GENETIC STUDIES OF SERUM ANTIGENS IN SPECIES HYBRIDS¹

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Received July 13, 1959

STUDIES of serum proteins in taxonomy had their beginnings with the classical work of NUTTALL (1904) who used the antigenic properties of sera as criteria for classification. Subsequently, numerous studies were made of serum differences between species. In these studies *in vitro* precipitation of serum-antiserum mixtures was employed to distinguish sera either by the degree of flocculation or the presence or absence of an interfacial ring. The results of these investigations were summarized by LANDSTEINER and VAN DER SCHEER (1924, 1940). The differences found in the sera of species were quantitative in that antisera reacted more strongly with the serum of the homologous than of heterologous species.

Relatively few studies were made in which the serum antigens of one species could be sharply differentiated from those of another. SASAKI (1937) reported that he was able to differentiate the serum of the White Leghorn chicken (*Gallus gallus*) from that of the guinea fowl (*Numida meleagris*) by absorbing antisera to one species with the serum of the heterologous species. According to SASAKI (1935) similar results had been obtained previously by ISHIHARA and MISAO in their studies of carp and by SASAKI in an investigation of a species cross in ducks.

In a series of papers CUMLEY and IRWIN (1940, 1942, 1943, 1952) reported that segregation of serum antigens occurs among backcross progeny of species and generic hybrids in accordance with an hypothesis of unifactorial inheritance. The genes responsible for the serum specificities are not the same as the causative genes for the differentiating cellular substances and may be independent of or loosely linked to these genes. More recently there have been a number of intra-specific studies implicating genes in the formation or properties of serum proteins. THOMPSON, FOSTER, GOWEN, and TAUBER (1954) reported differences among strains of mice with respect to the globulin component as determined electrophoretically. There were also differences among the mouse strains with respect to the total serum protein content. A protein with hemoglobin binding properties, i.e. haptoglobin, has been studied in man by SMITHIES and WALKER (1956). They presented family data to support their hypothesis that the synthesis of the haptoglobins is under the control of a two allele system. Evidence in support of this hypothesis has also been obtained by F. GALATIUS-JENSEN (1957). Genetic control of protein synthesis is also indicated in the suggestive evidence that the

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absence of globulin in man (agammaglobulinemia) is an inherent metabolic error, GITLIN, HITZIG, and JANEWAY (1956). Genetic variation may also account for the differences among individual rabbits in the antigenic components of serum observed by DRAY and YOUNG (1958).

The present paper deals with analyses of the sera of species among the Bovidae and Columbidae. Differences between species, species hybrids and backcross hybrids were detected with specific antisera in flocculation tests. Electrophoretic analyses were also made of the sera of species and their hybrids. These studies provide additional evidence for the genetic control of antigenic specificity among the serum antigens.

MATERIALS AND METHODS

Samples of sera were obtained from cattle (*Bos taurus*), bison (*Bos bison*), pigeons (*Columba livia*), ringdoves (*Streptopelia risoria*), dwarf turtledoves (*Streptopelia humilis*, or *tranquebarica* hereinafter referred to as *humilis*) and hybrids and backcross hybrids of the following species crosses: bison $\times$ cattle; ringdove $\times$ *humilis* and ringdove $\times$ pigeon. In the Bovidae, cattle were the recurrent species used to obtain the backcross progeny, in the other matings the ringdove was the recurrent parental species.

Blood samples from the Bovidae were obtained by jugular puncture and from the Columbidae by brachial venipuncture. Serum was obtained from the clotted blood and stored at -20°C until used. In order to reduce the lipid content of the sera of birds and rabbits the animals were starved for 24 hours before bleeding. Prior to immunization the sera of the rabbits were tested for the presence of naturally occurring antibodies. Such antibodies were found to a titer of two to three ($\log_2$) for both cattle and bison and also for *humilis*, pigeon and ringdove. Eighteen rabbit normal sera were tested with the sera of Columbidae and six rabbit normal sera with the sera of the Bovidae.

