p*H, the same mobility as that of ovalbumin.[14] If now the conventional separation is made into "albumin" and "globulin" fractions by adding to the egg white an equal volume of saturated ammonium sulfate and filtering, the resulting electrophoretic pattern of the soluble fraction is shown in (b) of Fig. 3. The pattern shows peaks corresponding to all of the constituents indicated in (a) with the possible exception of globulin G_2. The pattern for the corresponding insoluble or "globulin" fraction is given in Fig. 3(d). That material also shows peaks corresponding to all of the constituents appearing in the pattern for egg white.[15] However, there has been concentration of the albumin components in the filtrate and of the globulins in the precipitate.

(13) La Rosa, *Chemist-Analyst*, **16**, 3 (1927).

(14) This is only one of several examples we have encountered which indicate that the number of components in a mixture must not be judged from the number of peaks in the electrophoretic patterns (or the number of schlieren bands) obtained at a single *p*H value.

(15) Panormoff (*Jahresber. Fortschr. Tierchem.*, **28**, 6 (1898); see also *Chem. Centr.*, **69**, 11, 487 (1898)), early found that the "globulin" fraction contained ovalbumin.

Figure 3(f) shows the effect of crystallization of the "albumin" fraction, after bringing this solution to *p*H 4.6 by adding acetic acid. The globulin G_1 has disappeared, and conalbumin, C_1, is present in much decreased amount. That the ovomucoid, O, is reduced is shown by comparing the O + ϵ peaks in (a) and (b) with the ϵ peak in (f). The electrophoretic patterns, Fig. 3(g) and (h), show the effect of two further crystallizations. In the former, a scarcely perceptible amount of conalbumin is present, and in the latter it has practically disappeared. It will be observed that the peaks marked δ and ϵ persist unchanged in patterns (g) and (h). As fully discussed in a previous paper[8] these are due to concentration changes near the original position of the boundaries and do not arise from any single component. (Conclusive evidence that the δ and ϵ effects in (g) and (h) of Fig. 3 are not due to ovomucoid is given by the absence of a peak due to that constituent in patterns at *p*H values at which the peak due to that protein separates from the δ and ϵ maxima.) After three crystallizations, therefore, the patterns show that substantial purification of the ovalbumin has been attained. This is in agreement with the observations of Sørensen and Høyrup,[16] and Hektoen and Cole.[1]

