Analysis of the Proteins of Egg-white as an aid to the Classification of Birds

BY

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(*With one text-figure*)

Proteins are complex organic compounds and are present in all living things. Their importance is indicated by the fact that both enzymes and genes are proteins. Proteins are also found in muscle, feathers, internal organs, etc., and form the principal components of blood. There are a vast number of different proteins and every species has a different protein structure. The differences and similarities are exploited in the use of protein analysis for classification.

In order to see the use of protein analysis as an aid to classification in perspective it is perhaps worthwhile to consider briefly the ideas and difficulties of classification. Classification is an attempt to map out evolutionary history. Unfortunately the fossil record of avian history is very poor. The major evolution of birds occurred in the Cretaceous period, and virtually no avian fossils have been found belonging to this period. There are the early fossil birds, the Archaeopteryx, which show that different parts evolve at different rates since the feathers were avian but the rest largely reptilian (de Beer, 1954). But, in general, fossil records have been of little aid in classification.

The Darwinian concept was that evolution proceeded from the lower to the higher, that is to say from the simpler to the more complex. It is now considered that this rule is not invariable, in any case it is difficult to know what is primitive and what is advanced. Many, one might almost say all, taxonomic characters have been used. It is difficult to know which characters are of major importance, that is, have generic value, and which are easily modified by external conditions. This is one of the great difficulties of classification, to distinguish between those characters which are similar because of recent descent from a common ancestor and those which are similar because of changes caused by adaptation to a common environment, even though they have had a quite distinct evolutionary history. For a fuller account of these problems the reader is referred to a recent review by Mayr (1959).

Besides what we may call classical methods, several others have been suggested. All have some value, and all their limitations. The study of parasites has been suggested (Clay, 1951). Parasites evolve together with their hosts and, being simpler animals, may have changed less. However, transfer is possible (Mayr, 1957) and the method must be used with caution.

Behaviour is being used to an increasing extent in the study of evolution (Lorenz, 1958; Cullen, 1959; Tinbergen, 1959). It seems that behaviour characters are as conservative as taxonomic charcters, although care must be taken to distinguish between true differences in behaviour and those caused by the local environment.

The relationship of protein analysis to classification has been known for some time. Nuttall (1901) used the analysis of blood serum to examine the zoological classification of many animals. These early investigations, although interesting, were too crude to give any significant results. There have been more recent investigations, but serology has not been widely used in avian systematics. Immunological studies show that each species has its specific antigens[1]. The studies of Irwin and co-workers [for example Irwin (1953)] have given information on the classification of the doves (Columbidae).

The most convenient source of proteins is egg-white, which consists mainly of water and proteins. The specific nature of the proteins in egg-white was first shown by immunological studies (Landsteiner & van der Scheer, 1940), but the use of electrophoresis has given a more convenient and reliable method. This method was first used in classification studies by McCabe & Deutsch (1952) and since then Sibley, using a much improved apparatus, has obtained greater accuracy and has examined the electrophoretic pattern of a large number of species. Sibley (1960) gives a full account of this work and considers in detail the validity of this method and the results obtained to date.

The formidable-looking word electrophoresis (from Gr. *phoros* to bear, *electro* by electricity) is to bear or carry something by electricity. In more scientific terms it is the transportation of charged bodies in an electrical field. Thus, if proteins are placed in an electrical field they will migrate because they have an electrical charge. Because of the different size of the molecules and the differing electrical charge, different proteins move at different speeds and thus a separation is achieved. The usual method is to place a few drops of protein on a

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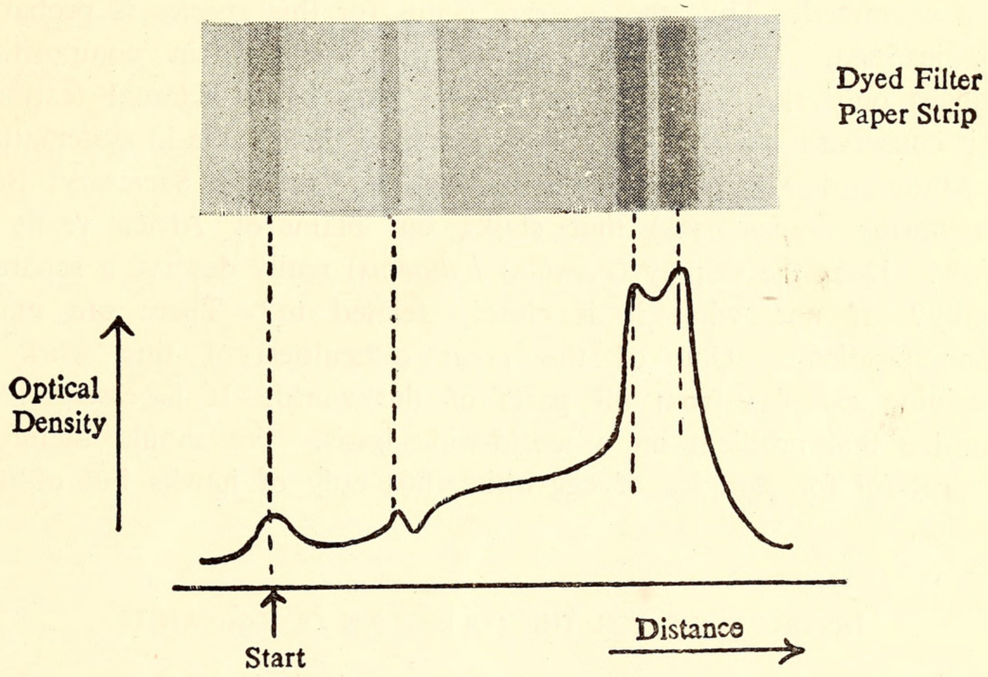
[1] Substances which stimulate the formation of antibodies.—Eds.