All immunizations were made with the pooled sera or plasma with the number included in each pool as follows: bison-93, ringdove-100, *humilis*-30, pigeon-50. Injections were made with a total of 6 cc of fluid, 0.2 cc for the first injection and 0.4 cc every other day until the series was completed. Ten days following the final injection the rabbits were bled providing the titer was five ($\log_2$) or greater. Blood was obtained from the marginal ear vein early in the day and a second bleeding was made intracardially later in the same day and the samples of sera were pooled. This procedure resulted in greater yields of blood than the use of either method alone.

All fluids used in the flocculation tests were preserved with the addition of merthiolate 1:3000. Tubes were made up with equal quantities of antigen and antibody solution, incubated at 37°C and scored macroscopically. Preliminary flocculation tests were carried out to determine the optimal proportion of antigen to antibody at which absorptions were performed. Antisera were exhaustively absorbed until the heterologous antigen solution failed to react with the antisera. Mixtures of antigen and antibody were incubated at 37°C for periods of one to

two hours and then centrifuged for 15 minutes at 2500 rpm in a refrigerated centrifuge. In successive absorptions the amount of antigen solution used for absorption was halved until the antisera failed to react with the absorbing antigen.

Free electrophoresis was carried out in the Spinco electrophoresis apparatus using the Albery cell (ALBERTY 1948). The duration of the run was 10,800 seconds and the imposed current was 16 milliamperes across a potential difference of 250 volts. Prior to electrophoresis the sera were dialyzed at 4°C for 24 hours against sodium veronal, pH 8.6 and 0.1 ionic strength. The concentration of protein was initially determined with the use of a Bausch and Lomb dipping refractometer. Buffer conductivity was measured with a Wheatstone bridge assembly for each run. Measurements for mobility and relative concentration were made on the descending portion of the electrophoretic pattern in the case of the bison and bovine sera. Since all bird sera examined possessed the β anomaly in the descending pattern which precluded the use of this pattern in the analysis, the ascending pattern was used. The relative proportions of each serum component were determined from the areas as measured with a planimeter. Each component was delimited so as to form a gaussian distribution. The procedure of SMITHIES and WALKER (1956) was used in making the starch gel electrophoresis runs.

RESULTS

Serum antigens differentiating humilis and ringdove

In flocculation tests anti-*humilis* sera reacted strongly with the serum antigens of ringdove as well as with the homologous sera of *humilis*. The sera of the two species share specificities but can be differentiated following absorption. The $\log_2$ of antigen titer, that is, the titration of antigen with constant antibody concentration averaged nine prior to absorption for the 11 sera or plasma and averaged five following absorption. The corresponding values for antibody titer were 5.4 and 1.3.³ These data are summarized in Table 1.

Paired comparisons of absorbed antisera for their reactivity with the sera of a given group of hybrids and backcross hybrids were made to determine whether any two or more of the antisera were identical. Identical antisera would be expected to show complete correspondence in reactivities when comparisons would be made of a given hybrid serum with the two antisera in question whereas non-correspondence would indicate nonidentity of the antisera. Two antisera which showed a high percentage of correspondence would be presumed to share antibody specificities or to possess some measure of cross-reactivity, or both. In all the comparisons made there were none in which there was complete correspondence. There were 28 comparisons, 25 of which showed a noncorrespondence of 20 percent or greater, the remaining five did not correspond in 7, 13, 13, 14 and 18 percent of comparisons, respectively (Table 2). The correspondence of the latter comparisons was not restricted to one or two antisera but included five of the eight antisera tested. It is concluded that no two of the antisera were identical, and all will be treated as if they detect different antigenic substances in the serum.