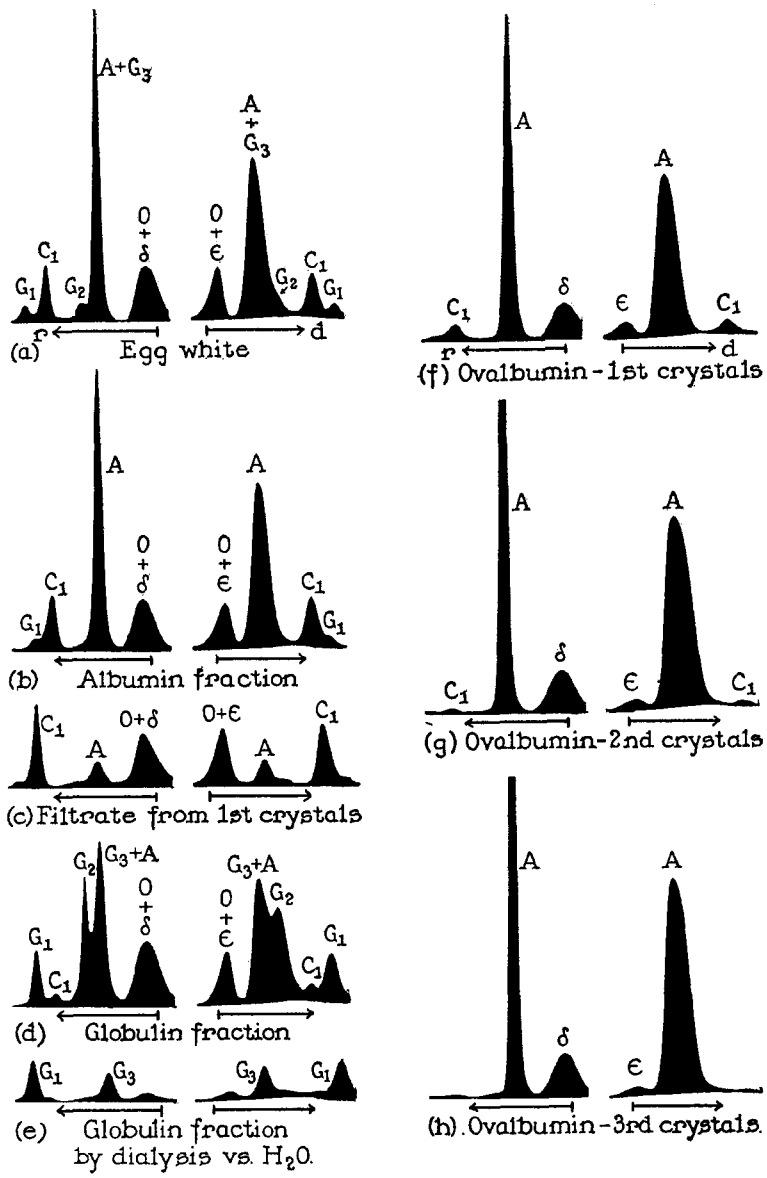


Fig. 3.—Electrophoretic patterns of the various fractions arising in the preparation of crystalline ovalbumin.

Of interest in this connection is the pattern of the filtrate obtained after crystallization of the "albumin" fraction, Fig. 3(b). This is shown in Fig. 3(c), and indicates relatively large proportions of the conalbumin, C_1, and ovomucoid, O, and a correspondingly small amount of ovalbumin, A, indicating, once more, that the crystallization serves as an effective means of separation of the chief constituent from its impurities.

The Preparation of Conalbumin and Ovomucoid.—By half saturation of egg white with ammonium sulfate or by a suitable addition of sodium sulfate[17] the mucin and globulins may be removed. From the filtrate the greater part of the ovalbumin may be crystallized at about *p*H 4.6. The residual ovalbumin may be removed together with the conalbumin by heat coagulation. The remaining protein, *ovomucoid*, may, then, be precipitated either by excess of alcohol, by saturation with ammonium sulfate, or by addition of sodium sulfate at 30° to a concentration of 320 g. per liter of water. A dialyzed solution of ovomucoid prepared by the latter method was

(16) Sørensen and Høyrup, *Compt. rend. Lab. Carlsberg*, **12**, 12 (1917).

(17) Kekwick and Cannan, *Biochem. J.*, **30**, 227 (1936).

used in this work. The homogeneity of this material will be discussed below.

Wu and Ling[18] have found that ovalbumin is capable of denaturation by shaking, while conalbumin is not. By this means ovalbumin may be removed from the conalbumin and ovomucoid. No method hitherto has been available for the preparation of uncoagulated conalbumin free from ovalbumin and ovomucoid. However, one of us (R. K. C.) has observed that when the albumin fraction is acidified to a *p*H below 4 a precipitate forms more or less slowly. On examination it was found to be *conalbumin*, or to be derived from that protein. The precipitate redissolves in water on the addition of alkali to a *p*H above 6 and is, then, soluble at any *p*H value above 4. It has a nitrogen content of 16%, a coagulation temperature below 55° and is not denatured by shaking. These are the recorded characteristics of conalbumin. Further details of the method of preparation and the properties of this protein will be reported elsewhere.

The Preparation of the Globulins.—There is no unanimity as to the globulin components of egg white which may be chemically separated from one another. The following procedure yields a reproducible product. Egg white was diluted with an equal volume of 0.25% sodium chloride and acidified to *p*H 6. The precipitate of mucin was discarded. The filtrate was half saturated with ammonium sulfate and the precipitate was redissolved in a small volume of dilute salt. When this was dialyzed against 0.25% sodium chloride more of the mucin separated. A subsequent dialysis against water gave a sirupy precipitate which readily dissolved in 0.25% sodium chloride to give a fairly clear solution. This was further purified by repeated salting out and dialysis. The protein was completely coagulated by heat at 80°, was insoluble in water over the *p*H range 5.5 to 7.5, but was freely soluble in quite dilute salt. This globulin preparation was found to have a mobility corresponding to G_2 at *p*H 4.64, but showed components G_2 and G_3 at the *p*H values 5.34 and 6.83.

Dilute solutions of globulins were also obtained by extracting with various buffers the precipitate obtained by dialyzing whole egg white against distilled water. On extracting with a buffer of *p*H 3.94 the solution contained G_1 and G_3 but no G_2, as is shown in the pattern given in Fig. 3(e), and at the *p*H values 4.64, 5.34 and 7.84 only G_1 was present.

The reasonable inference from these observations is that G_2 is a component which precipitates on dialysis only from concentrated solutions and only when the greater part of the other proteins has been removed. On the other hand, G_1 and G_3 both precipitate in the usual mucin fraction but can be extracted from it by dilute salt.

We have made no attempt to obtain mucin in a form suitable for electrophoretic studies because its insolubility would seem to preclude this. Whether there are in fact three globulins as well as a mucin, or whether one of the globulins, G_1 or G_3, corresponds with a slight solubility or decomposition of mucin, is a question that needs further investigation.

