AN ELECTROPHORETIC STUDY OF THE EGG WHITE PROTEINS OF VARIOUS BIRDS*

BY J. A. BAIN† AND H. F. DEUTSCH

(From the Departments of Chemistry and Physiological Chemistry, University of Wisconsin, Madison)

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The ovalbumins of chickens, turkeys, guinea-hens, ducks, and geese show varying immunological similarities, although it is possible to distinguish the five albumins with selected sera against chicken ovalbumin (1). Furthermore, mixtures of two albumins, such as guinea-hen and duck ovalbumin, can be clearly distinguished electrophoretically (2).

Marked specificities are shown by the electrophoretic diagrams of the blood serum (3) and plasma proteins (4) as well as by the milk whey proteins (5) of various animals. Such previous findings prompted an investigation of the proteins of the egg white of a number of birds. It was found that the egg white proteins of each species can be identified by their electrophoretic properties, although only relatively slight differences are observed between species in which previous studies have shown that the ovalbumins are closely related immunologically.

EXPERIMENTAL

The egg whites of the various species studied were separated as thoroughly as possible from the yolks, extreme care being taken to keep the vitellin membrane intact. 3 volumes of diethyl barbiturate buffer of pH 8.6 and ionic strength of 0.1 were added to the egg white, and the solutions were stirred so that all of the clumps were dispersed. Small amounts of mucous strands and the chalaza were then removed by centrifugation. The clear supernatant solutions were dialyzed from 48 to 96 hours at 0° against several changes of buffer and were analyzed electrophoretically. The duration of the electrophoretic experiments was 10,800 seconds with a constant potential gradient of between 5.8 and 6.3 volts per cm. All analytical measurements were made on the descending patterns. In so far as it was possible, the various electrophoretic components were designated according to the terminology used by Longsworth, Cannan, and MacInnes (6) for the chicken.

* This work was supported by grants from the Rockefeller Foundation and the Wisconsin Alumni Research Foundation.
† Postdoctorate fellow in chemistry. Present address, Department of Pharmacology, University of Illinois College of Medicine, Chicago, Illinois.
In this respect the slowest of the well defined components possessing a negative mobility in the buffer used have been termed conalbumins and their electrophoretic areas designated as $C_1$ and $C_2$ in order of their decreasing mobilities, as is shown in Fig. 1 for the chicken egg white. If this area is made up of only one component, it is designated $C$. In many of the egg whites examined, there appeared varying amounts of protein which migrated between the conalbumins and the salt boundary. Such slowly migrating material is indicated in the electrophoretic diagrams by an $S$. In most instances it is poorly defined electrophoretically and often is closely associated with the salt boundaries. For this reason no mobilities have been given for this area.

The electrophoretic components between the conalbumins and albumins have been referred to as globulins and designated as $G$. Included in this region is probably the ovomucoid. This region was relatively heterogeneous electrophoretically in most of the egg whites examined, and again no mobilities are reported for this area.

The components of higher mobility, which in chicken egg white comprise the major portions of the proteins and are known to represent the albumin, have been designated as $A$. Thus in Fig. 1 the areas designated as $A_1$ and $A_2$ represent two albumins closely related in mobility. A small amount of an electrophoretic component which has a higher mobility than the albumin but resolves well from these proteins has been referred to as a "fast" component and designated $F$ in this diagram (Fig. 1). When such protein material was present in any egg white in amounts less than 1 per cent of the total proteins, its area was included with the albumin.

The chicken, turkey, guinea hen, pheasant, mallard duck, and goose showed the presence of small amounts (0.2 to 1.5 per cent) of a protein isoelectric above pH 8.6, and hence with a positive electrophoretic mobility in the buffer used. No attempt was made to include this component as a part of the analytical data. Its presence in any noticeable amount is indicated by asymmetries in the ascending salt boundary.

The interaction of oppositely charged protein molecules in electrophoretic experiments has been noted previously (6-8). In the egg whites containing this protein of high isoelectric point, it is likely that the mobilities of the proteins having a negative net charge in the buffer used are lowered somewhat by interaction with the above component. Longsworth, Cannan, and MacInnes (6) have previously referred to this component in the egg white of the chicken as $G_1$ (globulin).

The marked variations in the electrophoretic patterns of the species studied make any such nomenclature as the above arbitrary until the several components have been separated and characterized. However, the system of numbering components used in this study will serve a refer-
ence function until a separation of the proteins in these egg white systems has been accomplished. In the discussion of the experimental results, the electrophoretic patterns of the various species will be discussed, with the chicken pattern as the basis of comparison.

Results

Chicken (White Leghorn)—The electrophoretic pattern of the egg white of this species is shown in Fig. 1. This figure is quite similar in appearance to the diagram obtained by Longsworth, Cannan, and MacInnes (6) at pH 8.0, except for the somewhat better resolution that would be expected at a higher pH. In this and other patterns no attempt has been made to designate individual components in the relatively heterogeneous area labeled G (globulins). A small amount of the relatively heterogeneous F component is present. Analytical data for all species studied are shown in Table I.

