GENETIC STUDIES ON SERUM PROTEIN IN PHASIANIDAE.
III. IMMUNOELECTROPHORETIC COMPARISON OF THE SERA OF
DOMESTIC FOWL, GUINEA FOWL AND THEIR HYBRIDS

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Recent advances in electrophoretic techniques have greatly facilitated the possibilities of the investigation on the protein constituents of the serum. By means of immunoelectrophoresis, the human serum has been separated into 40 fractions, and the Mendelian inheritance with some of these components has been reported. Moreover, similar results have been found in such animals as mice, rabbits, sheep and horses.

In working with avian species, several workers (Sanders *et al.* 1944; Deutsch *et al.* 1945; Brandt *et al.* 1952 and Sibley *et al.* 1959) reported the inter-specific and intra-specific differences of electrophoretic bands regarding with some protein components. Recently, serum albumin polymorphism was reported in domestic fowls (McIndoe 1962), in turkey (Quinteros *et al.* 1964) and in Japanese quails and quail-chicken hybrids (Haley 1965). The esterase polymorphism was also found from the experimental results using 18 avian species (Beckman *et al.* 1965).

In order to determine the genetical relationships among some species belonging to *Phasianidae*, the authors have carried out immunoelectrophoretic studies on the serum protein using some species which had been collected, regardless of whether these species had been used or not by the previous investigators.

The present paper is a report on part of these studies in which the immunoelectrophoretic patterns of sera were adopted as standard criteria of the comparisons. The materials in this paper are restricted to domestic fowls, guinea fowls and the genus hybrids involved with these two species.

MATERIALS AND METHODS

Five cocks of domestic fowl (*Gallus gallus* var. *domesticus*) including two Single Comb White Leghorn, one Rhode Island Red, one Plymouth Rock and one Gifu¹⁾ and seven hens of guinea fowl (*Numida meleagris*) were used as materials of the present studies. All of these individuals were employed as the parents in the inter-generic crossings. Ten genus hybrids (guinea fowl ♀ X domestic fowl ♂) used in the present

1) Japanese native variety in Gifu prefecture.

studies had been obtained with artificial insemination (Ishii *et al.* 1964), and are still employed for the embryological and immunological studies at the Institute for Breeding Research, Tokyo University of Agriculture.

Furthermore, in order to examine the sexual or varietal variations on serum components, 30 domestic fowls and five guinea fowls which have not any genealogical relationships to the individuals above-described were also used in this study. However, there was no notable complication in the results, i.e., the sexual or varietal variation in immunoelectrophoretic patterns of sera could not find at all. Therefore, the data described in the present paper are concerned with the cocks of WL (Single Comb White Leghorn), the hens of guinea fowl and their hybrids.

The following process was adopted for making serum samples. And it was the same for all materials used. The blood which was bled from cephalic vein was pooled, allowed to clot at 38°C for one hour, chilled at 0°C for 24 hours and the serum separated by centrifugation at 3,500g for five minutes. The following process for making the antisera was all the same in both of the parental species, domestic fowls and guinea fowls. Namely, antigen for immunization was prepared from serum diluted with an equal volume of 0.9 per cent saline solution and it contained 20~25mg protein per one ml. A milliliter of the antigen was initially injected with Freund's adjuvant into foot pads of rabbit. The same amount of the antigen was injected twice subcutaneously at a week intervals. The other injection of the antigen without adjuvant was given as booster at the time of three weeks after the last injection above-described. The immunized rabbits were killed within two weeks after the boost. In this way, anti-domestic fowl sera and anti-guinea fowl sera were obtained.

The immunoelectrophoretic technique used in the present study was a modification of the technique developed by Williams and Grabar (1955): The supporting medium of one per cent agar gel (Difco's Special Agar Noble) was pipetted on a glass plate at a thickness of 1.5mm. Veronal-HCl buffer solution (pH, 8.4; ionic strength 0.05) was used in the gel as well as in the electrode compartments. And a constant current of 2.5mA per cm width of the gel layer was applied for 200 minutes. Under such conditions, serum albumine migrated 4.5cm from the origin to the anode.

