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## IDENTIFICATION OF IMMUNOGLOBULINS IN CHICKEN EGGS AND THEIR ANTIBODY ACTIVITY

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The presence of immunoglobulins and a secretory component (SC) in the hen's egg and oviduct washings was investigated. In addition, the existence of transferred antibodies in the yolk and white of eggs from chickens immunized with sheep red cells (SRC) was examined by using the haemagglutination test.

Immunoglobulins G, M, and A were detected in the egg yolk, the egg white, and the oviduct washings; however, no SC was detected.

The transferred antibodies for SRC were detected in the egg yolks and whites. The agglutinin activity for SRC in the egg yolks was eluted from both the IgM and IgG regions on the Sephadex G-200 gel filtration; however, in the egg whites it was detectable only in the IgM region.

### INTRODUCTION

There has been ample evidence that the transmission of passive immunity can occur only by way of the yolk, not the white, of the egg (BRAMBELL, 1970). For example, antibodies against a variety of antigens (viruses, bacteria, toxins, and bovine serum albumin) are transmissible to the chicken via the egg yolk (RAMON, 1928; BUXTON, 1952; PATTERSON et al., 1962). Recently, IgM and IgA were detected in the white of an unembryonated egg, although IgG was found only in the yolk (ROSE et al., 1974). KRAMER & CHO (1970) reported that IgG could not be detected in fresh egg albumin but appeared in the albumin from the 4th day of embryogenesis and persisted through the 16th day. Chicken oviduct washings, however, were found to contain 3 immunoglobulin classes of IgG, IgM and IgA (ORLANS & ROSE, 1972).

Our object in the present study was to detect 3 classes of immunoglobulins in the egg yolk, the egg white, and the oviduct washings of chickens and to examine the antibody activity against sheep red cells (SRC) of the immuno-

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globulins which existed in the egg yolk and the white. In addition, the presence of secretory component (SC) in the chicken samples was examined.

#### MATERIALS AND METHODS

##### Chickens

Hens were obtained from our breeding flock of White Leghorn chickens kept in isolation (MIYAMAE, 1974).

##### Preparation of samples from normal chickens

Thirty eggs from our flock were used for preparation of normal globulin samples.

Crude yolk extract was prepared from the pooled yolks by a method similar to that described by WILLIAMS (1962). The clear layer formed after high speed centrifugation of the pooled yolks homogenized in 4 volumes of 16% saturated ammonium sulphate was used as crude yolk extract in this experiment. The crude yolk extract was precipitated with 50% saturated ammonium sulphate. The resultant precipitates were dissolved and dialyzed against phosphate-buffered saline (PBS, pH 7.2), and then adjusted to 10 times the concentration of the original volume. This preparation was used for the yolk globulin solutions.

The whites were thoroughly mixed with an equal volume of PBS. The oviducts of 5 adult hens were removed and their mucosae were washed through a total of 25 ml of PBS. The white solution and oviduct washings were precipitated with 50% saturated ammonium sulphate. The resultant precipitates from the white solutions and oviduct washings were dissolved and dialyzed against the PBS and concentrated 50 and 3 times of the original volumes, respectively. These globulin solutions were used for immunodiffusion and immunoelectrophoresis.

Intestinal IgA was prepared as described by WATANABE & KOBAYASHI (1974).

##### Immunization of chickens with SRC

Five 7-month-old hens were given 4 ml of 10% SRC suspensions intravenously 3 times daily. Blood was collected from the hens every other day for the first 10 days of immunization and every 3 days thereafter. The eggs were collected every other day and stored at 4°C until used. The sera and the crude yolk extract and white globulin solutions were prepared from these eggs by the method described in the previous section, and examined in the presence of antibodies to SRC using a direct haemagglutination (HA) test as described by ORLANS (1967) with a minor modification. We used 0.5% SRC suspension instead of 0.25%. The crude yolk extract and white globulin solutions without concentration were used as starting materials for the HA test.

#### Sephadex G-200 gel filtration

One ml of serum, 2 ml of crude yolk extract (5 times concentration), and 2 ml of white globulin solutions from 5 hens immunized with SRC were applied to the top of a 2.5×90 cm column of Sephadex G-200 and eluted with 0.16 M of borate-buffered saline (pH 8.2). The elution profile was determined by measuring 4.2 ml of each eluted fraction for the optical density at 280 nm.