TABLE 1

Titres (to log of base) of sera and plasma prior to and following absorption with pooled sera of ringdove

No. of serum and plasma	Antigen titer		Antibody titer	
	Before	After absorption	Before	After absorption
<i>anti-humilis</i>				
37F ₃	9	5	6	1
42F ₁	9	4	6	1
43F ₁	9	5	5	2
48F ₂	9	5	5	1
48F ₃	9	5	5	2
51F ₃	6	4	6	1
947F ₁	10	6	7	1
949F ₂	10	6	5	1
961F ₁	10	—	5	1
961F ₅	10	7	5	2
965F ₁	10	5	5	2
antiringdove (not absorbed)				
956F ₁	7	.	6	.
962F ₄	7	.	6	.
965F ₂	7	.	6	.
<i>antipigeon</i>				
36F ₅	10	5	—	—
42F ₄	8	5	—	—
43F ₄	8	5	—	—
48F ₁	8	7	—	—
51F ₁	8	5	—	—
51F ₃	—	—	8	5
959F ₁	10	7	5	1
961F ₆	—	—	—	—

— indicates test was not performed.

Future studies should indicate whether any of the antisera share antibody specificities.

The absorbed fluids were tested with the sera of the *humilis* × ringdove hybrids and backcross hybrids to determine whether the antigens were distributed according to Mendelian expectation. These results are summarized in Table 3. The reactions of individual hybrids are included in detailed tables which will be furnished upon request³. The sera of the F₁ hybrids, except two for three antisera and one for three other antisera, reacted with the *humilis* specific antisera as expected if the *humilis* parental species has contributed genes to the hybrid which are responsible for the serum specificities which distinguish the *humilis* serum from the ringdove serum. If more than one independent gene contributed by the *humilis* parental species is responsible for the antigenic differences between the species, then the first backcross generation is expected to contain more birds with

³ Detailed data for which reference is made in the text will be furnished on request.

reactive sera than birds lacking the serum antigens. These would be in the general proportion of 2^n-1 reactive to one nonreactive bird where "n" is the number of independent genes and the antisera cannot be used to discriminate between the antigenic products of the genes. In subsequent backcross generations the number of progeny whose sera are not reactive will increase depending on the number of *humilis* genes present and the genetic constitution of the parents available to form the next backcross generation. The number of progeny whose sera are non-reactive with *humilis* specific antisera No. 947F₁, 949F₂, 961F₁ and 961F₅ increases steadily with each successive backcross generation as would be expected with the continuous replacement of *humilis* genes with ringdove genes.

The data obtained from the reactions of the sera of backcross families possessing both reactive and nonreactive progeny were tested under the hypothesis that segregation had occurred with a ratio of one reactive to one nonreactive progeny,

TABLE 2
*Degree of identity of anti-humilis sera**

Percent noncorrespondence (number of sera compared, in parenthesis)	Antiserum designation						
	37F ₃	43F ₁	48F ₂	48F ₃	947F ₁	949F ₂	961F ₅
37 (43)	X	X					
40 (25)	X		X				
26 (27)		X	X				
32 (19)	X			X			
24 (21)		X		X			
13 (23)			X	X			
45 (20)	X				X		
35 (20)		X			X		
27 (11)			X		X		
27 (11)				X	X		
52 (29)	X					X	
57 (30)		X				X	
24 (17)			X			X	
18 (11)				X		X	
22 (47)					X	X	
64 (11)	X						X
30 (10)		X					X
20 (10)			X				X
20 (10)				X			X
24 (17)					X		X
7 (14)						X	X
60 (20)	X						X
42 (21)		X					X
35 (20)			X				X
31 (16)				X			X
14 (21)					X		X
27 (30)						X	X
13 (16)							X

* The comparison of two antisera is indicated by the presence of two "X's" on the same horizontal line.

TABLE 3

Summary of flocculation tests of specific anti-humilis sera or plasma with the sera of humilis-ringdove hybrids and backcross hybrids

Group	(Absorbed with sera of ringdove)*															
	Anti-humilis sera								Anti-humilis plasma*							
	37F ₃		43F ₁		48F ₂		48F ₃		947F ₁		949F ₂		961F ₁		961F ₅	
	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
F ₁	7	2	8	2	9	1	9	0	7	1	4	1	6	2	6	0
1st Backcross	3	3	3	4	4	3	3	4	10	0	10	0	8	1	10	0
2nd Backcross	3	8	5	7	8	4	3	4	--	--	7	3	2	0	10	4
3rd Backcross	0	10	3	7	1	0	1	0	6	9	13	4	2	0	3	3
4th Backcross	0	8	3	5	--	--	--	--	7	4	7	4	--	--	--	--

+ indicates progeny whose sera are reactive.

