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CHICKEN IMMUNOGLOBULINS IN SERUM AND ASCITIC FLUID

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Three subclasses of chicken IgG were found, each of them contained in chicken serum and ascitic fluid. IgG1 was the main subclass of serum IgG, but only a very small quantity of IgG1 was present in ascitic fluid. The sedimentation coefficient of both IgG2 and IgG3 was approximately 6S, being smaller than that of IgG1, 7S. An immunoglobulin considered to be the chicken homologue of mammalian IgA was shown to be contained in serum and ascitic fluid, which had a sedimentation coefficient of 6.5S.

INTRODUCTION

For a number of years, workers have stated the characteristics of IgG and IgM in an avian serum[1,4,6,8,13,14,17,23]. Only 2 reports[18,26] have dealt with the subclasses of avian IgG which were found 2, while mammalian IgG has often been described to have several subclasses, such as 4 of human IgG[3]. It has been reported, meanwhile, that avian IgG differs from mammalian IgG in certain physicochemical properties[2,9,12,14,23]. The probable existence of IgA in an avian serum has been suggested in some reports[4,5,7,11,24].

In this report, the identification of 3 subclasses of chicken IgG and of an immunoglobulin considered to be chicken IgA in serum and ascitic fluid will be described.

MATERIALS AND METHODS

Chickens

White Leghorn chickens were used unless otherwise stated.

Antigens

Chicken whole serum and its globulins Sera were collected from numerous adult chickens of different sorts. Most of the sera were collected from adult White Rock chickens which had a high concentration of IgM antibodies after inoculation with *Salmonella pullorum* antigens. The sera were pooled and salted out at successive 50, 20 and 50% ammonium sulphate at pH 7.0. The resultant

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precipitate dissolved and dialyzed in phosphate-buffered saline, pH 7.5, was found to be concentrated twice as much as the original volume.

Chicken ascitic fluid globulins Adult chickens having ascites as the result of serious peritonitis (unknown the causal agents) were used for collecting ascitic fluids and the globulins were prepared by salting out as described above.

The chicken IgG and IgM preparations These preparations were kindly supplied by Dr. C. KUNIYASU (Natl Inst. Anim. Hlth, Tokyo, Japan).

Antisera

Anti-chicken whole serum This antiserum was prepared in adult rabbits by several subcutaneous injections of 5 ml of the pooled whole serum before salting out.

The anti-chicken IgG and anti-chicken IgM preparations These rabbit antisera were supplied by Dr. C. KUNIYASU.

Immunoelectrophoresis

Microimmunoelectrophoresis and the comparative immunoelectrophoresis[19] were performed in one % agarose, using barbital buffer, ionic strength =0.05, pH 8.4, on microscope slides. 1.6 mA/cm were applied for 2 hours.

Sucrose density gradient ultracentrifugation

This technique was performed essentially as described by MARTIN & AMES (1961), using a linear sucrose gradient of 5~20 %. The Hitachi 65P ultracentrifuge was used and run at 35,000 rpm for 15 hours. At the conclusion of the run, fractions of 6 drops each were immediately collected after piercing the bottom of the tube with a needle. The fractions were applied to Lowry-Folin method[15] to determine the absorbance at 750 mμ. The sedimentation coefficient was determined by the interpolation method.

SDS-polyacrylamide gel electrophoresis

This electrophoresis was performed in 7 % of acrylamide at pH 7.2 according to the method of KATO (1970). Gels were electrophoresed at 6.5 mA/tube for 6.5 hours. The molecular weight was determined by the interpolation method.

