ELECTROPHORETIC STUDY OF BLOOD PROTEINS OF SOME PROCELLARIIFORM BIRDS

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LITTLE is known of the comparative histology or biochemistry of the blood of birds. The application of biochemical techniques to the study of avian systematics, a development of the last 20 years, is contributing an increasing amount to our knowledge in this field. Of major significance in introducing this type of investigation to ornithologists were the reports by Bain and Deutsch (1947) and McCabe and Deutsch (1952) on egg-white proteins. Sibley (1960), Gysels (1962, 1963), and Gysels and Rabaey (1962) emphasized and expanded this method of approach. The blood proteins of but few species of birds have been studied with a view to aiding in the determination of phylogenetic relationships.

Presented here are the results of electrophoretic analyses of the blood proteins of certain species of the order Procellariiformes. An effort has been made to ascertain the biochemical affinities of various members of the order.

MATERIALS AND METHODS

Samples of serum, plasma, and hemoglobin were obtained from eight Laysan Albatrosses (*Diomedea immutabilis*), seven Black-footed Albatrosses (*D. nigripes*), four Christmas Island Shearwaters (*Puffinus nativitatus*), six Wedge-tailed Shearwaters (*Puffinus pacificus*), and six Bonin Island Petrels (*Pterodroma leucoptera*). One individual thought to be a natural hybrid, *D. immutabilis* × *D. nigripes*, was also examined. The albatrosses and petrels were bled on Midway Atoll and the refrigerated blood flown to the laboratory; the shearwaters were shipped to Carbondale, Illinois, and bled.

Blood was taken from the brachial artery and the samples were subjected to centrifugation to precipitate cells from the serum and plasma. A solution of 0.7 per cent potassium oxalate was used as an anticoagulant when plasma samples were obtained.

The red blood cells were washed three times in saline and then hemolyzed by adding an equal volume of distilled water. Cell membranes were removed by centrifugation and the resulting hemoglobin solution was drawn off. Albumins were detected by precipitating the globulins from the serum in half-saturated ammonium sulfate.

The electrophoretic technique employed was the hanging strip method (Block, Durrum, and Zweig, 1958) using a Spinco model R, series B Durrum cell and a Spinco Duostat constant-voltage power supply. Samples were applied to No. 3 MM Whatman filter paper strips; sample size varied with the components of the blood to be resolved (serum, 30λ; serum lipoprotein, 70λ; plasma, 40λ; hemoglobin, 10λ). As a control, 15λ of human plasma were applied to one strip in each run. The electrolyte was one liter of barbiturate buffer with an ionic strength of 0.05 and pH 8.6. A constant current of 15 ma was maintained in each six-hour run; the d.c. voltage was 300 to 400 v.

After the completion of a run, the strips were dried at 120° C for 30 minutes and

then stained in a bromophenol blue-zinc sulfate preparation. Proteins carrying lipid components were detected by staining in an Oil Red O solution. A Spinco Analytrol, model RB, was used for scanning the strips to determine the concentration of proteins in the serum.

RESULTS AND DISCUSSION

Typical electrophoretic patterns of the blood proteins of the five species are shown in Figure 1. Six distinct protein bands were resolved from the serum of *D. immutabilis*. Protein band L1 stains as a lipoprotein and has a mobility faster than that of human albumin. The albumin, band L2, moves more slowly than human albumin. The third fastest protein band, 3, moves at a rate between that of human alpha 1 and alpha 2 globulins; in a number of animals it appears as a wide diffuse band and may be heterogeneous. Protein band 4 travels to a point between human beta globulin and the area of sample application. Although protein band 4 stains strongly with Oil Red O as a lipoprotein, it shows up in rather low concentration on strips stained with bromophenol blue. Protein band 5 is found at the area of sample application. Band 6 is slightly slower than human gamma globulin in its movement toward the cathode.

Proteins from the serum of *D. nigripes* travel at the same rates and stain similarly to the six bands found in the serum of *D. immutabilis*; presumably they are similar compounds. However, although variable, protein band 3 in most *D. nigripes* did not show up as a wide diffuse band as in *D. immutabilis*.

The blood of the natural hybrid between these two species had electrophoretic patterns (not illustrated) similar to the parental species.

Six distinct protein bands were also resolved from the serum of *Puffinus nativitatus*. Protein band S1 stains as a lipoprotein and is slower in movement than human albumin. Protein band S2, the albumin, travels at a speed between that of human alpha 1 globulin and human albumin. Protein bands 3, 4, 5, and 6 stain similarly and travel at the same rates as the corresponding bands in the albatrosses. Protein band 3 appears wide, although not as wide as in the albatrosses.

The electrophoretic patterns of the protein bands resolved from *Puffinus pacificus* are similar to those of *P. nativitatus*, with two exceptions. An additional band, J3, moves at a rate between those of human alpha 2 and beta globulins. Protein band 3 is not as wide as in *P. nativitatus*.

