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Passive Cutaneous Anaphylaxis in the Chicken

BIOLOGICAL FRACTIONATION OF THE MEDIATING ANTIBODY POPULATION

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Summary. The studies reported here demonstrated that adult chickens, when immunized with bovine serum albumin, respond with the production of serum antibodies which will mediate passive cutaneous anaphylactic (PCA) reactions in 10-day-old chicks but not in adults. This difference is at least in part due to differences in histamine sensitivity between chicks and adults. The molecule responsible for this PCA activity was indistinguishable from the predominant 7S chicken immunoglobulin antigenically and by a combination of gel filtration on Sephadex G-200 and ion exchange chromatography on DEAE-cellulose. However, the PCA activity is apparently biologically separable from portions of the 7S serum immunoglobulin population since this activity has never been elicited by immunoglobulins derived from yolk. On the basis of stability to denaturing treatments and short tissue fixation time periods, the chicken PCA-mediating antibodies appear to resemble the γG_1 homocytotrophic antibodies of mice and guinea-pigs instead of the IgE-type reagins found in some mammals. Therefore, PCA-mediating antibodies appear to be a subpopulation of the chicken 7S immunoglobulins, distinguishable by its inability to pass into the egg yolk but indistinguishable by standard chemical and immunochemical criteria.

INTRODUCTION

The chicken is of interest immunologically in that it is a submammalian vertebrate and has evolved on a line divergent from that which leads to man. The chicken has been shown to possess at least two classes of immunoglobulins. One is a high molecular weight immunoglobulin which resembles mammalian IgM and may be termed IgM. The other is a low molecular weight (7S) immunoglobulin which is commonly referred to as IgG. Recently an IgA-like class of immunoglobulin has been described in the chicken (Lebacq-Verheyden, Vaerman and Heremans, 1972; Orlans and Rose, 1972). During his evolution man has acquired five immunoglobulin classes with diversified functions. Studies on the immune responses of chickens and other lower vertebrates may lend insight into the reason(s) for the diversified multiple classes of immunoglobulins in man.

Passive cutaneous anaphylaxis (PCA) has been demonstrated in the chicken (Celada and Ramos, 1961; Conway, Van Alten and Hirata, 1968; Kubo and Benedict, 1968), and found to be specific in nature and obtainable in young birds but not in adult birds. More

recently, Ettinger, Hirata and Van Alten (1970) have shown through histological examination of reaction sites that these reactions are indeed PCA reactions and not reverse Arthus phenomena. The chicken PCA-mediating antibodies have been shown to remain fixed to skin for relatively short periods of time and electrophoretically reside in the fast γ-globulin region. In addition, antihistamines have been shown to block PCA reactions in young chicks.

The studies reported here were undertaken to further characterize chicken skin-fixing antibodies and to compare the 7S antibodies and immunoglobulins found in chicken serum and egg yolk. The studies on chicken skin-fixing antibodies were directed towards determining the following: (1) the population of molecules which mediate PCA reactions to bovine serum albumin (BSA); (2) whether this population is a previously unreported class of chicken immunoglobulins or a subclass of one of the two known chicken immunoglobulin classes; and (3) whether this population is similar or dissimilar to mammalian IgE. The comparative studies of serum and yolk 7S immunoglobulins were undertaken to determine if the whole spectrum of chicken 7S immunoglobulins are transferred to yolk or if this transfer is selective in nature as previously indicated by Orlans (1968). The main emphasis of these comparative studies was placed on the passage of antibody activity from hen to yolk and revealed a dichotomy wherein BSA-combining activity readily passed to yolk but PCA activity did not.

MATERIALS AND METHODS

Animals

Adult chickens (*Gallus domesticus*), White Leghorn hens and ruby crested Brown Leghorn roosters were acquired from the department of Poultry Science, University of Florida, Gainesville, Florida, U.S.A. Ten-day-old White Leghorn chicks were purchased from Brownlee Feed and Seed Co., Gainesville, Florida, U.S.A. Coturnix quail (*Coturnix coturnix*) were kindly donated by Dr Carlo Moscovici, V. A. Hospital, Gainesville, Florida, U.S.A. Domestic mallard ducks (*Anas platyrhynchos*) were hatched in the Division of Comparative Medicine, University of Florida, Gainesville, Florida, U.S.A.

Antigens

The antigen utilized in this study was bovine serum albumin (BSA) (Armour Co., Kankakee, Ill., U.S.A.). [^{125}I]BSA was prepared by trace labelling BSA by the chloramine T method of McConahey and Dixon (1966) with carrier-free ^{125}I (New England Nuclear, Boston, Mass., U.S.A.).

