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## ELECTROPHORETIC PATTERNS OF PLASMA PROTEINS AND HEMOGLOBIN OF THE PIGEON *COLUMBA LIVA DOMESTICA*

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### INTRODUCTION

Electrophoresis is being used with increasing frequency by vertebrate taxonomists. This technique takes advantage of the different migration rates of protein molecules in an electric field.

The objectives of this study were to: (1) determine if breeds of pigeons could be distinguished by studying plasma protein components and (2) determine if hemoglobin is useful by this technique in separating various breeds of pigeons. If so, this technique could be useful in studying molecular evolution during the development of present pigeon breeds. According to Darwin (1897) the Rock Pigeon (*Columba liva*) may be confidently viewed as the common parent pigeon stock. It is from this parent stock that numerous breeds and varieties have been developed. For this study three distinct breeds were used; namely the Modena, English Trumpeter and German Beauty Homer (Die Deutsche Schautaube). These breeds differ much in body characteristics, origin and time of development, and their "gene pools" have been somewhat isolated for many years.

The Modena (Fig. 1) is an Italian creation which had its beginnings as far back as 1328. The English Trumpeter (Fig. 2) is also an old breed. Aldrovandi in 1603 apparently described it as a variety of the Runt. The breed was well recognized in England by 1735 (Levi, 1957). The German Beauty Homer (Fig. 3) is a German creation which owes its origin to German breeders who developed a show pigeon from crosses of Racing Homers, Antwerps and others around 1908 (Weger, 1954).

### ACKNOWLEDGEMENTS

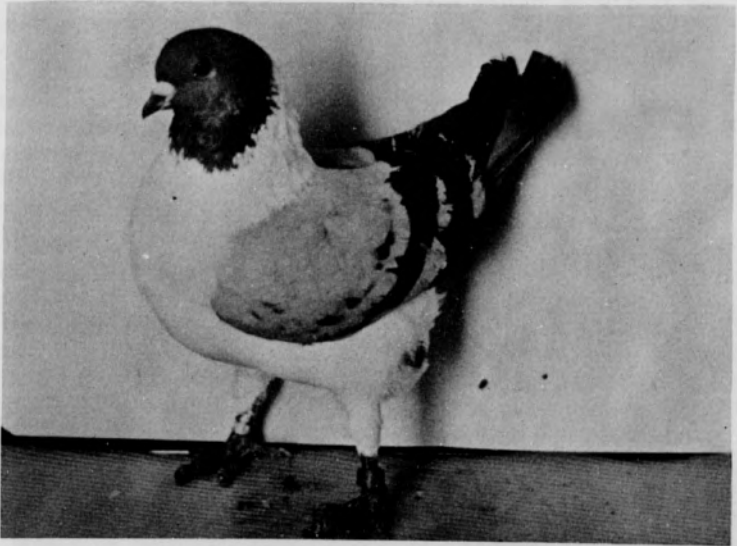
This study was primarily supported by a grant from the Faculty Research Committee of Arkansas State University and partly by NIH Grant ES 00275-02. Photographic work was done by Jeneane Saunders.

### MATERIALS AND METHODS

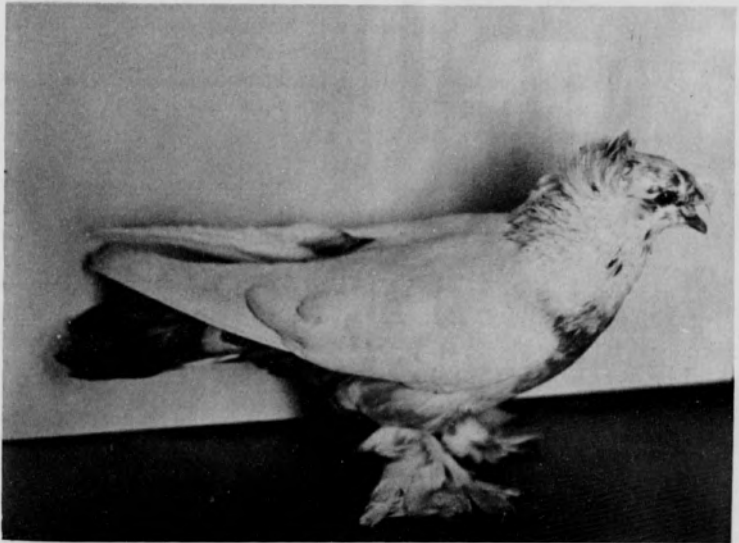
Fifty samples of 1.5 ml of whole blood were taken from the wing vein on the inside of the elbow joint from individuals of Modenas, English Trumpeters and German Beauty Homers. The pigeon was held with its back downward and the wing laterally spread. Removal of a few feathers made the vein visible (Schermer, 1967).

