Segregation of Plasma-Albumin Types from a Species Cross.* (31971)

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Modern gene theory postulates that the sequence of bases in DNA codes the construction of corresponding sequences of amino acids. Examples of inherited differences in proteins are therefore of special interest for the study of gene action. Species differences may have further significance to evolution and physiology. The present report deals with plasma-albumin types in certain species of doves and pigeons and their hybrids.

Birds of this order have been used extensively in immunogenetic studies, but electrophoretic patterns have received less attention. Scheinberg(1) studied the serum antigens of pigeon-rink neck dove hybrids and backcross hybrids and of *Streptopelia humilis* × *S. risoria* F_1 and backcrosses by serological methods and by free electrophoresis. He concluded that several genetically distinct serum antigens distinguish *S. humilis* from *risoria*. Evidently, free electrophoresis only distinguished relative concentrations of albumin to globulin.

β-globulin (transferrin) differences were reported by Mueller *et al*(2) between domestic pigeons (*Columba livia*) and the African triangular spotted pigeon (*C. guinea*); some intraspecific polymorphism also was detected. Beckman *et al*(3) studied a number gens of pigeon-ring neck dove hybrids and stated, "In avian serum the main fast-moving protein component is rather variable in electrophoretic mobility. This protein has been our main marker." *Columba livia, C. albitorques,* and *Streptopelia risoria* each had a distinctive band of this protein (albumin), and hybrids had both bands. Beckman *et al* found no intraspecific polymorphism of this protein.

In the studies just mentioned, starch-gel electrophoresis was employed. Desborough and Irwin(4) used acrylamide gel. They found some variation in transferrins within *Streptopelia humilis,* one type being shared with *S. risoria*. They also found a single wide band or 2 narrow bands in the albumin region. These two species and their hybrid descendants have been further studied for the present report. Acrylamide gel proved less useful in distinguishing the species differences than starch gel and was little used in the present study. Albumin differences were the most distinctive, as indicated by Beckman *et al*(3).

Materials and methods. Five species of doves and pigeons were included:

(a) *Streptopelia risoria,* the common ringdove (229 samples tested, from various sources in the U.S.A. and Europe).
(b) *S. humilis* (= *tranquebarica*), the red-backed or dwarf turtle dove (8 samples tested).
(c) *Columba livia,* the domestic pigeon (236 samples tested from various sources).
(d) *Zenaidura macroura,* the mourning dove (22 samples tested, from both Western and Eastern U.S.A. sources).
(e) *Geopelia cuneata,* the Australian diamond dove (1 sample).

Three types of F_1 hybrids were obtained:
(a) ♀ × (b) ♂—one fertile ♀, 5 juveniles
(a) ♀ × (b) ♂—one fertile ♀, 5 juveniles (♂♂)
(a) ♀ × (d) ♂—four examples, sterile (♂♂).

The *Streptopelia* (*humilis* × *risoria*) hybrid appeared completely fertile in a backcross to *S. risoria,* and segregation of at least 6 genetic character pairs was reported by Miller(5). These included sex (male *vs* female); silky *vs* normal plumage; erythrocyte agglutination by peanut lectin (P+) *vs* non-agglutination (P—); and presence *vs* absence of the species antigens, hu-1, hu-4, and hu-8, of *humilis*. Linkage between the genes for silky and for hu-8 was indicated. Electrophoretic pattern of albumin, as demonstrated in this report, constitutes a seventh genetic character in hybrid populations for

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inclusion in linkage tests with the previous 6, and several generations of backcross descendants are included.

Starch-gel electrophoresis of plasma was employed much as in the study by Mueller *et al*(2) although the horizontal method sufficed in this study. From 11 to 17 samples were compared in any one test with a maximum gel size of 20 × 24 × 0.6 cm. Electrophoresis was maintained for 3-5 hours at 200 v giving a current of 55-75 mA. A hemoglobin sample from a cow of type AB, mixed with a solution of brom-thymol blue, was used as a visual control during the gel run. The A band of cattle hemoglobin was allowed to travel 3-6 cm for best separation of the albumin types under these conditions. Buffalo black from 0.1 to 0.5% was the stain used.

Squabs were sampled by incising the wing vein and allowing several (20 or more) drops of blood to fall in ¼ ml of an anticoagulant solution (sodium citrate 2%, NaCl 1½%). Older birds were similarly sampled but in larger volumes. A single sample then could be used for several tests of the cells as well as of the plasma. No difference was noted between plasma and serum in these tests except that plasma naturally tended to stain less intensely when rather dilute.

Results. The starch-gel photographed by transmitted light for Fig. 1 shows the parental species and the 3 F_1 types. Each species has a single major albumin band, different in position from those of all the other species. The mourning dove band is the slowest, the pigeon and *humilis* dove, closely similar, next, and the *risoria* the fastest. The hybrids show 2 bands, corresponding to those of the parents.