Immunizations and sample collections

Adult chickens were immunized by i.v. injection of 40 mg BSA in normal saline on days 0, 46 and 79.

Blood samples were collected by jugular venipuncture and serum was separated after standing at room temperature for 1 hour followed by overnight refrigeration. Eggs were collected on the day of laying and the yolks harvested and extracted according to the method of Aulisio and Shelokov (1967). Briefly, the entire yolk was mixed with 1 vol. of normal saline and 2 vol. of chloroform in screw cap tubes. These tubes were inverted at 1/2-hour intervals for at least 3 hours and then refrigerated overnight. The tubes were then

centrifuged at 1500 times gravity for 15 minutes at 4°. The upper saline layer was used as a 1:2 dilution of extracted yolk.

All serum and yolk samples were stored at −40° until used.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylactic (PCA) reactions were normally performed using 10-day-old chicks (for some studies these reactions were performed using 10-day-old coturnix quail and 10-day-old mallard ducks as discussed below). The reactions were carried out by injecting 0·025 ml of the sample to be tested intradermally in the lateral thoracic skin of the test animal. After a 3–4-hour latent period, except where otherwise stated, the test animal was injected i.v. with 0·5 ml of a saline solution of BSA (5 mg/ml) containing 0·5 per cent Evans' blue. The reactions were read at 15 and 30 minutes post-challenge. Definite blueing of an area >4 mm in diameter was recorded as a positive reaction. Controls include replacing test serum with either normal chicken serum or 0·15 M saline.

Determination of sensitivity to histamine

The sensitivity of young and adult chickens and guinea-pigs to i.d. injection of histamine was determined by injecting varying dilutions of histamine phosphate (Fisher Scientific, New York, N.Y., U.S.A.) intradermally. Immediately following the i.d. injections the animals were injected i.v. with 0·25 ml of a 1 per cent solution of Evans' blue. Thirty minutes after the injections, the skin sites were observed for blueing. Areas of blueing >4 mm in diameter were scored as positive reactions.

Antigen-binding capacity

Antigen-binding capacity was determined by the method of Farr (1958). In brief, serial five-fold dilutions of the samples to be tested were incubated with [^{125}I]BSA for 24 hours. The initial dilution was 1:10 in borate buffer, pH 8·0, the serial five-fold dilutions beyond this point were in 10 per cent normal chicken serum in borate buffer, pH 8·0. At the end of the 24-hour incubation period the immune complexes were precipitated with 50 per cent saturated ammonium sulphate and centrifuged at 4° for 30 minutes at 1500 ***g***. The precipitates were washed with 50 per cent saturated ammoniumsulphate and the percentage of antigen bound was determined by counting in a sodium iodide crystal scintillation counter (Baird Atomic, Cambridge, Mass., U.S.A.).

Dissociation rates of antigen–antibody complexes

Dissociation rates of selected serum and yolk samples were determined by the method of Talmage (1960) as modified by Grey (1962). Five millilitres of a dilution of the sample to be tested which would bind 30–50 per cent of a solution of [^{125}I]BSA (0·5 μg/ml) was incubated at room temperature for 18 hours with 5 ml of the [^{125}I]BSA. At the end of this incubation period, 1 ml of a solution containing 100 μg/ml unlabelled BSA was added to the reaction mixture. Duplicate 1-ml aliquots were taken at this time and at intervals thereafter. To these aliquots 1 ml of saturated ammonium sulphate was immediately added and the aliquots placed at 4° for 1 hour, then centrifuged at 1500 ***g*** for 30 minutes at 4°, washed with 1 ml of 50 per cent ammonium sulphate and counted for ^{125}I.

Fractionation of serum and yolk extract

Serum and yolk samples were fractionated by gel filtration on Sephadex G-200 employing a 2·5 × 100 cm upward flow column equilibrated with 0·15 M sodium chloride, 0·15 M Tris—HCl, pH 7·4. In some cases, fractions obtained from gel filtration were further fractionated by DEAE (diethylaminoethyl) cellulose chromatography. These columns were eluted either in a stepwise manner or with a linearly increasing sodium chloride gradient.

Antisera

The antisera to whole chicken serum and to individual chicken serum proteins utilized in this study were kindly provided by Dr Gerrie Leslie, Tulane University, School of Medicine, New Orleans, Louisiana, and were prepared as described elsewhere (Leslie and Clem, 1969).

