STUDIES OF ELECTROPHORETIC PATTERNS OF THICK AND THIN WHITE OF THE CHICKEN EGG

by

BERNHARDT VICTOR ANDERSEN

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TABLE OF CONTENTS

INTRODUCTION

Egg white has been the subject of many researches because of its availability, its importance in nutrition, and since it is a cheap source of proteins for biological investigations.

The various major divisions or parts of the egg have been known for many years by the majority of people. One of the first divisions of the egg into its constituent parts was made by Romanoff (1) in 1929. He divided the egg into five parts; the outer white, middle white, chalaziferous layer, yolk, and air sac.

Hughes and Scott (2) were among the first to recognize the present divisions of egg white. They divided egg white into the outer thin, middle thick, and inner thin layers. Their work was concerned with the relative amounts of mucin, globulin, and albumin in the three respective layers. It was found that there was a significantly larger amount of mucin in the middle thick layer. The inherent viscosity of the middle thick layer was ascribed to the relatively large amount of mucin found in that layer.

Between 1936 and 1943 there apparently was no work done on the separate layers of egg white. A considerable amount of work was done on whole egg white. Longsworth, Cannon and MacInnes (3) made a comprehensive study of the number and kinds of proteins in whole egg white using a combination of electrophoresis and chemical means. Electrophoretic separation showed at least seven separate proteins in egg white. When they employed sodium acetate or sodium phosphate as a buffer, the proteins found were two

albumins, three globulins, conalbumin, and mucin.

Bain and Deutsch (4) have confirmed the work of Longsworth et al. (3). They obtained essentially the same electrophoretic patterns for whole egg white of chicken eggs in their investigation of the electrophoretic patterns of the whole egg white of 13 species of birds. A characteristic electrophoretic pattern was found for each species. It has been noted that all of this work was on whole egg white without any differentiation of the egg white layers.

In 1943, Romanoff (5) changed his conception of the morphology of the hens egg and divided the egg white into four layers. Of these, three were divided as Hughes and Scott (2) had done previously plus a fourth layer which also has been named the chalaziferous layer. This fourth layer has not been considered in this paper.

A complete book on the avian egg was published by Romanoff and Romanoff (18) in 1949. This book contains among other things a schematic drawing of the morphological structure of the chicken egg which was found to be very helpful.

Frampton and Romanoff (6) have attempted to characterize the proteins in egg white by electrophoretic analysis of each of the three main layers of chicken egg white. Their patterns were obtained after one hour of electrophoresis in 0.1 M acetate buffer at pH 4.45 and 1.6 per cent sodium chloride, at a potential gradient of 1.7 volts/cm. They concluded "that no major protein components in any one layer of egg albumin may be identified with

any major protein component in either of the other two layers". Another point of interest was that whereas the electrophoretic data obtained by Longsworth et al. (3) indicated the presence of 7 protein components in the whole white of the chicken egg, the data obtained Frampton and Romanoff with the three layers at pH 4.45 indicated a minimum of 10 separate protein components.

The following electrophoretic investigation of the three layers of chicken egg white was undertaken to continue the work of Frampton and Romanoff (6). This has been attempted by obtaining the mobilities of the various protein components in the three layers of egg white at various pH's and plotting them to obtain the isoelectric points. In this way it might be possible to compare more completely the results of work on whole egg white and work on the various layers of egg white.

MATERIALS AND METHODS

Egg White Separation Method

All of the eggs that were used in this work were obtained from the Kansas State College poultry farm. The eggs were obtained from one flock of White Leghorn chickens that were fed a normal diet. It was found to be essential that the eggs used were as fresh as possible. The eggs were all broken for separation within one hour of the time of laying. In this way it was possible to keep deterioration between the three layers of egg white to a minimum as well as making possible a more clear-cut separation of the three layers from each other.

It was found that a relatively complete separation could be effected by breaking the egg carefully upon a specially prepared glass plate. The glass plate was prepared with a large circle of paraffin deposited in the center. A small opening in the paraffin circle was used to remove the yolk after the separation had been effected. The ridge of paraffin was ¼ inch high and the diameter of the circle was about 7 inches. When the egg was broken on the glass, the outer thin white immediately flowed out away from the rest of the egg, and was collected most easily by means of a 10 ml glass hypodermic syringe. After collection of the outer thin egg white layer the glass was very carefully wiped off around the thick white envelope. The inner thin layer was then separated by carefully slitting the middle thick envelope which contains the inner thin egg white layer. This was then collected by means of a syringe in the same manner as was the outer thin egg white layer. After complete removal of the inner thin egg white the middle thick layer was removed by cutting it away from the yolk and sliding it off the plate into a suitable recepticle.

Preparation of the Buffered Solutions of the Proteins to be Run at Various pH Values

The various layers from 6 to 8 eggs formed a pooled sample of each of the three layers as described in the preceeding section. Each sample was then diluted somewhat below the final concentration desired with sodium acetate-acetic acid buffer solutions of constant ionic strength 0.1, prepared at various pH's by varying the amount of acetic acid added. For the accuracy desired in

electrophoretic work, the pH was always checked with a pH meter. If an exact value of pH; e.g., 7.5, must be obtained, it was better to add less acetic acid than necessary and make the final adjustment to the pH required by adding acetic acid dropwise while measuring the change with a pH meter.

Each sample was dialyzed against three changes of buffer which contained 1, 2, and 3 liters, respectively. The sample was dialyzed for 24 hours in the first two portions of buffer and for 48 hours in the third change of buffer. The sample was diluted to the desired concentration just before the third change in buffer. The extended period of dialysis made certain that equilibrium had been reached. The last buffer solution against which the sample was dialyzed was used as the buffer against which the boundaries were formed in the electrophoresis cells.