strip of wet filter paper, across which an electrical field is placed. This field is of high voltage but of low current. After the proteins have been under the influence of this field for some hours, the strip of paper is dried and then dyed. This dye reacts with the protein to give a coloured compound which enables the position of the colourless proteins to be seen. The amount of protein present in the various bands can be determined by the optical density, the blackness, of the paper strip. Machines are available that translate the optical density of paper into a curve of optical density plotted against the distance that the protein has been moved by the electrical field.

The figure below shows a drawing of a dyed filter paper strip and the optical density curve obtained from it. The curve shown above was obtained by Spofford and is of a Redtailed Buzzard (*Buteo jamaicensis*) which is found in North America and is similar to the



Desert Buzzard (*B. desertorum*). It is in the classification of the hawks that we are especially interested. We should be most grateful for any egg-white specimens of this family.

The use of protein analysis in determining the classification of birds can best be shown by a few examples.

One interesting problem in classification is posed by the flamingos (Phoenicopteridae). Are these spectacular birds related to the ducks (Anatidae) as their webbed feet, bill structure, and swimming habits suggest but have evolved long legs, or are they more closely related to the storks (Ciconiidae) but have evolved some duck-like characters because of their environment? The analysis of egg-white protein supports the view that the flamingos are more closely related to the storks than to the ducks.

Another case that illustrates the fact that protein structure changes only slowly is in the Brown Hawk of Australia. Apparently, because there are no true buzzards in Australia, this species has altered its feeding habits from those of a typical falcon and now resembles those of the slow-moving buzzard. The species has been considered by some to belong to the true falcons and has been called *Falco berigora*, whilst others have placed it in a separate genus and named it *Ieracidae berigora*. When I first saw this species, in Tasmania, I thought that it was a buzzard both by stance and hunting, although the wings were more pointed than in the buzzards. The analysis of the egg-white proteins indicates that the Brown Hawk is very closely related to the five other species of the *Falco* that have been examined. Thus the separate genus for this species is probably not justified. This example shows that the protein composition changes only slowly; here more slowly than some external features. This conservative nature of proteins increases their value in systematics.

Many other problems remain. Is the strange Secretary Bird (*Sagittarius serpentarius*) that stalks the plains of Africa really a hawk? Does the Osprey (*Pandion haliaetus*) really deserve a separate family? If not, what is it closely related to? There are many other problems. One of the great difficulties of this work is obtaining samples from all parts of the world. It is essential to examine this problem on a world-wide basis. We should therefore be grateful for samples of egg-white, not only of hawks but of any species.

Instructions for the collection of egg-white

If the egg-shell is not required for oological purposes, a large piece of the shell is removed and the egg-white can be tipped into a small container. It is important that the egg-white is not contaminated with yolk. If the egg shell is required for an oological collection the shell is drilled as usual. While blowing the egg the contents are collected in small portions and those contaminated with yolk are thrown away, the rest put into the collecting vessel.

Egg-white has only a limited life at normal temperatures and it is necessary either to keep the samples refrigerated or to send them off within a few days. It is requested that the samples are sent airmail; postage will be refunded.

It is possible to make a satisfactory analysis on a small sample, i.e. the contents of one small egg. However if the species is abundant, it is useful to have the contents of several eggs, as a more detailed analysis can then be made.

The egg-white can be sent in any clean, convenient vessel, but anyone who thinks that he may be able to help is asked to write to Dr. Walter Spofford, Upstate Medical Center, 766 Irving Avenue, Syracuse, 10, New York, U.S.A., and a collecting kit will be sent.[1]

It is important that the eggs are identified with certainty, and that every sample is clearly labelled.

SUMMARY

Protein analysis is considered to be a useful addition to the characters used in determining the classification of birds. The method of analysis is briefly described and some account is given of the results already obtained. It is hoped that members of the Society will aid this work by sending in specimens of egg-white protein. Details of how this should be done are given at the end of the article.

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[1] Some kit has already been received by the Bombay Natural History Society and the Honorary Secretary will be glad to distribute it among those interested.—EDS.