Determination of polypeptide chain molecular weights

The molecular weights of yolk 7S immunoglobulin heavy and light chains were determined by gel filtration (Saluk and Clem, 1971). Twenty micrograms of either serum or yolk 7S immunoglobulin labelled with ^{125}I was mixed with 50 mg of the other immunoglobulin. These proteins were then extensively reduced and alkylated, and chromatographed on a 100 × 2·3-cm Sephadex G-200 column equilibrated with 5 M guanidine. The ^{125}I labelling of these proteins was also performed by the chloramine T method (McConahey and Dixon, 1966).

Determination of precipitating antibody

Samples were screened for the presence of precipitins by simple capillary tube precipitation reactions.

RESULTS

RESPONSE OF CHICKENS TO IMMUNIZATION WITH BSA

All of the adult chickens immunized as described under Materials and Methods responded with the production of precipitating antibodies and antibodies which would mediate PCA reactions in 10-day-old chicks. In general, the production of PCA mediating and precipitating antibodies was parallel.

DIFFERENTIAL SUSCEPTIBILITY OF YOUNG AND ADULT CHICKENS TO PCA REACTIONS

Both Conway *et al.* (1968) and Kubo and Benedict (1968) have reported that while young chickens are susceptible to PCA reactions, adult chickens are not. The refractory state of adult chicken to PCA reactions could be explained by one or more of the following possibilities: (1) all skin receptor sites in the adult birds are occupied by immunoglobulins produced by the adult and, therefore, passively administered antibody cannot fix to the adult skin; (2) adults are less sensitive to histamine or other vasoactive compounds than are the young (this could possibly be due to high levels of histaminase in adults); (3) in development from young to adult the chicken skin receptor sites are lost and the adult skin does not have the ability to fix antibody; or (4) mast cells in the adult are not triggered to release histamine by antigen–antibody interaction. To investigate the first two of these possibilities the following approaches were used.

First PCA reactions were attempted in adult birds by i.d. injection of a known positive serum sample ranging from 0·025 to 0·10 ml. After latent periods ranging from 3 to 24 hours, these birds were challenged by i.v. injection of 1 ml of a solution containing 5 mg/ml BSA in 0·5 per cent Evans' blue. All of these tests gave negative reactions. These results are in agreement with the earlier reports of other investigators (Conway *et al.*, 1968; Kubo and Benedict, 1968; Ettinger *et al.*, 1970), and indicate that adult chickens are refractory to PCA reactions mediated by passively administered antibody. In addition, adult chickens that were producing anti-BSA antibodies which would mediate PCA reactions in young chickens were challenged by i.d. injections of BSA, and, at the same time, given Evans' blue i.v. These reactions were negative and indicate that the adults are resistant to direct cutaneous anaphylaxis.

TABLE 1

SENSITIVITY OF YOUNG AND ADULT CHICKENS TO HISTAMINE

Histamine (μg in 0·1 ml)	Skin reactions		
	Young chickens	Adult chickens	Guinea-pigs
100	ND	5/7	6/6
10	ND	3/7	6/6
1	7/7*	3/7	6/6
0·1	7/7	0/7	6/6
0·01	0/7	0/7	6/6
0·001	0/7	0/7	3/6

* Reactions recorded as number of positive reactors (blueing > 4 mm) over number of animals tested. ND = not done.

A more sensitive technique to test the ability of animals to display PCA reactions is the challenge of skin by i.d. injection of anti-light chain or anti-7S heavy chain antisera. By this method chicken antibodies with homocytotrophic capabilities of all specificities might be expected to participate in antigen–antibody interactions and possibly trigger degranulation of mast cells. Adult chickens were injected i.d. with varying dilutions of anti-light chain and anti-7S heavy chain antisera. Immediately following the i.d. injections these birds were injected i.v. with 1 ml of 0·5 per cent Evans' blue. Skin sites were observed for blueing at 15 and 30 minutes post-challenge. All such tests were negative. Similar reactions carried out in 10-day-old chicks were also negative. Subsequent studies indicated that if the young chicks were 'primed' with adult chicken 7S immunoglobulin about 3 hours prior to the administration of rabbit anti-chicken L or H chain antisera, then strong PCA-like reactions were observed at the site of priming. The results of these studies tend to indicate that adult chicken skin either lacks receptor sites or is unresponsive to the PCA mediators liberated by antibody–antigen reactions.

To investigate the possibility that adult chickens are less sensitive to histamine than are young chickens the following experiment was undertaken. Adult and young chickens were injected i.d. with varying dilutions of histamine and immediately thereafter i.v. with Evans' blue. The same procedure was employed in guinea-pigs to compare the sensitivity to histamine of chickens and guinea-pigs. These studies indicated the young chickens to be more sensitive to histamine than were adult chickens (Table 1). In addition, both young and adult chickens were found to be much less sensitive to histamine than were guinea-pigs.