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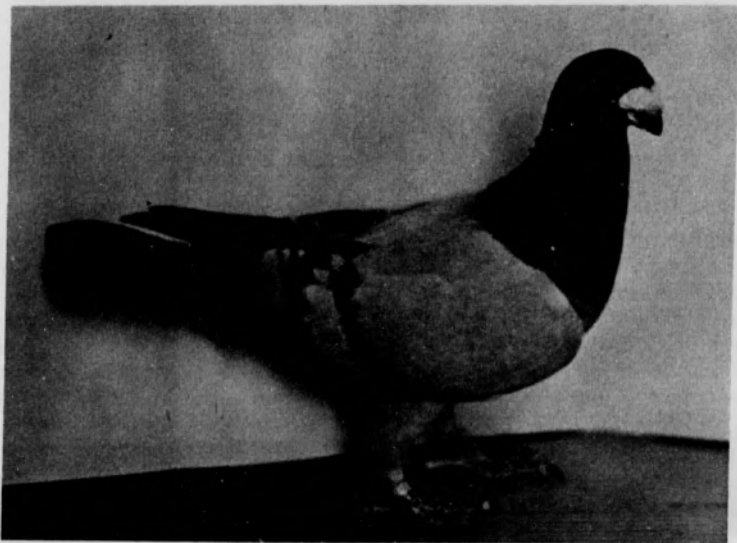
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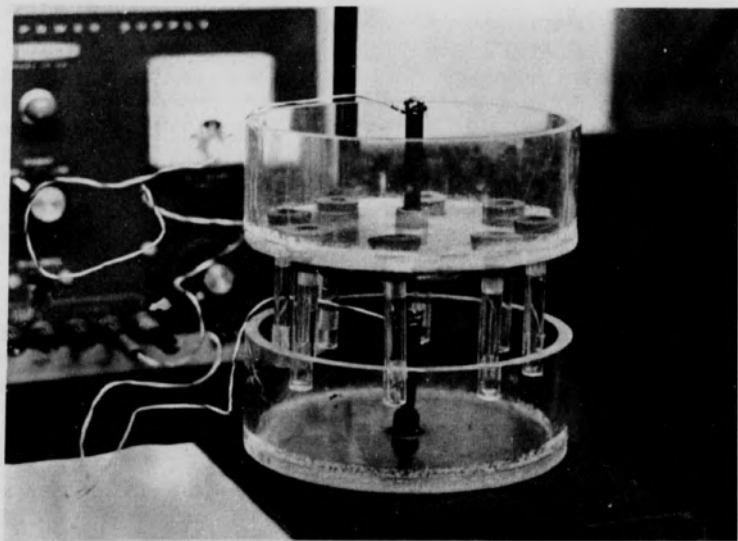
**Fig. 1. The Blue Gazzi Modena Pigeon.**



**Fig. 2. The English Trumpeter.**



**Fig. 3.** The German Beauty Homer (Die Deutsche Schautaube).



**Fig. 4.** Eight Column Electrophoresis Apparatus.

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Whole blood was drawn from each pigeon by a B-D insulin syringe needle and put in a solution of 3.8 percent sodium citrate (4 parts blood and 1 part sodium citrate) to prevent clotting. The blood was centrifuged for 5 minutes. The plasma was removed by a transfer pipette and frozen. The RBC's were washed six times in a 0.75 per cent bird physiological saline solution (Humason, 1967). A ml of distilled water was added to the RBC's to cause hemolysis. Hemoglobin was drawn off with a transfer pipette and frozen until used.

Disc electrophoresis procedures closely followed those of Davis (1961). The analyses were performed with an eight column electrophoresis apparatus (Fig 4) utilizing graphite electrodes and two circular reservoirs (2 in. deep and 6 in. in diameter) constructed from 6 in. acrylic tubing. Acrylamide gels (0.5 cm x 4.8 cm) and TRIS buffer at pH 8.3 were used throughout. Separation of plasma proteins was conducted at 25°C and at 12 mA for 150 min at 100 volts. After separation the staining was accomplished by using Naphthol Blue Black. The most distinct electrophoretic patterns were obtained with 15 ul of plasma. The gels were then destained and stored according to procedures outlined by Davis (1961).