A trough for antiserum was cut at a distance of 7mm from antigen pit. In order to obtain the fully developed immunoprecipitin lines, the glass plates were kept in a moist chamber at room temperature. The precipitin line developed fully at the third day of immuno-reaction. Accordingly, the immunoelectrophoretic patterns at the third day were adopted as the standard criteria of the comparison.

Furthermore, with the purpose of certification of the chemical properties of the individual protein components of antigen, selective absorption methods, histochemical staining and gel filtration with Sephadex G-200 column were employed. For the staining of proteins, lipoproteins and glycoproteins, amino black 10B, Sudan black B and Schiff's reagent were used respectively.

RESULTS

From the results of the Biulet's method, the authors found that the concentration of total serum proteins in domestic fowls (mean value was 53.5 ± 6.4 mg/ml, ranged from 41 to 60 mg/ml) was much greater than that in guinea fowls (mean value was 37.0 ± 1.4 mg/ml, ranged from 36 to 39 mg/ml).

The migrating rate in electrophoresis, the shape of the individual protein components in immuno-reaction, the results from the histochemical staining methods, gel filtration method and the specific absorption methods were taken as the base for the identification of components.

As shown in Fig. 1, the serum of domestic fowls was separated into 12 fractions. In detail, four or five lines among 12 precipitin lines were observed in α_1 -region, and the other four lines, two lines and a line were also observed in α_2 -, β - and γ -region respectively.

From the results of the examination of the mobility and the quantitative condition of fraction, the authors were able to recognize that the second line in α_1 -region was considered to be albumin. By means of the histochemical staining with Sudan black B, the fifth line in α_1 -region was certified to be lipoprotein (Fig. 3). Furthermore, the results of the gel filtration method used in the present study confirmed the twelfth line in γ -region to be immunoglobulin-G (IgG).

The conditions of experiment for the serum of guinea fowls were quite the same as those for the serum of domestic fowls. And the authors confirmed that the general appearance of immunoelectrophoretic pattern in guinea fowls was nearly the same as that in domestic fowls, although there was a few species differences which must be taken into account (Fig. 2). In the first place, there was a difference of electrophoretic mobility of albumin components between two species concerned. Without exception, albumin in domestic fowls migrated with a surpassing rate comparing with that in guinea fowls (Fig. 4).

With regard to the fifth precipitin line, the other remarkable difference was observed. Namely, the fifth line in domestic fowls was observed as a single line with a strong precipitin arc, while that in guinea fowls appeared as a compound line. In order to settle this problem, the diluted domestic fowl serum of which total protein concentration was quite the same as that of guinea fowl serum was reacted to the anti-domestic fowl serum used in the preceding experiments. Even under this condition, the fifth line in domestic fowls did not change its distinct characteristic of a single line. As shown in Figs. 2 and 3, the precipitation of this component in guinea fowls was less intensity than that in domestic fowls.

In γ -region, a remarkable difference regarding the intensity in the precipitation of IgG was also observed, i.e., the intensity of IgG precipitin line in domestic fowls was much stronger than that in guinea fowls (Fig. 2).

Fig. 1

Fig. 3 α_1 -Lipoprotein

Fig. 5

Fig. 6

Fig. 7

Fig. 2

Fig. 4

Fig. 8

Plate 1. Immunoelectrophoretic analysis of serum protein. Fig. 1. The immunoelectrophoretic pattern of domestic fowl and its schematic drawing. Fig. 2. Comparison of the patterns between the parental species and their hybrid. Fig. 3. Comparison of lines of α_1 -lipoprotein stained with Sudan black B. Fig. 4. Comparison of the migrating rate of albumin. Fig. 5. Pattern of domestic fowl serum to the anti-domestic fowl serum absorbed with guinea fowl serum. Fig. 6. Comparison of lines of albumin; reaction of anti-domestic fowl serum to serum samples obtained from the parental species and genus hybrid. Fig. 7. Comparison of lines of albumin; reaction of anti-guinea fowl serum to serum samples above-described. Fig. 8. Line of IgG precipitated with antiserum obtained from the rabbit of No. 16 is compared with that from the rabbit of No. 15; and they were immunized with the same treatment. A: Trough of antiserum obtained from the rabbit of No. 16. B: Trough of antiserum obtained from the rabbit of No. 15. a: domestic fowl, b: genus hybrid, c: guinea fowl.