Experimental Apparatus

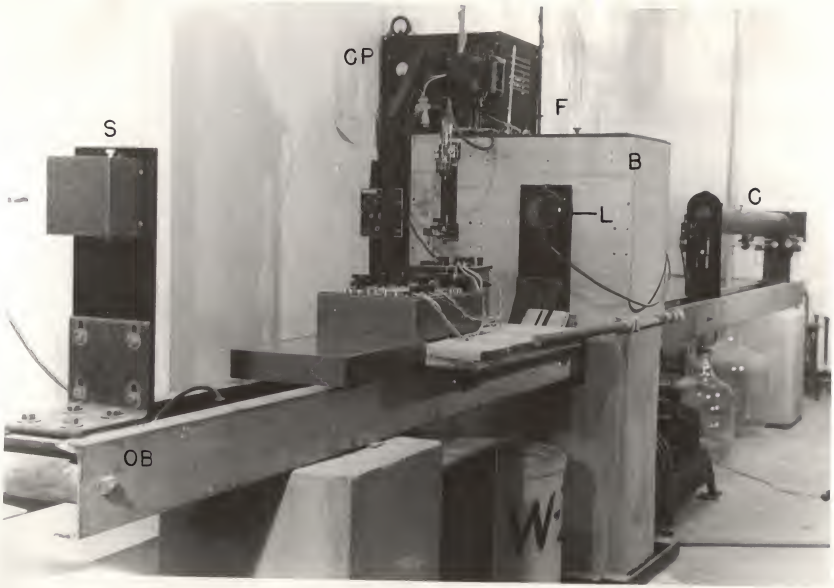
The electrophoretic measurements were carried out in a modified Tiselius apparatus manufactured by the Klett Manufacturing Company, New York City. A photograph of the instrument that has been installed at Kansas State College is shown in Plate I. The instrument is made up of 6 main parts. The light source, S, fitted with a green Wratten B filter has been mounted at one end of the optical bench. The optical bench, OB, has been constructed of two five inch channel irons resting on concrete piers which were imbedded on solid earth to obtain a minimum of vibration. The low temperature water bath, B, into which the "schlieren" lens, L, has been mounted, rests on the center pier but not on the

EXPLANATION OF PLATE I

The electroporesis apparatus

S	Light source
OB	Optical bench
L	"Schlieren lens
B	Water bath
C	Camera
CP	Control panel
F	Framework supporting the electrophoresis cell

PLATE I



optical bench so that any vibrations from the agitation of the water are not transmitted to the optical system. The camera, C, rested upon the opposite end of the optical bench from the light source. The control panel, CP, has also been pictured. It has the function of maintaining a constant current of 20 ma flowing through the electrophoresis cell.

The literature has many articles on the methods and equipment used in electrophoresis (3, 7, 8, 9, 10, 11, 12, 13, 14). Because of this complete coverage the principles of electrophoresis have not been more completely covered here. A discussion of the observations of boundaries, some boundary customs, and a detailed description of the setting of a "metastatic" mercury thermoregulator, only, have been covered in the following sections.

Boundary Observations and Customs

There have been developed two main methods of boundary observation which have become known as the "schlieren" and cylindrical lens methods.

The "schlieren" method was devised by Toepler from a device of Foucault who used it as an accurate method of testing lenses for chromatic and spherical aberrations. Plate II, Fig. 1, illustrates the Foucault lens test. Foucault employed light from a distant source, L, which was focused on the lens to be tested. He placed a screen, S, at the focal point of the lens which just covered the image formed at this point. By focusing a telescope, T, on the lens from behind the screen it was possible to detect imperfections because if the lens was perfect no light would reach

the telescope; but if there was a slight imperfection, A, at any point in the lens some light would have reached its focal point either before or behind the screen and this would cause illumination in the telescope. Toepler has extended this method to detect small variations in refractive index in a given medium. A discussion of this work has been published by Schardin (15).

The present scanning moving boundary schlieren method has been worked out by Longsworth (10, 11) and Longsworth and MacInnes (7) from the work of Foucault and Toepler. This method has been illustrated in Plate II, Fig. 2. A lamp, L, has been used to illuminate a thin horizontal slit, S, which was focused on the schlieren lens, D, and projected by the lens to the point, P, still in a horizontal plane. The schlieren knife edge, Q, was a screen with a sharp horizontal upper edge in the same plane as P. The telescope was replaced with a camera with objective, O, and ground glass, G, focused on the cell, E. When no refractive element or boundary was present in the electrophoretic cell, all the light was brought to focus to form an image of the slit at P. The image of the cell at G was then uniformly illuminated. The presence of a boundary in the cell produced a change in the refractive index. It must be remembered that the boundary is not an infinitely thin plane but a region of continuously varying composition. Some place in the boundary a maximum change of refractive index was found with a gradual diminishing of refractive index on each side of the maximum change. The values of the gradient plotted against distance would produce a normal Gaussian

EXPLANATION OF PLATE II

Basic optical systems for the electrophoresis apparatus

Fig. 1. The Foucault lens test

L light source

S screen

T telescope

A imperfection in lens

D lens

Fig. 2. The Longsworth moving boundary method

L lamp

S slit

D schlieren lens

Q schlieren knife edge

G ground glass

E cell

Fig. 3. The cylindrical lens method

R light source

L schlieren lens

G cell

D slit

O objective lens

CL cylindrical lens

G ground glass or film

PLATE II

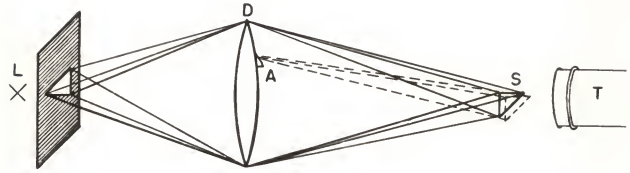


Fig. 1.

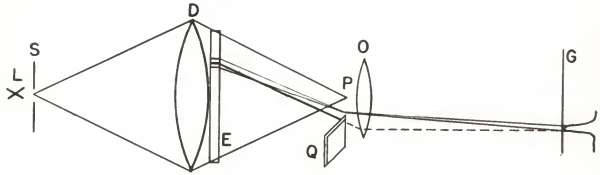


Fig. 2.

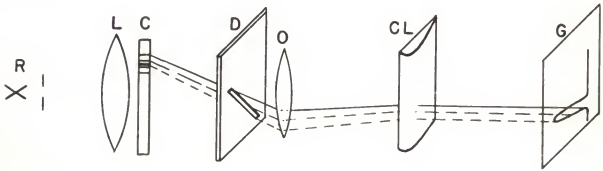


Fig. 3.

distribution curve in the ideal case. If the knife edge was raised to intercept the maximally refracted pencil of light, which would be the point of greatest refractive index change, a dark band appeared on G. In Longsworth's method a photographic plate was driven at a constant rate past a narrow vertical slit placed at G. A system of gears was arranged to drive the knife edge Q up at the same time as the plate was traveling across the slit. The portion of the diagram to the right of G, has been depicted in a plane perpendicular to the page and shows the record produced on positive film.