After gel filtration, the immunoglobulin components present in fractions I and II (described in the results) of these samples were examined to determine the nature of the immunoglobulins by treatment with 2-mercaptoethanol (2-ME) as described by DELHANTY & SOLOMON (1966), and they were heated at 65°C for 30 minutes in a water bath or absorbed with an equal volumes of anti- $\gamma$ ,  $\mu$ , or  $\alpha$ -chain sera.

#### Immunodiffusion and immunoelectrophoresis

Double diffusion was performed in phosphate buffer (pH 7.2) containing 1% agar and 3% NaCl. Immunoelectrophoresis was performed in Veronal buffer (pH 8.4,  $\mu=0.05$ ) containing 1% agar for 70 minutes at 1.6 mA/cm.

#### Immunoglobulins and antisera

Chicken IgG and IgM sera and biliary IgA were purified as described by WATANABE & KOBAYASHI (1974).

Anti-chicken whole serum and the monospecific antisera of IgG, IgM and biliary IgA were prepared in rabbits by several intramuscular injections of the mixtures of Freund's complete adjuvant (Iatron Ltd, Tokyo) and chicken whole serum, purified IgG, IgM, or biliary IgA. The  $\gamma$ ,  $\mu$ , and  $\alpha$ -chain antisera were prepared after suitable absorption of the antisera with either the IgG or IgM.

The anti-SC serum was prepared as described by WATANABE & KOBAYASHI (1974).

### RESULTS

#### Identification of immunoglobulins and secretory components in eggs and oviduct washings from normal chickens

The distribution of IgG, IgM, IgA, and SC was evaluated in the globulin solutions of chicken egg yolks, whites, and oviduct washings by means of double diffusion with anti- $\gamma$ ,  $\mu$ ,  $\alpha$  and SC sera (fig. 1). It was observed that the IgG, IgM, or IgA in each solution shared identical antigenic determinants. SC was not present in the egg yolks, whites, and oviduct washings, but it was present in the intestinal IgA. These results are summarized in table 1. In immunoelectrophoresis of each solution the IgA precipitin arcs were formed in the  $\beta$ -region with the anti- $\alpha$ -chain serum (fig. 2).

TABLE 1 *Distribution of immunoglobulins and secretory component in egg yolk, egg white, oviduct washings and intestinal IgA*<sup>\*1</sup>

ANTISERUM	SAMPLES <sup>*2</sup>			
	Egg yolk	Egg white	Oviduct washings	Intestinal IgA
Anti- $\gamma$	+	+	+	ND <sup>*3</sup>
Anti- $\mu$	+	+	+	ND
Anti- $\alpha$	+	+	+	ND
Anti-SC	-	-	-	+

<sup>\*1</sup> Determined in double diffusion with the specific antisera of H-chain and SC

<sup>\*2</sup> Prepared with 50% saturated  $(\text{NH}_4)_2\text{SO}_4$

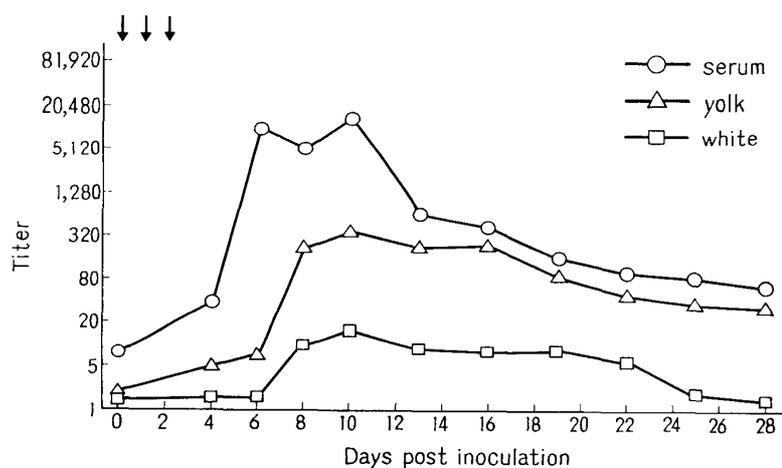
<sup>\*3</sup> ND=not done, + =detected

#### Antibody activity of chicken sera, egg yolks and whites

The HA titer (geometric mean) of the sera obtained from 5 hens inoculated with SRC and that of the crude yolk extract or white globulin solutions prepared from eggs from the same birds are shown in figure. 3.