RESULTS

The 5 components of a whole serum which had a reaction of identity between the IgG preparation were shown by the comparative immunoelectrophoresis (fig. 1). The 5 components of an immunoglobulin were supposed from the anode to the cathode as IgM, 'IgA', IgG1, IgG2 and IgG3 respectively judging from the immunoelectrophoretic pattern. 'IgA' was provisionally designated for the precipitin line corresponding to the mammalian IgA line. IgG1 was considered to be the main subclass of chicken serum IgG showing the broadest precipitin line, which was identical with the IgG preparation. In the immunoelectrophoretic

test using the specific anti-γ1 made by absorbing the anti-IgG preparation, that is anti-IgG1, with an excess of the IgM preparation, γ1-chains did not share common antigenicity with IgM, 'IgA', IgG2 and IgG3. Only IgM reacted with anti-μ made by absorbing the anti-IgM with an excess of the IgG. The 5 components were sensitive to 0.3 M 2-mercaptoethanol (reduced with the 2-mercaptoethanol at room temperature for one hour, then alkylated with iodoacetamide) like mammalian immunoglobulins.

The 3 subclass precipitin lines of serum IgG were demonstrated in the above-mentioned experiments, but the only IgG1 line was formed when the whole serum used in the experiments was reacted with another sort of antiserum. The IgG2 and IgG3 lines in addition to the IgG1 line were, however, revealed in a reaction by the whole serum with the latter antiserum concentrated 3-fold.

Each serum fraction obtained by sucrose density gradient ultracentrifugation, known the sedimentation coefficients, was used in immunoelectrophoresis. The sedimentation coefficients of the fractions were applied to immunoglobulins when their precipitin lines appeared. The IgG preparation, IgG1 (given 7.1S, determined by LESLIE & CLEM, 1969), was chosen as one of the standard proteins in the interpolation method. The strongest precipitin lines of IgM, 'IgA', IgG2 and IgG3 were revealed respectively by the 25.5S, 6.2S~6.8S, 5.5S~6.2S and 5.5S~6.2S fractions.

Ascitic fluid globulins did not show the IgG1 line in the immunoelectrophoresis using anti-whole serum (fig. 2). The precipitin line of IgG1 was, nevertheless, formed with anti-seminal plasma, anti-tracheobronchial wash and anti-

TABLE 1 *Distribution of immunoglobulins in chicken serum and secretions*

ANTISERA	PREPARATIONS AND IMMUNOGLOBULIN CLASSES									
	Whole serum globulins					Ascitic fluid globulins				
	M	'A'	G1	G2	G3	M	'A'	G1	G2	G3
Anti-whole serum	+	+	+	+	+	+	+	−*	+	+
Anti-seminal plasma	−	−	+	−	−	+	+	+	−	−
Anti-tracheobronchial wash	+	+	+	−	−	+	−	+	−	−
Anti-intestinal globulins	−	+	+	−	−	−	+	+	−	−

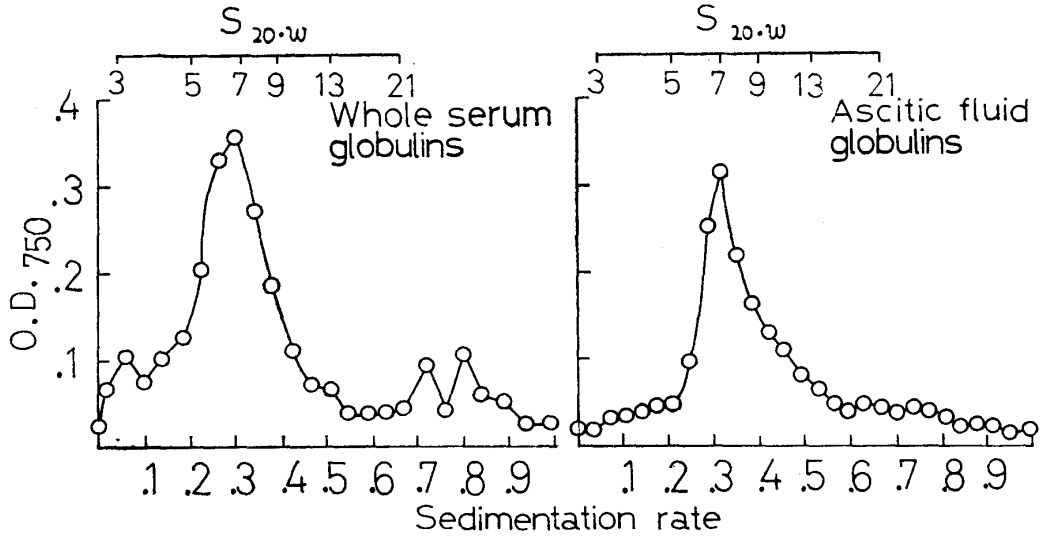
The precipitin lines were detected by immunoelectrophoresis which was performed in 3 ml of one % agarose, using barbital buffer, ionic strength = 0.05, pH 8.4, on microscope slides. 1.6 mA/cm were applied for 2 hours.