A second method for the observation of boundaries that has been used at Kansas State College is the cylindrical lens method developed by Philpot (16) and Svensson (17). The main advantage of this method is that it provides visual observation of the boundaries during the course of an experiment. That was the only purpose for which it was used in this present work. A schematic drawing has been included, Plate II, Fig. 3. The optical system includes the schlieren lens, L, with the cell, C, illuminated from the same light source. A slit, D, was placed before the objective lens, O, and a cylindrical lens, CL, was placed between the objective and the film with the slit in front of the film omitted. By this means a picture can be obtained either visually or on a photographic plate. The slit, D, may be revolved at different angles to obtain Gaussian curves of varying height and clarity.

There are several boundary customs or conventions that have

been generally accepted. These may be best explained through a description of the Tiselius electrophoresis cell and the action of a protein solution in the cell. The cell consists of two rectangular channels in which the protein migration was carried out and the refractive index gradients photographed. When a direct current was applied across the cell, the two boundaries split into a series of boundaries which travel in one side in an ascending direction. These boundaries were designated as ascending boundaries with the boundary that remains at or near the original boundary designated as the δ (delta) boundary. The splitting boundaries in the other side of the cell that were descending have been designated as descending boundaries with the boundary that remains near or at the original boundary designated as the ϵ (epsilon) boundary. The delta and epsilon boundaries do not represent protein components and have been designated by some investigators as salt boundaries.

There were two physical properties of proteins determined by electrophoretic means. These were the mobility and isoelectric point of each protein. The electrical nature of the protein particle in solution is based on the fact that proteins are inherently amphoteric and able to produce upon ionization either positive or negative protein ions, depending on the character of the external medium. The isoelectric point has been defined as the pH of a protein at its isoelectric point is a characteristic of that protein. The velocity of electrophoresis was determined by measuring the rate of movement of the boundary. The mobility

was then calculated from the formula

$$\mu = vqk/i$$

where μ is the mobility in $cm^2\ sec^{-1}\ volt^{-1}$, v is the velocity of migration of the protein component in cm/sec, q is the cross sectional area of the cell in sq cm, k is the specific conductance of the protein solution in mho/cm, and i is the current in amperes passing into the cell.

Mobilities in this experiment were computed by the salt boundary method. The values of v, equation 1, were obtained on the basis of the lateral displacements of the protein components from the associated salt boundary. Mobilities were generally computed from the patterns of the ascending boundaries because these exhibited much better resolution of the protein components than did the descending boundaries. The descending boundaries were obtained in some cases for the purpose of comparison only.

The photograph of the ascending boundary was projected on a large piece of paper with a magnification factor of 5.0. Any degree of magnification was permissible but the exact value must be known. The position of the vertex of each protein component, the salt boundary, the reference line, and a base line were indicated by means of a hard lead pencil. This procedure was followed for the ascending boundary pictures taken at several different periods of time for each electrophoretic run.

Measurements were then made and tabulated for the lateral distance in cms, that each protein had traveled from the salt boundary. The time T of movement was recorded in minutes. If

M represents the magnification factor and d the distance of protein component movement then v may be expressed

$$v = d/MT(60) \qquad (2)$$

k in equation 1 may be defined as

$$k = J/R \qquad (3)$$

where J is the cell constant which in this experiment was 1.117. R is the resistance of the protein solution. Substitution of these factors into equation 1 results in a general equation

$$\mu = (d/MT)\ (qJ/iR) \qquad (4)$$

In this experiment q was 0.836, i was 0.020 ampere, and the magnification factor, M, was 5. Substitution of these constant figures into equation 4 resulted in the working equation

$$\mu = d/(5)\ (60)\ (T) \cdot (0.836)\ (1.117)/(0.020)\ (R) \qquad (5)$$

By substitution of experimental values of d,T, and R into equation 5 the final values of μ were obtained.

It was necessary to obtain mobilities, for each protein component migrating toward both the cathode and anode, in order to obtain the isoelectric point of each protein. In this paper all mobilities of proteins, when migrating toward the cathode were designated as positive and those proteins migrating toward the anode were designated as having negative mobility values.

The mobility values were very important not only for the determination of isoelectric points; but also for the identification of the individual protein components as albumins, globulins, conalbumin, and mucin. Identification of the protein components was aided by close comparison with similar experiments

performed by Longsworth et al. (3). The protein components were labeled to correspond as closely as possible with the components found by Longsworth.

The "Metastatic" Mercury Thermoregulator

There is one instrument on the apparatus that has not been mentioned in any of the previous papers written. It is the water bath temperature regulator which has a trade name of "Metastatic" mercury thermoregulator. The thermoregulator, which has been depicted in Plate III has been connected on the instrument to a Fischer-Serfass electronic relay system which operates the refrigeration unit.

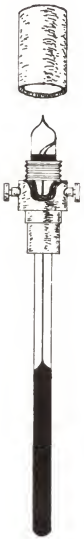
The thermoregulator opens and closes an electrical relay circuit by means of the rise or fall of a column of mercury in a capillary. Electrical contact is made as the top of the mercury column reaches the upper platinum contact wire. Contact is broken as the mercury column recedes from the upper contact wire. The contact wire may be seen through the small observation window located in the bakelite neck of the regulator. The amount of mercury in the capillary determines the temperature at which contact will be made or broken. Setting the regulator, therefore, consists of leaving just enough mercury in the capillary to cause the top of the column to touch the contact wire at the exact temperature to be maintained.

The regulator contains two mercury reserviors. The lower reservoir feeds mercury to the capillary in the same manner as

EXPLANATION OF PLATE III

Metastatic Mercury Thermoregulator

PLATE III



the bulb in a standard mercury thermometer. The upper reservoir, which is covered by the bakelite head, retains whatever quantity of mercury is excessive for a given temperature.

To set the regulator, the bakelite cap is removed by pulling out the spring set in the side slots, then the cap is carefully lifted upward with a twisting motion. The upper reservoir and enlarged end of the capillary will now be visible. The regulator bulb (lower reservoir) is immersed in a bath operating at the exact temperature desired. Observe the height to which the mercury may spill out of the open end of the capillary into the upper reservoir. This constitutes an excess not required for control at such temperatures. If the column stops above the visible contact, the mercury above that point must be expelled from the capillary. To do this, remove the regulator from the bath, dry the bulb and stem, and heat the bulb slowly over a hot-plate or soft gas flame until a droplet of mercury of the proper size protrudes from the tip of the capillary. A sharp tap with the finger against the bakelite neck, and immediate withdrawal of the regulator from the source of heat, will cause the droplet to be thrown off into the upper reservoir, and will prevent additional mercury from being expelled. If, after immersion in the constant-temperature bath, the mercury column comes to rest below the platinum contact, mercury must be re-admitted to the capillary. To accomplish this the bulb and stem are dried and then heated until the mercury just begins to protrude from the open tip of the capillary. Then the regulator is removed from the source of heat

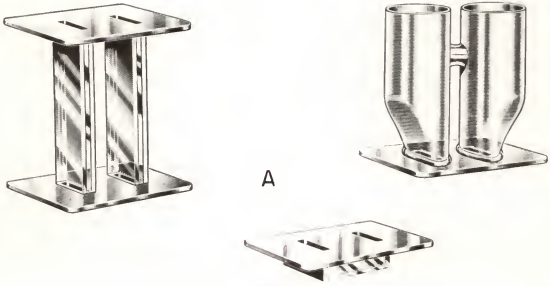
and quickly inverted in order that the open tip of the capillary dips into the mercury in the top reservoir. By allowing the mercury to cool in this position, any desired quantity of mercury can be drawn back into the capillary. If too much mercury is drawn into the capillary, it can be expelled by repeating this whole process.