Turkey (Bronze)—From Fig. 2 it can be seen that the electrophoretic components of turkey egg white closely resemble those of the chicken. The conalbumins have somewhat higher electrophoretic mobilities and comprise a smaller per cent of the total egg white proteins, as compared to those in chicken egg white. The area immediately following C1 is, however, noticeably different. Furthermore the A2 and A1 components are not as well resolved, and, as shown in Table I, the analytical data differ somewhat from those for the chicken.

Guinea Hen—As seen from Fig. 3, this species shows marked deviations from the chicken in the electrophoretic diagram of its egg white proteins. As in the case of turkey egg white, the conalbumins have slightly higher mobilities than those of the chicken egg white. A larger amount of the C1 component is present and the globulin area does not extend over as wide a mobility range as the analogous area of chicken egg white. A much higher ratio of the area A2 to A1 is another distinguishing feature.
Pheasant (Ring-Neck)—A further departure from the electrophoretic pattern of chicken egg white is shown by the egg white of this species (Fig. 4). Three proteins of closely related electric charge occupy the area

<table>
<thead>
<tr>
<th>Species</th>
<th>C4</th>
<th>C3</th>
<th>C1</th>
<th>G</th>
<th>A2</th>
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* Mobilities expressed as cm² volt⁻¹ sec⁻¹ × 10⁶.

FIG. 2. Electrophoretic diagram of turkey egg white proteins
of the chicken. A conalbumin area showing only slight electrophoretic resolution and a larger amount of the S component further distinguishes the egg white proteins of this species. Inspection of the analytical data of Table I reveals this variation.

**Fig. 3.** Electrophoretic diagram of guinea hen egg white proteins

**Fig. 4.** Electrophoretic diagram of pheasant egg white proteins

**Fig. 5.** Electrophoretic diagram of goose egg white proteins

**Goose (Toulouse)**—In Fig. 5, the electrophoretic patterns of the egg white proteins of the goose reveal a closely related complex of proteins in the conalbumin area. The two albumins possess higher mobilities than is the case for the analogous chicken egg white proteins. In addition, the
A2 component is present in larger amounts than the A1 component. A relatively heterogeneous globulin area which resembles that of turkey egg white more closely than that of the chicken further characterizes this protein system.

**Duck (Mallard)**—The electrophoretic diagram of the egg white of this species (Fig. 6) is somewhat similar to that of the goose. However, there is more of the A1 than of the A2 component, and the material designated S is present in larger amounts than in the egg white systems of the species discussed above, making up some 2.5 per cent of the total proteins. In goose egg white, the A2 component predominates.

**Duck (Muscovy)**—The electrophoretic pattern of the egg white of this species (Fig. 7) can be clearly distinguished from its close relative, the mallard duck. A larger amount of the heterogeneous S component and deviations tending toward more homogeneity in the conalbumin and albumin areas permit such a differentiation. With the exception of somewhat higher electrophoretic mobilities the pattern of the albumin and globulin areas closely resembles the analogous proteins of turkey egg white.

**American Coot (Mud Hen)**—The electrophoretic diagram for the egg white of this species is shown in Fig. 8. It differs markedly from the egg
white of the other water-fowl studied in all of the electrophoretic areas, particularly in the \( F \) component. Present in this egg white are three well defined conalbumins, two closely related albumins which are present in equal amounts, and a relatively large and well defined \( F \) component. The albumins have markedly lower mobilities as compared to those found in the egg whites of the other water-fowl; indeed, the \( F \) component has an electrophoretic mobility more closely related to the \( A_1 \) than to the \( F \) component of the egg white of the other water-fowl.

**Black Tern**—The electrophoretic pattern of the egg white proteins of the black tern is shown in Fig. 9. Two conalbumins are well resolved from a relatively heterogeneous globulin area which merges with the slower moving portion of the albumin area. The \( A_1 \) component of the albumin area makes up only a small percentage of these proteins and has a mobility analogous to the \( F \) component in the egg white protein systems of the Galliformes species (chicken, turkey, guinea hen, and pheasant). Such data are reported in detail in Table I.

**Common Pigeon**—The electrophoretic pattern of pigeon egg white proteins (Fig. 10) and the patterns of the corresponding proteins of the
two types of dove and the English sparrow exhibit certain similarities. A well defined conalbumin is the only feature of the electrophoretic diagram of the pigeon egg white which is comparable to that found in the eggs of the species shown in Figs. 1 to 9 inclusive. The area designated G is the largest component and is more or less merged with the proteins of higher mobility which are designated as albumin (A). Unlike the previously discussed species, the egg albumin component of the egg white of the pigeon does not resolve into two components as seen from Table I, and makes up a smaller percentage of the total egg white proteins. A well defined \( F \) component is also present.

**FIG. 10.** Electrophoretic diagram of pigeon egg white proteins

![Electrophoretic diagram of pigeon egg white proteins](image)

**FIG. 11.** Electrophoretic diagram of ringed turtle-dove egg white proteins

![Electrophoretic diagram of ringed turtle-dove egg white proteins](image)

*Ringed Turtle-Dove*—The electrophoretic pattern of the egg white of this species (Fig. 11) shows a marked difference from that of the closely related common pigeon (Fig. 10). A distinguishing feature is the resolution of the \( A \) component from the \( G \) area. The mobility of the \( A \) component of the egg white of the ring dove approaches that of the \( F \) component of the pigeon. No analogous electrophoretic area is present in the egg white proteins of the ring dove.