Furthermore, by means of the selective absorption method, the authors were able to confirm the facts that only two lines, α_1 -line and IgG line were observed in the reaction of domestic fowl serum to the anti-domestic fowl serum absorbed with guinea fowl serum, while none of line was observed in the reaction of guinea fowl serum to this absorbed antiserum (Fig. 5).

For making the studies on the genetic properties of the serum components, the immunoelectrophoretic pattern in the genus hybrids was compared with those in the parental species. It was recognized that the electrophoretic pattern in the hybrids was different in some points from those in both of the parents, although nearly all the immuno-precipitin lines were in common with those of the parents. Namely, the fifth line in the hybrids was observed as a single line as shown in the pattern of domestic fowls. And such figure may be more clearly observed in the preparations stained with Sudan black B (Fig. 3).

There was a distinctive figure regarding the second line in the hybrids. Without exception, the second line in the hybrids was composed of two lines of which electrophoretic mobilities were different from each other. And one of these lines migrated with a surpassing rate as high as that in domestic fowls, and the other line migrated with an inferior rate which exhibited in guinea fowls (Fig. 6).

As shown in Fig. 7, this condition was supported completely by the results of experiments in which the sera of parental species and their hybrids were reacted to anti-guinea fowl serum.

The intensity of precipitation of IgG in the hybrids was intermediate between those in the parents, i.e., it was weaker than that in domestic fowls and was stronger than that in guinea fowls.

Conclusively, it is able to consider that the antigenic components in serum of the two species concerned have much the same general constituents, but in detail, they have minute constitutional differences with regard to the following precipitin lines: the second line (albumin), the fifth line (α_1 -lipoprotein) and the twelfth line (IgG).

DISCUSSION

In the present studies, the immunoelectrophoretic pattern of serum of the two species, domestic fowls and guinea fowls was adopted as the standard criterion in the comparison of constitutional characteristics of their sera. According to the experimental results involved with their genus hybrids, some genetic considerations were introduced herein. However, because of the lack of the notable difference about the other serum components, the discussion in this paper may be restricted to the following three immuno-precipitin lines which exhibited the difference between the species concerned. From the results of the following experiments, i.e., histochemical staining method, absorption method and gel filtration method, three lines described above were confirmed as albumin, α_1 -lipoprotein and IgG respectively.

Albumin polymorphism in chicken serum was first reported by McIndoe (1962) and was essentially supported by Beckman *et al.* (1963). Recently, Haley (1965) confirmed that Japanese quails have three phenotypes regarding the serum albumin which are controlled by two codominant autosomal alleles, while only one phenotype is considered in chicken serum albumin. And he observed the fact that two electrophoretic bands regarding this component exhibited in the chicken-quail hybrids.

The results presented in this paper indicate that the albumin of the two species concerned are different only in their electrophoretic mobility, but are not different immunologically and they are able to cross-react. Therefore, it is possible to consider that the albumin of these species include the parts which are composed of common determinant groups and the other parts which are not concerned with immunological determinant possessing a different net charge. At any rate, one of the two species exhibited a different albumin phenotype from the other.

With regard to the albumin component in the genus hybrids, two precipitin lines whose electrophoretic mobilities were different from each other were always observed immunoelectrophoretically. From their mobility, it is able to consider that the line which migrates with a surpassing rate may correspond to the line observed in domestic fowls, and the other line which migrates with an inferior rate may correspond to that in guinea fowls. And this consideration is supported by the results from the experiments in which the serum of two species and the genus hybrids were reacted to the anti-guinea fowl serum. Although there is a difference of parental species used in the hybridization, the authors like to recognize the phenotypical agreement between our results and the results presented by Haley. Further investigations of this albumin phenotype should be conducted. However, because of the difficulties for succession of the generations in this hybridization, the further genetic studies have not been carried out.

The following two presumptions regarding the species difference of α_1 -lipoprotein can be presented: in the first place, such specificity will be caused by the quantitative difference of serum α_1 -lipoprotein of each species; in the second place, the difference will be due to its qualitative difference. In other words, each of the species will be possessed of the species specific serum α_1 -lipoprotein.

The immunoprecipitin line of α_1 -lipoprotein of domestic fowls was different from that of guinea fowls in the shape of precipitation and in the electrophoretic mobility. It seems that the second presumption is more acceptable. On the contrary, the first presumption may be denied with the results from experiments in which the concentration of serum protein of domestic fowls was diluted to the same degree of that of guinea fowls, and this diluted serum was reacted to the antiserum.