0 indicates progeny whose sera are not reactive.

-- indicates tests were not performed.

* For all these antisera, the pooled sera of *humilis* were reactive and the sera used for absorption, that of ringdoves, were not reactive.

TABLE 4

A priori test of hypothesis of 1:1 segregation among backcross hybrids of humilis with ringdove and bison and cattle

Designation of antiserum	Number of families	Expected		Observed		Probability of greater value of χ^2
		With reactive sera	With nonreactive sera	With reactive sera	With nonreactive sera	
Anti-humilis						
37F ₃	3	7.0	8.0	6.0	9.0	<.7>.5
43F ₁	7	10.4	13.6	12.0	12.0	<.7>.5
48F ₂	5	7.3	9.7	11.0	6.0	<.1>.05
48F ₃	4	5.1	6.9	6.0	6.0	<.7>.5
947F ₁	4	4.5	6.5	4.0	7.0	<.7>.5
949F ₂	5	8.5	10.5	11.0	8.0	<.3>.2
961F ₅	3	6.5	7.5	8.0	6.0	<.5>.3
Antibison						
43F ₃	11	7.9	15.0	10.0	13.0	<.5>.3

the conventional backcross test. The *a priori* method of BERNSTEIN (STERN 1950) was used to determine the validity of the hypothesis. Table 4 lists the observed and expected numbers on an hypothesis of a one to one segregation. In all cases there was no statistically significant departure from the expected values which supports the hypothesis of single gene segregation among the backcross progeny for the serum antigens tested.

The parents of different families were not always alive at the time the progeny were tested. It has therefore been necessary to assume that the serum of the backcross hybrid parent likewise would have been reactive. In all cases but one where the backcross parents were available their sera were found to be reactive.

The one exception involved a progeny whose sera reacted faintly (score of one with a maximum possible score of seven) at one serum dilution with antiserum 949F₂, whereas the parent serum did not react. It was not possible to test the serum of the parental hybrid at a lower serum dilution to determine whether the serum was completely without activity.

The small volume of serum which was obtained from each dove precluded the absorption of the *humilis* specific antisera with the reactive serum of each of the backcross hybrids. Consequently it is not known whether the same antigen, and hence the same gene, is involved in all the families tested with a given absorbed antiserum. With antisera 37F₃, 43F₁, 48F₂ and 48F₃ there was apparent segregation in each backcross generation so that it appears likely that only a single gene is involved. With antisera 947F₁, 949F₂, 961F₁ and 961F₅, it seems likely that more than one antigenic substance was identified by the antisera since the first and second backcross generations possess a preponderance of hybrids whose sera are reactive. It is, therefore, less likely that in these cases the same gene is segregating in each backcross family but absorption experiments should provide evidence concerning this question.

All of the antisera tested differ one from the other when comparisons of the flocculation reactions are made and imply as many different serum antigens as there are antisera. Through the kindness of PROFESSOR M. R. IRWIN who furnished data on the erythrocyte antigens it has been possible to make comparisons of serum antigens with erythrocyte antigens. The evidence available does not indicate a common gene origin for a serum antigen and a cellular antigen; the segregations in fact suggest loose linkage or independence (Table 5).

Serum antigens of pigeons and ringdoves

Additional evidence of segregation of serum antigens was obtained from flocculation tests of the sera of hybrids and backcross hybrids of pigeons and ringdoves. Seven antisera to sera or plasma of pigeons were obtained which possessed an average antigen titer ($\log_2$) of 8.6 prior to and 5.8 following absorption with serum of ringdove (Table 1). The respective antibody titers for two of the antisera which differentiated the sera of pigeon and ringdove, respectively, were eight and five prior to, and five and one following absorption.

