Immunology, 1974, **27**, 683.

Quantification and Distribution of Chicken Immunoglobulins IgA, IgM and IgG in Serum and Secretions

ANNE-MARIE LEBACQ-VERHEYDEN, J.-P. VAERMAN AND J. F. HEREMANS

Department of Experimental Medicine, University of Louvain, B-1200 Brussels, Belgium

(*Received 21st January* 1974; *accepted for publication 13th February* 1974)

Summary. IgA was found to be present in chicken serum in a concentration of 0·33 mg/ml, thus representing less than 4 per cent of total immunoglobulins. Of this amount, about 20 per cent appeared to be monomeric, most of the rest consisting of polymers greater than dimers.

The average concentration of IgM in chicken serum was found to be 2·55 mg/ml. This comprised a small, hitherto undetected, monomeric fraction.

IgA predominated in absolute amounts over other immunoglobulins in chicken bile and intestinal secretions, but not in saliva, tears and seminal plasma. In relative terms, however, every external secretion investigated was selectively enriched in IgA, as evidenced by a (IgA secretion)/(IgA serum):(IgG secretion)/(IgG serum) ratio greater than one.

INTRODUCTION

Chicken serum has long been known to contain two classes of immunoglobulins, IgM and IgG (Leslie and Clem, 1969). We first identified a third class of fowl immunoglobulin, which we tentatively named 'Iga' on the basis of its predominance in digestive secretions and bile (Lebacq-Verheyden, Vaerman and Heremans, 1972a). This protein was renamed IgA when it was found to be present in the overwhelming majority of the intestinal plasma cells, a feature typical of mammalian IgA (Lebacq-Verheyden, Vaerman and Heremans, 1972b). These findings were soon confirmed by other authors (Orlans and Rose, 1972; Bienenstock, Perey, Gauldie and Underdown, 1972, 1973; Bienenstock, Gauldie and Perey, 1973) but were at variance with other reports (Kincade and Cooper, 1971; Leslie, Wilson and Clem, 1971; Leslie, Crandall and Crandall, 1971), according to which IgG (or IgY) would represent the secretory immunoglobulin of the fowl. More recently, however, the latter authors also correctly identified IgA in chicken serum, secretions (Leslie and Martin, 1973) and gut plasma cells (Kincade and Cooper, 1973).

The present investigation was concerned with the quantitative distribution of immunoglobulins in chicken serum and secretions before and after fractionation based on molecular size, electric charge and solubility.

MATERIALS AND METHODS

Animals

White Leghorn chickens, 7–9 weeks old, were used.

Correspondence: Dr A.-M. Lebacq-Verheyden, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS.

Sera

Individual samples and pools were collected, made 0·1 per cent in NaN_3 and stored at −20° until use. Serum globulins were salted out with 45 per cent saturated ammonium sulphate.

Secretions

Intestinal secretions were collected and the proteins, precipitable by 45 per cent saturated $(NH_4)_2SO_4$, termed 'intestinal globulins', were obtained as described previously (Lebacq-Verheyden *et al.*, 1972a).

Bile was collected post-mortem from the gall-bladder, centrifuged, extensively dialysed and further diluted with saline to twice the original volume.

Individual samples of semen, obtained by manipulation of a rooster, were pooled, centrifuged and stored at −20° until use.

One millilitre of tears and 10 ml of saliva were collected after intramuscular injection of 0·2 ml of neostigmine or carbachol (Aitken and Parry, 1972).

Antisera

The preparation of rabbit antisera specific for the heavy chains of IgM, IgA and IgG has already been described (Lebacq-Verheyden *et al.*, 1972a). Rabbit antiserum to chicken bile was obtained after several foot-pad injections of 0·1 ml of bile emulsified in Freund's complete adjuvant.

Quantitation and purification of immunoglobulins

Immunoglobulins were quantitated by radial immunodiffusion (Mancini, Carbonara and Heremans, 1965) with class-specific antisera, using purified chicken immunoglobulin standards (see Discussion section). All samples were tested in triplicate. Results are expressed in mg/ml.

IgG was isolated from serum globulins (salted out with 30 per cent saturated ammonium sulphate) by DEAE–cellulose chromatography followed by two Sephadex G-200 gel filtrations (Lebacq-Verheyden *et al.*, 1972a).