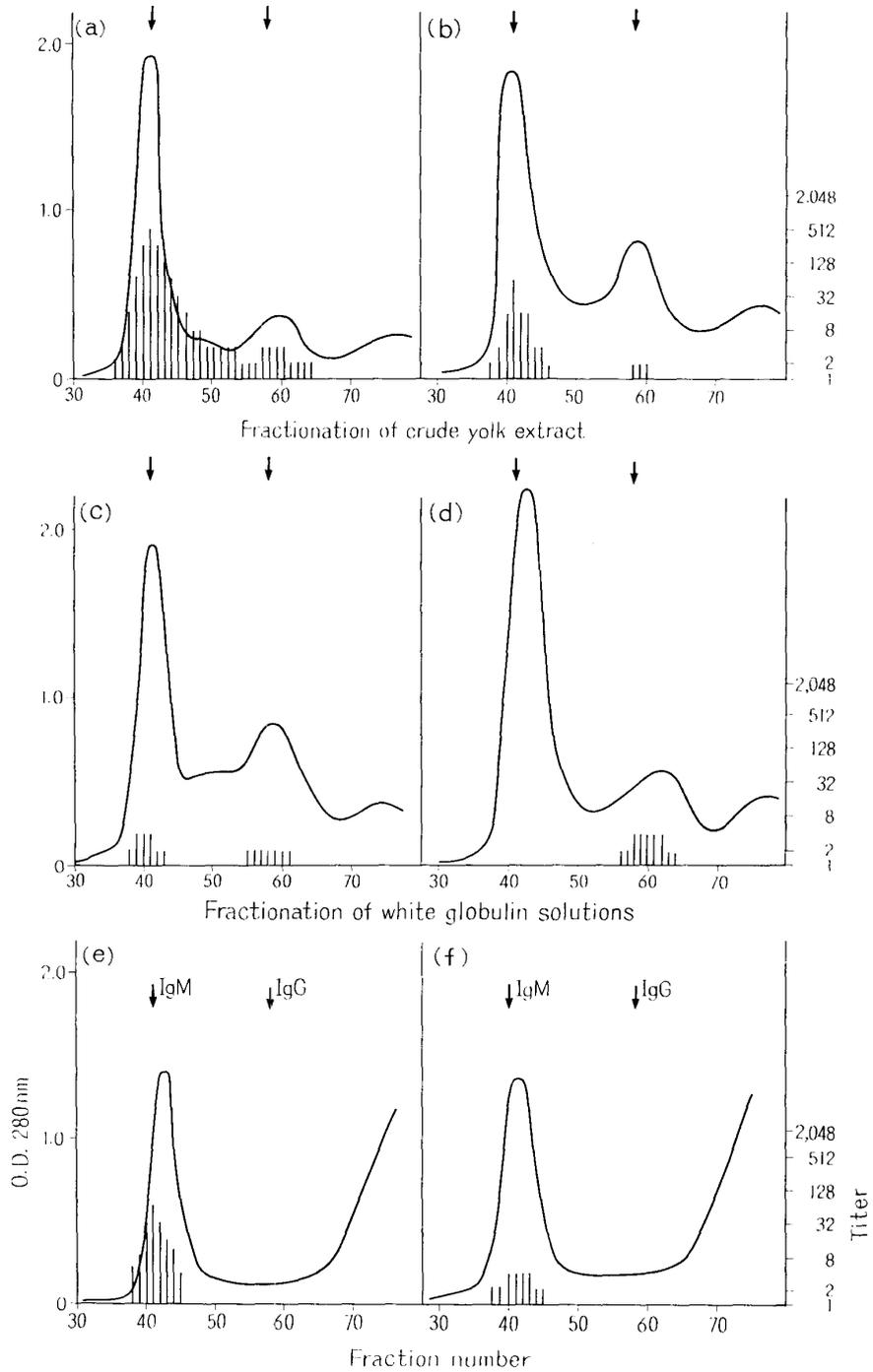
The titer of the serum increased rapidly from the 4th day of immunization and reached a peak on the 10th day. On the other hand, the titers of the egg yolk and white rapidly increased 2 days later than the serum did.

A naturally occurring antibody to SRC was detected in sera from pre-immunized chickens at a dilution of 1:10 but not in the crude yolk extract or white globulin solutions prepared from eggs of the same birds.