EFFECT OF LATENT PERIODS ON PCA REACTIONS

Although Kubo and Benedict (1968) have reported on the effect of latent periods on PCA reactions in young chicks, it was felt that similar information should be included in this study. The reason for this duplication was that the test animals used in this study were 10-day-old chicks while the youngest chicks used by Kubo and Benedict (1968) were 3 weeks old. To study the effect of latent periods in 10-day-old chicks, four groups of chicks were sensitized by i.d. injection of 0·025 ml of a known positive serum sample and then challenged at various times after sensitization by i.v. injection of a solution of BSA in Evans' blue. The results of this experiment (Table 2) showed that the short latent period (i.e. 4 hours) gives maximal results. This finding is in agreement with the findings of Kubo and Benedict (1968).

TABLE 2

EFFECT OF LATENT PERIODS ON PCA REACTIONS IN 10-DAY-OLD CHICKS

	Latent period (hours)	PCA reactions
Group 1	4	7/7*
Group 2	24	5/10
Group 3	48	3/10
Group 4	72	1/13

* Indicates the number of positive reactors (blueing > 4 mm in diameter) over the number of animals tested.

HETEROCYTOTROPIC EFFECTS OF CHICKEN SKIN-FIXING ANTIBODY

There are two types of mammalian immunoglobulins that have skin-fixing properties: (1) those that fix the skin of heterologous species, such as human IgG fixing to guinea-pig skin; and (2) those that fix only to homologous skin and the skin of closely related species. Celada and Ramos (1961) have reported that chicken antibodies do not fix to mouse skin. It was of interest in this study to determine if chicken antibody would fix to the skin of species more closely related to chickens than mice. For this purpose, chicken antibodies were used to produce PCA reactions in 10-day-old Japanese quail (*Coturnix coturnix*) belonging to the same order as chickens, and 10-day-old mallard ducks (*Anas platyrhynchos*) belonging to a different order than chickens. PCA reactions were carried out in these birds using various dilutions of chicken antiserum as described under Materials and Methods. The results of these studies (Table 3) show that chicken antiserum is efficient in fixing to quail skin while it does not fix to the skin of ducks, the more distantly related species. While chicken antibody will fix to quail skin, positive reactions are obtained at higher dilutions in 10-day-old chicks than in 10-day-old quail.

STUDIES OF YOLK ANTIBODIES

In mammals the transfer of antibodies across the placenta is selective in nature (Gitlen, 1966; Smith, 1966). IgG is the only immunoglobulin class which crosses the placenta. It is of special interest to this study that mammalian reagins do not cross the placenta. Several

groups of workers have shown that chicken antibodies cross into yolk (Orlans, 1968; Aulisio and Shelokov, 1967; Aulisio and Shelokov, 1969; Kramer and Cho, 1970; Malkinson, 1965; Patterson, Younger, Weigle and Dixon, 1962). Inasmuch as this transfer is analogous to placental transfer in mammals it was of interest to compare the 7S immunoglobulins found in chicken serum and yolk especially regarding the antigen-binding capacity, PCA activity, dissociation rates and size.

At no time during the course of this study was a yolk sample shown to mediate PCA reactions. This fact, taken by itself, could be explained in several ways. Yolk samples might give negative PCA reactions because: (1) they lack that population of molecules which mediates PCA reactions; (2) they have insufficient amounts of antibodies to mediate PCA reactions; (3) they contain antibodies of such low avidity that PCA reactions are not mediated, or (4) PCA-mediating antibodies are in some way altered as they cross into yolk.

TABLE 3

PCA REACTIONS IN 10-DAY-OLD QUAIL AND DUCKS COMPARED TO 10-DAY-OLD CHICKS

Sensitizing treatment	PCA reactions		
	Chicks	Quail	Ducks
0·025 ml neat*	10/10	5/6	0/3
0·025 ml 1:10 dilution	10/10	4/6	0/3
0·025 ml 1:100 dilution	8/10	0/6	ND
0·025 ml 1:1000 dilution	1/10	ND	ND

* Serum used for sensitization in these studies was from a chicken hyperimmunized with BSA.

† Reactions are recorded as the number of positive reactors (blueing >4 mm in diameter) over the number of animals tested.

ND = not done.

As it is conceivable that the extraction procedure for yolk samples was denaturing PCA activity if present, the following experiment was performed. A known positive serum sample (PCA titre of 1:200) was added to a normal yolk sample and extracted as described in Materials and Methods. After the extraction procedure this sample was used in PCA reactions and shown to retain strong PCA activity at a titre of at greater than 1:90.