The Complexity of Ovalbumin.—Although ovalbumin forms small, but well-defined, crystals it has two components, A_1 and A_2, which have different electrophoretic mobilities at most *p*H values. This was first pointed out by Longsworth[7] and has been observed independently by other workers.[19] Some electrophoretic patterns of at least three times recrystallized ovalbumin are shown in Fig. 4. It will be seen that at low *p*H values and at very high ones there is only one peak or maximum in the patterns, but at intermediate *p*H values there are two. The mobilities of both components are given, for a range of *p*H values, later in this paper. In Fig. 2, showing the patterns for whole egg white, the second component, A_2, can be seen at *p*H 6.12 as a shoulder on the side of the peak A_1 for the main component, and at *p*H 8.00 as a separate peak. (Albumins prepared from the eggs of other species showed only one component, at the single *p*H value (5.2) used.[20]) There appears to be some variability in the relative amount of the component, A_2, but it has been found in all of the many samples of ovalbumin that we have examined, although the material has been prepared from eggs from diverse sources, and with some variations in the modes of preparation.

The component A_2 does not appear to be, as was at first conjectured, an intermediate step in the usual processes of denaturation. The native recrystallized ovalbumin remaining after a large part of the material had been denatured by means of heat, acid, alkali and surface denaturation

(18) Wu and Ling, *Chinese J. Physiol.*, **1**, 407 (1927).

(19) Tiselius and Eriksson, *Biochem. J.*, **33**, 1752 (1939).

(20) Landsteiner, Longsworth and van der Scheer, *Science*, **88**, 83 (1938).

yielded patterns practically indistinguishable from the starting material. The subject should be studied further, especially with relation to the sources of the material, and, possibly, other methods of denaturation. It is of interest that the product of complete heat denaturation gave a single boundary differing but slightly in mobility, at the *p*H values 6.8 and 9.7, from that of the main component of unaltered ovalbumin A_1.

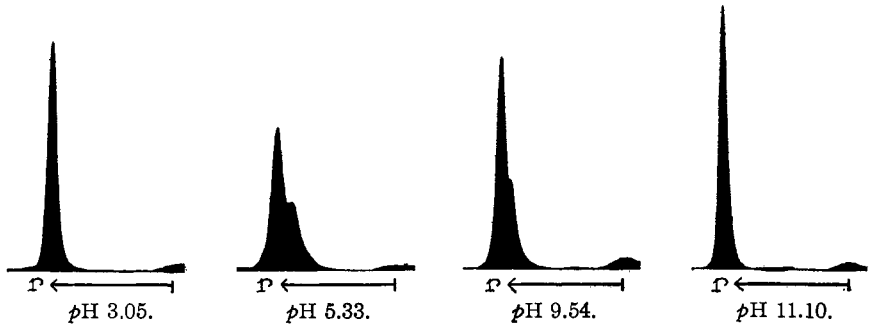


Fig. 4.—Electrophoretic patterns illustrating the complexity of crystalline ovalbumin.

The Complexity of Conalbumin.—Conalbumin, prepared as has been described above, was studied electrophoretically at a series of *p*H values, with the results, for the rising boundaries, shown in Fig. 5. At *p*H 3.92 only one component is indicated, whereas at the higher *p*H values there are two maxima (other than that due to the δ effect), indicating two components which we will designate by C_1 and C_2. In obtaining these patterns a salt-free stock solution of the protein at *p*H 6.2 was diluted to 0.9% of the material and dialyzed for three days at a given *p*H before electrolysis. It will be seen from Fig. 5 that the proportion of the component C_2 decreases with increasing *p*H. Since the patterns for the rising and falling boundaries are reasonably symmetrical (a consideration to be discussed in a later paper) it does not appear likely that the relative concentrations of the two forms of the protein change appreciably during the time of electrolysis *i. e.*, three to four hours. However, since the proportions of C_1 and C_2 change with *p*H it is necessary to assume that a measurable shift of composition occurs during the three-day period of dialysis. Further evidence on this point was obtained by dialyzing a sample of the protein at *p*H 3.6, during which time it was fully converted to the C_2 form. One portion was then dialyzed at *p*H 4.6 for eight hours, and another for three days prior to electrolysis. The second sample contained a much larger percentage of C_1 than the first. This suggests that the fraction of C_1 observed at a particular value of the *p*H does not represent an equilibrium concentration, but merely a transition stage, which is, however, quite reproducible if the experimental conditions are duplicated.