At the present stage of research, it may be difficult to decide the genetic conclusion with the α_1 -lipoprotein. Because the immunoelectrophoretic pattern of α_1 -lipoprotein in the genus hybrids was quite the same as that in domestic fowls, it seems reasonable to assume that the phenotype of α_1 -lipoprotein in the hybrids was dominantly controlled

by the gene inherited from domestic fowls.

There was necessity of a preliminary experiment in which the utmost attention was paid in the dietary condition of all materials to avoid the serious effect of dietary lipoids on the immunoelectrophoretic pattern of α_1 -lipoprotein. From the results of this experiment, it was clear that within the scope of investigation, this disturbance was not so serious as to confuse the results.

An interspecific difference in intensity of immuno-precipitation of IgG was confirmed in the present study. From the results obtained, the authors prefer to accept that this difference is due to the difference of the antigenic property of serum γ -globulin.

It is generally recognized that there is an individual variation in the ability of response for immunization in rabbits even if they were immunized under the same conditions. From the results of the present study, the authors confirmed such fact concerning the immuno-reaction of IgG. Data in this paper was restricted to the data with the reaction of the samples to the anti-domestic fowl serum obtained from the rabbit of No. 16. As shown in Fig. 7, however, when the antiserum obtained from the rabbit of No. 15 which was immunized with the same treatment as that for the rabbit of No. 16 reacted to the domestic fowl serum, the line of IgG was precipitated as a matter of course. On the other hand, this antiserum reacted to the guinea fowl serum without any precipitin line of IgG.

The above-mentioned result suggests that because of the difference of antigenic construction of IgG between two species concerned, some antisera included in anti-domestic fowl serum can not cross-react with IgG of the guinea fowls. In other words, the IgG molecule is possessed of high specificity in the immuno determinant groups.

The present assumption for the antigenic relation of IgG shown between the two species concerned may be supported by the relation of hetero-homologous antigen reported by Jennings (1959 d). Namely, the IgG of domestic fowls and guinea fowls corresponds to his homologous antigen and cross-reacting antigen respectively. Conclusively, it is possible to consider that the IgG of domestic fowls contains all determinant groups required for the combination with anti-domestic fowl serum, while the IgG of guinea fowls lacks some of the determinant groups concerned.

SUMMARY

By means of electrophoresis, sera of domestic fowls, guinea fowls and their hybrids were fractionized. And their immunoelectrophoretic patterns were used as the standard criteria for the interspecific comparisons. The authors were able to confirm the following results, and they presented some genetic considerations.

1. The concentration of serum proteins of 53.5 ± 6.4 mg/ml was estimated in domestic fowls and also that of 37.0 ± 1.4 mg/ml was estimated in guinea fowls.
2. Serum of domestic fowls was separated into 12 fractions. Excepting the

fractions in α -region and γ -region, the other fractions in domestic fowl serum were in common with those in guinea fowl serum. And the components which exhibited the uncommon characteristics in α -region and γ -region were identified as α_1 -lipoprotein and immunoglobulin-G (IgG) respectively.

3. The species difference in albumin is due to the difference of electrophoretic migrating rate of this component, i.e., the rate in domestic fowls surpassed that in guinea fowls.

4. Two precipitin lines of albumin were observed in electrophoretic pattern of serum of the genus hybrids. Among them, the line migrated with a surpassing rate corresponds to the albumin in domestic fowls, and the other line migrated with an inferior rate corresponds to that in guinea fowls. And the authors assumed that these three albumin phenotypes are controlled by a pair of codominant allele.

5. The precipitin line of α_1 -lipoprotein in domestic fowls was observed as a single line, while that in guinea fowls was observed as a compound line. And that in the hybrids was a single line and rather the same as that in domestic fowls. The authors assumed that this interspecific difference is due to the difference of the antigenic construction of α_1 -lipoprotein.

6. The precipitin line of IgG showed a remarkable specific difference between two species concerned. And in their hybrids, the precipitation of IgG exhibited an intermediate characteristics. The authors consider that the interspecific difference of IgG is also due to the difference of the antigenic construction of this component.

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