Four of the absorbed fluids were present in sufficient quantities to test the hybrids and backcross hybrids for the presence of segregation. These results are presented in Table 6. JTPDD₅ serum reacted with antiserum 965F₃ unexpectedly since that of his sire JTPD did not. Unfortunately further tests could not be made because of death of the bird. The segregations observed in the backcross hybrids confirm and extend the findings of IRWIN and CUMLEY (1942) of differences in the serum antigens between pigeons and ringdoves. As with the anti-*humilis* sera all of the antipigeon sera appeared to identify different antigens. When antiserum 42F₄ was absorbed with the serum of each of the four reactive sera of the backcross hybrids, or in the case of 43F₄ with the reactive sera of two of the backcross hybrids, all activity was removed for the other reactive backcross progeny

TABLE 5

Relation of serum antigens to cellular antigens in humilis-ringdove hybrids and backcross hybrids

Antiserum designation	Classes*				Erythrocyte antigenic factor
	++	+0	0+	00	
37F ₃	1	(1)	1	3(1)	1
43F ₁	(1)	1(1)	3(1)	1(1)	.
48F ₂	1(1)	2(1)	1	2(2)	.
48F ₃	1(1)	(1)	2	3(2)	.
51F ₃	1	0	0	1	.
949F ₂	1	1	0	0	.
961F ₁	0	2	1	0	.
961F ₅	0	1	1	0	.
37F ₃	5	2	1	6	4
43F ₁	5	3	3	8	.
48F ₂	8	2	4	4	.
48F ₃	5	3	1	3	.
51F ₃	0	2	1	6	.
949F ₂	2	2	5	3	.
961F ₁	1	3	8	1	.
37F ₃	4	1	2	7	8
43F ₁	3	2	3	6	.
48F ₂	4	2	6	3	.
48F ₃	4	2	2	4	.
949F ₂	1	2	4	1	.
961F ₁	0	0	2	1	.
961F ₅	2	2	4	1	.
37F ₃	1	1	1	2	X
43F ₁	2	0	1	2	.
48F ₂	2	0	1	2	.
48F ₃	1	1	1	2	.
961F ₁	0	0	2	1	.

* Classes:

++ sera reacting with antisera to erythrocytes and to serum.

+0 sera reacting with antisera to serum but not with antisera to erythrocytes.

0+ sera not reacting with antisera to serum but does react with antisera to erythrocytes.

00 sera not reacting with either antiserum.

Numbers in parenthesis refer to doubtful reactions.

and the F₁ parent; however reactivity was still present for the sera of pigeons. The antipigeon sera are therefore complex but each recognized only a common substance among the backcross progeny.

Serum antigens of bison and cattle

The unique population of hybrids and backcross hybrids of bison and cattle at the Range Dominion Experiment Station, Manyberries, Canada made it possible to test for segregation of serum antigens in successive generations following the species cross. Extensive absorption of five antibison sera with the pooled normal sera of cattle yielded one antiserum which reacted specifically with bison serum.

TABLE 6

Flocculation tests of specific antipigeon sera with the sera of pigeon-ringdove hybrids and backcross hybrids

Group	Sire	Dam	Progeny	Antiserum designation			
				42F ₄	43F ₄	961F ₆	965F ₃
F ₁	Unknown		G587Y	+	—	+	+
			G587D ₂	+	—	+	+
			G587F ₂	—	—	+	+
			G587F ₃	—	—	+	+
			G587α ₃	+	—	+	+
			PD ₁	+	—	+	+
			PD ₃	—	—	+	—
			PD ₅	—	—	0	—
		JTPD	+	+	—	0	
1st backcross	JTPD(F ₁)	Ringdove	JTPDD ₂	+	+	+	0
			JTPDD ₃	+	+	+	0
			JTPDD ₄	+	0	+	0
			JTPDD ₅	—	—	—	+
			JTPDD ₆	+	+	0	0
			JTPDD ₇	—	—	0	0
			JTPDD ₈	—	—	0	0
Pooled ringdove sera			0	0	0	0	
Pooled pigeon sera			+	+	+	+	

