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Received October 22, 1965. P.S.E.B.M., 1966, v121.

Molecular Weight Differences in Chicken Isoantibodies.* (30718)

SISTER DANILE KEILY,† L. W. SCHIERMAN‡ AND A. W. NORDSKOG
(Introduced by W. F. Hollander)
Poultry Science Department, Iowa State University, Ames

A number of factors are known to influence the molecular weight of antibodies produced by an immunized animal although several questions regarding the regulation and heterogeneity of the immune response remain unanswered(1). Whether antibodies of high or low molecular weight are formed may depend, in part, on the nature and physical state of the antigen. That this may be particularly true with chickens has been reported(2,3,4). The present study was undertaken to investigate molecular weight differences in chicken isoantibodies specific for 5 blood group systems. Two of the systems are associated with histocompatibility(5). Details of the chicken blood groups may be found in recent reports (6,7).

Materials and methods. Gel filtration. Using Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Rochester, Minn.), the macroglobulins were directly separated from the lower molecular weight γ-globulins of 18 different isoantisera. That the Sephadex method is efficient for fractionating chicken as well as human sera is indicated by several studies(2,4,8,9). The Sephadex column measured 3.5 × 35 cm. Three ml of serum were placed in the column and eluted at room temperature, with a 0.1 M Tris-HCL in 0.2 M NaCl (pH 8) solution. A 0.2 M NaCl (pH 7) solution, used with some of the sera, also proved to be a satisfactory eluant. The eluate was collected in 4 ml portions with a flow rate of approximately 24 ml per hour so that elution was usually completed in 10 hours.

Antisera. The 18 sera were obtained from adult partially inbred chickens that had been hyperimmunized by giving at least 3 intravenous injections of washed erythrocytes at one-week intervals. All of the sera came from birds bled after one or more series of injections. Thus, the antisera were collected from one month to about one year after the first erythrocyte injection. Specificity of the antisera was previously determined by testing with erythrocytes from birds of known blood group genotypes.

Testing the eluate fractions. Two sera, specific for antigens of different blood group systems, were mixed and then passed through the column. Antibody activity of the eluate fractions was determined by agglutination tests. Separate tests were made with erythrocytes from 2 birds differing in blood type for the respective systems, so that each would give a positive reaction with only one antiserum. The tests were made in small tubes by using 0.05 ml of a 2% erythrocyte suspension and 0.1 ml of eluate from each collection tube. The mixture was incubated at

* Journal Paper No. J-5145 of Iowa Agr. and Home Economics Exp. Station, Ames, Project No. 1039. This work was supported in part by National Science Foundation Grant GP318.

† Summer participant in the N.S.F. Research Participation Program. Present address: Mary College, Bismarck, N. Dak.

‡ Present address: Dept. of Surgery, Mount Sinai Hospital, New York.

TABLE I. Antibody Activity of Chicken Isoantisera Following Fractionation with Sephadex G-200.

Blood group system	Serum No.	Protein peak I (High molecular wt fraction)	Protein peak II (Low molecular wt fraction)
A	1	+++*	+++
	2	+++	+
	3	+++	—
	4	++	+++
B	5	+	+++
	6	+	+++
	7	—	+++
	8	+	+++
	9	+	+++
	10	+	++
C	11	++	++
	12	+	+
D	13	+++	—
	14	+++	—
	15	+++	—
L	16	+++	—
	17	++	—
	18	++	—

* Hemagglutinating activity from tubes at center of peak: — none, + weak, ++ moderate, +++ strong.

room temperature for an hour and observed macroscopically for agglutination.

Protein determination. Presence of the two globulin and the albumin portions as they appeared in the eluate tubes, was indicated by measuring the protein concentration. One ml of eluate from each tube was mixed with 4 ml of biuret reagent and the optical density determined at 520 mμ.

Results and discussion. In the typical case, 3 fairly distinct protein peaks were represented in the eluate fractions. In a few cases, fractionation was repeated to obtain good separation. The 3 protein peaks, in order of elution, were assumed to be 19S macroglobulin (Peak I), 7S γ-globulin (Peak II) and albumin (Peak III). The molecular weights of chicken antibodies have been determined by others using Sephadex fractionation(2,4).

