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Differentiation of Sera of Two Species of Doves and Their Hybrid.*

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Following the studies of Nuttall,¹ the precipitin-reaction has come into wide use as a means of correlating the taxonomic relationships of species with the serological properties of their serum-proteins. For papers citing the numerous reports of work of this nature, the reader is referred to other sources.²⁻⁶ A few workers have employed the method of absorption of precipitins to differentiate in precipitin reactions, the serum-proteins of species that are distinguishable either not at all or with difficulty by direct precipitation. These are summarized by Cumley.⁷ (Landsteiner and van der Scheer⁸ have pointed out limitations in the principles of absorption of precipitins, which affect the interpretation of the results obtained by this technic.) We are familiar with only a few reports⁹⁻¹¹ dealing with the antigenic relationships of the serum of a species-hybrid to that of each of the parents. In the present tests, the serums of the Pearlneck (*Streptopelia chinensis*), Ring dove (*St. risoria*) and their hybrids (F_1 -P.N./R.D.) were compared.

The precipitins were produced by injecting 0.6 cc of the serum of a species into rabbits 3 times per week until 6 cc of serum had been injected. The immune sera were collected on the tenth and eleventh days after the last injection. For absorptions, the undiluted anti-

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¹ Nuttall, G. H. F., *Blood Immunity and Blood Relationship*, 1904, Cambridge, The University Press.

² Baier, J. C., Jr., *Physiol. Zool.*, 1933, **6**, 91.

³ Boyden, A., *Am. Nat.*, 1934, **68**, 516; *Sigma Xi Quart.*, 1936, **24**, 152.

⁴ Hicks, R. A., and Little, C. C., *Genetics*, 1931, **16**, 397.

⁵ Hektoen, L., and Cole, A. G., *J. Inf. Dis.*, 1932, **49**, 29.

⁶ Wolfe, H. R., *Biol. Bull.*, 1939, **1**, 108; *Zoologica*, 1939, **24**, 309.

⁷ Cumley, R. W., *Am. Nat.*, 1939, **73**, 375.

⁸ Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1924, **40**, 91.

⁹ Ishihara, M., and Misao, T., *Jap. J. Gen.*, 1929, **4**, 147.

¹⁰ Kraus, R., and Prizbram, H., *Zentralbl. f. Physiol.*, 1907, **21**, 258.

¹¹ Sasaki, K., *Jap. J. Zootechn. Sci.*, 1926, **2**, 1; *Z. f. Tierzuchtung und Zuchungsbiol. (B)*, 1937, **38**, 361.

serum to one species was mixed with an equal volume of the serum of the other species, and stored at 2-5°C for 24 hours. The process was repeated, using smaller volumes of the absorbing serum, if, after centrifugation of the mixture, the supernatant fluid produced a ring when tested with this serum. For the precipitin-tests, the antiserum was placed in each of a series of capillary tubes of about 2 mm diameter, to a height of approximately 2 mm, and the antigen in its successive dilutions was carefully layered above. The appearance of a ring at the interface within 2 hours was taken as indication of a precipitate.

TABLE I.
Results of Ring-Precipitin Tests, with Anti-Pearlneck and Anti-Ring Dove Sera.

Antiserum No.	Antiserum	Absorbed by serum of	Antigens tested	Highest dilution of antigen giving a precipitate
39S3	Pearlneck	—	Pearlneck	1:16,384
"	"		Ring dove	1:16,384
"	"		F ₁ P.N./R.D.	1:16,384
39S3	"	Ring dove	Pearlneck	1:1024
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	1:32
39S3	"	F ₁ P.N./R.D.	Pearlneck	None
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	None
198S3	"	—	Pearlneck	1:32,768
"	"		Ring dove	1:32,768
"	"		F ₁ P.N./R.D.	1:32,768
198S3	"	Ring dove	Pearlneck	1:2048
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	1:64
198S3	"	F ₁ P.N./R.D.	Pearlneck	None
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	None
263S1	Ring dove	—	Pearlneck	1:16,384
"	"		Ring dove	1:16,384
"	"		F ₁ P.N./R.D.	1:16,384
263S1	"	Pearlneck	Pearlneck	None
"	"	"	Ring dove	1:512
"	"	"	F ₁ P.N./R.D.	1:128
263S1	"	F ₁ P.N./R.D.	Pearlneck	None
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	None
287S2	"	—	Pearlneck	1:16,384
"	"		Ring dove	1:16,384
"	"		F ₁ P.N./R.D.	1:16,384
287S2	"	Pearlneck	Pearlneck	None
"	"	"	Ring dove	1:1024
"	"	"	F ₁ P.N./R.D.	1:128
287S2	"	F ₁ P.N./R.D.	Pearlneck	None
"	"	"	Ring dove	None
"	"	"	F ₁ P.N./R.D.	None

Concentrated antiserum was used in the tests if the antisera were not absorbed. In the absorption-tests, the antisera were diluted in varying degrees, depending upon the amount of antigen required for absorption.

The results of the tests involving anti-Pearlneck and anti-Ring dove sera, either unabsorbed or following absorption by the serum of the other species or the hybrid, with the various dilutions of the sera of Pearlneck, Ring dove and their F_1 hybrid, respectively, are given in the table. It will be noted that the sera of Pearlneck, Ring dove, and their F_1 , respectively, reacted at the same dilution with each of the various unabsorbed antisera, and that no distinction between them could be made by these tests.

However, when anti-Pearlneck serum was absorbed by Ring dove serum, the reagent thus produced precipitated the serum of both Pearlneck and the F_1 , but not that of Ring dove. Likewise, following absorption of Ring dove antiserum by the serum of Pearlneck, the test fluid reacted with the serum of Ring dove and the hybrid, but not of Pearlneck. The serum of the species-hybrid invariably showed a precipitate at a lower dilution with the absorbed fluids than did the serum of either Pearlneck or Ring dove with their respective homologous antisera. Therefore, we may reasonably conclude that the serum of the species-hybrid contains a part, possibly all, of the same, or of related proteins that make for the species-specific qualities of the sera of each of the parental species. Additional evidence as to the possible relationship of the serum-proteins of the hybrids with those of each parent is furnished by the reactivity of the antisera for either parent following the respective absorptions by the hybrid serum. As will be noted, such reagents produced no precipitates at all with any of the 3 kinds of serum. Thus, since the hybrid serum by absorption could remove all the precipitins from the antiserum for either parent, it would seem that the antigenic components of the serum of this species-hybrid were very similar to, if not identical with, those of the serum of both parental species.

Investigations are now under way to determine the reactivity of the serum of backcross individuals (to Ring dove), representing the different unit-cellular characters of Pearlneck.¹²

Summary. The serum proteins of two dove species, Pearlneck and Ring dove, and their hybrid were indistinguishable by direct precipitin-tests; *i.e.* the three kinds of serum reacted at the same dilution with antisera for each of the two species. Following absorption, however, of the antiserum to one species by the serum of the other, a differentiation of the serum-proteins was readily made. The serum of the species-hybrid appeared to possess a combination of the precipitinogens of both parental species.

¹² Irwin, M. R., *Genetics*, 1939, **24**, 709.