IgM, as prepared according to Benedict (1967) or Leslie (Leslie and Benedict, 1968), was found to be contaminated by polymeric IgA. Most of the latter was removed by an additional zone electrophoresis on Pevikon.

IgA was isolated from bile by two successive gel filtrations, one on Sephadex G-200 and the other on Sepharose 4B. The main peak was then freed of mucinous contaminants (Alcian Blue-positive material) by zone electrophoresis on Pevikon. Details will be presented elsewhere (Lebacq-Verheyden, Vaerman and Heremans, in preparation).

The purity of the immunoglobulin samples, whose protein concentration (Biuret) had been adjusted to 30 mg/ml, was satisfactory when checked by agarose gel electrophoresis and immunoelectrophoresis using polyvalent antisera to bile or serum. Upon radial immunodiffusion, IgG or IgA samples only reacted with anti-γ or anti-α reagents, whereas the IgM preparation still contained about 0·1 mg/ml of IgA and 0·03 mg/ml of IgG. This minor correction was applied to the IgM standard. The lowest concentrations measurable by these techniques were 10 μg/ml of IgM, 10 μg/ml of IgA, and 30 μg/ml of IgG.

Fractionation procedures

Salting-out with ammonium sulphate. Four millilitres of serum were mixed at room tempera-

ture with various amounts of a 4·1 M solution of ammonium sulphate (pH 6·8) and distilled water, achieving final volumes of 10 ml and molarities in ammonium sulphate ranging from 1·0 M to 2·1 M. The mixtures were left overnight at 4° and were then centrifuged for 30 minutes at 25,000 *g*. The precipitates were carefully separated from the supernatants, washed once with 10 ml of the appropriate solution of ammonium sulphate and dissolved in 1 ml of distilled water.

DEAE–cellulose chromatography. Ten millilitres of two-fold concentrated serum were dialysed against the starting buffer, 0·01 M Tris–HCl, pH 8·0, containing 0·01 M NaCl, and applied on top of a 3 × 20 cm column of DEAE–cellulose (Whatman De32). Elution was performed with a linear gradient of increasing NaCl concentration (0·01 M–0·30 M) in the same buffer; 10-ml fractions were collected and mixed with an equal volume of a saturated $(NH_4)_2SO_4$ solution. After centrifugation, the resulting precipitates were dissolved in 1 ml of distilled water.

Zone electrophoresis on Pevikon. Ten millilitres of serum dialysed against 0·03 M Tris–HCl buffer, pH 8·0, were applied on a block of Pevikon (50 × 25 × 1 cm) and run for 24 hours in the same buffer at 4° under a voltage gradient of 9 $V.cm^{-1}$. After the run, the block was cut into 2-cm segments which were eluted with 20 ml of saline.

Gel-filtration on Sephadex G-200. Three-millilitre samples were applied on top of 2·5 × 100 cm column of Sephadex G-200 and eluted with 2 per cent NaCl buffered at pH 8·0 with 0·01 M Tris–HCl, containing 0·1 per cent NaN_3. Fractions of 3 ml were collected for quantitative analysis.

Density gradient ultracentrifugation. A linear gradient of sucrose (10–40 per cent) in borate-buffered saline (pH 8·4) was used. Two hundred microlitres of serum, containing marker proteins, *viz.* human IgG (7S), human secretory IgA (11S), human IgM (19S) and canine serum IgA (9S) were layered on top of the 5 ml gradient. The SE39 rotor of the Spinco Model L ultracentrifuge was run for 20 hours at 70,000 *g* at 4°, after which the tubes were punctured and drained from the bottom in two-drop fractions, which were assayed for their immunoglobulin contents.

Reduction and alkylation. Serum samples were reduced in 0·1 M Tris–HCl, pH 8·0, with 0·02 M dithiothreitol, followed by alkylation with 0·05 M iodoacetamide. Solid Tris was added to maintain pH at approximately 8.