The remaining four sera were of such low titer following absorption that they could not be used to type the backcross sera. Among the F₁ hybrids the sera of 23 reacted with the antibison specific sera and that of one failed to react. Among the first backcross, 28 sera reacted and seven did not; in the second backcross population 13 reacted and six did not. These results are indicative of a successive replacement with genes from the recurrent cattle parent of genes contributed by the bison. Typing results with families in which the hybrid parent possessed reactive sera and possessed at least one nonreactive progeny were treated in the same way as the data from the *humilis* × ringdove families. The test for segregation among the hybrids as presented in Table 3 provides evidence for the hypothesis that segregation of serum antigens has occurred among the backcross progeny in accordance with a unifactorial mode of inheritance. The presence of large numbers of progeny in each generation with reactive sera indicates the presence of more than one antigenic difference which differentiates bison from cattle sera.

DISCUSSION

The results obtained in this study indicate that the antigenic substances of the serum segregate in backcrosses in accordance with a Mendelian mode of inheritance. Hybridizing species possess serum antigens in common but in addition contain species specific antigens. This confirms and extends the results already obtained by CUMLEY and IRWIN (1942, 1952) and CUMLEY, IRWIN and COLE (1941, 1943).

If there are species specific antigens, it is expected that all members of the species will possess them and that species hybrids would likewise contain the substance in their sera. It is also possible that some antigens may be restricted to a given species even though these substances are not found in all members of that species. It is shown in Table 3 that some F_1 hybrids possessed sera which did not result in flocculation with one or more absorbed antisera. The failure to react does not appear to be random with respect to the hybrids involved but the data are meagre with regard to this question. There were 56 mixtures of plasma or serum and antiserum, of these ten did not result in flocculation. Three hybrids together accounted for seven of these and the remaining three were distributed among the other hybrid sera.

The occurrence of F_1 progeny whose sera are not reactive with the different antisera suggests segregation of some antigenic substances existing within the *humilis* parental group. An alternative is that quantitative differences are present among the hybrids with respect to the amount of antigenic material they possess. Some evidence which bears on this proposal was obtained from measurements of the total serum protein of hybrids using a refractometer. The mean percent protein of the 38 hybrids and backcross hybrids determined in this way was 4.07 ± 1.4 percent. There was no obvious association between failure to react and low serum protein concentration since all F_1 sera failing to react were either average or above average in concentration. This indicates that total serum protein content is not a consideration. In order to determine whether the concentration of a specific antigen is involved, isolation of the specific antigen which differentiates the *humilis* from the ringdove sera will be necessary. A specific antiserum which promotes the optimum production of precipitate could be used to test for heterogeneity of antigen concentration among the F_1 hybrids and the parental species and to compare their respective antigen titers. Lacking such a serum, nitrogen determinations of the precipitates of F_1 hybrid sera at constant antibody concentrations may provide evidence for heterogeneity.

An analysis of the sera of members of the parental species would also show whether some individuals completely lack the antigen, which would suggest heterozygosity. Further evidence would come from intraspecific progeny tests of individuals which produce hybrids with nonreactive sera. Intraspecific heterogeneity may also result from the presence of inhibitors. Inhibitors in the serum could be detected by testing whether the serum in question inhibits a known reactive serum. An hypothesis of interallelic interaction resulting in inhibition at a much earlier stage than the formation of serum antigens would require progeny tests and analyses of embryonic materials for inhibition.