Comparisons of antigen-binding capacity and PCA titres in serum and yolk samples taken from the same hens are shown in Table 4 and Fig. 1. As can be seen, the antibodies in yolk follow those in serum by about 5–6 days. This is in agreement with previous reports (Aulisio and Shelokov, 1967; Kramer and Cho, 1970; Patterson *et al.*, 1962; Orlans, 1967). The differences in antigen-binding capacity between serum and yolk samples are apparently insufficient to explain lack of PCA activity in yolk samples. In fact, two yolk samples from hen 3H had antigen-binding capacities greater than did a serum sample which had considerable PCA activity.

IDENTIFICATION OF IMMUNOGLOBULIN FRACTION CONTAINING PCA ACTIVITY

Orlans (1968) has reported that chicken PCA activity resides in the 7S immunoglobulin fraction of chicken serum. Kubo and Benedict (1968) have reported that chicken PCA activity migrates as a fast γ-globulin. It was felt that for the purposes of this study an

attempt to isolate the PCA activity from other antibody activities in immune sera should be undertaken. For this purpose a sample which had a high PCA titre (1:500) was fractionated by gel filtration on Sephadex G-200 (Fig. 2). The PCA activity was found in the 7S fraction. This was concentrated by pressure dialysis and chromatographed by

TABLE 4

COMPARISON OF ANTIGEN-BINDING CAPACITY AND PCA TITRES IN SERUM AND YOLK FROM THE SAME HEN

Sample	Reciprocal of titre binding 33 per cent antigen*	Reciprocal of PCA titre	Sample	Reciprocal of titre binding 33 per cent antigen	Reciprocal of PCA titre
Chicken 1H			Chicken 3H		
3-day serum	0†	0‡	3-day serum	0	0
7-day serum	1370	100	7-day serum	450	50
10-day serum	1090	10	10-day serum	450	1
16-day serum	120	0	16-day serum	240	1
6-day yolk	0	0	6-day yolk	0	0
7-day yolk	0	0	7-day yolk	0	0
11-day yolk	220	0	8-day yolk	2	0
13-day yolk	400	0	9-day yolk	180	0
16-day yolk	240	0	13-day yolk	360	0
18-day yolk	0	0	16-day yolk	270	0
			17-day yolk	180	0

* Antigen-binding capacity was determined at an antigen concentration of 0·5 μg BSA/ml.

† 0 indicated < 33 per cent binding with undilute sample.

‡ 0 indicates undetectable PCA activity with undilute serum sample or yolk sample that is diluted 1:2 in the extraction procedure.

stepwise elution on DEAE-cellulose. Three peaks were eluted that contained PCA activity. These peaks contained protein which reacted with antiserum to 7S immunoglobulin heavy chain. The peaks immediately preceding and immediately following the peaks with PCA activity also contained protein which reacted with anti-7S heavy chain. The PCA activity could therefore not be separated from other 7S immunoglobulins by this combination of gel filtration and ion exchange chromatography.

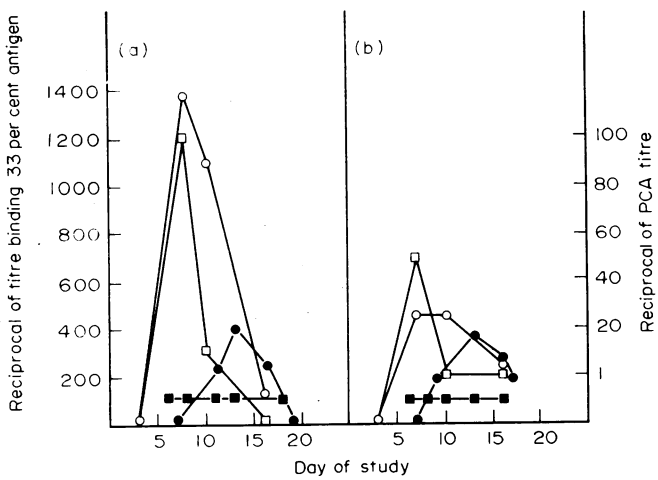

FIG. 1. Comparison of antigen-binding capacity and PCA titres in serum and yolk samples from the same hen. (a) Data from chicken 1H. (b) Data from chicken 3H. (○) Serum antigen-binding capacity; (●) yolk antigen-binding capacity; (□) serum PCA titres; and (■) yolk PCA titres.