FIGURE 3 *Haemagglutination titer of serum, egg yolk and white*

NB Arrow indicates the time of SRC injection days.

FIGURE 4 Haemagglutination titer of Sephadex G-200  
Fractionation of serum



NB serum: 1 ml of pooled serum from five chickens sampled on the 10th (a) and 19th (b) day was applied; crude yolk extract: 2 ml of pooled crude yolk extract (concentrated 5 times) from each egg of 5 chickens collected on the 10th (c) and 19th (d) was applied; white globulin solutions: 2 ml of pooled white globulin solutions from each egg of five chickens collected on the 10th (e) and 19th (f) day was applied. fraction size: 4.2 ml; rate of flow: 8.4 ml/hours; vertical bars: HA titers of the each fractionation; arrows: the peaks of the elution profile of serum IgM and IgG

Identification of immunoglobulin classes of HA antibodies in the sera, egg yolks and whites

The sera, egg crude yolk extract and white globulin solutions were obtained from 5 chickens on the 10th and 19th day after inoculation. The preparations pooled were subjected to Sephadex G-200 gel filtration. The resulting elution profiles determined by absorption at 280 nm are shown in figure. 4 (sera: a, b, crude yolk extract: c, d, white globulin solutions: e, f). Distribution of SRC antibodies in the fractions of the serum determined by the HA test seen as two peaks coinciding closely with the first and second protein peaks, which were the IgM and IgG elution positions, respectively. The former antibody peaks were always higher than the latter.

In the crude yolk extract, on the other hand, the HA antibodies eluted at the regions equivalent to the first and second protein peaks on the 10th day, but only to the second protein peak on the 19th day.

In the white globulin solutions, HA antibody activity was distributed only in an area of the first protein peak on the 10th and 19th day, respectively.

These results indicate that there are two molecular sizes of HA antibodies in the sera and eggs, and that one of them is similar in size to IgM and the other to IgG.

Tests were made to examine the properties and classes of immunoglobulins of HA antibodies in the serum, crude yolk extract and white globulin solutions by treatment with 2-ME or heat and by the cross-absorption test.

The fractions of the first and second protein peaks of the gel filtration of the samples obtained on the 10th day both coincided closely with the 2 peaks of the HA titer, and they were separately pooled (only the first protein peaks were pooled in the case of the white globulin solutions) and designated as fraction I (fr. I) and fraction II (fr. II), respectively.

In the heat-stability test, the presence of heat-stable agglutinins was not confirmed. The agglutinins in fr. I of the serum and the crude yolk extract and white globulin solutions, and fr. II of the serum and crude yolk extract were heat-labile (tab. 2).

After 2-ME treatment, each fraction was examined for HA activities; the results are shown in table 2. The agglutinins of fr. I of the three samples were 2-ME susceptible.

The results of the HA titers of the absorbed samples in the cross-absorption experiment are shown in table 3. Absorption by specific anti- $\mu$ -chain serum exerted a great deal of influence on the HA titers of fr. I (prepared from the serum, egg yolk and white globulin solutions), while absorption by anti- $\gamma$ -chain serum decreased the titers of fr. II from the sera or egg yolk extract.

TABLE 2 *Haemagglutination activity of serum, egg yolk and egg white preparations before and after treatment with 2-ME and heat\**

TREATMENT	HAEMAGGLUTINATION TITER				
	Serum		Egg yolk		Egg white
	fr. I	fr. II	fr. I	fr. II	fr. I
Before 2-ME or heat	10,240	80	40	20	10
After 2-ME	10	40	0	10	0
After heat	80	20	10	0	0

\* Serum and eggs were sampled on the 10th day post inoculation.

TABLE 3 *Haemagglutination activity of immunoglobulins in serum, egg yolk and egg white after absorption with specific anti- $\gamma$ ,  $\mu$ , and  $\alpha$ -chain antiserum\**

ANTISERUM USED FOR ABSORPTION	HAEMAGGLUTINATION TITER				
	Serum		Egg yolk		Egg white
	fr. I	fr. II	fr. I	fr. II	fr. I
Anti- $\gamma$	1,280	40	40	5	10
Anti- $\mu$	20	80	5	20	0
Anti- $\alpha$	2,560	80	20	20	5
PBS	5,120	80	40	20	10

\* Serum and eggs were sampled on the 10th day post inoculation.