The serum antigens which distinguish one species from another are in all likelihood associated with the serum proteins although there is no direct evidence for this postulate. In the event that such is found to be the case the differences will most likely be manifested in the serum globulins. When free electrophoresis of the sera of cattle, bison and their hybrids was conducted, differences were observed in the number of globulin components which distinguish bison from cattle and

the hybrid from each parent. These differences are most clearly seen in the electrophoretic patterns of Figure 1 in which the diagonal slit angle used was 55° for bison and cattle sera and was 65° for the hybrid sera. The complete patterns at a lower angle are shown in Figure 2. The details concerning these patterns are summarized in Table 7. Eight peaks could be distinguished in the globulin pattern of pooled cattle sera whereas only six were seen in the electrophoretic pattern of pooled bison sera. Some of the hybrid serum patterns contained eight distinguishable globulin peaks. The mobilities of two of the peaks do not coincide with either of the parental sera, moving more slowly than the gamma globulin components of cattle or bison sera. The sera of three of the hybrids as well as bison serum also possessed serum components moving more slowly than albumin, the so-called prealbumins. The number of these components in the hybrids is quite variable, one hybrid sera possessed three of these components, another possessed two and a third possessed one as did the serum of bison. The amount of these components is extremely small as judged by the magnitude of the peaks, and therefore slight

FIGURE 1.—Electrophoretic patterns of sera, descending boundary. a. pooled cattle sera. b. Pooled bison sera. c. Serum of bison-cattle hybrids.

FIGURE 2.—Electrophoretic patterns of sera, descending boundary. a. Pooled cattle sera. b. Pooled bison sera. c. Serum of bison-cattle hybrid.

disturbances in the preparation of the serum for electrophoresis will result in the absence of these peaks from the electrophoretic pattern. In Figure 3 are presented the starch gel patterns of the sera of cattle, bison and the hybrid. The pattern of the hybrid possesses more distinguishable components than the sera of either parental species. This was not evident from the results of free electrophoresis and may be accounted for by the superior resolving power of the starch gel procedure. The electrophoretic patterns of the hybrids suggest that the serum contains all of the proteins present in each parental serum as well as additional components only present in the hybrid serum.

The electrophoretic patterns of *humilis*-ringdove sera and pigeon-ringdove sera are shown in Figures 4 and 5. These are tracings of the electrophoretic patterns which were made because the photographs contained components too faint

TABLE 7
Mobilities of electrophoretic components of species and species hybrids*

Sera	Prealbumins			Albumin	Globulins						Concentration (%)					
Pool from 70 cows	...	...	...	6.98	6.07	5.53	4.92	4.38	3.98	...	3.38	2.50	1.61	...	...	2.66
Pool from 17 female bison	...	...	...	6.51	...	5.56	...	4.42	3.78	3.46	3.25	...	1.61	...	...	2.31
Pool from 93 male bison	...	8.4	...	6.90	6.00	...	4.80	...	3.60	3.40	...	2.05	...	...	...	2.35
Pool from 93 male bison	...	...	...	7.00	6.00	...	4.80	4.15	3.80	3.40	...	1.90	...	...	...	1.39
Bison cattle hybrid no. 2	...	...	...	6.35	5.57	...	4.40	...	3.75	...	2.92	2.28	1.58	...	...	1.91
Bison cattle hybrid no. 21	...	...	...	6.70	...	...	4.68	4.23	3.92	...	3.22	2.49	1.91	1.45	1.03	2.13
Bison cattle hybrid no. 50	8.86	8.70	7.88	6.54	5.78	...	4.67	...	3.86	...	3.02	2.19	1.73	1.34	0.90	2.38
Bison cattle hybrid no. 65	...	...	7.52	6.59	...	...	4.58	...	3.90	...	3.13	2.42	1.84	1.37	0.80	2.86
Pool from 100 ringdove†	...	...	...	7.28	5.89	...	4.22	...	...	...	3.09	2.09	...	1.54	...	1.07
Pool from 30 <i>humilis</i> †	...	...	7.90	6.4	...	...	4.92	4.50	...	3.41	...	2.40	1.93	...	...	1.11
Pool from 10 <i>humilis</i> × ringdove hybrids†	...	...	7.75	6.25	5.46	...	4.71	...	...	3.38	2.86	2.23	1.74	...	...	1.09
Pool from 23 pigeons	...	...	...	6.21	...	5.32	4.90	...	3.83	...	2.81	2.34	1.72	...	...	1.50
Pool of two pigeon × ringdove hybrids†	...	...	7.26	5.89	...	...	4.64	...	...	3.35	3.16	2.23	1.57	...	...	1.19

* Mobilities × 10⁻⁵.

