VARIATION IN PLASMA PROTEINS OF SUBOSCINE BIRDS

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Information obtained from biochemical data has now been used extensively to aid in uncovering the evolutionary relationships of animals. Because many biochemical characters can be shown to be conservative (vary little within taxonomic groups) they are thought to be very good indicators of relationships (see Sibley, 1960, and Zuckerkandl and Pauling, 1965).

Most of the studies of electrophoretic patterns of avian blood serum and plasma proteins (Brandt, Clegg, and Andrew, 1951; Common, McKinley, and Maw, 1953; Wall and Schlumberger, 1957; Sibley and Johnsgard, 1959; and Sibley and Hendrickson, 1970) have led to the almost universal conclusion that although there is a basic and conservative overall pattern, there is so much variation due to sex, age, health, polymorphisms, and unknown factors as to render the information of little value in uncovering the evolutionary relationships of birds.

As most of the studies to date have utilized filter paper, cellulose acetate, or starch gel electrophoresis, it is of interest here to consider the electrophoretic patterns of avian plasma proteins in suboscine birds as delimited by the more refined acrylamide gel electrophoresis. It should be pointed out here that the patterns obtained from both plasma and serum (without clotting factors) are very similar and Wall and Schlumberger (1957) reported, ". . . no essential differences were discernible between the electrophoretic patterns of serum and plasma from the same birds."

MATERIALS AND METHODS

Owing to the difficulty in obtaining live material of suboscine birds, the birds were shot and the blood extracted from cuts in either the wing or the neck. The blood was placed in a culture tube containing a tablet of potassium oxalate dissolved in 2.0 ml of a 1.0 per cent saline solution. The tubes were then placed on ice. Red blood cells were precipitated by centrifugation and the supernatant liquid used in the electrophoretic analysis. Disc electrophoresis similar to that described by Davis (1964), and Ornstein (1964), was used. This technique has been used previously for avian material by Desborough and Irwin (1966). A tris-glycine buffer at pH 8.5 with brom-phenol blue added as a marker was utilized. Ten tubes with acrylamide gel were run simultaneously in a cold room at a constant 50° F, with each tube conducting 5 ma. The current was terminated when the brom-phenol blue front had migrated 32 mm (approximately 30 minutes time). The gels were then removed from the tubes and stained for total protein by 0.2 per cent Amido Black in a 5:5:1 solution of water, methanol, and acetic acid for 30 minutes. The gels were then destained in an 8 per cent acetic acid solution and stored in a solution of the same composition. One tube of human plasma obtained from the author was included in each electrophoretic run

