Absence of Alpha Globulin from the Egg White of the Domestic Turkey.* (31001)

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Quantitative differences in the egg white protein complex of several avian species have been observed by many investigators(1-8). Similar *intra*-species differences have been demonstrated in the domestic chicken(9-13). A general agreement exists now that in that species at least, the protein complex of the whole egg white is made up of one or more fractions of albumin, conalbumin, ovomucoid, globulin, and lysozyme(11,14-20).

Embryonic mortality in domestic turkeys is, on the average, higher than in domestic chickens. The hatchability levels (of fertile eggs) in the two species are of the order of 65% and 80% respectively(21). Because h^2 of hatchability in turkeys is low(22), one might expect, on theoretical grounds, the maternal "C effect" (*cf* Lerner, 23), mediated through the extranuclear components of the ovum, to be important in avian embryogenesis. Although some seemingly conflicting experimental evidence exists on this score(24, 25), it should not be unreasonable to expect both *qualitative* as well as the already referred to *quantitative* differences in these components to be related to the developmental potential of the avian embryo. The work reported below demonstrated the existence of at least one qualitative difference between the chicken and the turkey in the egg white protein.

Materials and methods. The original source material for the study was provided by freshly laid White Leghorn (chicken) and Broad Breasted Bronze (turkey) eggs. The antiserum came from one-year-old male rabbits. All of the immunological analyses were based on the double-diffusion Ouchterlony agar plate method(26).

The antisera were produced in the rabbits by injecting them, *via* the ear vein route, with homogenized whole egg white obtained from an egg laid by a specific bird. Ten injections in all were administered on alternate days to each rabbit: the first injection was 1 cc and the subsequent ones were 2 cc each. Before initiating an injection series, the egg white was homogenized, filtered through gauze and then diluted 1:7.5 in a 0.9% saline solution. The egg white-saline mixture was stored at —20°C until used. A test blood sample was taken *via* heart puncture 10 days after the last injection. The definitive antiserum was collected the following day in the same way. Four rabbits were involved in each specific series of injections, *i.e.,* the animals in such a group were injected with the same egg white. Each antiserum within the series was identified by the rabbit donor and the antiserum that gave the most clear-cut precipitin lines within a pilot agar plate test was selected for further use.

Upon being drawn from the rabbit, the blood was set aside, at room temperature, to coagulate. The sample was then stored overnight at 5°C. The next morning the serum was centrifuged, the complement inactivated (30 minutes' heating at 56°C), 0.01% of merthiolate added and the specimen was then stored at —20°C until needed.

The initial series of observations on the pattern of precipitin lines (on the "within-hen" basis) involved a White Leghorn, No. 1: different eggs laid by this hen were used to provide the antigen and to produce the antiserum. In subsequent series in which the inter-species comparison (*i.e.,* chicken *vs* turkey) were undertaken, another White Leghorn hen, No. 337, and a Broad Breasted Bronze hen, No. 474, were also involved as sources of egg white for the production of antisera.

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Reactants against the 3 antisera (induced by the egg white of hens No. 1, 337 and 474) were the 3 original egg white specimens used to induce these antisera, the egg white obtained from other eggs laid by these hens, and the egg white from one egg laid by each of 11 Broad Breasted Bronze hens taken at random from a breeding flock. Genetically, these hens were closely related to hen No. 474.

The agar plates for the tests were made of 1.25% agar, 0.9% NaCl, and 0.01% merthiolate. The mixture was buffered at pH 7.0 with a phosphate buffer. The distance between the reactant wells, each of which was 3 mm deep and 8 mm wide, was 8-10 mm. The egg white antigen dilutions (in a 0.9% saline solution) started at 1:5, going in some instances to 1:2560. The serum was either undiluted or diluted 1:2. The charged agar plates were kept for 2 weeks in an incubator at 37°C and 85% relative humidity before being "read." Later, the observed pattern of precipitin lines in the plate was analyzed with the help of 3 egg white fractions obtained with the ammonium sulfate fractionating procedure. The 3 concentrations of ammonium sulfate used were: 31% (fraction I), 39% (fraction II), and 50% (fraction III).‡

The analysis was further expanded through the use of 3 commercially prepared egg white proteins: ovalbumin and ovoconalbumin, each recrystallized five times, and ovomucin, repurified twice. As will be seen, the latter point may have been responsible for a doubtful interpretation regarding the ovomucin reaction.