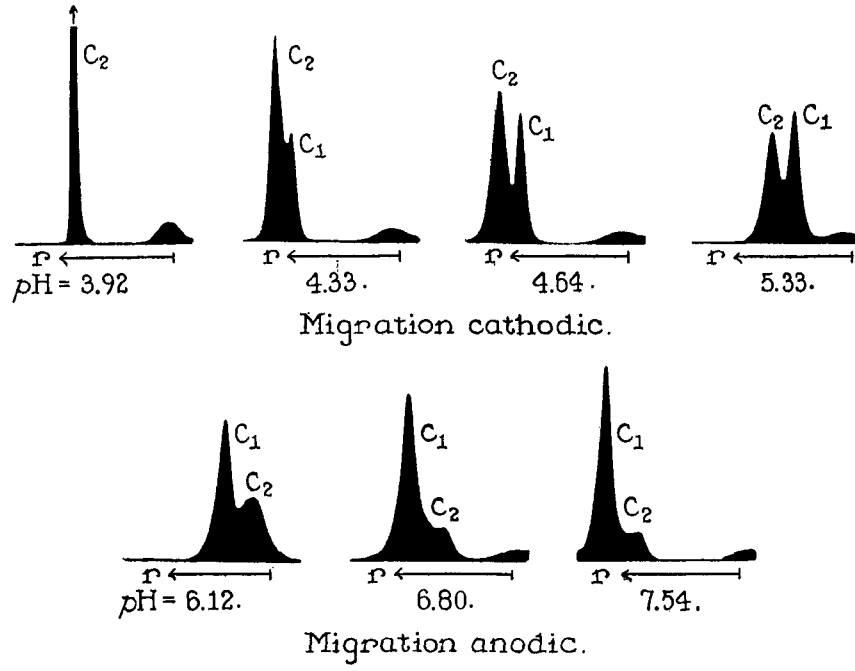


Fig. 5.—Electrophoretic patterns illustrating the complexity of conalbumin as prepared from egg white.

Conalbumin in egg white itself, at least above *p*H 4, shows only one component with mobilities corresponding to the constituent C_1. That component will therefore be referred to as unmodified conalbumin, whereas C_2 will be called the modified

form.[21] However, if the diluted egg white is dialyzed for three days at *p*H 3.6 and then again dialyzed at *p*H 4.6 and an electrophoretic pattern obtained, maxima corresponding to the two components, C_1 and C_2, are observed. It thus appears that in egg white, as well as with the separated conalbumin, the transformation from C_1 to C_2 takes place below *p*H 4, the latter being the stable form below that *p*H value and the former being stable above it, at least in dilute salt solutions.

Evidence that there are two components of different molecular weight in the purified conalbumin is furnished by the sedimentation pattern obtained with the Rockefeller Institute supercentrifuge through the kindness of Dr. Alexandre Rothen. This is shown in Fig. 6, and indicates a main component, presumably C_1, which has a sedimentation velocity corresponding to a molecular weight of about 70,000, and a second component with a higher sedimentation constant.

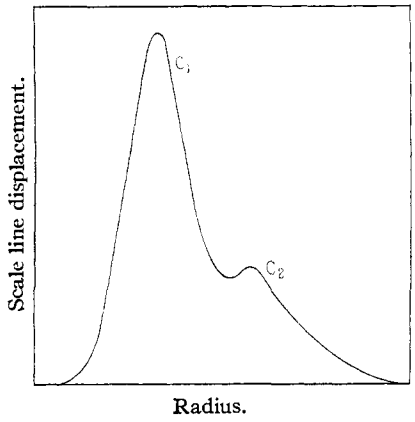


Fig. 6.—Sedimentation pattern of conalbumin.

This suggests that the modified conalbumin C_2 may be a polymer of C_1. Although both ovalbumin and conalbumin are complex, it has not been found possible to change the proportions of the two components of ovalbumin by changing the *p*H, whereas in the case of conalbumin the proportions change markedly with that variable.

The Electrophoretic Inhomogeneity of Ovomucoid.—Tiselius and Horsfall[22] have found that certain proteins show the phenomenon of "reversible boundary spreading." In such cases an initially sharp boundary will become diffuse as the electrolysis proceeds but if the direction of the current is reversed will progressively recover most of its initial sharpness. The obvious explanation of this phenomenon is that the protein molecules forming the boundary have a range of mobilities instead of a single value. The boundaries of the ovomucoid of egg white exhibit this type of spreading to a marked extent. This is illustrated by the superimposed tracings, shown in Fig. 7, of the electrophoretic patterns obtained during the electrolysis of a 1.18% solution of ovomucoid in a 0.1 *N* sodium acetate buffer at *p*H 5.32. The

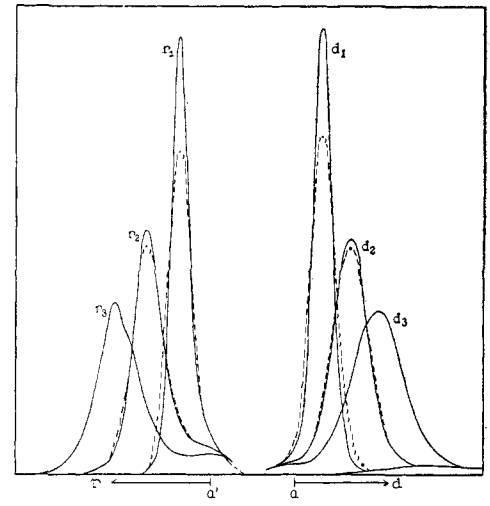


Fig. 7.—Electrophoretic patterns illustrating the reversible boundary spreading of ovomucoid.