Experimental Procedure

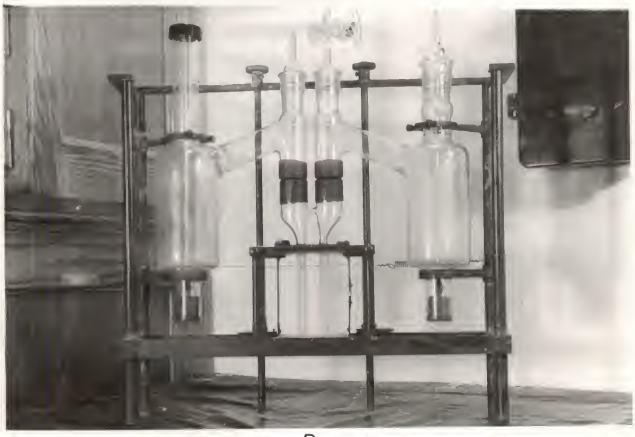
A separate cold room, which maintains a temperature of from 5 to 10 degrees centigrade, has been set up especially for this work. This room has been used as a dialysis room and also for loading the electrophoretic cell to reduce to a minimum the tendency that many proteins have of being easily denatured. It has the added advantage that the strain on the electrophoretic cells from sudden cooling, when they were placed in the 0.5 degree centigrade water bath in making a run, was diminished. The cells, rack and assembly are shown in Plate III.

The electrophoresis cells used were of the type holding a total of 11 ml of liquid. The ground glass junction surfaces of the bottom plate, center section and the upper plate were greased with a 1:1 mixture of petrolatum and mineral oil. These three sections were placed together and moved into alignment so that the opening in the three pieces all coincided exactly. When the ground surfaces were joined together, a continuous seal must be obtained with complete exclusion of air. This cell assembly was then placed in the support rack and clamped in position very loosely.

PLATE IV



A



B

Using a 5 ml syringe fitted with a 10-inch hypodermic needle, 5 ml of the protein solution was placed in the cell assembly. The needle was placed in the right hand channel of the cell with the point resting on the bottom plate. The protein solution was then allowed to run out of the syringe at the slowest rate possible, in most cases due to the weight of the syringe plunger alone. In the great majority of cases this procedure has been found to introduce the protein solution with the exclusion of all air bubbles.

The ground glass junctions were then displaced laterally to the right so that the bottom plate and center section did not coincide. The protein solution in the bottom plate was examined for air bubbles, and if any were found the process was repeated. If there were no air bubbles in the bottom cell, the right hand channel of the center section was then carefully filled with protein until a slight excess was observed in the upper plate. All air bubbles were carefully removed from the sides of the center section with the tip of the hypodermic needle. The excess protein solution in the left hand channel was removed.

The left-hand channel of the center section, which was to be filled with buffer solution, was rinsed twice with 5 ml portions of cold distilled water. This channel was then washed thrice with portions of the buffer it was to contain, and finally carefully filled with the pure buffer to a level well into the upper plate. Bubbles were again carefully removed. Any bubbles, if left in the cell, might become dislodged during the experiment, float

upward, and cause the boundary to be destroyed. Extreme care was taken to keep the tip of the hypodermic needle clean. Grease may be picked up when the needle passes through the ground glass junctions. If this grease was transmitted to the edges of the center cell sections, light passing through the cell during an experiment would be distorted and the experiment useless. Several such experiments were performed in which this error appeared.

The top section was then moved to the left while the center section was held in a static position, until the top plate was in perfect alignment with the bottom plate. The cell assembly was then clamped firmly in position in the rack by thumb screws. The right hand side of the top section, which had some protein solution in it, was rinsed in the conventional manner and was then filled with 5 ml of buffer.

The upper cell arms were then connected to the top plate of the cell by means of gum rubber tubing. When making a large series of runs, it was found that the gum rubber tubing could be left fastened over the top plate of the cell and only needed to be disengaged from the upper cell arms of the electrode vessels. The electrode vessels and the upper cell arms were filled with buffer to within an inch of the position for the ground glass fittings. The electrodes were then inserted in the electrode arms, after which the three ground glass fittings were lubricated and fitted. Approximately 20 ml of saturated sodium chloride were added, by means of the hypodermic syringe, through the bore of the copper tubing of the electrodes, into the electrode vessel

and were layered around the electrodes.

The cell and frame were then moved from the cold room to the water bath of the electrophoresis instrument where it was placed with the cell as close to the "schlieren" lens as possible. While the temperature of the system was becoming equilibrated, the syringe on the side of the water bath was filled with buffer. Buffer was added to each electrode vessel with the three way stopcock turned so that the excess buffer could escape through the horizontal opening. The rubber tube leading from the syringe was then connected to the horizontal opening of the three way stopcock. With this rubber tubing in place the plunger of the manually operated syringe was driven upward until all of the air bubbles had been expelled through the top of the three way stopcock. The three way stopcock was once more rotated 180 degrees so that the buffer in the syringe was connected to the buffer in the electrode vessel.

When the temperature had become equilibrated, the boundaries were formed. This was done by moving the center section of the cell carefully into alignment with the top and bottom plates. The center section was moved manually by control rods built for that purpose into the cell rack. The boundaries thus formed are hidden by the ground glass junction surfaces. They were moved into view by manually forcing buffer slowly from the syringe into one side of the electrode vessel. In order not to break the boundaries the operation of the syringe was carried out over a period of at least five minutes. It has been found safe to allow

one minute per revolution of the gear that drives the syringe plunger.