Results. Several precipitin lines (A_1, A_2, B, C_1, C_2) and the D "complex" were observed when the egg white of White Leghorn hen No. 1 was tested with homologous antisera. The relative position of these lines from the respective antigen wells corresponds, of course, to decreasing molecular weight and concentration of the antigens represented by them. With increased dilution of the antigen, lines A, B and C became less distinct; by contrast, the D complex became resolved into at least 2 lines (Fig. 1 and 2). Reducing the antibody concentration by one-half yielded essentially the same pattern as above. However, the precipitin lines now were not as sharp in their outline (*cf* Fig. 1 and 2 with Fig. 3 and 4).

Comparative tests involving the egg white of chicken No. 337 and the egg white of turkey No. 474 against the antiserum induced by the former antigen source showed that lines A and C and the D group were common to both birds. However, line B was absent on the turkey side (Fig. 5). Line A on occasions was observed to separate into lines A_1 and A_2. An example of this is seen in Fig. 8, in which fraction I and a chicken whole egg white reacted with a rabbit antiserum against the latter. It can be seen that line A on the left side (in the antigen-fraction I area) was joined by lines A_1 and A_2, developed on the right side (antigen-chicken whole egg white). Immunological similarity of antigens in the two species, represented by lines A in particular, is shown clearly in Fig. 6. Furthermore, as one would expect, line(s) B failed to materialize at all with respect to either chicken or turkey antigens when an anti-turkey antiserum was used in the center well (Fig. 7). The antigen represented by this line was absent in the egg white of all 12 turkey hens used in this study. The specificity of line B for the chicken egg white was further demonstrated by first absorbing the anti-chicken antiserum with the turkey egg white and then exposing the absorbed serum in a diffusion plate to a chicken egg white. Under such circumstances, only line B was in evidence.

Tests involving paired antigens, one a chicken egg white and the other a specimen of fractionated egg white, and an antiserum against the chicken egg white clearly showed that the antigen represented by line B, as well as the D complex were absent in fraction I. The line and the complex reappeared in fractions II and III (Fig. 8, 9, 10). The D complex in the chicken was analyzed by testing it against known concentrations of ovalbumin, ovoconalbumin and ovomucin. Strong lines for the first two (Fig. 11 and 12) clear-

‡ Complete details on the fractionation procedure are given in Progress Reports for 1964, Poultry Council of Washington State Univ., pp109-116. These reports are available at W.S.U. Library.

ly indicated that at least these 2 components are found in this protein complex. The presence of a weak line opposite the ovomucin well (Fig. 13) is probably attributable to the