initial boundary positions in the two sides of the channel were at a and a′. The pattern contours after electrolysis for successive periods of 4500 seconds at 5.81 volts/cm. are indicated by the full curves r_1, r_2 and r_3 for the rising boundary and d_1, d_2 and d_3 for the descending boundary, respectively. After the third period the current was reversed and the dashed curves represent the contours after two more intervals of 4500 seconds. A comparison of the full and dashed curves for each time interval indicates that, although the boundaries spread as they migrate away from their initial positions, this spreading is nearly completely reversed on changing the direction of the current. Since some spreading of a boundary, due to diffusion, occurs independently of the electrophoresis, the boundaries do not recover all of their original sharpness. The substantial recovery that is effected indicates, however, that most of the boundary spreading is due to electrical inhomogeneity of the ovomucoid. Similar spreading of the ovomucoid boundaries has been observed

(21) We are avoiding the terms "native" and "denatured" since C_2 retains its solubility at its isoelectric point.

(22) Tiselius and Horsfall, *Ark. Kemi, Mineral. Geol.*, **13A**, No. 18 (1939).

at other *p*H values. However, in no instance has there been indication of resolution into two or more separate boundaries.

It is essential, however, to distinguish between boundary spreading due to electrical inhomogeneity of the protein and that due to variations of the electric field strength, and possibly of *p*H, in a boundary. It has been found experimentally that such variations tend to produce a spreading of a descending boundary, an effect which is reversed on changing the direction of the current. The same variations tend, however, to sharpen a rising boundary. If, as is usually the case, the rising boundary is initially sharp, the additional effect tends to maintain that sharpness. Consequently, when the current is reversed, and the rising boundary becomes the descending one, a spreading due to the gradients occurs. A tentative explanation of these phenomena is given in a recent paper from this Laboratory.[8] The superposition of these field gradient effects upon reversible spreading due to electrical inhomogeneity is clearly shown in the patterns of Tiselius and Horsfall. Thus their patterns of the rising boundary indicate a smaller decrease of sharpness on electrolysis than those of the descending boundary and a correspondingly smaller recovery on reversal of the current. Confusion as to the true extent of reversible spreading due to inhomogeneity can be avoided by electrolysis under conditions such that the refractive index gradients in the two sides of the channel are nearly the same at any given time. These conditions can be realized experimentally at or near the (average) isoelectric point of the protein. This was the condition under which the experiment illustrated in Fig. 7 was carried out. The fact that the curves for the rising and falling boundaries are very nearly mirror images indicates that little of the spreading is due to the variations of field gradients just discussed, and must therefore be ascribed to electrophoretic inhomogeneity of the ovomucoid.

The Mobilities of the Protein Components of Egg White as Functions of the *p*H.—A considerable portion of this research has been directed toward the determination of the variation with the *p*H values of the mobilities of the protein constituents of egg white. The results of these measurements are given in Table I, and, at least for the experiments to be discussed in this paper, were all at an ionic strength of 0.1. For the

TABLE I

THE MOBILITIES, $u \times 10^5$, AT 0°, OF THE PROTEIN CONSTITUENTS OF EGG WHITE IN BUFFER SOLUTIONS OF SODIUM SALTS AT AN IONIC STRENGTH OF 0.1

Buffers	Acetate					Phosphate				Veronal, chloride	Phosphate, chloride
*p*H	3.93	4.33	4.45	4.64	5.33	6.12	6.80	7.54	8.00	7.82	6.71
Ovomucoid, O	0.61		−0.27		−1.75		−3.42				
Ovomucoid, O in egg white	.63		− .34		−1.83	−2.95	−3.51				
Ovomucoid, O Hesselvik	1.05		.11		−1.44		−3.42				
Conalbumin, C_1		4.19		2.89	1.35	−0.78	−1.59	−2.16			
Conalbumin, C_1 in egg white	5.65		3.30		1.19	− .81	−1.57		−2.27		
Conalbumin, C_2	5.81	4.83		3.71	1.89	− .34	−1.05	−1.58			
Ovalbumin, A_1	2.63			−0.18	−2.88		−6.07			−5.99	−5.50
Ovalbumin, A_1 in egg white	2.52		0.36		−2.51	−5.05	−5.79		−6.49		
Ovalbumin, A_2					−2.59		−5.57			−5.50	−5.04
Ovalbumin, A_2 in egg white					−2.21	−4.60	−5.27		−5.96		
Globulin, G_1	7.41			7.23	7.00		3.72			4.35	
Globulin, G_1 in egg white	7.29		7.32		7.09		3.46				
Globulin, G_2	3.70			2.03	1.06		−1.21				
Globulin, G_2 in egg white	3.67		2.40		0.44	−2.29					
Globulin, G_3	2.89			1.30	0.69		−2.05				
Globulin, G_3 in egg white			1.49		−0.37	−1.82					