RESULTS

SALTING-OUT WITH AMMONIUM SULPHATE

Chicken serum was salted out with concentrations of $(NH_4)_2SO_4$ ranging from 1·0 M to 2·1 M, and the three immunoglobulins, IgG, IgM and IgA, were quantified in the precipitates (Fig. 1). When the recovery of each immunoglobulin was expressed as a percentage of its concentration in the original serum (Fig. 1a),IgA appeared to be much more soluble than IgM or IgG. However, when the results were expressed in mg/ml (Fig. 1b), it was obvious that the small amounts of IgA present in serum did not allow an extensive use of this differential solubility for isolation purposes.

DEAE–CELLULOSE CHROMATOGRAPHY AND ZONE ELECTROPHORESIS ON PEVIKON

DEAE–cellulose chromatography of chicken serum resulted in a poor separation of

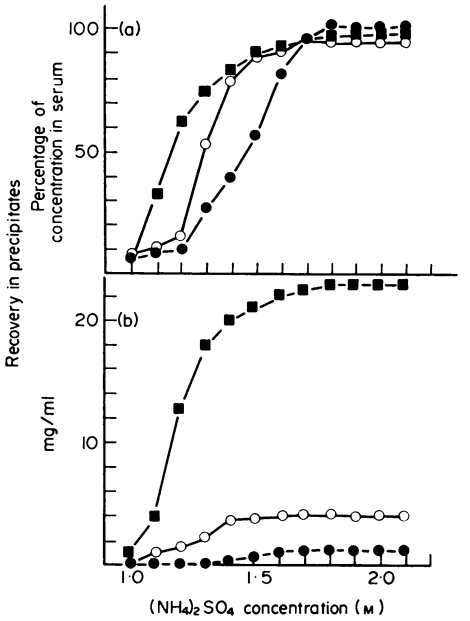

FIG. 1. Solubility of serum immunoglobulins in ammonium sulphate solutions. Quantitation of chicken serum IgG (■), IgM (○) and IgA (●) fractions salted out at various molarities in ammonium sulphate. Recoveries of immunoglobulins are expressed both as percentages of the original concentration (a) in serum and (b) in mg/ml.

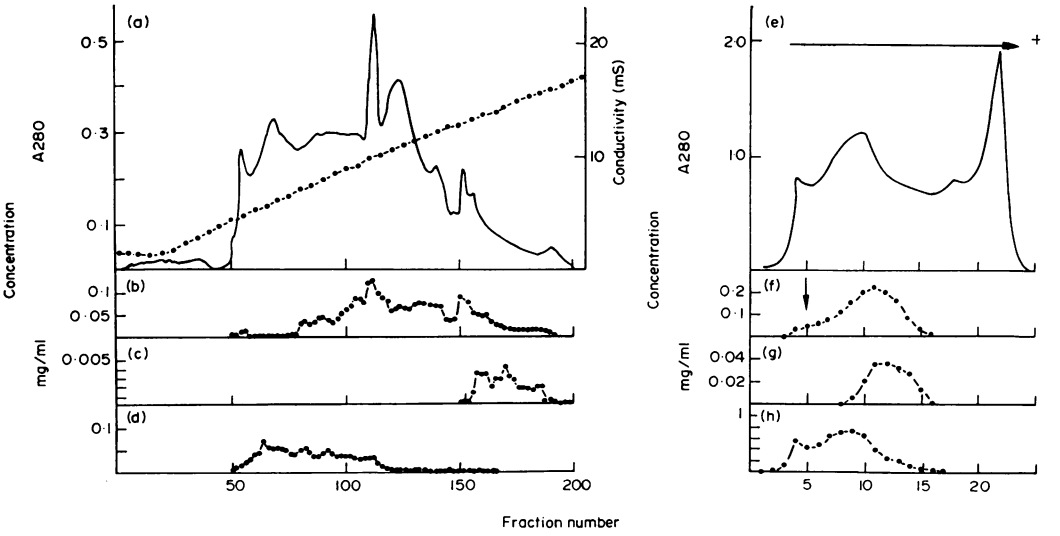

FIG. 2. Electric charge of serum immunoglobulins. (a)–(d) DEAE–cellulose chromatography and (e)–(h) block electrophoresis on Pevikon of chicken serum, with indication of the distributions of (b) and (f) IgM, (c) and (g) IgA and (d) and (h) IgG. Conductivity is expressed in milli-siemens (mS). The arrow indicates the point of application of sample.

proteins, unless large amounts of eluting buffer were used (Fig. 2a). In contrast to mammalian IgG, very little fowl IgG was obtained at the lowest ionic strengths, and this was associated with some IgM. Small amounts of both immunoglobulins persisted even in the last eluates of the gradient, which contained IgA.

