Immunogenic and antigenic epitopes of immunoglobulins

I. CROSS-REACTIVITY OF MURINE MONOCLONAL ANTIBODIES TO HUMAN IgG WITH THE IMMUNOGLOBULINS OF CERTAIN ANIMAL SPECIES

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Summary. Antibody-producing hybridoma clones have been isolated following immunization of mice with human IgG. Twenty-five monoclonal antibodies (nine anti-Cγ3, fourteen anti-Cγ2, one anti-κ and one anti-λ) were selected for study of their cross-reactivity with the IgG of fifteen mammalian species and chicken immunoglobulin. Each antibody exhibited a unique reaction profile suggesting that human IgG expresses a very large repertoire of immunogenic epitopes. Whilst some antibodies showed a very restricted cross-reactivity profile for others a very wide reactivity profile was observed—including two clones producing autoantibodies. Antibodies demonstrating cross-reactivity between human Fcγ and 7S chicken immunoglobulin allow its definitive assignment as a homologue of human IgG. Four clones demonstrated specificity for bovine IgG subclass γ1 and γ2 and the degree of reactivity allows their application to qualitative and quantitative assay systems. These studies suggest new perspectives for the characterization of immunoglobulins and the standardization of anti-immunoglobulin reagents.

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INTRODUCTION

A number of studies of the cross-reactivity of conventional polyclonal antisera with homologous proteins derived from different animal species have been reported. The degree of cross-reactivity usually reflects the phylogenetic relationship between the species studied and structural homology between individual antigens; thus yielding information on species and protein evolution. A broad and systematic study of cross-reactivity between human and animal plasma proteins provided a basis for assignment to evolutionary groups (Bauer, 1970, 1971a, 1974).

Cross-reactivities of anti-immunoglobulin towards immunoglobulins of different species have been reported, (Bauer, 1970, 1971b; Esteves & Binaghi 1972; Neoh, Jahoda, Rowe & Voller 1974; Vaerman, Heremans & Van Kerckhoven, 1969) and these have been exploited to detect, isolate and define Ig classes and subclasses, (Porter, 1979). Thus Leslie & Martin (1978) recently defined IgD in the chicken, using an antiserum raised in the frog to human IgD; however, the same group earlier proposed that the predominant 7S serum antibody in the chicken be designated IgY on the basis that no antigenic cross-reactivity with human IgG could be detected (Leslie & Clem, 1969).

When the primary and tertiary structure of the protein under study is available it may be possible to

define structurally the epitopes responsible for cross-reactivity. Such detailed studies have been made of cytochrome C, (Urbanski & Margoliash, 1977), sperm whale myglobin (Atassi & Habeeb, 1977), lysozyme (Atassi, 1978) and serum albumin (Sakata & Atassi, 1980).

A most important but unfortunately variable factor in studies of antigenic cross-reactivity of animal immunoglobulins has been the antiserum employed (Bauer, 1974; Arnon & Geiger, 1977). The advent of monoclonal antibodies gives new impetus to such studies since the expression of individual epitopes may be examined using specific, defined and reproducible reagents.

The present report demonstrates that individual monoclonal antibodies to human IgG have characteristic profiles of cross-reactivity with IgG derived from a wide variety of species. Certain antibodies allow the definition of IgG in the chicken and others show specificity for bovine $\gamma 1$ and $\gamma 2$ IgG subclasses. Several antibodies exhibit cross-reactions that demonstrate that they have potential for application to the qualitative and quantitative analysis of IgG in species other than man.

MATERIALS AND METHODS

Sera and IgG preparations
Gorilla, baboon and New World monkey sera were kindly supplied by Dr A. Voller, Nuffield Laboratories of Comparative Medicine, Institute of Zoology, London. IgG1 and IgG2 were isolated from adult bovine serum essentially according to the method of Porter (1971) but using DEAE-sephacel rather than DEAE cellulose. Purity was demonstrated by polyacrylamide gel electrophoresis and immunoelectrophoresis using specific antisera raised for $\gamma 1$, $\gamma 2$ and α-chain Porter (1971).

Chicken IgG was isolated according to the method of Leslie & Clem (1969) and purity checked using rabbit anti-γ, α and μ-chain antisera (Porter & Parry, 1976). All other sera were obtained locally. IgG was isolated following dialysis against 0·03 M phosphate buffer, pH 7·0 and passage over a column of DEAE cellulose equilibrated to the same buffer.