* Positive when the ascitic fluid concentrated 8-fold was used.

intestinal globulins (tab. 1), the preparation of whose antisera will be described in another report. Non-detection of the IgG1 line in ascitic fluid globulins in a reaction with anti-whole serum might be caused by the excess of antibodies. Evidence of this assumption is as follows. The IgG1 line was formed with anti-whole serum by the ascitic fluid globulins concentrated 8-fold with polyethylene glycol.

The components smaller than the sedimentation coefficient of 7S were shown to be rather poor in ascitic fluid than in a whole serum by the density ultracentrifugation (fig. 3). This finding was also confirmed by SDS-polyacrylamide

FIGURE 3 *Patterns of sucrose density ultracentrifugation*



Linear gradient 5~20 per cent Run at 35,000 rpm for 15 hours The fractions were monitered by absorbance 750 mμ by Lowry-Folin method. The sedimentation coefficients were determined by the interpolation method using a plot of the standard proteins; chicken egg albumin, 3.4S. bovine serum albumin, 4.4S. chicken serum IgG1, 7.1S.* equine catalase, 11.2S. chicken serum IgM, 17S.* (*LESLIE & CLEM, 1969).

gel electrophoresis (fig. 4). These results reflected the poorness of IgG in ascitic fluid as shown in immunoelectrophoresis.

All the 3 subclasses of IgG appeared in sera of many chickens, but on the other hand only IgG1 and IgG2 were revealed in yolk extracts (tab. 2). The IgG2 and IgG3 precipitin lines were not demonstrated when serum and ascitic fluid were reacted with anti-seminal plasma, anti-tracheobronchial wash and anti-intestinal globulins (tab. 1). IgG3 was especially noticed to be lacking in its precipitin line in a reaction by a secretion with anti-secretion, although IgG2 was

TABLE 2 *Appearance of the 3 IgG subclasses in chicken serum and yolk extract*

SOURCES	SUBCLASSES		
	IgG1	IgG2	IgG3
Whole serum	26/26	26/26	21/26
Yolk extract	24/24	23/24	0/24

The precipitin lines were detected as described in table 1. Rabbit antiserum against the pooled chicken whole serum containing a high concentration of IgM was used.
Positive sample/applied sample The applied sera and yolks were collected at random from chickens and chicken eggs respectively.

rarely demonstrated. These results suggest that the content of IgG2 and IgG3 is low in chicken yolk and secretions.

Discussion

The 3rd subclass of IgG, IgG3, was found for the first time in a chicken whole serum and ascitic fluid in the present study. Only a little IgG3 was contained in serum and acitic fluid, but was not demonstrated in yolk and secretions. As Only a very small quantity of IgG3 is present in serum, ascitic fluid, yolk and secretions of almost all chickens, non-detection of the subclass might have occurred in previous studies. This assumption was evidenced by the appearance of the IgG3 line in a reaction by a whole serum with the concentrated anti-whole serum, which did not form the subclass line before concentration. IgG1 was shown to be the main subclass of chicken serum IgG, in contrast that IgG2 is main or on a level with IgG1 in many mammalian species[3,20~22,25], and was widespread in chicken serum, ascitic fluid, yolk and secretions. The existence of 2 subclasses of IgG in chicken serum and yolk agrees with the findings reported by Wilkinson & French (1969) and Orlans & Rose (1972). The sedimentation coefficient of both IgG2 and IgG3 was approximately 6S, being smaller than that of IgG1, 7S.

The high anodal electrophoretic mobility of chicken immunoglobulins is more pronounced than in many mammalian species. This finding is especially noticeable in 'IgA' relative to IgG or IgM.