Zone electrophoresis of chicken serum on Pevikon yielded similar results (Fig. 2b). IgM was already detected in the most cathodal fractions, whereas IgA was more anodically restricted. IgG and IgM were distributed throughout the α_2, β and γ electrophoretic fractions.

GEL FILTRATION ON SEPHADEX G-200 AND SUCROSE DENSITY GRADIENT ULTRACENTRIFUGATION

Serum

Fractionation of serum on a Sephadex G-200 column yielded three protein peaks (Fig. 3a). IgM was eluted, as expected, in the first peak; however, a small amount of IgM was

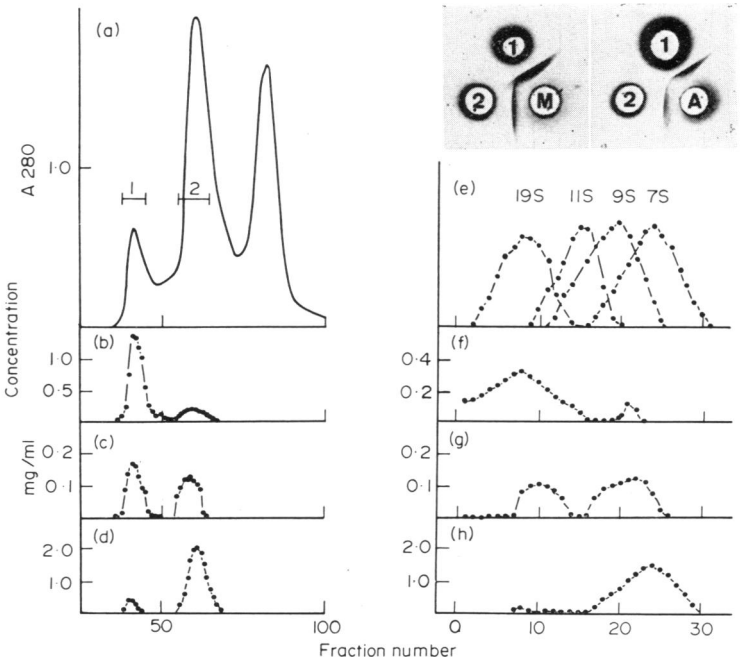

FIG. 3. Molecular size of serum immunoglobulins. (a)–(d) Sephadex G-200 chromatography and (c)–(h) sucrose density gradient ultracentrifugation of chicken serum, with indication of the distributions of (b) and (f) IgM, (c) and (g) IgA and (d) and (h) IgG. Markers: 19S = human IgM; 11S = human secretory IgA; 9S = canine serum IgA; 7S = human IgG.

Insert: Ouchterlony analyses of pooled and concentrated fractions 1 and 2 of Sephadex G-200, with anti-μ (M), and anti-α (A) sera. Note the spur of IgM 19S over that of IgM 7S.

consistently detected in the ascending part of the second peak. IgA was eluted roughly at the same locations. The first IgA fraction seemed predominant in most, but not all, of the serum pools tested. IgG was chiefly eluted in the second peak, though traces of heavy IgG—probably aggregates—were also present in the first peak. Immunochemical analyses of the pooled and concentrated fractions 1 and 2 revealed that upon immunoelectropho-

resis (not shown), the 'light' IgM was cathodically restricted. On an Ouchterlony plate, 'heavy' IgM slightly spurred over 'light' IgM, indicating the presence of some additional antigenic determinants (possibly J chain) in the polymeric molecule (Fig. 3, insert). No evidence of spurring of 'heavy' IgA over 'light' IgA could be detected (Fig. 3, insert).