Since the PCA activity could not be separated from other 7S immunoglobulins as above, an attempt was made to separate this activity on the basis of antigenicity. As yolk apparently contains no PCA activity it was felt that an antigenic difference might be shown between serum and yolk 7S immunoglobulins, and if such a difference was shown it might be due to the PCA-mediating population. Initially serum and yolk samples were tested by Ouchterlony technique with antiserum to 7S heavy chain. The antiserum employed showed no antigenic difference between the serum and yolk 7S immunoglobulins. To further investigate the above mentioned possibility, 7S immunoglobulins were obtained in pure form, as adjudged by immunoelectrophoresis, Ouchterlony technique, and ultracentrifugation, from serum and yolk by a combination of gel filtration and ion exchange chromatography. These proteins were used to immunize guinea-pigs by the technique of Henney and Ishizaka (1969). One group of four guinea-pigs was made tolerant to serum

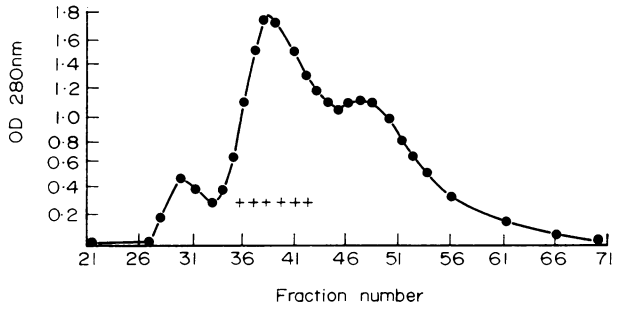

FIG. 2. Sephadex G-200 gel filtration of a chicken serum sample containing PCA activity. + + + indicates the presence of PCA activity.

7S immunoglobulins and immunized with yolk 7S immunoglobulins, another group of four was made tolerant to yolk 7S immunoglobulins and immunized with serum 7S immunoglobulins. All of the animals immunized in this manner, except one from the first group mentioned, failed to respond. This one animal responded with the production of antibodies which gave a single line of identity between yolk and serum 7S immunoglobulins. No antigenic difference between serum 7S and yolk 7S immunoglobulins was therefore detected by this approach.

EFFECT OF DENATURING TREATMENTS ON PCA ACTIVITY

The reaginic antibodies of mammals have been shown to be sensitive to various denaturing treatments such as heating or treatment with 2-mercaptoethanol (Ishizaka, Ishizaka and Menzel, 1967; Ishizaka and Ishizaka, 1968; Mota and Peixoto, 1966; Nussenzweig, Merryman and Benacerraf, 1964; Stanworth, 1963; Zvaifler and Becker, 1966). In order further to characterize chicken PCA-mediating antibodies and more fully compare these antibodies with mammalian reagins, the effect of denaturing treatments on these antibodies was studied.

In initial experiments it was discovered that chicken PCA activity is resistant to heating at 56° for periods of 30 minutes and 1 hour. As Zvaifler and Becker (1966) have shown that rabbit reagin may take as long as 4 hours of heating at 56° for complete inactivation, it was decided to do timed heating studies. Aliquots of a known positive serum sample (titre

of 1:20) were heated at 56° for periods of 1, 2, 4, 12 and 24 hours. These samples were then used for PCA reactions and determination of antigen-binding capacity. The results of these studies (Fig. 3) show that up to 4 hours of heating at 56° has no detectable effect on PCA titre, while heating for this period of time reduces the antigen-binding capacity by 6 per cent. However, additional heating for 24 hours at 56° completely abolished the PCA activity while it only decreased the antigen-binding capacity by 34 per cent. These studies show skin-fixing properties of chicken antibodies to be relatively heat stable but nonetheless more susceptible to heating than are the antigen-binding properties.

The effects of a reducing agent on PCA activity were investigated in the following manner. A known positive serum sample (titre of 1:200) was mixed with an equal volume

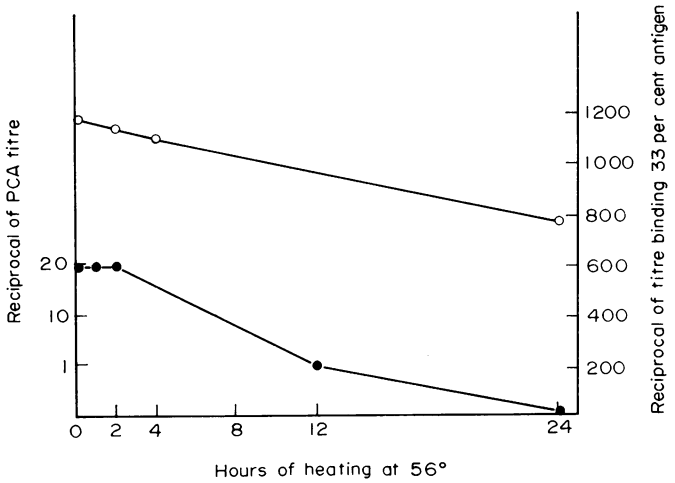

FIG. 3. Effect of heating at 56° on (●) PCA and (○) antigen-binding capacity titres of chicken antiserum to BSA.

of 0·2 molar 2-mercaptoethanol and incubated at room temperature for 1 hour. At the end of this period of time the sample was used to elicit PCA reactions in 10-day-old chicks. This treatment failed to abolish the PCA activity of the sample (the sample remained positive at a titre of 1:200).