Determination of the sedimentation coefficient and molecular weight on chicken serum 'IgA' has not yet been studied. Nevertheless, a component probably corresponding to 'IgA' appeared in some reports[5,7,26] and the sedimentation coefficient might be given as approximately 7S. In the present study.

6.5S is given for the coefficient from the results of the density ultracentrifugation.

Ascitic fluid contained too small a quantity of IgG1 in spite of the fact that it was considered for the fluid to be produced as the result of infection with any pathogenic organisms. There are no sufficient reasons to interpret the finding in the present study.

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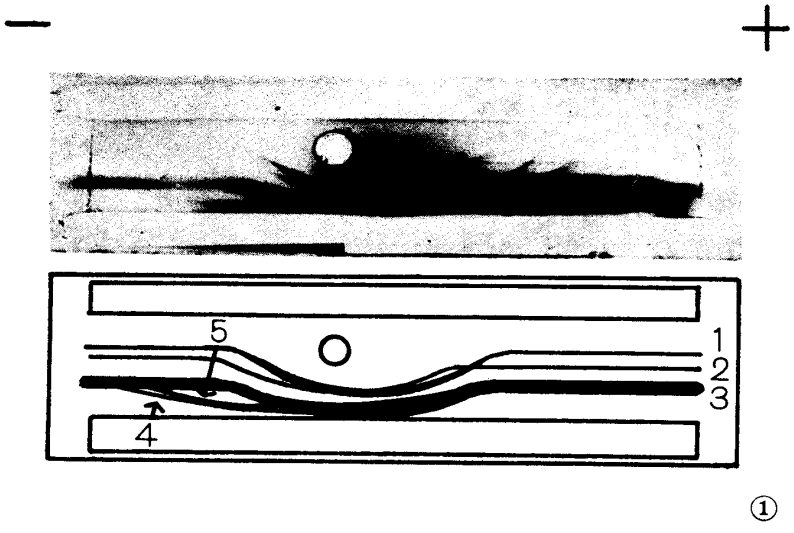
EXPLANATION OF PLATE

Fig. 1 Comparative immunoelectrophoresis of a chicken whole serum in relation to chicken serum IgG1 (the upper trough) The lower trough was filled with anti-chicken whole serum.
Performed in one % agarose, using barbital buffer, ionic strength =0.05, pH 8.4 1.6 mA/cm were applied for 2 hours.
1: IgM; 2: 'IgA'; 3: IgG1; 4: IgG2; 5: IgG3

Fig. 2 Immunoelectrophoresis of chicken ascitic fluid The upper trough was filled with anti-chicken whole serum.
The conditions were the same as fig. 1.
1: IgM; 2: 'IgA'; 3: IgG2; 4: IgG3

Fig. 4 Seven % of acrylamide was contained in the gels and 2-mercaptoethanol was not. The electrophoresis was performed at pH 7.2, 6.5 mA/tube for 6.5 hours.
The molecular weights were determined by the interpolation method; the standard proteins—chicken egg albumin, 45,000, bovine serum albumin, 67,000, chicken serum IgG1, 170,000*, equine catalase, 240,000. (* LESLIE & CLEM, 1969)



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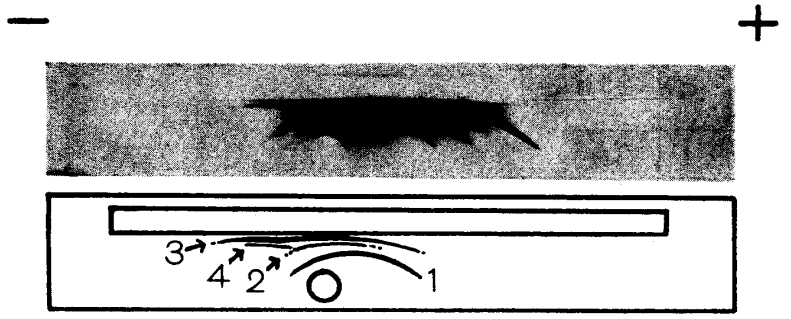

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