Similar results were obtained after density gradient ultracentrifugation (Fig. 3b). The bulk of IgM and IgG sedimented with their corresponding human counterparts. The minor fraction of IgM and the two fractions of IgA were not exactly associated with any of the markers used, although they sedimented close to the 7S and 19S positions. They will hereafter be termed IgM≃19S, IgM≃7S, IgA≃19S and IgA≃7S. When the serum

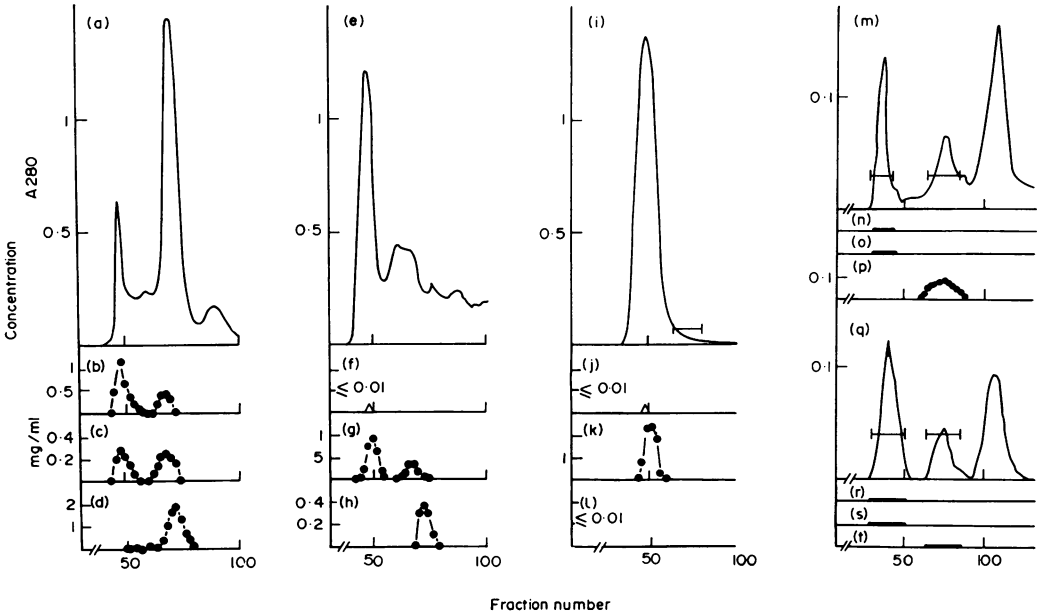

FIG. 4. Molecular size of immunoglobulins of chicken secretions. Sephadex G-200 chromatography of (a)–(d) serum globulins, (e)–(h) intestinal globulins, (i)–(l) bile proteins, (m)–(p) seminal plasma and (q)–(t) saliva on a column previously calibrated with serum globulins. The horizontal bars show the localization of immunoglobulins after pooling and concentration of the corresponding fractions. (b), (f), (j), (n) and (r) IgM. (c), (g), (k), (o) and (s) IgA. (d), (h), (l), (p) and (t) IgG.

samples were run for 15 hours instead of 20 hours, a fraction of IgM and IgA sedimented much faster than the 19S marker. Similar fractionation of chicken serum after reduction and alkylation in neutral buffer resulted in a single peak of 7S material for both IgM and IgA, with very little immunoglobulins detected in the 19S region.

To establish the relative contributions of 19S *versus* 7S serum IgA the 19S and 7S serum fractions prepared by density gradient ultracentrifugation were assayed for their IgA contents by means of radial immunodiffusion, after reduction and alkylation in neutral buffer. Reduced and alkylated serum globulins were used to construct a reference curve. In this way, IgA≃7S was found to contribute about 20 per cent to the total serum IgA concentration, whereas it appeared to contribute as much as 40–50 per cent to the mixture analysed in Fig. 3a and 3b (see Discussion section).