different *p*H ranges buffers were used which were near their maximum buffer capacity. The measurements were made on the purified components when possible but, as explained above, preparations of the separate globulins have not yet been made available. In addition, mobilities have been computed from measurements on the electrophoretic patterns of whole egg white. These are also recorded in the table.

It will be observed that the two sets of values, though in general agreement, frequently differ by more than the experimental error. The differences, which may be positive or negative, appear to depend upon the relative viscosities of the egg white solution and of those of the purified proteins. Also, due to uncertainties as to the field gradients effective in the case of protein mixtures, certain approximations were necessary in computing the mobilities from the egg white patterns.[23]

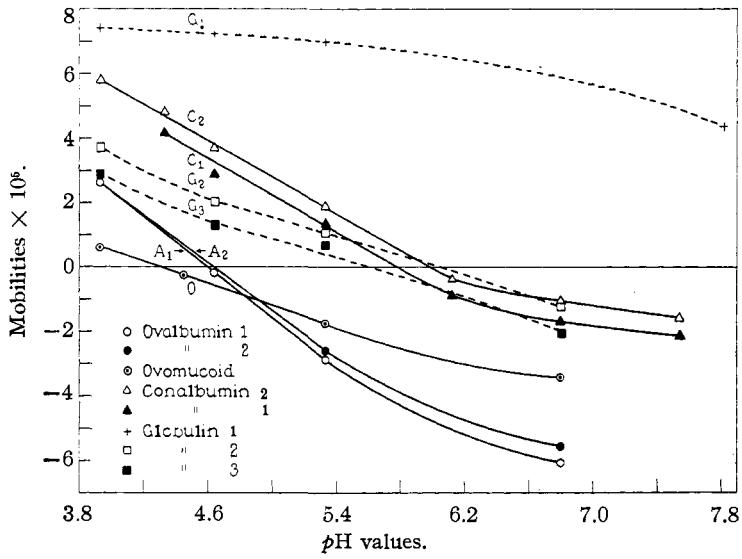


Fig. 8.—The electrophoretic mobilities of the constituents of egg white as functions of the *p*H.

The only published measurements of the mobilities of a constituent of egg white over a range of *p*H values are those of Hesselvik[24] on ovomucoid. Values interpolated from his results are included in Table I and indicate a somewhat higher isoelectric point, *p*H 4.5, than that, *p*H 4.3, obtained in this research. His material was obtained by alcohol precipitation and the basis for his mobility computations is not clearly stated.

A plot of the mobilities of the various purified, or partially purified, proteins as functions of the *p*H is given in Fig. 8. It will be observed that, with the exception of G_1, all of the components have isoelectric points but at quite different *p*H values. This is of significance in connection with the protein complexes, indicated by the patterns for the higer *p*H values, which will be discussed in the next section.

Protein-Protein Interaction.—Very early in the course of this work certain abnormalities in the patterns of egg white were observed that, we now believe, may be ascribed to interaction between the constituents. Thus mobility measurements on our globulin preparations in the neighborhood of *p*H 6 gave values for G_2 and G_3 similar to those of conalbumin, C_1, as may be noted in Fig. 8. The patterns shown in Fig. 2(b) indicate, however, that in the native egg white at *p*H 6.12 these globulins are migrating between the conalbumin and the ovomucoid, and that separation from the latter is incomplete. Although the peaks corresponding to the globulin G_1 had moved out of the field at the time the patterns of Figs. 2(b) and 2(c) were obtained, their areas and positions in the two sides of the channel were far from being mirror images of each other, suggesting that this component, carrying as it does a charge opposite to that of the other egg white constituents at this *p*H, may have been involved in the apparent interaction between the ovomucoid and the other globulins.

That a difference in the sign of the charge carried by the interacting proteins is an important, although possibly not essential, factor is indicated by the patterns of Fig. 9. These were obtained from the two sides of the channel on electrolysis of an egg white solution, diluted 3 ×, in a 0.1 *N* sodium acetate buffer at *p*H 4.45. These patterns deviate much more from each other than can be explained on the basis of the δ and ϵ effects alone. It will be noted that the area of the peak corresponding to ovomucoid in

(23) These mobilities have been obtained from the displacements of the maxima of the descending boundaries except for components migrating between the A_1 and the δ, or ϵ, boundaries. In the latter case mean values of U_r^* and U_d^3 have been used. This procedure makes an approximate correction for the change of the field strength at the A_1 boundary. Moreover, the mobility of G_1 has been computed from data on the rising boundary and the conductance of the buffer solution, since the patterns for the descending boundaries indicate, at the higher *p*H values, complexes with other proteins.