When the boundary was in position, the peaks were photographed on Kodak 9 by 12 cm metallographic plates and designated as zero time pictures. The leads from the control panel were then attached to the electrodes and the current and timer turned on. A current of 20 ma was used in all experiments and a thermostat temperature of 0.5 degree centigrade was employed. After suitable intervals of time, depending upon the rapidity of boundary splitting, the current was turned off and scanning photographs were taken of both channels of the cell. The exposed plates were developed in Eastman Kodak DK-50 commercial developer and fixed in ordinary commercial thiosulfate fixing solution.

RESULTS AND DISCUSSION

Electrophoretic Patterns from the three Egg White Layers and Whole Egg White

Electrophoretic patterns were obtained for the three egg white layers of the hen egg. These patterns were obtained over a wide range of pH's in sodium acetate-acetic acid buffer at 0.1 ionic strength for varying periods of electrophoresis. The patterns taken at the longest intervals are shown in Plates V, VI, and VII. All of the protein constituents were not completely resolved at every pH. At pH 3.92 the two albumin peaks and the slowest moving ovoglobulin component were all found under one peak. The ovomucin peak was found to cause the salt boundary to

EXPLANATION OF PLATE V

Patterns of outer thin egg white in acetate buffer at various pH's and 0.1 ionic strength

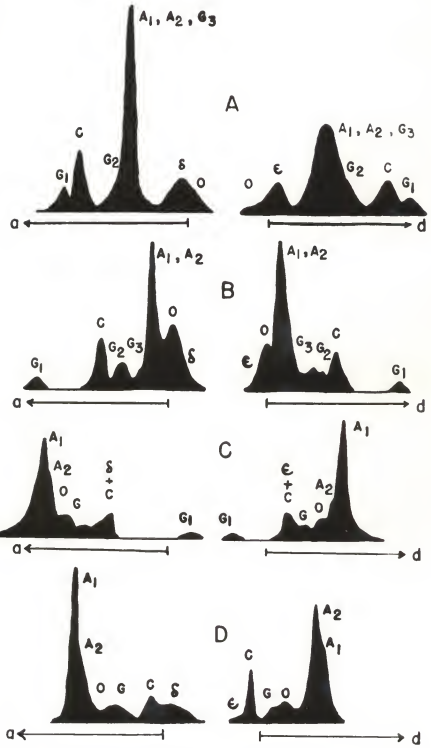
A Pattern taken at pH 3.92 after 130 minutes

B Pattern taken at pH 4.45 after 120 minutes

C Pattern taken at pH 5.85 after 96 minutes

D Pattern taken at pH 7.5 after 100 minutes

PLATE V


A
A1, A2, G3
C
G2
G1
δ
O
a
A1, A2, G3
ε
G2
C
G1
O
d
B
A1, A2
C
G2
G3
O
δ
G1
a
A1, A2
O
G3
G2
C
ε
G1
d
C
A1
A2
O
G
δ + C
G1
a
A1
ε + C
A2
G
O
G1
d
D
A1
A2
O
G
C
δ
a
A2
A1
C
ε
G
O
d

EXPLANATION OF PLATE VI

Patterns of inner thin egg white in acetate buffer at various pH's and 0.1 ionic strength

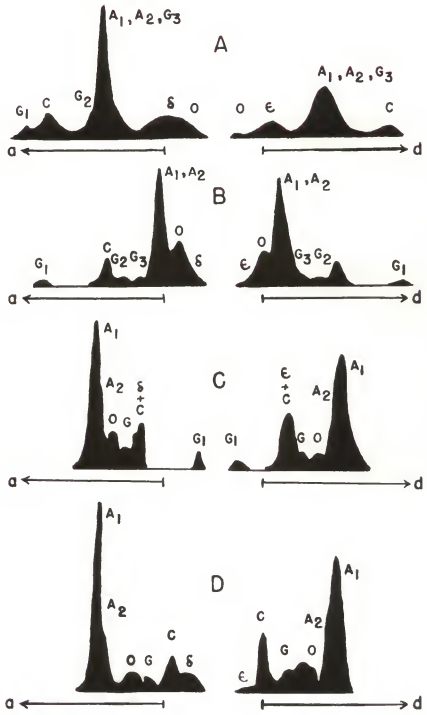
A Patterns taken at pH 3.92 after 130 minutes

B Patterns taken at pH 4.45 after 120 minutes

C Patterns taken at pH 5.85 after 70 minutes

D Patterns taken at pH 7.5 after 100 minutes

PLATE VI


A
A1, A2, G3
G1
C
G2
δ
O
a
O
ε
A1, A2, G3
C
d
B
A1, A2
G1
C
G2
G3
O
δ
a
A1, A2
O
ε
G3
G2
G1
d
C
A1
A2
δ + C
O
G
G1
a
ε + C
A1
A2
G1
G
O
d
D
A1
A2
C
O
G
δ
a
A1
C
A2
G
O
ε
d

EXPLANATION OF PLATE VII

Patterns of thick egg white in
acetate buffer at various pH's and 0.1 ionic strength

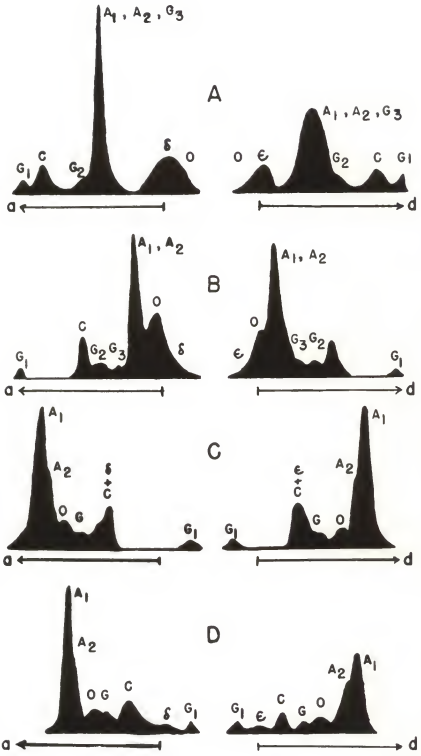
A Patterns taken at pH 3.92 after 130 minutes

B Patterns taken at pH 4.45 after 120 minutes

C Patterns taken at pH 5.85 after 96 minutes

C Patterns taken at pH 7.5 after 75 minutes

PLATE VII


A
A1 , A2 , G3
G1
C
G2
δ
O
a
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ε
G2
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A1 , A2
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G3
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a
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G3
G2
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A1
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D
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δ
G1
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A2
A1
G1
ε
C
G
O
d

be very large and also rather wide spread.