Secretions

Three millilitres of intestinal globulins, adjusted to 10 mg/ml, were fractionated on the Sephadex G-200 column previously calibrated with serum globulins at the same protein concentration (Fig. 4). The immunoglobulins of intestinal secretions were eluted at the same place as their serum counterparts. However, no IgM≃7S was detected, and polymeric IgA clearly predominated over the monomeric form. In fractions of bile, virtually

TABLE 1

VARIABILITY OF IMMUNOGLOBULIN LEVELS IN INDIVIDUAL AND POOLED SERA

Individual serum number	Immunoglobulins (mg/ml)		
	IgA	IgM	IgG
1	0·32	2·10	4·8
2	0·34	3·22	6·6
3	0·22	1·60	6·6
4	0·36	2·62	7·6
5	0·28	2·10	4·4
6	0·47	1·26	5·6
7	0·19	2·10	6·2
8	1·28	2·84	7·2
9	0·28	2·46	6·2
10	0·12	2·84	6·6
11	0·43	3·62	7·2
12	0·84	2·84	10·0
13	0·30	2·10	7·6
14	0·19	1·42	5·4
15	0·03	3·60	6·8
16	0·28	2·46	10·0
17	0·34	4·04	10·0
18	0·36	2·84	8·2
19	0·47	2·46	4·4
20	0·34	1·60	6·6
Mean	0·377	2·506	6·90
s.d.	0·268	0·754	1·684
s.d. of mean	0·013	0·038	0·084
Pooled sera approximately (n = 100)			
1	0·45	2·46	6·0
2	0·22	2·64	5·0
Mean	0·33	2·55	5·5

all the IgA was polymeric. The fractions of seminal plasma and saliva had to be pooled and concentrated to allow detection of IgA, which was clearly present only in the heaviest fractions. Density gradient ultracentrifugation of the intestinal globulins and bile (not shown) confirmed the results of Sephadex G-200 chromatography and in addition revealed the existence of small amounts of IgA≃7S in bile.

IgG, IgM AND IgA CONTENT OF SERUM AND SECRETIONS

The three immunoglobulins were determined in two different pools of about a hundred sera each, as well as in twenty individual samples. The results are summarized in Table 1

and illustrate the great variability of IgA levels which ranged from 0·03 to 1·28 mg/ml in individual sera and even showed different averages in the two pools. The concentrations of immunoglobulins in serum and secretions are compared in Table 2. The digestive secretions, bile and intestinal fluid, contained respectively 15·7 and 2·7 times more IgA than IgG. The corresponding ratios of secretory IgA *versus* serum IgA were 9·5 for bile and 4·5 for intestinal globulins.

TABLE 2

COMPARISON OF SERUM AND SECRETORY IMMUNOGLOBULINS

	Immunoglobulins (mg/ml)			Ratio	Ratio of (Ig secretion)/(Ig serum)			IgA secretion/IgA serum
	IgA	IgM	IgG	IgA/IgG	IgA	IgM	IgG	IgG secretion/IgG serum
Serum (mean of pools)	0·33	2·55	5·50	0·06				
Serum globulins (10 mg/ml)	0·37	2·43	5·30	0·07				
Intestinal globulins (10 mg/ml)	1·66	<0·01	0·61	2·72	4·49*	≤0·004*	0·11*	40·8
Bile	3·15	0·28	0·20	157·5	9·54	0·11	0·04	238·5
Seminal plasma	0·03	≤0·01	0·44	0·07	0·09	≤0·004	0·08	1·1
Saliva	0·09	≤0·01	0·28	0·32	0·27	≤0·004	0·05	5·4
Tears	0·15	≤0·01	0·87	0·17	0·45	≤0·004	0·16	2·8

* Intestinal globulins were compared to serum globulins.

DISCUSSION

Our results illustrate the striking heterogeneity of the fowl serum immunoglobulins. Purification of any single class of immunoglobulins by current fractionation procedures was found to be very difficult. Immunoadsorption of serum or bile on class-specific antibodies might be envisaged, but has not been found entirely satisfactory in our hands (in preparation).