From these results, the immunoglobulin classes of HA antibodies in fr. I of the sera and eggs are considered to be mainly IgM, but those in fr. II seemed to be mostly IgG. The IgA which existed in the sera, egg yolk extract and white globulin solutions probably does little in HA activity.

#### DISCUSSION

ROSE et al. (1974) reported the presence of IgA and IgM in chicken egg white and ORLANS & ROSE (1972) detected three classes of immunoglobulin (IgG, IgM and IgA) in chicken oviduct washings. Up to date, however, there are no definite experimental findings of the existence of IgG in chicken egg whites and IgM and IgA in egg yolks.

In the present study we found the presence of three classes of immunoglobulins in concentrated preparations from egg yolk, white and oviduct washings of chickens by using double diffusion and immunoelectrophoresis with the specific antisera to each class of H-chains. The failure to find IgA and

IgM in the egg yolk and IgG in the egg white by ROSE et al. (1974) can be attributed to low concentration of their preparations.

WATANABE & KOBAYASHI (1974) reported that IgA associated with SC is only present in chicken intestinal secretions but not in other exocrine secretions. In the present experiment, SC was also present in intestinal IgA but not in egg yolk, egg white, and oviduct washings.

KRAMER & CHO (1970) demonstrated the antibody against *E. coli* and Newcastle disease virus in egg yolk and white, but they did not identify the immunoglobulin classes. DELHANTY & SOLOMON (1966) reported the completely heat-stable agglutinins 'probably IgA' in chicken serum immunized with goat red cells.

After Sephadex G-200 gel filtration of crude yolk extract and egg white globulin solutions from chickens immunized with SRC, the agglutinins for SRC were detectable in fractions of serum IgM region (fr. I) in both preparations and in those of serum IgG region (fr. II) of the crude yolk extract. However, in spite of the presence of antigenic identification of IgG in the fr. II of the egg white by immunodiffusion and immunoelectrophoresis, the absence of antibody activity in the fractions might be due to a low concentration of the agglutinins.

From the results of heat-stability and cross-absorption tests and treatment of 2-ME, the agglutinins of fr. I are probably IgM and those of fr. II are IgG.

It is reported that the transfer of IgG from the hen via the yolk to the circulation of the embryo is analogous to cross-placental transmission in mammals (ROSE et al., 1974). Furthermore, maternal IgM passes to the rabbits foetus (HEMMINGS & JONES, 1962; KAPLAN et al., 1965). Although there is some evidence that IgM in birds can reach the embryo through the yolk sac (CHO, 1970; STRATIL, 1967), this has not been definitely proved. Therefore, it is interesting to speculate the origin of IgM in egg yolk or egg white. Since we found the existence of IgM in egg yolk or white globulin solutions and the presence of SRC agglutinins in fr. I of crude yolk extract or white globulin solutions, it may be that IgM in yolk can be transferred from chicken serum through the yolk sac during development of ovarian oocytes, and that IgM in the egg white is probably transferred from the serum through the epithelial cells of the oviduct by means of secretion (PORTER & ALLEN, 1972), or diffusion. These speculations of the transfer mechanism of IgM in the egg yolk or white from chicken serum were further supported from results shown in figure. 4, in which the profiles of the HA titer of serum was similar to those of the HA titers of egg yolks or whites, and the titer of the serum increased rapidly a few days earlier than that of the yolk or white.

With regard to the origin of IgA present in egg white and oviduct washings, LEBACQ-VERHEYDEN et al. (1972) reported that numerous IgA containing cells were located underneath the epithelium of the oviduct. Their results suggested that IgA in the egg white might be transferred through the epithelium. To elucidate the origin and transferred mechanism of immunoglobulins in egg yolk and white and the absence of SC in the oviduct, immunohistochemical studies are now in progress.

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#### EXPLANATION OF PLATE

- Fig. 1 Immunodiffusion plates of chicken serum (s), egg yolk (y), egg white (w), oviduct washings (o) preparations and intestinal IgA (i). G = chicken serum IgG, M=chicken serum IgM, B=chicken bile globulins. 1, 2, 3, and 4=the  $\gamma$ ,  $\mu$ ,  $\alpha$  chain and SC antisera, respectively
- Fig. 2 Immunoelectrophoresis of chicken serum (s), egg yolk (y), egg white (w) and oviduct washings (o) preparations. a. B=the H-chain antiserum of biliary IgA prepared by absorption of the biliary IgA antiserum with IgG. Anode to the right