(24) Hesselvik, *Z. Physiol.*, **254**, 144 (1938).

the left-hand pattern has been markedly increased at the expense of the ovalbumin when compared with the corresponding peaks of the right-hand pattern. Reference to Fig. 8 indicates that between *p*H 4.3 and 4.6 ovomucoid and ovalbumin are oppositely charged. It is only in this narrow *p*H interval that abnormal pattern asymmetries involving these two constituents are observed. That such asymmetries due to interaction may arise in all cases involving oppositely charged constituents has not yet been established, however. Thus in the *p*H interval from 4.6 to 5.8 there is evidence of but little interaction between ovalbumin and conalbumin. The tendency for the proteins rich in carbohydrate, of which ovomucoid is an example, to form complexes with other proteins and to promote protein interaction has already been noted by Pedersen and MacFarland[25] in connection with the sedimentation patterns of protein mixtures.

The patterns shown in Fig. 9 were obtained with the aid of a double length center section as described earlier in this paper and the more complete separation of the egg white constituents that is possible in this type of cell, when compared with patterns previously published, illustrates one of the chief advantages of the double length cell.[26] The feature of the patterns, however, that is of interest in connection with protein–protein interaction is the contrast between the asymmetry of the ovalbumin–ovomucoid portions of the patterns and the corresponding portions of the patterns of Fig. 2(a) which were obtained in the same buffer but at a lower protein concentration. This suggests that, at a given *p*H and ionic strength, interaction is favored by high protein concentration. Similar experiments in which the *p*H and protein concentration were kept constant showed that interaction is also favored by low ionic strength.

The Electrophoretic Analysis of Egg White.—The preceding discussion of protein–protein interaction is a necessary prelude to a consideration of the electrophoretic analysis of egg white. If interaction can occur between oppositely charged components, patterns in which the relative magnitudes of the areas due to each constituent are not confused by such interaction can only be obtained at *p*H values at which all constituents carry charges of the same sign. In the case of egg white all of the components migrate cathodically at *p*H values below the isoelectric point of ovomucoid, *i. e.*, 4.3. Owing to the rather unusual electrochemical behavior of G_1 there is, apparently, no *p*H above which all of the constituents migrate anodically. Consequently, of the patterns available for analysis most weight should be given to that obtained at *p*H 3.92. Analysis with the aid of this pattern is complicated, however, by the fact that G_3 and (A_1 and A_2) are not resolved at this *p*H and it has therefore been necessary to use patterns obtained at other *p*H values. The latter were selected for sym-

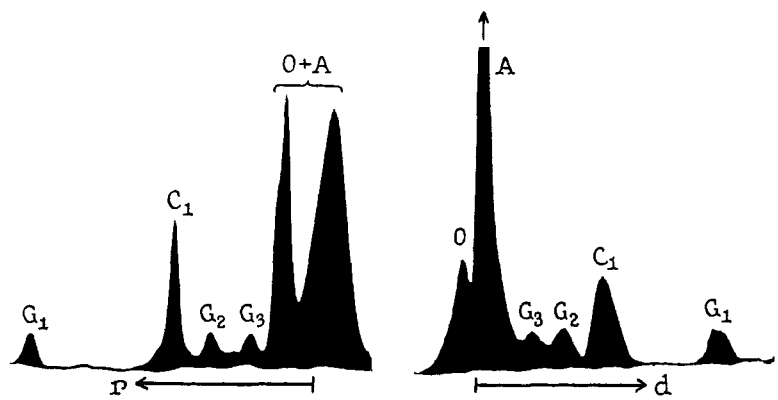


Fig. 9.—Electrophoretic patterns of egg white, diluted 3×, in a 0.1 *N* sodium acetate buffer at *p*H 4.45.

TABLE II

THE COMPOSITION OF EGG WHITE BY ELECTROPHORETIC ANALYSIS

	Per cent. of total proteins						
	Ovalbumin	Conalbumin	Ovomucoid	Globulins			Pattern[a]
*p*H	A_1 A_2	C_1	O	G_1	G_2	G_3	
3.92	..	13.4	15.3	3.8	4.6	..	A
	..	13.3	13.6	3.4	..	..	B
4.45	60.2	15.3	13.5	2.9	4.4	3.6	A
	59.8	14.3	13.6	2.2	5.4	4.7	B
5.32	..	15.3	..	2.6	4.5	4.3	A
	..	13.5	..	1.7	4.1	4.6	B
Av. (electrophoretic)	60.0	13.8	14.0	2.8	4.6	4.3	
By chem. methods	70	9	13		6.7		

[a] A, pattern in which A_1 boundary was ascending. B, pattern in which A_1 boundary was descending.

