# AN INVESTIGATION OF INTRASPECIFIC DIFFERENCES IN PLASMA ALBUMIN OF RING NECK DOVES

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## Introduction

Inherited albumin differences, as determined by gel electrophoresis of plasma and serum of different animals, have been demonstrated or suggested by the results of various studies. (McIndoe 1962; Desborough and Irwin 1966; Quinteros et al. 1964). Individual and species differences have been indicated. Starch gel electrophoresis of the plasma of an inbred line of Brown Leghorn chickens (McIndoe 1962) demonstrated the inheritance of a fast and a slow albumin band. Some birds exhibited either one fast band or a single slow band. Others had both the fast and slow bands. Ring neck doves, Streptopelia risoria, and dwarf turtle doves, Streptopelia humilis, were reported (Desborough and Irwin 1966) as having individuals in their populations that indicated a single albumin band and some that indicated double albumin bands as shown by acrylamide gel electrophoresis. Albumin differences in species hybrids of doves and pigeons as determined by starch gel electrophoresis have been found by Dr. W. J. Miller. Similar findings were reported in turkey hybrids (Quinteros et al. 1964).

This report describes a study of the plasma of a large colony of ring neck doves of diverse sources to determine if plasma albumin differences as shown by acrylamide gel electrophoresis could be verified within the species and, if so demonstrated, establish the mode of inheritance.

## Materials and Methods

The horizontal acrylamide gel electrophoresis technique used is a slight modification, by W. J. Miller and the author, of a previously reported technique (Raymond and Weintraub 1959). This nearly "continuous" buffer system had a pH of 9. Fresh tray buffer was given a 3-hour pre-run and changed only after marked evaporation or after about 20 runs.

The current was transmitted through the gel at a potential of 150 volts. The period of time of the run was determined by a visual control of a mixture of longhorn cattle hemoglobin and brom-thymol blue stain. The operation was terminated when the brom-thymol blue stain had progressed 8 cm toward the positive pole and the cattle hemoglobin A band 3 cm. This migration was usually completed in

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two hours' time. The gel was removed and placed in a solution of 0.5% naphthol blue black stain (Buffalo Black) for a period of two minutes, then destained.

Plasma of 782 doves was analysed by the above method. Most plasma samples had been frozen and stored up to a period of two years. The other samples were taken as young birds developed in the population. The blood was extracted by brachial venipuncture and collected in test tubes containing an anticoagulant (citrate) in a ratio of approximately four volumes of blood to one of citrate. The components of the anticoagulant were two per cent sodium citrate and one and a half per cent sodium chloride in distilled water. The mixture was centrifuged and the plasma drawn off. The initial frozen samples of plasma and most fresh samples were taken when the birds were at an age of two to four weeks. Some adults were again bled, and this plasma compared to previous samples taken at an earlier age.

The albumin bands were first determined by the intensity of the stain (E-C Apparatus Corporation 1965) and the position on the gel. The common protein stains, such as naphthol blue black, stain albumins more intensely than the other plasma proteins. Except for the prealbumins, which can be differentiated by their light staining, the regular albumins progress more rapidly toward the anode than the other plasma proteins.

The albumin was better verified by precipitation and dialysis of the plasma to isolate the albumins (Korner and Debro 1956). By this method the plasma proteins were precipitated with 10% trichloroacetic acid. The acid was poured off and absolute ethanol was added to the remaining solids to dissolve the albumin. The undissolved protein was spun down by centrifugation and the clear supernatant drawn off. The alcohol and dissolved albumin were dialysed against distilled water for twenty-four hours. The dialysate was compared to samples of unaltered plasma by acrylamide gel electrophoresis.

## Results and Discussion

Upon examination of the gels it was discovered that, depending upon the sample of plasma examined, one or two deeply stained bands appeared in the presumed vicinity of expected albumin appearance (See Figure 1). The second band always appeared just in front of the cattle hemoglobin B zone, which was included in the visual control. The fastest albumin band which was signified as type A was present in all plasma tested. The slower second band called B appeared frequently in the population. The results of the 782 plasma samples showed a ratio of 402 doves having the single A band to 380 doves having the double band. It was noticed that a predominance of individuals with double banded albumin began to appear in the more recent matings.

A check on family data was made for the determination of a mode of inheritance. Plasma samples to be tested were available for complete family groups of parents and offspring of only 26 matings.

FIGURE 1. A COMPARISON OF ALBUMIN A AND B BANDS AND THEIR RELATIONSHIP TO CATTLE AB HEMOGLOBIN AS SHOWN BY ACRYLAMIDE GEL ELECTROPHORESIS