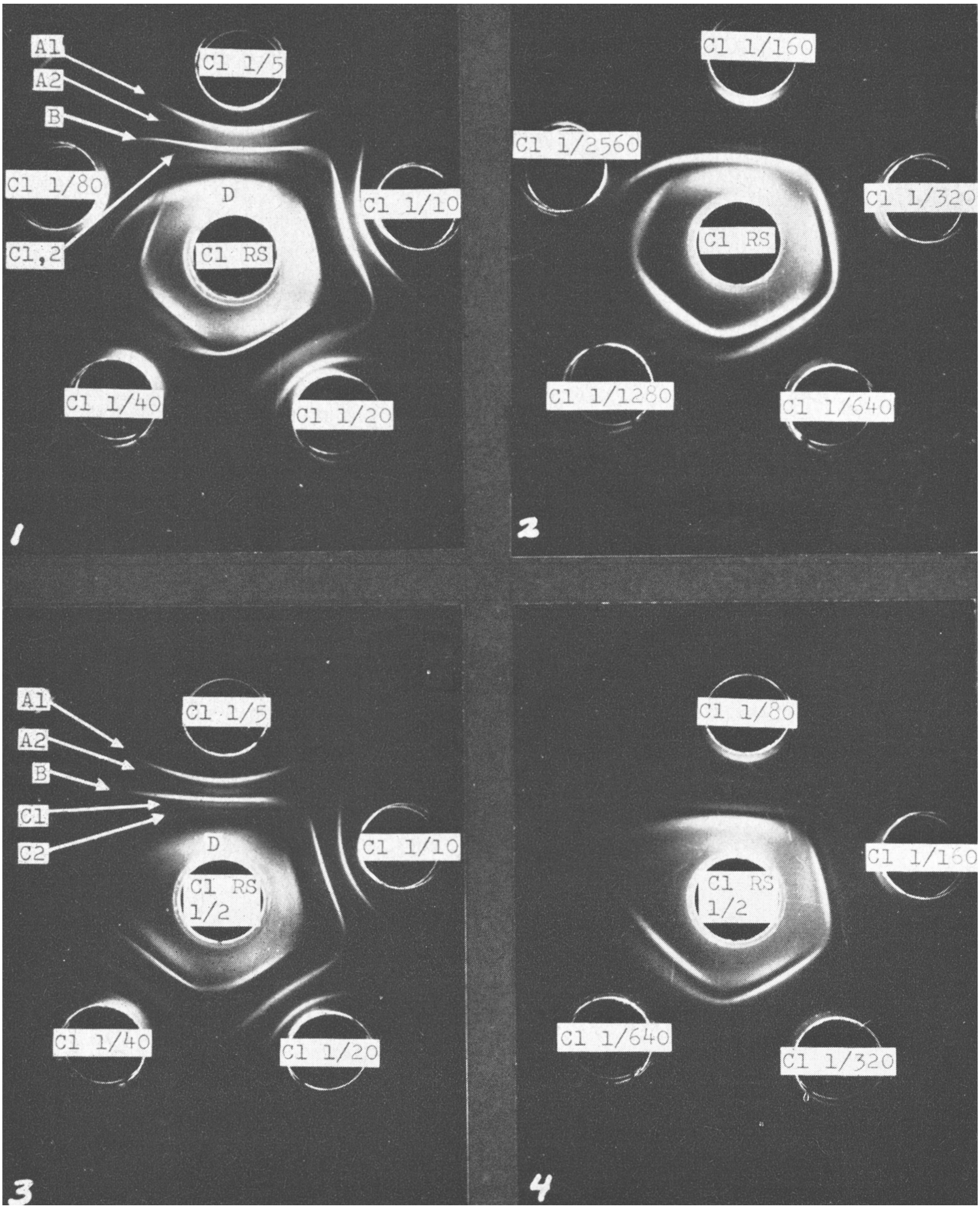

FIG. 1-4. Basic patterns of precipitin lines resulting from a reaction between the chicken egg white (C1) and the antiserum (C1RS) against it after the plates were incubated for 14 days. White Leghorn hen No. 1 was the source of the egg white in both instances.

FIG. 1 and 2. The egg white dilutions ranged between 1:5 and 1:2560 while the antiserum was undiluted.

FIG. 3 and 4. The egg white dilutions ranged between 1:5 and 1:640; the antiserum was diluted 1:2.