DETERMINATION OF DISSOCIATION RATES OF ANTIBODY–ANTIGEN COMPLEXES

As stated above, one possible explanation for the lack of detectable PCA activity in yolk samples is that only low avidity antibodies are transferred to yolk. To explore this possibility the dissociation rates of selected serum and yolk samples were determined as described in Materials and Methods. The results of these studies are presented in Fig. 4. It appears that the lack of PCA activity in yolk samples cannot be explained by a more rapid dissociation of immune complexes formed by yolk antibodies than those formed by serum antibodies (i.e. lower avidity of yolk antibody). The dissociation rates of serum and yolk samples are not greatly different, and some serum samples have even greater dissociation rates than some yolk samples.

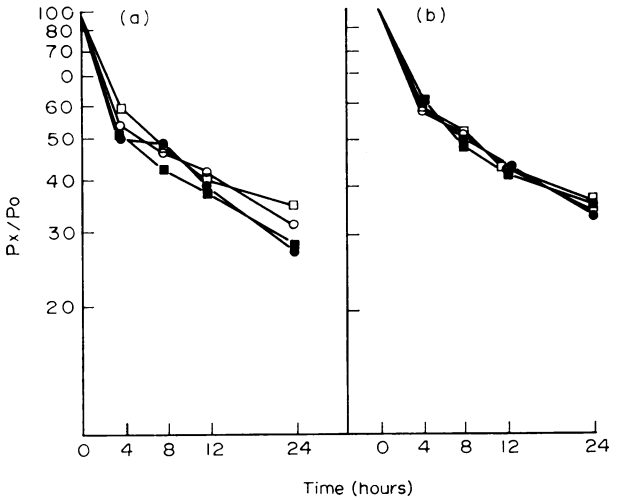

FIG. 4. Dissociation rates of serum and yolk samples from the same hen. (a) Represents samples from chicken 1H and (b) represents samples from chicken 4H. (○) 7-Day serum sample; (●) 10-day serum sample; (□) 11-day yolk sample in (a) and 13-day yolk sample in (b); and (■) 13-day yolk sample in (a) and 17-day yolk sample in (b). Px/Po = per cent antigen bound at time X.

DETERMINATION OF MOLECULAR WEIGHTS OF YOLK 7S IMMUNOGLOBULIN HEAVY AND LIGHT CHAINS

As mentioned above, alterations of PCA-mediating antibodies by passage into yolk is one possible explanation for the lack of PCA activity in yolk samples. One possible form of alteration would be the cleaving of a polypeptide from the antibody molecule as it crosses into yolk. To explore this possibility the yolk immunoglobulin heavy and light chain molecular weights were determined by gel filtration. These studies show both heavy and light chains to be the same in yolk and serum 7S immunoglobulins (Fig. 5). Therefore, if the serum immunoglobulin responsible for PCA activity is of a different molecular weight than the predominant 7S immunoglobulin its concentration must also be significantly lower.

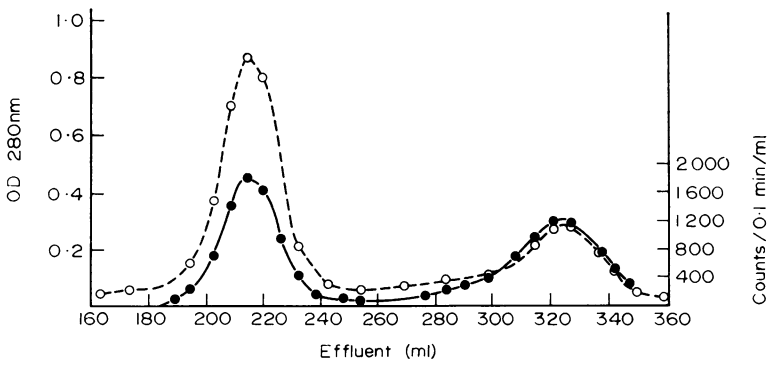

FIG. 5. Sephadex G-200 (equilibrated with 5 M guanidine-HCl) fractionation of extensively reduced and alkylated serum 7S immunoglobulin. (□) Optical density at 280 nm; and (●) counts per 0·1 min/ml.