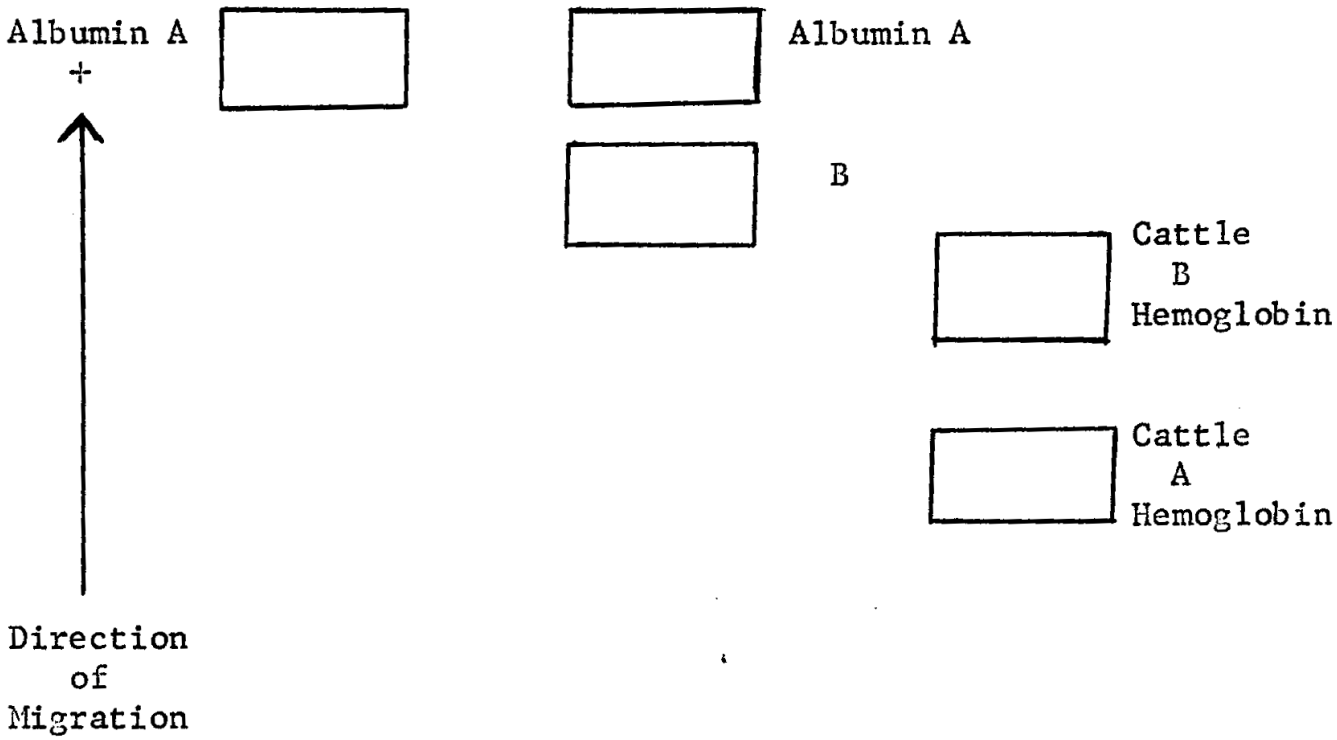


TABLE 1. TEST FOR ALBUMIN INHERITANCE IN RING NECK DOVES, Streptopelia risoria

| Mating type | | Number of progeny in class | | |
|---|---|---|---|---|
| Male x Female | Number of matings | A | AB | |
| A x A | 11 | 19 | 23 | |
| A x AB | 1 | 6 | 1 | |
| AB x A | 8 | 15 | 20 | |
| AB x AB | 6 | 6 | 33 | |
| Total | 26 | 46 | 77 | 123 |

Debro 1956) in order to isolate the albumin. This method is a more accurate indication of albumin than position in the gel. Isolated albumin was extracted from the combined plasma samples of 25 doves exhibiting AB bands. When this albumin was examined by acrylamide gel electrophoresis and compared to unaltered samples of the same plasma, differences appeared. The unaltered plasma showed the two dark staining zones, but the extracted albumin showed only a single band in the area of the A band. This seems to indicate that the B band is not albumin.

One hypothesis for the inconsistent appearance of the slow band may be that a pathological alteration of some serum proteins has caused the second dark staining band. It is known that this has occurred in the gamma and beta globulin regions of plasma (E-C Apparatus Corporation 1965). This could be possible since in 1966 a paratyphoid epidemic (Samonella typhimurium var. Copenhagen) again swept through the dove colony having been present endemically from a previous epidemic (Miller 1965). This hypothesis would require Samonella organisms to be present in the Wisconsin dove colony, too.

Another possibility may be that the constituents of the frozen plasma change over a long period of storage. It was noted that a higher percentage of single band plasma samples in comparison to double band samples occurred in the plasma that had been stored for over one year's duration. It should be noted that Miller has never noted this phenomenon in starch gel electrophoresis of the plasma of doves in the same colony.

It was noticed that occasionally the appearance of two bands in the A albumin zone occurred. Upon careful examination, it was discovered that this was not caused by two different types of albumin. Instead, in some gels the proteins migrated more rapidly near the upper surface than at the bottom. This often gives an impression of two distinct bands of albumin in this zone.

At the present time the appearance of the second dark staining zone in the acrylamide gels cannot be explained. More study should be made to determine the origin of the zone and reason for varying results from different bleedings of doves.

## Summary

The plasma of 782 ring neck doves was analysed by acrylamide gel electrophoresis. The investigation was to determine if a variation in the plasma albumin could be verified and the mode of inheritance of such variation.

Two dark staining bands were noted in the assumed albumin region of many stained gels. The more rapidly moving dark staining band, A, was always present and the other band, B, occurred frequently. A simple inheritance pattern for the B band could not be determined. A second sampling of previously tested birds often gave

different band results, although repeated tests with any one sample were consistent.

Upon analysis of the plasma by the extraction of the albumin, there was an indication that the B band is not albumin. As far as can be determined, only one albumin type appears in this colony of ring neck doves of diverse origin.

## References

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