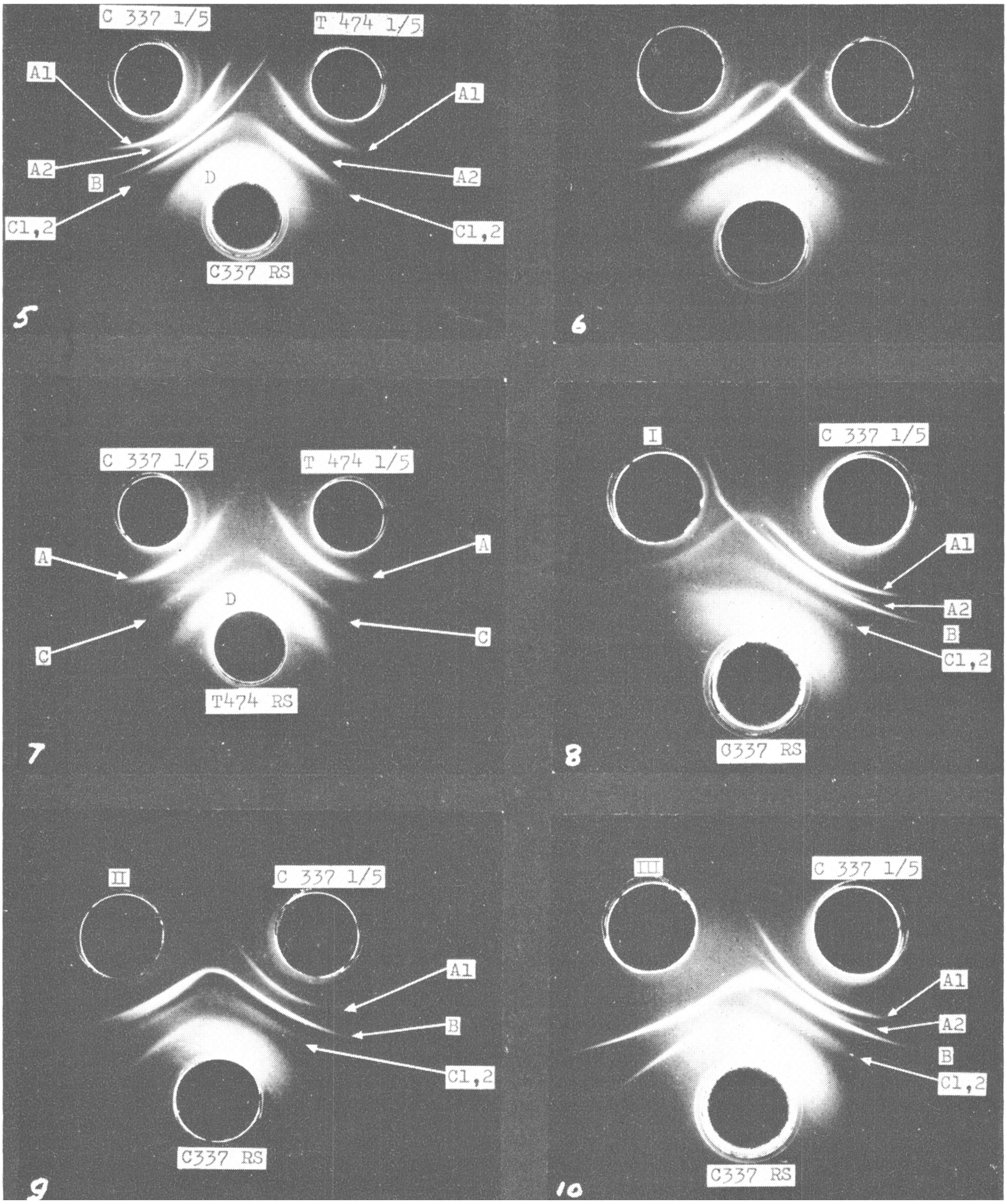

FIG. 5. A reaction between antichicken egg white antiserum C337RS and egg white from White Leghorn hen No. 337 and Broad Breasted Bronze hen No. 474.

FIG. 6. A test similar to that shown in Fig. 5, but involving antiserum C1RS and chicken egg white C1 (in left well) and turkey egg white T474 (in right well).

FIG. 7. A reaction between antiturkey egg white antiserum T474RS and the chicken and turkey egg white in left and right antigen wells, respectively. Note absence of line B (compare with Fig. 5).

FIG. 8-10. Comparative precipitin reactions involving fractions I, II, III and chicken egg white C337 and antichicken egg white antiserum C337RS. Note absence of line B and of complex D in the area facing the well containing fraction I (Fig. 8), and their presence in Fig. 9 and 10, where the test antigens were fractions II and III.