No hemagglutinins were found in the Peak III portion of any of the sera. Distribution of the hemagglutinating activity among the heavy and light fractions of the 18 sera is shown in Table I. For the *B* system, the antibodies were found primarily in the low molecular weight fraction. When *B* antisera were mixed with *D* or *L* antisera, prior to passing through the column, the *D* and *L* antibodies were found only in Peak I while most of the *B* antibody activity was present in Peak II. The fact that the *D* and *L* antibodies were present only in the high molecular weight fraction represents an exception to the more common observation in which 7S antibodies replace 19S antibodies upon prolonged immunization. In one case, the *D* antiserum came from the 4th bleeding of a bird that had been producing *D* antibodies for nearly a year after the initial erythrocyte injection. Although only macromolecular *D* and *L* antibodies were found in this study, further investigation may show that they may also occur as 7S antibodies.

With the *A* and *C* antisera the hemagglutinins were usually found in Peak I and Peak II. It is possible that the hemagglutinins in the eluate from the second protein fraction of serums 2 and 11 (Table I) may have been due to contamination by small quantities of 19S antibody. Studies with rabbits(10,11) indicate that 19S antibody is much more efficient than 7S antibody in causing hemagglutination. That two antibody fractions were present in serums 1, 4 and 12 was suggested by a lower titer of hemagglutinins in the eluate collected between the tubes at the center of Peak I and Peak II. Additional evidence that two fractions were present in these sera comes from the observation that the sedimentation pattern in the hemagglutination reaction is determined by the molecular weight of the isoantibody. With chicken sera 3 distinct types of positive reactions may occur when the cells are allowed to settle in the test tube. The agglutinated cells may: (*a*) spread out over the base of the tube and firmly adhere to the glass surface, (*b*) cover the base of the tube but not stick to glass, or (*c*) settle into a small uneven button without sticking to glass. In our laboratory we have observed the *a* pattern associated with *B* and *C* antisera while the *b* and *c* patterns are typical *D* and *L* reactions. The *A* antisera may be of all 3 patterns although only one is displayed by a single *A* specific serum. In the present study, with the fractionated sera, only the fraction

obtained in Peak II caused the agglutinated cells to adhere to glass. Thus, the property of sticking to glass appears uniquely associated with the 7S fraction of chicken serum.

An additional observation, in agreement with that reported for rabbit antibody(11), revealed that the incubation period necessary for agglutination is related to antibody molecular weight. For the macroglobulin fraction, strong agglutination took place in about 45 minutes, while for the low molecular weight antibodies having the same specificity, 10 minutes was sufficient.

With regard to the molecular weight of the isoantibodies, no clear-cut distinction between the histocompatibility (*B* and *C*) and the non-histocompatibility (*A*, *D* and *L*) blood group systems was found. However, the rate of 7S antibody production may be related to the antigenicity of the particular system. With our stock, nearly all individuals produce *B* antibodies upon immunization so that the *B* antigens are typically strong antigens. Relatively few individuals produce detectable *D* or *L* antibodies to the antigens we have tested. The *A* and *C* antigens in our population are intermediate in antigenicity.

Summary. Eighteen chicken blood typing antisera were fractionated into different molecular weight classes by passing through a column of Sephadex G-200 gel. These antisera, prepared by isoimmunization, were specific for antigens of 5 blood group systems. Antibody activity in sera specific for two systems (*D* and *L*) was present in the high but not the low molecular weight fraction. With *A*, *B* and *C* sera, antibody activity was usually present in both fractions although *B* antibodies were primarily of low molecular weight. The property of the agglutinated erythrocytes to stick to glass was associated with the low molecular weight chicken antibodies. Differences in the time required for strong agglutination were also associated with molecular weight. With the macroglobulins the incubation period required for agglutination was about 4 times longer than with low molecular weight antibodies.

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Received July 23, 1965. P.S.E.B.M., 1966, v121.

Effect of Plasmin on Outflow Resistance in the Primate Eye.* (30719)

MAURIZIO PANDOLFI AND TAGE ASTRUP
James F. Mitchell Foundation, Institute for Medical Research, Washington, D.C.

The mechanism regulating outflow of aqueous humor from the eye is incompletely understood. Hyaluronic acid, present in tissues of the angle of the anterior chamber of the eye, has been suggested as a regulator of filter resistance since application of hyaluronidase increases the outflow rate(1). However, hyaluronidase is active in some animal species and not in others(2). In the human eye its effect has been reported as equivocal(3). In a histochemical study of fibrinolytic active sites in the eye, the canal of Schlemm was

* Supported by grant HE-05020, USPHS, Nat. Heart Inst.