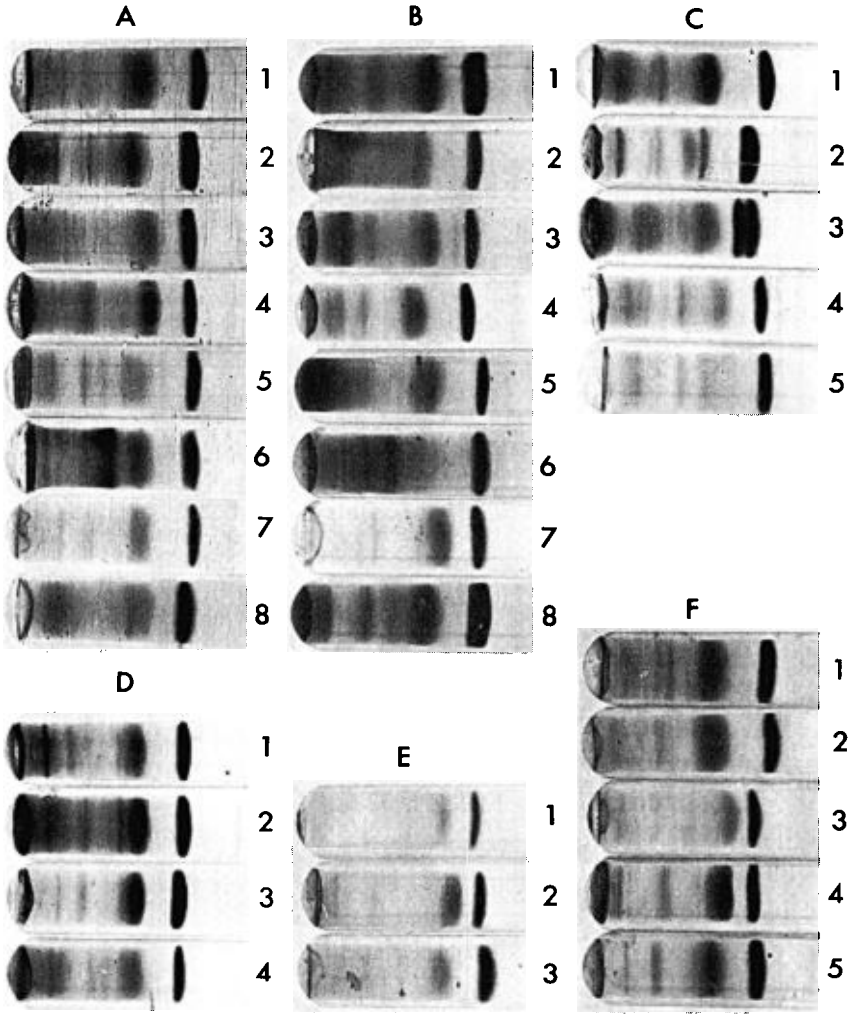

FIG. 1. Acrylamide gel electrophoresis patterns of plasma proteins of: (A) Furnariidae: 1. *Pseudocolaptes lawrencii*, 2. *Hyloctistes subulatus*, 3. *Automolus ochrolaemus*, 4. *Thripadectes rufobrunneus*, 5. *Xenops minutus*, 6. *Cranioleuca erythrops*, 7. *Synallaxis albescens*, 8. *Margarornis rubiginosus*; (B) Dendrocolaptidae: 1. *Dendrocincla anabatina*, 2. *Glyphorhynchus spirurus*, 3. *Deconychura longicauda*, 4. *Sittasomus griseicapillus*, 5. *Xiphorhynchus erythropygius*, 6. *Campylorhamphus pusillus*, 7. *Lepidocolaptes souleyetii*, 8. *Dendrocolaptes certhia*; (C) Formicariidae: 1. *Microrhopias quixensis*, 2. *Taraba major*, 3. *Grallaria perspicillata*, 4. *Thamnophilus doliatus*, 5. *Thamnophilus bridgesi*; (D) Cotingindae: 1. *Querula purpurata*, 2. *Lipaugus unirufus*, 3. *Rhytipterna holerythra*, 4. *Pachyramphus polychopterus*; (E) Pipridae: 1. *Pipra mentalis*, 2. *Manacus candei*, 3. *Chiroxiphia linearis*; (F) Tyrannidae: 1. *Myiarchus* sp., 2. *Contopus* sp., 3. *Myiozetetes similis*, 4. *Pipromorpha oleaginea*, 5. *Onychorhynchus mexicanus*.

as a test for consistency. During the study approximately 100 bird specimens representing a broad spectrum of the suboscines were examined. All individuals were run several times.

Identification of the serum and plasma proteins in birds is based primarily on comparisons with human serum electrophoretic patterns (see Davis, 1964; Clarke, 1964; and Sibley and Hendrickson, 1970). The fastest migrating and most prominent band is the serum albumin. The other more prominent bands are alpha-, beta-, and gamma-globulins, which include a great variety of different proteins.

RESULTS AND DISCUSSION

The comparisons made indicate a high degree of variation among individuals and closely related species, often more than between distantly related forms. This variation is probably due to such factors as age, sex, health, and unknown factors. However, once the small variations are not considered there appears to be a basic pattern for the major components, the serum albumin and major globulin band (closest to the albumin), throughout suboscine birds. These results confirm Sibley's and Hendrickson's results from electrophoretic patterns of plasma proteins that, ". . . once we eliminate the taxonomically non-significant variation from the patterns, we find that they are remarkably similar in all birds." "The variation superimposed upon this basic pattern mainly reflects the physiological functions of the plasma proteins and is consonant with the known or suspected roles which these substances play in the life of the organism."

However, there are several points of interest in Figure 1. The position of the first two major bands, the serum albumin and globulin, is very constant, and although some variation is exhibited by the gels, this is likely due to individual variation. There was a pre-albumin band in some species, but not in others. Of interest in Figure 1 is the double band of serum albumin found in *Grallaria*. I found serum albumin to be polymorphic in both *Thamnophilus bridgesi*, and in *Xiphorhynchus lachrymosus*. The double band in *Grallaria* therefore seems to be of little importance.

Although difficulty in interpretation is encountered due to the almost endless variation in the smaller bands, it is of interest to consider the patterns obtained from the family Cotingidae, a family which has been thought to be quite heterogeneous. Here, the four forms examined, *Querula*, *Lipaugus*, *Rhytipterna*, and *Pachyramphus*, although they represent diverse morphological types, have plasma electrophoretic patterns which are almost identical (Fig. 1).

In summary, the results of this study confirm previous studies of serum and plasma proteins in birds. There is a basic pattern which is similar in all birds, superimposed on a great variety of taxonomically meaningless variation. Where patterns do seem to indicate relationships (as in the Cotingidae), one is hesitant to advocate the validity of the data owing to the otherwise great variation.

ACKNOWLEDGMENTS

Many people assisted during various parts of this study. I would especially like to thank Gene K. Lindsay for instructing me in various biochemical techniques, the faculty of the Department of Biology of the University of Costa Rica and especially Douglass Robinson, who provided laboratory space during the Central American phase of this study. This study was supported by a grant from the Frank M. Chapman Memorial Fund of the American Museum of Natural History, a grant from the National

Science Foundation, GB-6230, to N. G. Hairston, The University of Michigan, for research in Systematics and Evolutionary Biology, and a grant from Southern Methodist University (No. 88-43) for evolutionary studies of suboscine birds.

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DEPARTMENT OF BIOLOGY AND SHULER MUSEUM OF PALEONTOLOGY, SOUTHERN METHODIST UNIVERSITY, DALLAS, TEXAS 75222, 20 FEBRUARY 1970.

PUBLICATION NOTES AND NOTICES

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