This study, in contrast to that reported by Desborough and Irwin(4) with acrylamide gels, has not revealed any albumin polymorphism within species, and each group of F_1 hybrids was uniform. However, the prealbumin and other serum components may need further study.

In Table I data are presented for the backcross derivatives of *Streptopelia risoria* × *S. humilis*, a total of 359 individuals in 59 sibships. The albumin band types are labeled R for *risoria* and H for *humilis*, the hybrid condition being designated HR. One

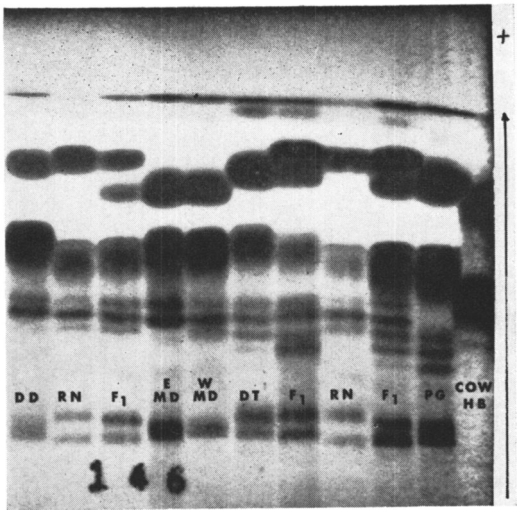

FIG. 1. Photograph by transmitted light of a starch gel of dove plasma from 5 species and 3 species hybrids. From left to right: DD = diamond dove, *Geopelia cuneata;* RN = ring neck dove, *Streptopelia risoria;* F_1 between ring neck and eastern mouring dove; EMD = Eastern mourning dove, *Zenaidura* risoria; F_1 between ring neck and eastern mourning dove, *Zenaidura macroura marginella;* DT = dwarf turtle dove, *S. tranquebarica;* F_1 between dwarf turtle dove and ring neck dove; RN = ring neck dove; F_1 between ring neck dove and the common pigeon, *Columba livia;* PG = the common pigeon.

may infer simple monohybrid co-dominant segregation from the results. Sex linkage was excluded because the F_1 ♀ had both bands, and in the HR × R matings with 10 or more progeny each, association of sex and band type was random. HR × R would be expected to give a 1:1 ratio; from 27 such matings, actually 76 HR and 72 R were obtained. HR × HR should produce a 1:2:1 ratio of R:HR:H, and the actual numbers from 12 matings were 16:42:20, a reasonable fit. R × R, either or both derived from hybrids, bred true, as expected. No matings of H × H were obtained since few H birds survived, and none has yet produced progeny. It is possible that differential mortality is involved, but it is presently considered unlikely.

Table II summarizes linkage test analyses for albumin type and each of the other 5 segregating factors. A more detailed division of the data shows no significant deviation from free recombination in any test, so that linkage can be considered improbable.

Certain adult females (pigeon, ring neck,

TABLE I. Albumin Phenotypes of Parents and Progeny from Matings of Dwarf Turtle Dove Hybrids.

No. of matings	Parents	Progeny: One band type R	Progeny: Two band type HR	Progeny: One band type H	Total
1	R* × HR(F_1)	15	9	–	24
27	HR × R	72	76	–	148
18	R × R	109	–	–	109
13	HR × HR	16	42	20	78
59		212	127	20	359

* Type R = *S. risoria* type band (faster).
H = *S. humilis* type band (slower).

mourning doves, dwarf turtle dove hybrids with ring necks, and 2 white Pekin ducks contrasted with 2 drakes of that breed) exhibited broad bands or blurring of the serum components behind the albumin. Although the albumins evidently were little affected, all the slower components might be deficient or hidden in the blur. Such blurring occurred *only* in some adult females. Most probably it is associated with egg laying since large quantities of albumin are mobilized at this time.

Discussion. The family segregation ratios of the albumin types (H of *S. humilis* and R of *S. risoria*) fit the scheme of co-dominant alleles, which may be symbolized Alb^H and Alb^R.

The present first backcross family exhibited an excess of birds having single bands, 15, to double bands, 9. The second or further backcross generations did not exhibit such a deviation. Since no new phenotypes are encountered and 15:9 is not significantly different from 1:1, it is likely that only one locus is involved; but other factors, such as gametic selection or selective zygotic survival, may occur in the first backcross. F_1 × F_1 matings and the reciprocal backcross likely would add useful data regarding this trend, but are not now available.

TABLE II. Recombinants from Test Cross Matings.*

Characters assorting	No. of matings	Fraction of recombinants†
Alb-H, silky	10	39/83
Alb-H, P-	6	28/62
Alb-H, hu-1	12	50/94
Alb-H, hu-4	9	44/79
Alb-H, hu-8	4	23/49

* Reverse phases of linkage with smaller numbers of progeny are not significantly different and are omitted.
† Values approximating 1/2 are expected if the controlling genes are independent.