The best resolution was obtained at pH 4.45, where all of the components were resolved except the two albumin components were still not separated from each other. At pH's 5.85 and 7.5 all of the components except one were found to have changed their direction of movement. It was found that the direction of movement of the G_1, ovoglobulin, component was towards the cathode over the entire pH range. This fact was also observed by Longsworth in his investigation. By comparison of Plates V, VI, VII, with each other there was found a very marked similarity of pattern at the same pH value.

This has been demonstrated in a more concrete manner in Plate VIII in which the patterns for three separate egg white layers and also whole egg white have been depicted under the same conditions of electrophoresis. Careful examination of this plate shows that the components in the three layers were identical except for a slight variation in the amount of each component present in any one layer. Thus by visual comparison of the egg white patterns it has been concluded that the protein components are identical in the three distinct egg white layers. Comparison of the patterns obtained for each layer with that of whole egg white was also used to further this conclusion.

A pictorial comparison of the pattern obtained from whole egg white in this investigation with that obtained by Longsworth et al. (3) has been shown on Plate IX. The components in Longsworth's patterns appeared to be identical with those found in this

EXPLANATION OF PLATE VIII

Patterns of various egg white layers at pH 4.45 in acetate buffer and 0.1 ionic strength, time: 120 minutes

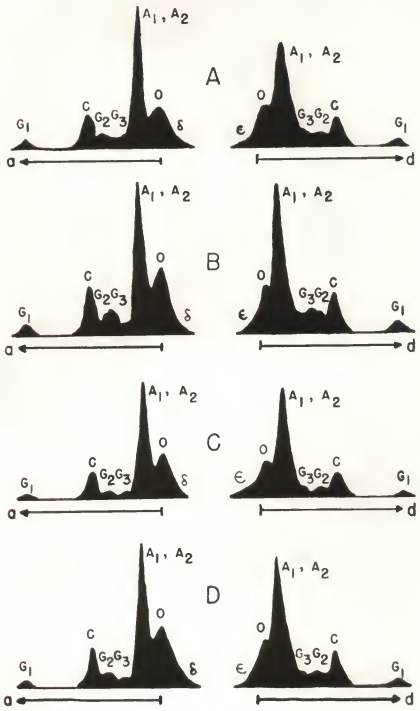
A Whole egg white

B Outer thin egg white

C Inner thin egg white

D Thick egg white

PLATE VIII



EXPLANATION OF PLATE IX

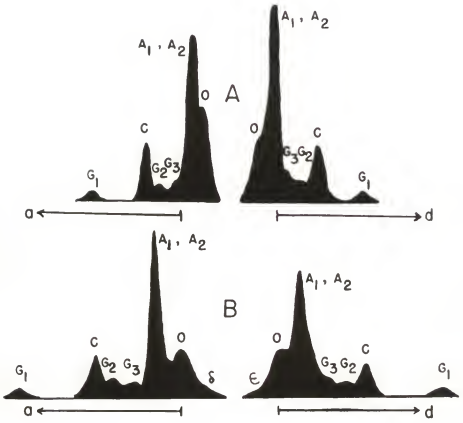
Patterns of whole egg white in acetate buffer at pH 4.45 and 0.1 ionic strength

A Pattern taken from Longsworth et al. (3)[a]

B Pattern obtained in this investigation after 120 minutes

[a]Longsworth did not report the time after which it was photographed

PLATE IX



investigation. The patterns from this investigation were probably taken after a longer period of time but for the purposes of visual comparison this was not important.

The paper by Frampton and Romanoff (6), in their work on the three egg white layers, reported that the components in any one layer cannot be identified with any components in any other layer. The scanning pictures in this paper were obtained after one hour of electrophoresis in 0.1M acetate buffer at pH 4.45 and 1.6 per cent sodium chloride, at a potential gradient of 1.7 volts/centimeters. By placing the patterns from the various layers one below the other with the zero times coinciding Frampton and Romanoff reported that it was plain that the mobilities of every fraction in each layer were different.

In this experiment there were obtained the scanning pictures found on Plate VIII under very similar conditions to those used by Frampton and Romanoff. It was found that the resolution and ionic strengths were greatly changed by the addition of 1.6 per cent sodium chloride. Without the sodium chloride in the buffer the electrophoretic patterns in this investigation indicated that the protein components in the three layers were identical.

The scanning pictures presented by Frampton and Romanoff in their publication were too small and indistinct to read. All attempts to duplicate the results indicated by Frampton were unsuccessful in this investigation.

Comparison of Mobility and Isoelectric Point Values of the Three Egg White Layers

Mobilities were calculated for the three layers of chicken egg white over a wide range of pH values in the manner described in previous part of this paper. Sodium acetate-acetic acid buffer was used to obtain all the pH values. Because of the inaccuracy inherent in using a series of buffers it was felt that even the extension of one type of buffer system to the extreme limits of its buffering action was more accurate. It has been shown by various investigators that the mobility and resolution of a protein in a protein mixture vary considerably in various buffer systems at the same pH. The sodium acetate-acetic acid buffer system gave good resolution and was used by Longsworth et al. (3) in a similar experiment. Thus use of this buffer gave good resolution and also allowed close comparison with other similar investigations.

Several series of electrophoretic runs were made of each egg white layer at each pH. The mobility values calculated for each pH were averaged to obtain a more correct value. The average mobilities thus obtained have been tabulated in Table I. The egg white protein components have been designated in accordance with the method of Longsworth et al. (3).

The mobility values of individual components in the three egg white layers have apparently very similar values. This would seem to indicate that the same protein components are present in each of the egg white layers. The slight discrepancies in mobility

values may be attributed to several factors. The amount of each protein component varies from layer to layer which may account for some differences. Also the total concentration of proteins in various layers varies even though the same dilution factor was used. Some of the variance in mobility has also been ascribed to experimental error in procedure and calculation. However it would seem probable from the general trend of the mobility values that the same protein components are present in each egg white layer.

The mobility values in Table 1 were plotted against their corresponding pH's and are shown in Figs. 4, 5, 6, and 7. The isoelectric point of a protein has been shown to be a characteristic physical constant for any one protein by many investigators. Table 2 is a tabulation of the isoelectric points of the various protein components and in whole egg white. These values were extropolated from the graphs shown on Figs. 4, 5, 6, and 7.