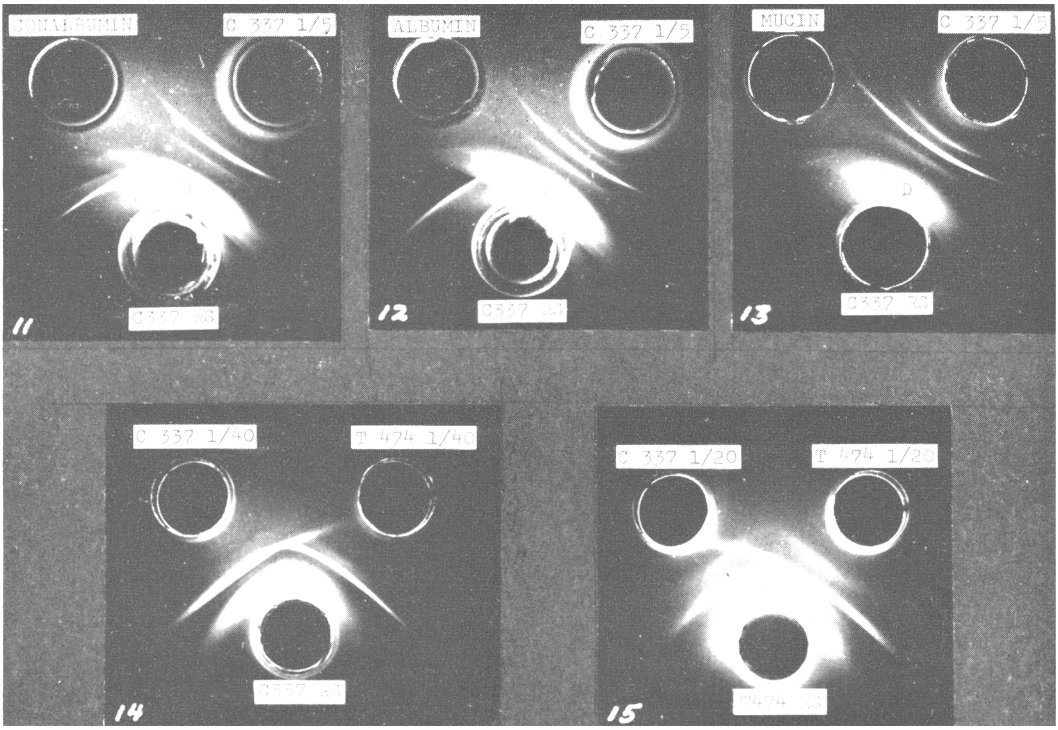

FIG. 11-13. A test demonstrating the presence of conalbumin and albumin (Fig. 11 and 12) and the probable absence of mucin (Fig. 13) in complex D.

FIG. 14 and 15. Th existence of a species difference in complex D; a spur in the complex precipitin line appears on the isogenic side of the reaction.

presence of either ovalbumin or conalbumin or both in the ovomucin fraction used in the analysis.

Discussion. Kaminski(15,17) demonstrated both by the double-diffusion agar plate method and immunoelectrophoresis the practical effectiveness of the ammonium sulfate procedure in fractionating chicken egg white proteins. According to Cohn *et al*(27), nearly all of the gamma globulin is "salted out" of the egg white when the concentration of ammonium sulfate is 34%. These workers concluded also that alpha and beta globulins, plus the remaining gamma globulin, are precipitated at the 40% saturation of ammonium sulfate. The balance of globulins is precipitated at 50%. This scheme would then indicate that fraction I in the present study (obtained at the 31% concentration of ammonium sulfate) was made up largely of gamma globulin, with some admixture of beta globulin, lacking, however, in alpha globulin. It will be recalled (Fig. 8) that fraction I was seen to be singularly deficient in the antigen responsible for the precipitin line B, the line that was absent in the agar plate analysis of the turkey egg white (Fig. 5). On the basis of the ammonium sulfate globulin fractionation procedure used in the study, the conclusion is inescapable that, in contrast to the chicken egg white, the turkey egg white lacks the alpha fraction of the globulin complex. The extent to which this "deficiency" is relevant to turkey's indifferent hatchability performance can be revealed only by further work involving, perhaps, a comparative study of the alpha globulin content of egg whites from "high" and "low" hatching chickens. Physico-chemical methods for protein analysis should prove advantageous in any comparative examination of the egg white protein structure in chicken and turkey breeding stocks selected for low embryonic mortality (*i.e.*, high hatchability). One is reminded in this regard that some evidence exists for the domestic fowl, pointing to chemically identifiable differences in birds grouped according to their reproductive efficiency and somatic growth rate(28-31).

A close immunological relationship between antigens represented by line A and, to a lesser extent, line C in the chicken and turkey, was clearly demonstrated when the egg white of both species was tested against an anti-chicken antiserum; the respective lines joined each other without any suggestion of overlapping or spur formation (*cf* Fig. 5 and 6). The same was not true for the D complex. At higher dilutions of antigens, the presence of a spur in the interaction lines between the isogenic (as to species) antigen and antibody and the lack of such a spur on the heterogenic side indicated species specific differences for that complex (*cf* Fig. 14 and 15).

Summary. 1. An immunological analysis of the egg white obtained from White Leghorn and Broad Breasted Bronze hens, and tested against both the homologous and heterologous rabbit antisera, was carried out by means of the Ouchterlony double diffusion agar plate method. 2. The turkey egg white was shown to be deficient of a protein fraction which, on the basis of the ammonium sulfate fractionation procedure, was identified as alpha globulin. With respect to other egg protein fractions, the chicken and the turkey were closely similar.

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