Six protein bands were noted in the serum of *Pterodroma leucoptera*. P1, the prealbumin, stains as a lipoprotein, is faster than human albumin, and travels at approximately the same speed as protein band L1 of *Diomedea*. However, in some *Pterodroma leucoptera* protein band P1 moved more slowly. Protein band P2, the albumin, moves at a rate between that for albumin of *Diomedea* and *Puffinus*, and between human alpha 1

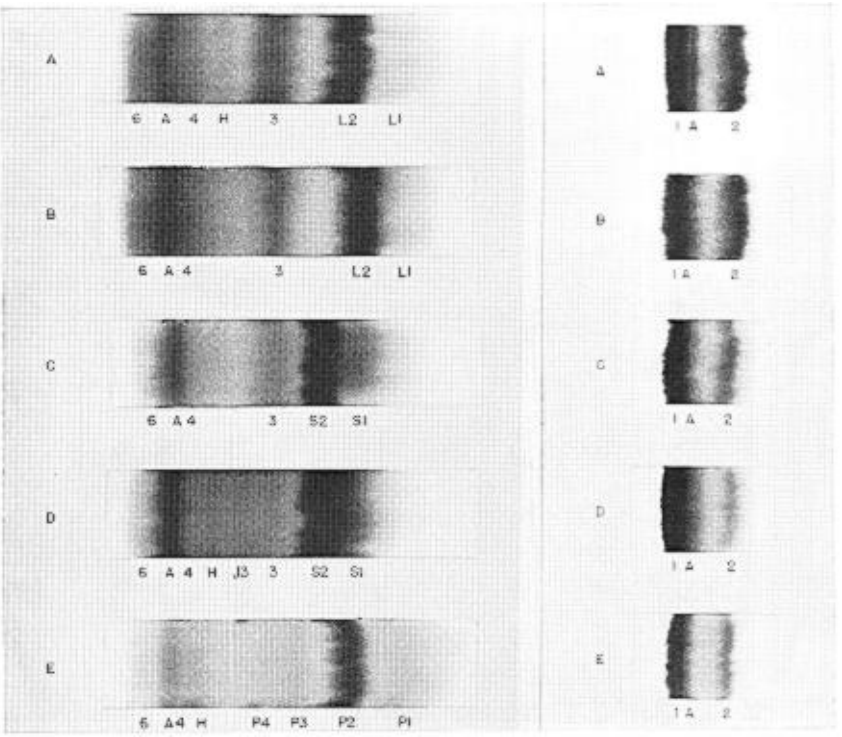

Figure 1. *Left.* Typical electrophoretic patterns of the sera of five species of the order Procellariiformes (see below). The anode is to the right and the area of sample application (and protein band 5) is indicated by A. Hemoglobin in hemolysed samples is indicated by H. For explanation of other notations, see text. *Right.* Typical electrophoretic patterns of hemoglobins of the same five species (see below). The area of sample application is indicated by A and the anode is to the right. For explanation of other notations see text.
Species represented: A, *Diomedea immutabilis*; B, *D. nigripes*; C, *Puffinus nativitatus*; D, *Puffinus pacificus*; E, *Pterodroma leucoptera.*

globulin and human albumin. Protein band P3 travels somewhat faster than human alpha 1 globulin, and band P4 moves slightly faster than human alpha 2 globulin. Bands 4, 5, and 6 move at rates similar to the corresponding protein bands in the other species. The concentration of all protein bands is less in *P. leucoptera* than in the other species.

One specimen of *P. leucoptera* had two albumins. Similar double albumins have been detected in unusual human plasma (Earle *et al.*, 1959).

All five species have two hemoglobins which move correspondingly the same. Hemoglobin 1 travels toward the cathode at a speed somewhat slower than human gamma globulin. Hemoglobin 2 is slightly slower than human beta globulin. In the albatrosses, hemoglobin 2 shows a stronger concentration, relative to hemoglobin 1, than is found in the shearwaters

and the petrel. Gratzer and Allison (1960) have reported two hemoglobins in a large number of birds.

Prealbumins have been reported in laying hens (Brandt, Clegg, and Andrews, 1951), chick embryos (Marshall and Deutsch, 1950), pigeons infected with malaria (Schinazi, 1957), and cockerels treated with diethylstilbestrol (Clegg *et al.*, 1951). All of our birds were bled in the nesting period, which may account for the presence of the prealbumin. There is some intraspecific variation in the concentrations and mobilities of the prealbumins. This may be the result of the birds being in slightly different stages of the nesting cycle. The possibility that this variation may indicate entirely different prealbumins cannot be ruled out. It would be of interest to know if there is some common physiological function associated with these various conditions that results in the presence of prealbumins.

In two of the *D. immutabilis* and two of the *P. leucoptera* examined, the albumins stained as lipoproteins. Lipids in domestic fowl (*Gallus domesticus*) are often bonded to albumins, although this has been shown not to be true in estrogenized pullets (McKinley *et al.*, 1953).

The taxonomic conclusions revealed by this study support the current systematic scheme reached by more classical methods. Members of the order show unity in protein bands 4, 5, 6, and the two hemoglobins. However, the families Diomedeidae (containing the genus *Diomedea*) and Procellariidae (containing *Puffinus* and *Pterodroma*) are not well separated, the only distinct difference being the relative concentration of hemoglobin 2. The value of this feature as a taxonomic character may be questionable; such quantitative differences often reflect temporal environmental differences.

The three genera are easily distinguishable, and the greatest similarities in blood proteins existed between the congeneric species (*D. immutabilis* and *D. nigripes*; *P. nativitatus* and *P. pacificus*). Some degree of close relationship may be indicated, through protein band 3, between the genera *Diomedea* and *Puffinus*, or perhaps between *Diomedea* and *Pterodroma* through protein bands L1 and P1.

The success in demonstrating the species specificity of the blood proteins of some birds of this order seems to indicate that further investigations may prove fruitful in comparative studies of the biochemistry of avian blood, and these studies may shed light on problems of avian systematics.

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SUMMARY

The blood proteins of 31 specimens of five species and one hybrid specimen of the order Procellariiformes were examined electrophoretically. The genera were well marked, and congeneric species exhibited the greatest similarities. The families Diomedeidae and Procellariidae did not show marked separation. Results of the study thus support the present systematic arrangement. The presence of prealbumins may be related to specific physiological conditions of the birds.

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