## RESULTS AND DISCUSSION

Studies prior to 1957 on electrophoresis of avian plasma or serum proteins have been limited to a few isolated birds. Wall and Schlumberger (1957) investigated the plasma electrophoretic patterns of the shell parakeet (*Melopsittacus undulatus*). Sibley and Johnsgard (1959) published on the variability in the electrophoretic patterns of avian serum proteins. Their studies included a variety of avian species. In this study the Rock Dove (*Columba livia*) was investigated. The histogram profiles of the serum proteins showed two large components for this species. Perkins (1964) published on electrophoretic patterns of the serum proteins and hemoglobins of the genus (*Larus*). He showed no more variation between the species than within a single species for this genus even though there is a wide variation in the size and coloration of these birds. It was concluded by Baker and Hanson (1965) that species and subspecies of geese cannot be distinguished on the basis of the blood proteins and that there are only minor differences between the genus (*Anser*) and (*Branta*). Eleven species including subspecies were used in their study. Rylander (1967) studied electrophoretic patterns of the serum proteins of two genera of the family Scolopacidae. He concluded that intraspecific variation is great and that there is no significant difference between the species or even genera in this family. Sibley and Johnsgard (1959) stated that if reliable measurements of avian sera are desired, sample size must be large, and birds must be separated by age and sex, just as in studies of morphological char-

The results from this study by using acrylamide gel electrophoresis techniques on three distinct breeds of domesticated pigeons show minor individual differences within the breed and also differences between individuals of different breeds. Because of these variations there was no one representative electropherogram for each breed of pigeon. Some samples from individuals within a breed were remarkably similar. Intra-breed variation is not always easy to explain. These variations may be caused by differences in experimental procedures, by sex, age and perhaps health of individual pigeons. In general, there was some uniformity in protein patterns among the pigeons studied. These patterns are shown in Figs. 5-8. Some protein components did not migrate from the starting zone of the small pore acrylamide gel. For all three breeds a pronounced disc appeared at 3 to 4 mm below the starting zone. In the region of 1 to 15 mm from the starting zone the breeds varied in the number of narrow slowly migrating protein discs. In this region there were 7 to 8 protein discs for the English Trumpeter; 8 to 9 for the German Beauty Homer; and 6 to 8 for the Modena. For all three breeds two wide rapidly migrating plasma protein discs appeared in the 15-30 mm region of the gels. In the lower 15-30 mm region the most significant variation between different breeds was found in the number of narrow rapidly migrating discs preceding each wide disc. English Trumpeters had 1 or 2 narrow protein components before the first wide protein disc and sometimes one small disc before the second wide disc. The German Beauty Homer usually had one narrow protein disc preceding each large one. Plasma from Modena's also had 1 or 2 narrow discs preceding each wide disc. The absence of the narrow disc in certain individuals was probably due to extremely faint discs caused by low concentrations of a plasma protein component and to masking by the wide fast moving disc, some of which migrated at different speeds between individual pigeons.

Because of technical difficulties hemoglobins of the breeds were not extensively studied by this electrophoretic technique. Hemoglobins from the above mentioned breeds seemed to have exceptionally low specific gravities. Hemoglobin from the White King breed showed one large component. Other studies have shown hemoglobin to be of no value in identifying bird species by electrophoresis techniques.

#### SUMMARY

Fifty pigeons of three distinct breeds (English Trumpeter, German Beauty Homer and Modena) were used in studying patterns of plasma proteins and hemoglobin with disc electrophoresis techniques. Individual variations of protein patterns were observed and described. Disc electrophoretic techniques were not useful in studying pigeon hemoglobins.

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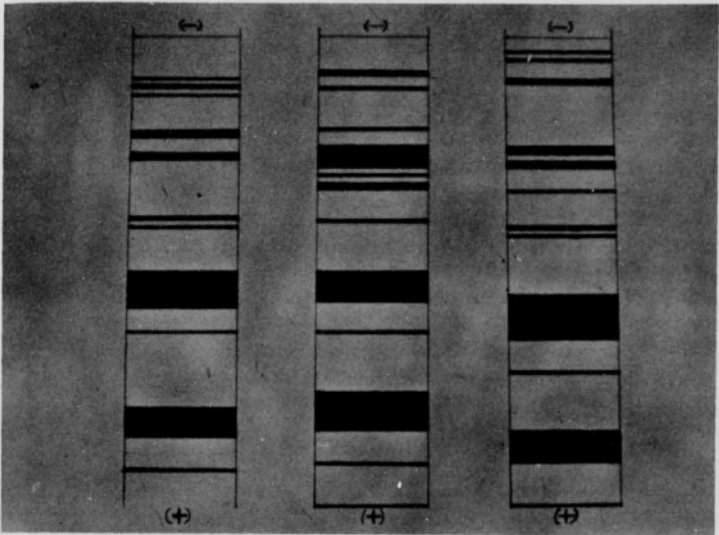


Fig. 5. Electrophoreograms Of Three Modenas.