Desborough and Irwin (loc. cit.) reported data in which an excess of single bands, 44, to double bands, 21, was evident in 4 first backcross families in which a 1:1 ratio otherwise was reasonable. However, their double bands referred to the intraspecies variation.

Two serum albumin phenotypes detected by gel electrophoresis have been described in species hybrids of turkeys(6) with results similar to those presented here.

Variations in the prealbumin serum protein of the hen due to egg formation were reported by Kristjansson, Taneja, and Gowe (7). Such variation and that noted for non-albumin components in this report probably are related to the estrogen-stimulated increase of non-ultrafilterable fraction of calcium and phosphorus noted by Riddle and McDonald (8).

Summary. Starch gel electrophoresis of the plasma of pigeons, ring neck doves, dwarf turtle doves, and mourning doves reveals constant distinct bands in the plasma albumin different for each species. In hybrids among some of these species, the albumin shows both parental components as double bands. The hybrids from dwarf turtle doves × ring neck doves are fertile. There were 24 progeny from one family in the first backcross plus 335 offispring from 58 second and later backcrosses and matings *inter se* of the backcross hybrids. The ratios conformed to expectations on the assumption of a monohybrid, co-dominant, autosomal gene pair. No linkage of the albumin types with the other

characters was noted. A blurring of the non-albumin components of the plasma was noted in certain adult females of 4 species, a probable association with egg production.

1. Scheinberg, S. L., Genetics, 1960, v45, 173.
2. Mueller, Joan O., Smithies, O., Irwin, M. R., ibid., 1962, v47, 1385.
3. Beckman, L., Conterio, F., Mainardi, D., Serological Museum Bull. 29, 1963, p5.
4. Desborough, Sharon, Irwin, M. R., Physiol. Zool., 1966, v39, 66.
5. Miller, W. J., Science, 1964, v143, 1179.
6. Quinteros, I. R., Stevens, R. W. C., Stormont, C., Asmundson, V. S., Genetics, 1964, v50, 579.
7. Kristjansson, R. K., Taneja, G. C., Gowe, R. S., Brit. Poult. Sci., 1963, v4, 239.
8. Riddle, O., McDonald, Margaret R., Endocrinology, 1945, v36, 48.

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Response of Guinea Pig to Sublethal X-Irradiation and Live Tularemia Vaccine. (31972)

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Animals exposed to ionizing irradiation are usually more susceptible to bacterial infection than nonirradiated animals. Also, irradiated animals have less capacity both for antibody formation and for the development of resistance to challenge following vaccination (1-3).

The anticipated increased utilization of live vaccines for immunization of man poses a problem with respect to the potential risk incurred by vaccinees if exposed to irradiation. This report concerns a study designed to determine the response of guinea pigs administered sublethal irradiation before or after administration of live tularemia vaccine.

Materials and methods. Experimental animals. Adult, male guinea pigs (Hartley strain) weighing 325- to 375-g, were caged in groups of 5 and fed water and Rockland guinea pig pellets *ad libitum*.

X-irradiation. A 1,000 KVP Maxitron apparatus was used as the source of irradiation. Guinea pigs were irradiated in groups of 10 at a distance of 100 cm; dose rates ranged from 56- to 73-r/minute.

Bacterial cultures. Pasteurella tularensis vaccine strain LVS(4) and highly virulent strain SCHU S4(5) were grown in modified casein partial hydrolyzate medium, MCPH (6).

Agglutination tests. A formalinized suspension of strain SCHU S4 was used as antigen and tests were performed and read according to the National Institutes of Health method(7).

Aerogenic vaccination. Organisms were nebulized from a 50% MCPH menstruum and dispersed as a small-particle aerosol with a Chicago-type atomizer in a 4,800-liter exposure chamber similar to that described by Wolfe(8). Guinea pigs inhaled approximately 10^5 viable cells of strain LVS.

Assessment of viable P. tularensis. Appropriate dilutions of homogenized tissues were prepared in gel-saline (0.1% gelatin in 0.85% NaCl), plated on glucose cysteine blood agar(9), and incubated 4 days at 37°C.

Results. Mortality resulting from irradiation. The whole-body X-irradiation $LD_{50/30}$ for the guinea pig was established at 290 r. The maximum sublethal dose was approximately 140 r. To ensure maximal irradiation effect with minimal irradiation-induced mortality, a dose of 140 r of whole-body irradiation was selected.

Combined irradiation-P tularensis *LVS vaccination.* The times between irradiation and later vaccination of guinea pigs were 12, 6, 3, or 1 day. The times between aerogenic vaccination and later irradiation were 2 to 4 hours, 1 day, or 3 days. Animals irradiated 3 days prior to vaccination showed the highest mortality (25%) (Table I). One of 40 irradiated controls died; all nonirradiated vaccinees survived.

Deaths in irradiated vaccinated guinea pigs