Monoclonal antibodies
Monoclonal antibodies were raised against human IgG by the technique of Kohler & Milstein (1975). The detailed laboratory protocol is published elsewhere

(Lowe, Hardie, Jefferis, Ling, Drysdale, Richardson, Rayundalia, Catty, Appleby, Drew & MacLennan, 1981). Monoclonal antibody products were selected by their ability to agglutinate sheep red blood cells sensitized with the immunogen and immunoglobulin fragments. Antibodies shown to be specific to the Fcγ fragment, were further tested for their ability to agglutinate pFc'-coated cells. Antibodies giving a positive titre were assumed to be directed against the C$\gamma 3$ homology region. Antibodies reactive with Fcγ but not pFc' were assumed to be directed against epitopes expressed within the C$\gamma 2$ homology region or dependent on the structural integrity of the Fc fragment (Table 1). Monoclonal antibodies directed against the kappa (6el) and lambda (C4) light chains were also included in the study.

Polyclonal sheep anti-human Fc antiserum was obtained from the IDRL laboratory of this department and is available from Seward Laboratory, Blackfriars Road, London SE1 9UG.

Turbidimetric titrations
The protocol for difference turbidimetric analysis was essentially as previously reported (Steensgaard, Jacobsen, Lowe, Hardie, Ling & Jefferis, 1980). Turbidity was determined at 260 nm using a Gilford 2000 recording spectrophotometer and tandem mixing cells. Reactions were carried out in 0·15 M sodium chloride and 5% polyethylene glycol mol. wt 3000 on mixing an antigen solution (1 ml) containing 0·04 mg/ml of IgG with an antibody solution (1 ml) containing antibodies F7c (10 μl) and G7c (10 μl).

Preparation of IgG linked red cells
Human ORh + red cells were trypsinized according to the protocol of Coombs, Wilson, Eremin, Gurner, Haegert, Lawson, Bright & Munro (1977). These cells were sensitized with IgG by the chromium chloride method (Ling, Bishop & Jefferis, 1977) and titrations carried out in microtitre plates with RPMI 2% human serum albumin as diluent.

RESULTS

The haemagglutination titres ($\log_2$) of twenty-five monoclonal antibodies and a polyclonal sheep anti-human Fcγ antiserum against seventeen IgG preparations are presented in Table 1. The polyclonal antiserum was reactive, at various titres, against all

Table 1. Haemagglutination titre ($\log_2$) of IgG-sensitized human red cells by antibody

Agglutinating antibody / Species origin of IgG	Human	Gorilla	Baboon	Rhesus	New world	Horse	Donkey	Bovine γ2	Bovine γ1	Sheep	Swine	Rabbit	Cat	Guinea pig	Mouse	Rat	Chicken
Anti-Fcγ†																	
1a1	22	21	16	17	21	17	16	9	9	—	13	11	13	12	11	7	7
Anti-pFc'γ																	
OB7	13	25	15	15	5	4	6	14	9	6	6	6	5	ND	—	—	—
X1a11	14	12	10	6	10	9	10	—	—	7	—	6	4	4	—	—	—
9a6	4	6*	4*	5	—	7	1	—	ND	4*	—	8	—	5	—	3	—
3e7	7	—	1	6	—	—	—	—	—	—	—	—	4	—	—	—	—
8a4	25	16	—	13	7	—	7	5	11	7	5	4*	—	—	—	—	—
Wel0	19	13	3	7	—	4	3	—	—	3	3	6	4	—	—	—	ND
A4L	4	3	—	10	6	4	—	—	—	—	—	4	—	—	—	—	—
X1e4	4	7	—	—	10	9	—	—	—	—	6	12	—	—	8	—	—
Anti-Cγ2‡																	
E1g	9	14	6	5	12	13	11	12	9	12	12	11	3	7	4*	6	6
F7c	14	13	10	9	—	—	12	9	11	13	—	14	4	6	—	9	—
3a11	12	—	—	12	4	—	2	—	—	—	3	—	3	—	—	—	—
X6e12	2	6	6	7	—	—	—	—	—	5	—	6	3	3	—	—	—
G1b	11	—	10	17	8	—	—	—	—	—	—	4	—	—	—	—	—
HHb	15	12	—	9	8	—	—	—	—	—	8	8	3	—	—	—	—
K1d	10	—	8	—	7	>7	—	4*	8*	ND	—	ND	ND	6	—	10	9
F1d	7	6	4*	14	12	—	1	7*	8	6*	3	6	—	7	—	7	4*
F10f	10	15	10	17	—	—	—	12	19	19	—	4*	4	—	—	—	—
G7c	>29	17	10	16	—	—	7	18	8	13	15	8	—	4	—	—	—
A55	>25	19	10	10	—	—	10	9	—	14	15	8	5	—	—	—	—
WC2	22	>20	10	10	5	3	—	—	8	15	—	7	5	—	ND	—	—
WA12(§)	22	>20	10	—	8	3	7	—	12	—	5*	7	ND	—	ND	—	—
J10d	7	—	—	14	—	—	—	—	ND	7	—	—	—	—	—	—	—
aκ 6e1	17	15	10	14	12	—	7	—	—	—	—	7	6	6	—	—	—
aλ C4	29	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Weak haemagglutination.
† Polyclonal sheep anti-Fcγ antibody.
‡ Presumed specificity—these antibodies agglutinate Fcγ but not pFc'γ sensitized cells.
§ Since shown to be the same clone as WC2.