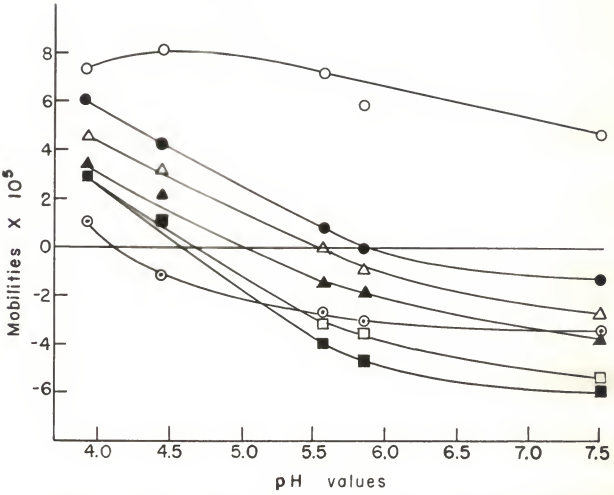


Fig. 4. The electrophoretic mobilities of the constituents of inner thin egg white as functions of the pH.

- ○ G_1, ovoglobulin
- ● C, conalbumin
- △ G_2, ovoglobulin
- ▲ G_3, ovoglobulin
- ■ A_1, ovalbumin
- □ A_2, ovalbumin
- ⊙ O, ovomucin

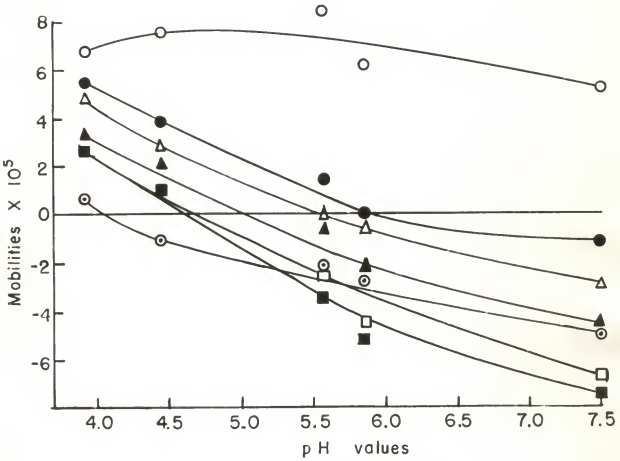


Fig. 5. The electrophoretic mobilities of the constituents of outer thin egg white as functions of the pH

- ○ G_1, ovoglobulin
- ● C, conalbumin
- △ G_2, ovoglobulin
- ▲ G_3, ovoglobulin
- ■ A_1, ovalbumin
- □ A_2, ovalbumin
- ⊙ O, ovomucin

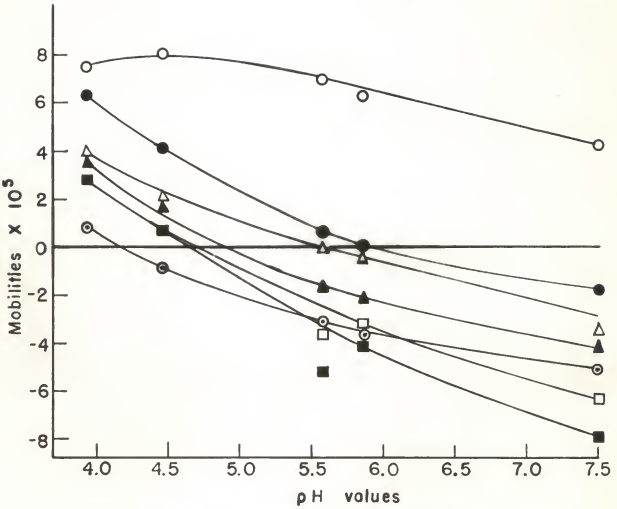


Fig. 6. The electrophoretic mobilities of the constituents of thick egg white as functions of the pH

- ○ G_1, ovoglobulin
- ● C, conalbumin
- △ G_2, ovoglobulin
- ▲ G_3, ovoglobulin
- ■ A_1, ovalbumin
- □ A_2, ovalbumin
- ⊙ O, ovomucin

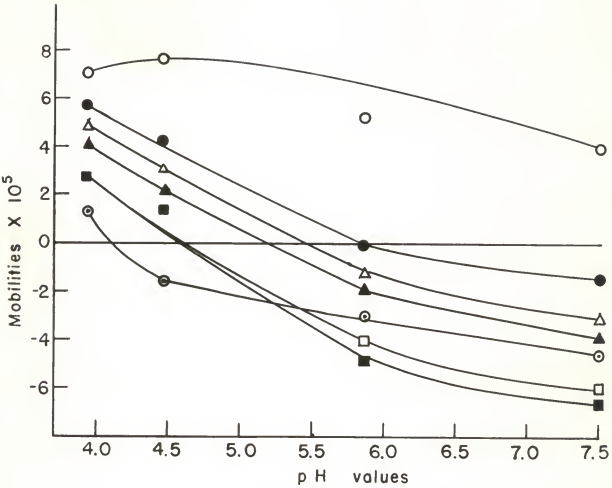


Fig. 7. The electrophoretic mobilities of the constituents of whole egg white as functions of the pH

- ⊙ G_1 , ovoglobulin
- ● C , conalbumin
- △ G_2 , ovoglobulin
- ▲ G_3 , ovoglobulin
- ■ A_1 , ovalbumin
- □ A_2 , ovalbumin
- ⊙ O , ovomucin

Table 1. The mobilities, X 10^5, at 0.5 C, of the protein constituents of various layers of egg white and whole egg white in acetate buffer.[a]

Ovoglobulin			Conalbumin	Ovoalbumin		Ovomucin	
G_1	G_2	G_3	C	A_1	A_2	Q	pH
			A. Inner thin egg white				
7.35	4.6	3.55	6.04	2.98	2.98	1.17	3.92
8.24	3.19	2.34	4.26	1.07	1.07	-1.08	4.45
7.1	0.00	-1.4	0.86	-3.94	-3.14	-2.8	5.58
5.98	-0.91	-1.81	0.00	-4.66	-3.57	-3.00	5.85
4.65	-2.77	-3.7	-1.24	-5.9	-5.36	-3.66	7.5
			B. Outer thin egg white				
6.81	4.84	3.37	5.48	2.73	2.73	0.69	3.92
7.6	2.82	2.12	3.89	1.05	1.05	-0.90	4.45
8.40	0.00	-0.46	1.42	-3.45	-2.46	-2.03	5.58
6.13	-0.61	-2.12	0.00	-5.2	-4.45	-3.25	5.85
5.14	-2.84	-4.43	-1.09	-7.41	-6.71	-4.75	7.5
			C. Thick egg white				
7.43	4.01	3.51	6.35	3.14	3.14	0.88	3.92
8.01	1.68	2.11	4.10	0.97	0.97	-0.90	4.45
6.98	0.00	-1.77	0.67	-5.2	-3.74	-3.16	5.58
6.2	-0.415	-2.12	0.00	-5.07	-4.3	-3.46	5.85
4.02	-3.5	-4.24	-1.84	-7.90	-6.37	-5.1	7.5
			D. Whole egg white				
7.14	4.94	4.04	5.72	2.78	2.78	1.45	3.92
7.75	3.2	2.33	4.32	1.32	1.32	-1.60	4.45
4.8	-1.2	-2.1	0.00	-4.86	-3.72	-2.92	5.85
4.00	-3.00	-3.80	-1.40	-6.60	-6.00	-4.60	7.5[b]

[a] Mobilities computed by the SB method, from ascending boundaries.
[b] The mobilities for whole egg white at pH 7.5 were obtained by extrapolation of the curve from the three other pH values.