† Birds starved for 24 hours prior to bleeding to diminish the lipid level in the serum.

to be reproduced. The patterns suggest that the hybrid possesses all of the serum protein found in the sera of each of the parental species.

The albumin-globulin ratios of the species sera as well as the hybrids are presented in Table 8. The variation in the albumin-globulin ratios among the hybrids presenting values: (a) close to one of the parents as in the case of the bison-cattle hybrid, (b) intermediate in value as in the case of the *humilis*-ringdove hybrid

FIGURE 3.—Starch gel electrophoresis patterns of sera of bison, cattle and their hybrid. a. Pooled sera of cattle. b. Pooled sera of bison. c. Serum of bison-cattle hybrid No. 68.

FIGURE 4.—Electrophoretic patterns of sera, ascending boundary. a. Pooled ringdove sera. b. Pooled *humilis* sera. c. Pooled *humilis* × ringdove sera.

FIGURE 5.—Electrophoretic patterns of sera, ascending boundary. a. Pooled ringdove sera. b. Pooled pigeon sera. c. Pooled sera of pigeon-ringdove hybrids.

TABLE 8

Relative concentrations of serum proteins in the sera of species and species hybrids

Species and number of animals in serum pool (in parentheses)	Relative concentration of albumin in percent	Relative concentration of globulin in percent	Ratio of albumin to globulin	Protein concentration in percent
Cattle, female (70) (mean of two determinations)	39.72	60.78	.65	2.66
Bison, female (17)	55.80	44.20	1.26	2.31
Bison, male (93)	52.38	47.62	1.10	1.39
Bison-cattle hybrids (mean of 4 hybrids of both sexes)	39.59 $\pm$ 4.7	60.41	.66	2.37
Ringdove (100)	60.43	39.57	1.53	1.07
<i>humilis</i> (30)	83.30	16.70	4.99	1.11
<i>humilis</i> $\times$ ringdove hybrids (10)	63.79	36.21	1.76	1.09
Pigeon (23)	61.19	38.81	1.45	1.50
Pigeon $\times$ ringdove (2)	68.84	31.16	2.28	1.19

and (c) greater than either parental value as it is for the pigeon-ringdove hybrid is analogous to the genetic instances of dominance, genes lacking dominance and the presence of overdominance. Whether one should expect all of these different gene mechanisms to operate on seemingly the same kind of synthesis is a subject for much future experimentation.

The data on electrophoresis indicate that the major genetic differences between species lies in their globulins since the hybrids differ from the parental species mainly in this serum fraction. Both THOMPSON *et al.* (1954) and SMITHIES and WALKER (1956) have found a genetic basis for the differences within species among the globulins they studied. Species may differ in the other serum components but it seems likely that an intensive genetic study of the globulins will be more rewarding at this time. This is especially appropriate for experiments in which purified serum constituents are the subject of study.

SUMMARY

Specific antisera have been prepared against the serum antigens of *humilis*, pigeon and bison. With these, segregation was observed among the backcross progeny of *humilis*-ringdove, pigeon-ringdove and bison-cattle crosses. An hypothesis of single gene inheritance for each serum antigen is proposed to account for the results obtained.

ACKNOWLEDGMENT

The author is pleased to acknowledge the helpful suggestions and criticisms in the preparation of this paper from PROFESSOR M. R. IRWIN.

The author was fortunate in having access to unique populations from which blood samples were obtained. He is indebted to PROFESSOR M. R. IRWIN, University of Wisconsin for the use of dove and pigeon hybrids and backcross hybrids; to

MR. JIM TELFORD, Madison, Wisconsin for use of hybrids and backcross hybrids of pigeon $\times$ ringdove; and to DR. H. PETERS and DR. J. McARTHUR, supervisors of the Dominion Agricultural Experiment Station, Manyberries, Alberta, Canada for assistance in obtaining blood samples from the bison $\times$ cattle hybrids and backcross hybrids.

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