DISCUSSION

The present study has confirmed earlier reports of a PCA-mediating population of chicken immunoglobulins (Celada and Ramos, 1961; Conway *et al.*, 1968; Kubo and Benedict, 1969), and has further characterized this population. The reagins of mammals, especially those of man, have several biological properties which set them apart from other immunoglobulins. In man, and other mammals that have been sufficiently studied, these immunoglobulins have been shown to belong to a separate class known as IgE. Among the distinguishing biological properties of mammalian IgE are: (1) its ability to mediate immediate hypersensitivity reactions; (2) its ability to remain 'fixed' to tissue receptor sites for prolonged periods (at least 6 weeks in the case of human IgE); (3) its lability to heat and other denaturing agents; and (4) its failure to cross the placenta. The results reported here and those reported by other investigators (Celada and Ramos, 1961; Conway *et al.*, 1968; Kubo and Benedict, 1968; Ettinger *et al.*, 1970) show there to be both similarities and differences between chicken PCA-mediating immunoglobulins and mammalian IgE.

Chicken PCA-mediating immunoglobulins are similar to mammalian IgE in that they mediate immediate hypersensitivity reactions, apparently are not passively transferred to the young, and give immediate hypersensitivity reactions which are blocked by antihistamines. This would suggest that the hypersensitivity reactions are mediated in the same manner in both mammals and chickens, presumably by degranulation of mast cells releasing histamine and other vasoactive compounds. The chicken PCA-mediating immunoglobulins differ from mammalian IgE in respect of several biological properties. The results reported here show chicken PCA-mediating immunoglobulins to be resistant to treatment with 2-mercaptoethanol and not particularly labile to heating. These studies also show that chicken PCA-mediating immunoglobulins do not remain fixed to tissues for extended periods (<72 hours) in contrast to mammalian IgE.

While the chicken PCA-mediating antibodies do not closely resemble mammalian IgE they do resemble PCA-mediating antibodies from some mammals. It has been shown that in guinea-pigs, rats and mice the $\gamma 1$ subclass of IgG immunoglobulins has the capacity to mediate PCA reactions (Nussenzweig *et al.*, 1964; Clausen, Munoz and Bergman, 1969; McVeigh and Voss, 1969; Morse, Austen and Block, 1969; Overy, Benacerraf and Block, 1963; Overy, Barth and Fahey, 1965; Thorbecke, Benacerraf and Ovary, 1963). These PCA-mediating antibodies have been shown to have the following characteristics: (1) they require a short latent period and do not remain fixed to tissue receptors for prolonged periods of time; (2) they are resistant to heating at 56°; (3) they are resistant to treatment with 2-mercaptoethanol; (4) they migrate with $\gamma 1$ mobility; and (5) they are a subpopulation of the major 7S class of immunoglobulins. The chicken PCA mediating antibodies have these same characteristics and, therefore, resemble these mammalian PCA-mediating antibodies instead of IgE.

Results presented in this study indicate that chicken PCA-mediating immunoglobulins are a subpopulation of the 7S immunoglobulins. The chicken 7S immunoglobulins appear to be a heterogeneous population of molecules with multiple functions including specific precipitation, skin fixing activity, and complement fixation. The work of Orlans (1967, 1968) has indicated the existence of subpopulations within the chicken 7S immunoglobulin class based on differences in resistance to heating, reduction with mercaptoethanol, salt requirement for specific precipitation, complement uptake, passive haemagglutination,

skin sensitizing activity, and transfer to yolk. Leslie (1968) has reported two populations of chicken 7S immunoglobulin based on susceptibility to proteolysis. In addition, there are two reports (Barabas and Barabas, 1969; Wilkinson and French, 1969), which show subpopulations of chicken 7S immunoglobulins based on immunoelectrophoretic properties. All of these data taken together conclusively indicate at least two, and possibly more, subpopulations which constitute the chicken 7S immunoglobulin population. These subpopulations are apt to be shown to be subclasses in the future.

Recently it has been reported that the chicken possesses an IgA-like immunoglobulin (Orlans and Rose, 1972; Lebracq-Verheyden *et al.*, 1972). It is conceivable that this immunoglobulin is responsible for the PCA activity in chicken serum samples but this is unlikely for the following reasons: (1) in mammals, IgA does not mediate immediate hypersensitivity reactions (Ishizaka and Ishizaka, 1968); (2) the anti-7S heavy chain antisera used in these studies was the same as that used by Leslie *et al.* (1971), and in their studies failed to detect IgA; and (3) Orlans and Rose (1972) report IgA to be present in crude yolk extract in levels approximating serum levels while the data obtained here showed yolk extracts to lack PCA-mediating immunoglobulins.

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