An interesting finding was the identification of three major forms of IgA and IgM, differing in molecular weight, namely IgA>19S, IgA≃19S, IgA≃7S, IgM>19S, IgM≃19S, IgM≃7S. The relative contributions of serum polymeric (19S and >19S) and monomeric (7S) IgA were measured on the pooled 19S and 7S serum fractions obtained after density gradient ultracentrifugation. Since reduction and alkylation in neutral buffer was shown to convert virtually all serum IgA into 7S subunits (also demonstrated by Leslie and Martin, 1973), we measured the relative contributions of polymeric and monomeric IgA by comparing the 19S and 7S serum fractions in their reduced and alkylated status. Thus, during radial immunodiffusion, all the standard and test samples contained immunoglobulins in the same state of aggregation, thereby eliminating the difficulty that IgA polymers yield smaller precipitate rings than do monomers (Tomasi and Bienenstock, 1968; Brandtzaeg, Fjellahger and Gjeruldsen, 1970; Van Munster, 1972). IgA≃7S did not contribute more than 20 per cent to the total serum IgA concentration. This proportion

may however be different in individual chicken sera, as shown by unpublished work. Our quantitations of IgA in serum and intestinal globulins are somewhat overestimated since we used biliary IgA$\simeq$19S as a standard.

IgM consisted mainly of heavy material. The small amount of lightweight chicken IgM encountered in our study has apparently not been reported before. It appeared very similar to the '7S non-haemagglutinating subunit of IgM antibody' obtained by Leslie and Benedict (1968) after mild reduction of purified IgM with 0·02 M cysteine. Accidental cleavage of polymeric IgM after collection of the blood was excluded as a possible origin of monomeric IgM in our samples, since monomeric IgM was found to occur in fresh serum treated with 10^{-3} M iodoacetamide (not mentioned in Results section).

Monomeric IgM has already been observed in human sera by several authors (reviewed by Solomon and McLaughlin, 1970). In addition, biosynthetic studies have demonstrated that human IgM$\sim$7S is neither an *in vitro* breakdown product nor a result of IgM$\sim$19S intra-cellular catabolism (Solomon and McLaughlin, 1970). The 'IgM' of primitive vertebrates like the sea lamprey, the dogfish and the lemon shark also exists in monomeric and polymeric forms (Marchalonis and Edelman, 1965, 1966; Clem and Small, 1967). As the immunoglobulins of the sea lamprey have non-covalently linked L-chains (Marchalonis and Edelman, 1966) like human IgA2 (Grey, Abel, Yount and Kunkel, 1968) and chicken IgA (Vaerman, Lebacq-Verheyden and Heremans, submitted for publication), some of these ancestral forms of immunoglobulins might be related to IgA rather than to IgM.

To conclude, IgA represents less than 4 per cent of total chicken immunoglobulins, among which it exists in predominantly polymeric form. In chicken bile, saliva, intestinal fluid and tears, the secretion *versus* serum ratio for IgA is always much higher than that of IgG, in conformity to what has been established in a variety of species. The fundamental association of IgA with mucous surfaces herewith appears to extend to classes of vertebrates other than mammals.

ACKNOWLEDGMENTS

This work was supported by Grant number 10013 from the Fonds de la Recherche Fondamentale Collective and Grant number 1192 from the Fonds de la Recherche Scientifique Médicale (Brussels, Belgium). One of us (A.-M.L.) is Aspirant du Fonds National de la Recherche Scientifique (Brussels, Belgium).

The excellent technical assistance of Mrs A. Derijck-Langendries was highly appreciated as well as the competent editorial assistance of Mr M. Delory.

REFERENCES

AITKEN, I. D. and PARRY, S. H. (1972). 'Stimulation of lachrymation and salivation in the adult fowl (*Gallus domesticus*).' *Res. vet. Sci.*, **13,** 482.

BENEDICT, A. A. (1967). *Purification of Chicken Immunoglobulins* (ed. by C. A. Williams and N. W. Chase), p. 235. Academic Press, New York.

BIENENSTOCK, J., GAULDIE, J. and PEREY, D. Y. E. (1973). 'Synthesis of IgG, IgA, IgM by chicken tissues: immunofluorescent and ^{14}C amino acid incorporation studies.' *J. Immunol.*, **111,** 1112.

BIENENSTOCK, J., PEREY, D. Y. E., GAULDIE, J. and UNDERDOWN, B. J. (1972). 'Chicken immunoglobulin resembling γA.' *J. Immunol.*, **109,** 403.

BIENENSTOCK, J., PEREY, D. Y. E., GAULDIE, J. and UNDERDOWN, B. J. (1973). 'Chicken γA: physicochemical and immunochemical characteristics.' *J. Immunol.*, **110,** 524.