(25) Pedersen and MacFarland in Svedberg and Pedersen, "The Ultracentrifuge," Oxford Univ. Press, New York, N. Y., 1940, p. 408.

(26) The double length cell used in this experiment was the first one produced by the Klett Manufacturing Co. (177 East 87th Street, New York, N. Y.). The optical imperfections in the cell windows, apparent in the distorted base line of the pattern, have been eliminated in subsequent cells.

metry, thus indicating comparative freedom from interaction, and for resolution. The results of these analyses are given in Table II. In these computations allowance for the δ and ϵ effects has been made by assuming that the latter are additive and are equal to the effects observed in solutions of the purified constituents at the same *p*H, ionic strength, and protein concentration as that of the corresponding component in egg white. While we have made no systematic study of the composition of egg white, we have observed some variation of material from different sources. Thus the percentage of ovalbumin has varied from 60 to 70% while the conalbumin has varied from 13 to 18%. The table also gives the results obtained by Sørensen[27] for the composition of egg white, using chemical methods.

Summary

Electrophoretic patterns of egg white have been obtained over a range of *p*H values. These patterns have indicated seven electrically distinguishable components: *viz.*, ovalbumin, A_1 and A_2, ovomucoid, O, conalbumin, C_1, and three globulins, G_1, G_2 and G_3. The identity of these components has been established by comparison with patterns obtained from the purified components, with the exception of the globulins which have not yet been separated.

Each step in a separation of ovalbumin has been followed electrophoretically, indicating in this process the relatively greater efficiency of crystallization over salting out. Ovalbumin has been shown to consist of two components both in egg white and in the purified state.

The separation of conalbumin involves a modification into a form, C_2, which slowly reverts, above *p*H 4, to the normal form, C_1, in which it exists in egg white.

Ovomucoid, though not electrically separable into more than one component, shows complexity as indicated by "reversible boundary spreading."

The mobilities of the components have been determined over the range of *p*H 3.9 to 8, which includes isoelectric points of all constituents except the globulin G_1.

Evidence is given of protein–protein complexes, especially when the components carry opposite electrical charges. The extent of this association increases with protein concentration and decreasing ionic strength.

An analysis of egg white has been obtained electrophoretically.

(27) M. Sørensen, *Compt. rend. trav. lab. Carlsberg*, **20**, no. 3 (1933-1935).

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

alpha-Bromo Secondary Alkyl Ketones. I. Reaction with Sodium Alcoholates. A New Synthesis of Tertiary Acids by Rearrangement[1,2]

BY J. G. ASTON AND R. B. GREENBURG

The problem of replacement of α-halogen in ketones by bases has been considered in detail by Kohler and co-workers.[3a,b] Kohler and Addinall[3a] called attention to the fact that in the synthesis of β-diketones by the action of alcoholates on α,β-dibromo ketones a second reaction can occur, namely, one producing β-alkoxy-α-hydroxy acetals. They formulated the reaction as occurring through the α-bromo-β-alkoxy ketone (which is supposed to be an intermediate in the production of the β-diketone) as follows

$$\mathrm{R{-}CH(OCH_3){-}CHBr{-}CO{-}R} \longrightarrow$$
$$\mathrm{R{-}CH(OCH_3){-}CHBr{-}C(OCH_3)(ONa){-}R} \xrightarrow{-\mathrm{NaBr}}$$
$$\mathrm{R{-}CH(OCH_3){-}CH(O){-}C(OCH_3){-}R} \xrightarrow{\mathrm{CH_3OH}}$$
$$\mathrm{R{-}CH(OCH_3){-}CHOH{-}C(OCH_3)_2{-}R} \quad (A)$$

This second reaction gave a clue to the reaction involved in the synthesis of the β-diketones. The precedent for the last step was supplied by Kohler and Brown,[3b] who studied the reaction of potassium cyanide on desyl chloride and thus obtained two stereoisomeric oxido-nitriles

$$\mathrm{C_6H_5{-}CH(O){-}C(C_6H_5){-}CN}$$

However, desyl chloride had already been shown

(1) From the doctorate thesis of R. B. Greenburg, The Pennsylvania State College, 1940.

(2) Certain preliminary reactions were carried out several years ago by W. D. Moyer and R. R. Heterick.

(3) (a) Kohler and Addinall, THIS JOURNAL, **52**, 3728 (1930); (b) Kohler and Brown, *ibid.*, **55**, 4299 (1933).