*Hybrid Dove*—As judged by electrophoresis, the egg white of hybrids resulting from the mating of a male ringed turtle-dove (*Streptopelia risoria*) and a female oriental dove (*Streptopelia orientalis*) shows a marked similarity to that of the ring dove. However, as seen in Fig. 12, an additional small component designated \( C_1 \) appears on the shoulder of the \( G \)
component and a better resolution of the G and A regions is evident. Certain of these differences are revealed by a comparison of the analytical data as shown in Table I. The eggs of *Streptopelia orientalis* could not be obtained, but by inference it can be assumed that the electrophoretic patterns of the egg white of this species would show variations from the analogous proteins of the ring dove.

*English Sparrow*—The electrophoretic pattern of the egg white proteins of this species resembles that of the pigeon quite closely. In Fig. 13, however, it can be seen that the albumin portion of the closely related G area is more heterogeneous and is present in smaller amount than is true in the case of the pigeon egg white protein system. No attempt has been made in Table I to calculate mobilities or percentage composition of the components in this area. The conalbumins are made up of two closely related proteins. The salt boundaries suggest the presence of protein of low or of no net charge.

**DISCUSSION**

In this study the egg white proteins of thirteen species of birds belonging to six different phylogenetic orders were examined electrophoretically. A characteristic pattern was obtained for each species. There are, however, greater similarities for the species within a given order than for less closely related species. Thus, the Galliformes have egg white proteins relatively
closely related electrophoretically. The same is true for the Anseriformes (goose, mallard duck, and muscovy duck). The difference in mobilities of the ovalbumins of these two orders, as noted by Landsteiner et al. (2), with absorption methods for the location of the boundaries, is well illustrated in Table I, where it can be seen that, while the mobilities of the $A_1$ and $A_2$ components of the Galliformes species are identical, the same is not true for the Anseriformes species. In the latter order the analogous albumin components have higher electrophoretic mobilities than the corresponding proteins of the Galliformes and they differ for each of the species.

The three members of the order Columbiformes (common pigeon, ringed turtle-dove, and a hybrid of the latter species) showed marked similarities in the electrophoretic patterns of their egg white proteins, but the group as a whole showed patterns of pronounced difference from all other species studied, with the exception of the English sparrow. In this respect the chief points of difference of the Columbiformes species were the presence of a single conalbumin area, a very high percentage of the area designated as globulin, and the presence of a relatively low percentage of albumin. In the case of the common pigeon this latter area showed poor electrophoretic resolution from the globulin area. The ringed turtle-dove (*Streptopelia risoria*) could be distinguished from the hybrid of this and an oriental species (*Streptopelia orientalis*), even though the differences in the electrophoretic patterns were not great. Colovos (9) has shown that the sera of the common pigeon and the ring dove can be distinguished electrophoretically, although he did not examine the sera of the hybrid.

The egg white of the English sparrow (Passeriformes) showed a closer electrophoretic resemblance to that of the common pigeon than to any of the other species examined, although the presence of two conalbumins, closely related electrophoretically, and a more heterogeneous and closely related globulin-albumin area allows one to distinguish these two egg white systems easily.

The electrophoretic patterns of the egg whites of the American coot or mud hen (Gruiformes) and the black tern (Charadriiformes) show certain similarities to the corresponding diagrams of the Galliformes and Anseriformes species. The conalbumins of these two species are, however, better resolved electrophoretically and, in the case of the egg white of the mud hen, three well resolved components appear in the diagrams. The albumin proteins of the egg white of the mud hen have the lowest electrophoretic mobilities of any observed in this study.

Unfortunately, eggs were obtained for only one species of the orders Passeriformes, Gruiformes, and Charadriiformes. A further study of additional species in each of these or in other orders would allow one to determine whether similarities such as those noted for the Galliformes species are common to other orders as well. Such work is being initiated.
This limited electrophoresis study reveals the same type of species specificities of egg white proteins that has been found for the proteins of other biological fluids (3-5). The greatest similarities of the electrophoretic patterns appear in the conalbumin area usually designated C2. In general, the areas designated as globulins were relatively heterogeneous. The albumins showed a relatively wide variation in mobilities and in percentage distribution into the two components designated as A1 and A2. The egg whites of most of the species showed the presence of electrophoretically ill defined proteins which migrate at a rate which is slower than that of the conalbumins under comparable conditions. In addition, a small amount of a protein isoelectric above pH 8.6 was present in the egg whites of the chicken, turkey, guinea hen, mallard duck, pheasant, and goose.

The authors wish to thank Dr. M. R. Irwin for gifts of the eggs of the Columbiformes species and to acknowledge the interest of Dr. J. W. Williams in this work.

SUMMARY

The egg white proteins of thirteen species of birds were examined electrophoretically. It is possible to differentiate each species by this technique.

BIBLIOGRAPHY

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