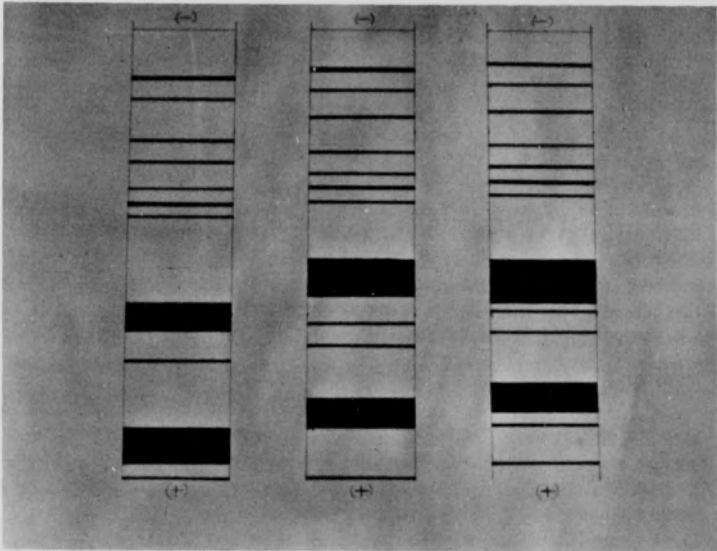
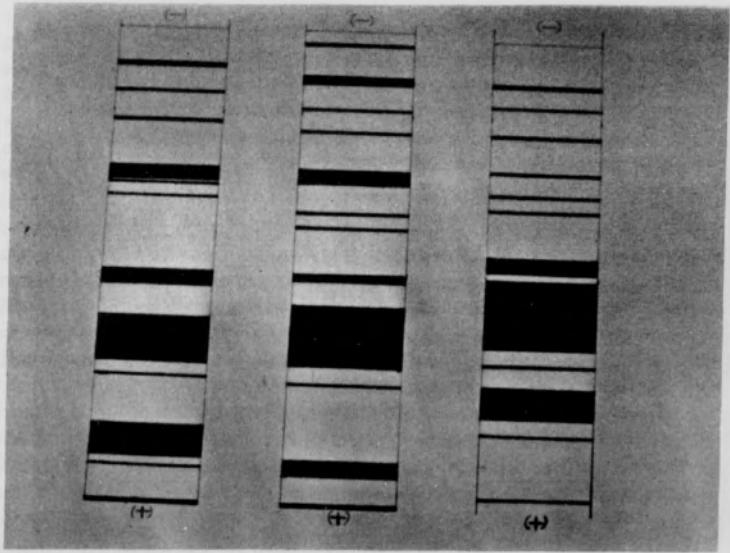
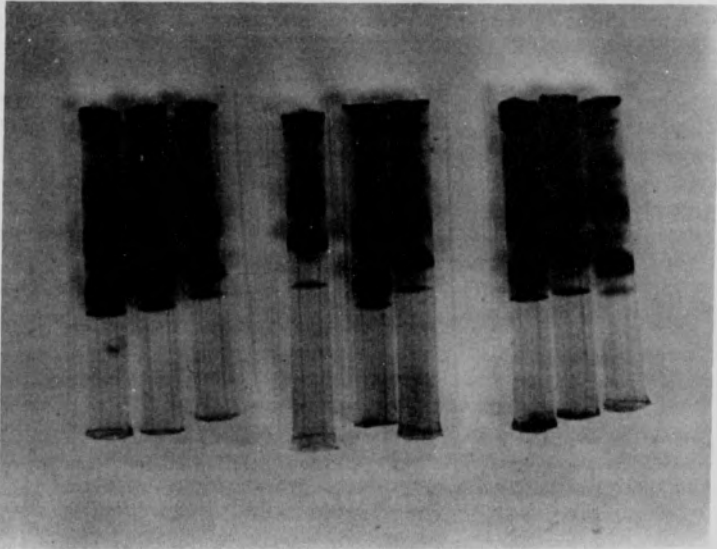


Fig. 6. Electrophoreograms Of Three English Trumpeters.



**Fig. 7. Electrophoreograms Of Three German Beauty Homers.**



**Fig. 8. Disc Electrophoresis Patterns Of Plasma Proteins On Acrylamide Gels (From left: English Trumpeters, German Beauty Homers and Modenas).**



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