Table 2. Isoelectric points of egg white proteins in the three layers of egg white and whole egg white as obtained from Plates IV and V.

Protein measured	Egg white layer				
	Inner	Outer	Thick	Whole	Whole[a]
Ovomucin, O	4.1	4.05	4.15	4.1	4.35
Ovalbumin, A_1	4.6	4.6	4.62	4.58	4.6
Ovalbumin, A_2	4.67	4.67	4.70	4.64	4.66
Ovoglobulin, G_2	5.58	5.58	5.58	5.48	6.1
Ovoglobulin, G_3	5.03	5.03	4.95	5.10	5.6
Conalbumin, C	5.85	5.85	5.85	5.85	5.8

[a] values taken from Longsworth et al. (3).

Comparison of the curves on the four graphs show a marked similarity with one another as should be the case if the protein components in the three egg white layers are identical. The curves, when compared with the work of Longsworth, were found to be very similar and show the same general characteristics and location. This fact was considered as another proof that the protein components in the three egg white layers are the same in every case.

An examination of Table 2 showed that the isoelectric points of the individual proteins in the three egg white layers are practically identical. In comparison with Longsworth's values it was found that the isoelectric point values for the two albumin fractions in this experiment are almost identical with the values obtained by Longsworth. The isoelectric point values for conalbumin also were found to be identical with that obtained by Longsworth. In the case of the globulins and mucin, the isoelectric point values in this experiment were consistently

somewhat lower than those values found by Longsworth for whole egg white. However, in every case the isoelectric points between the components in the three layers and also with whole egg white were almost identical. They were certainly within the range of experimental error. Thus from isoelectric point comparisons further proof that the protein components in the three egg white layers are identical is obtained.

No comparison of mobility values can be made with Frampton and Romanoff (6) since they did not conduct their investigation to that extent. However, it was indicated in their publication that the mobilities were different. Another point of interest is that they inferred that there were two albumin components only in the inner thin and one albumin component in the other two egg white layer. It has been shown in Plates V, VI, and VII, that two albumin components are present in all three layers and are resolved at pH 5.85 and 7.5.

CONCLUSIONS

Electrophoretic patterns, for the three layers of chicken egg white, have been obtained over the entire range of the sodium acetate-acetic acid buffer system. It was found that the best resolution of the components was at pH 4.45 for this buffer. A visual comparison of the patterns indicated that the identical protein components were present in each of the three egg white layers.

The mobilities of each protein component in the three egg

white layers and whole egg white were calculated over a range of pH values. An examination of these mobility values indicated that the protein components in the three egg white layers agreed when compared with each other and when compared with whole egg white. This was another indication that there was no difference in the protein components in the three egg white layers.

The mobilities were plotted as functions of pH. It was found that very similar curves were obtained, when a comparison was made between the three egg white layers and whole egg white curves. This is further evidence that the protein components are identical in the three egg white layers.

The isoelectric point was obtained for each protein component found. In this case excellent agreement was also found between the components in the three egg white layers.

Thus the results obtained in this investigation parallel those of Longsworth et al. (3) very closely. Comparison of the results of this investigation with a similar investigation by Frampton and Romanoff showed no agreement.

From the evidence already discussed, it has been concluded that the protein components in the three egg white layers are identical.

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STUDIES OF ELECTROPHORETIC PATTERNS OF THICK AND THIN WHITE OF THE CHICKEN EGG

by

BERNHARDT VICTOR ANDERSEN

B. S., Kansas State College
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The object of this investigation was to obtain electrophoretic scanning pictures for the three morphological layers of chicken egg white. It was also felt that a separate investigation might resolve some of the differences found in the literature of chicken egg white. Longsworth, Cannan, and MacInnes found 7 protein constituents in whole egg white while Frampton and Romanoff, analyzing separately each of the three layers, found at least 10 different constituents.

The investigation was conducted by electrophoretic experiments performed with a modified Tiselius apparatus manufactured by the Klett Manufacturing Company, New York City. Experiments were conducted on both whole egg white and on each of the three layers separated from the whole egg white. Scanning pictures were obtained over a range of pH's in a sodium acetate-acetic acid buffer system. The ionic strength used was 0.1 and the current introduced was 20 ma. The temperature of the water bath was maintained at 0.5 degree centigrade. The protein solution was diluted with 7 volumes of buffer and dialyzed against several changes of buffer for 4 days. Enough data was obtained to calculate mobilities and also to determine the isoelectric point of each protein component found. The mobility was calculated from the formula

$$\mu = vqk/i$$

where μ is the mobility in $cm^2\ sec^{-1}\ volt^{-1}$, v is the velocity of migration of the protein component in cm/sec, q is the cross sectional area of the cell in sq cm, k is the specific conductance

of the protein solution in mho/cm, and i is the current in amperes passing into the cell. The mobility and isoelectric points of a protein, under specified conditions, are accepted physical constants for that protein.

This investigation has greatly expanded the work begun by Frampton and Romanoff. Mobility and isoelectric point data have been determined for the proteins in the three egg white layers. These were obtained from a series of scanning pictures of the protein constituents in the three egg white layers.

Comparison of the scanning pictures (visually), mobility data, and isoelectric points, of the protein components of the three egg white layers and of whole egg white seemed to indicate that the protein components were practically the same in every case.

The results of this investigation parallel those found by Longsworth, Cannan, and MacInnes (their study was on whole egg white only) for whole egg white very closely. It was possible to identify seven protein components in whole egg white and in each of the three egg white layers. These components appear to be identical in every respect except concentration.

The conclusions reached by Frampton and Romanoff in their study of the three egg white layers could not be verified in this investigation.