BRANDTZAEG, P., FJELLANGER, I. and GJERULDSEN, S. T. (1970). 'Human secretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins.' *Scand. J. Haemat.*, supplement **12,** 83.

CLEM, L. W. and SMALL, P. A. (1967). 'Phylogeny of immunoglobulin structure and function. I. The immunoglobulins of the lemon shark.' *J. exp. Med.*, **125,** 893.

GREY, H. M., ABEL, C. A., YOUNT, W. J. and KUNKEL, H. G. (1968). 'A subclass of human γA-globulins (γA_2) which lacks the disulfide bonds linking heavy and light chains.' *J. exp. Med.*, **128,** 1223.

KINCADE, P. W. and COOPER, M. D. (1971). 'Development and distribution of immunoglobulin-containing cells in the chicken.' *J. Immunol.*, **106,** 371.

KINCADE, P. W. and COOPER, M. D. (1973). 'Immunoglobulin A: site and sequence of expression in developing chicks.' *Science*, **179,** 398.

LEBACQ-VERHEYDEN, A.-M., VAERMAN, J.-P. and HEREMANS, J. F. (1972a). 'A possible homologue of mammalian IgA in chicken serum and secretions.' *Immunology*, **22,** 165.

LEBACQ-VERHEYDEN, A.-M., VAERMAN, J.-P. and HEREMANS, J. F. (1972b). 'Immunohistologic distribution of the chicken immunoglobulins.' *J. Immunol.*, **109,** 652.

LESLIE, G. A. and BENEDICT, A. A. (1968). 'Non-hemagglutinating non-dissociable 7S subunits of chicken IgM antibody.' *Proc. Soc. exp. Biol. (N.Y.)*, **128,** 1012.

LESLIE, G. A. and CLEM, L. W. (1969). 'Phylogeny of immunoglobulin structure and function. III. Immunoglobulins of the chicken.' *J. exp. Med.*, **130,** 1337.

LESLIE, G. A., CRANDALL, R. B. and CRANDALL, C. A. (1971). 'Studies on the secretory immunologic system of fowl. II. Immunoglobulin-producing cells associated with mucous membranes.' *Immunology*, **21,** 983.

LESLIE, G. A. and MARTIN, L. N. (1973). 'Studies on the secretory immunologic system of fowl. III. Serum and secretory IgA of the chicken.' *J. Immunol.*, **110,** 1.

LESLIE, G. A., WILSON, H. R. and CLEM, L. W. (1971). 'Studies on the secretory immunologic system of fowl. I. Presence of immunoglobulins in chicken secretions.' *J. Immunol.*, **106,** 1441.

MANCINI, G., CARBONARA, A. O. and HEREMANS, J. F. (1965). 'Immunochemical quantitation of antigens by radial immunodiffusion.' *Immunochemistry*, **2,** 235.

MARCHALONIS, J. J. and EDELMAN, G. M. (1966). 'Phylogenetic origins of antibody structure. III. Antibodies in the primary response of the sea lamprey, *Petromyzon marinus*.' *J. exp. Med.*, **127,** 891.

MARCHALONIS, J. J. and EDELMAN, G. M. (1965). 'Phylogenetic origins of antibody structure. I. Multichain structure of immunoglobulins in the smooth dogfish (*Mustelis canis*).' *J. exp. Med.* **122,** 601.

ORLANS, E. and ROSE, M. E. (1972). 'An IgA-like immunoglobulin in the fowl.' *Immunochemistry*, **9,** 833.

SOLOMON, A. and MCLAUGHLIN, C. L. (1970). 'Biosynthesis of low molecular weight (7S) and high molecular weight (19S) immunoglobulin M.' *J. clin. Invest.*, **49,** 150.

TOMASI, T. B. and BIENENSTOCK, J. (1968). 'Secretory immunoglobulins.' *Advanc. Immunol.*, **9,** 1.

VAERMAN, J.-P., LEBACQ-VERHEYDEN, A.-M. and HEREMANS, J. F. (1974). 'Absence of disulfide bridges between heavy and light chains in IgA from chicken bile.' *Immunol. Comm.* (In press.)

VAN MUNSTER, P. J. J. (1972). '*De secretoire component.*' Thesis, Schippers, Nijmegen.