IgG preparations with the exception of the homologous sheep IgG. In contrast each monoclonal antibody has its own individual reaction profile. The only overlap was between antibodies WC2 and Wa12 produced by clones which have since been shown to be identical.

The haemagglutination titres ranged from 2 (3all) to > 29 for G7c. The ascitic fluids obtained with several clones gave a titre > 20. These high titres were confirmed by retitration following accurate external dilution by a factor of 10^5. End points > 12 were obtained demonstrating a positive haemagglutination reaction at a dilution 4×10^8. Whilst each antibody has its individual reactivity profile the composite pattern is indicated by the proportion of antibodies reactive with the IgG of a given species. Thus, for example, of the twenty-five antibodies tested the number reactive with gorilla, baboon, rhesus and new world monkeys, swine, horse, rat, chicken IgG, etc. was 19, 17, 19, 14, 11, 10, 5 and 3, respectively. However such a composite picture obscures the fundamental interest of the study and the following clones may be selected for special comment.

3e7

3e7 has a very high titre for an epitope expressed on human and rhesus IgG, but not baboon, gorilla or new world monkey IgG.

X1e4

X1e4 was reactive with an epitope expressed on human, gorilla, rhesus and new world monkey, but not baboon IgG.

E1g and F7c

E1g and F7c had an extremely wide spectrum of reactivity *including* mouse IgG.

We10 and WC2

We10 and WC2 were reactive with bovine $\gamma 1$, but not bovine $\gamma 2$.

A4L and A55

A4L and A55 were reactive with bovine $\gamma 2$, but not bovine $\gamma 1$.

C4

C4 had a very high titre against human and gorilla IgG, but was unreactive with the IgG of all other species tested.

F7c and K1d

F7c and K1d identified an IgG analogue in chicken immunoglobulin.

The antibodies F7c and G7c were applied to the technique of difference turbidimetry to determine whether they could, in combination, form insoluble complexes. The optimum conditions for development of turbidity with human IgG were determined and the IgG of the other species were used at the same concentration. As previously established (Steensgaard *et al.*, 1980) there was no development of turbidity on interaction of either of the antibodies, singly, with antigen. However, the combination F7c + G7c developed turbidity (Fig. 1) on interaction with human, baboon, gorilla, bovine $\gamma 2$, sheep ($\gamma 1 + \gamma 2$) and bovine $\gamma 1$ IgG. In contrast there was no development of turbidity with new world monkey IgG which is consistent with the haemagglutination data which showed that G7c is unreactive with the IgG of this species.

An interesting difference in the reaction characteristics of the bovine $\gamma 1$ and $\gamma 2$ preparations was apparent. Although the haemagglutination titres were essentially identical the bovine $\gamma 1$ sample exhibited slow reaction kinetics and had not reached its maximum over the duration of the experiment. However, the bovine $\gamma 2$ sample exhibited fast reaction kinetics
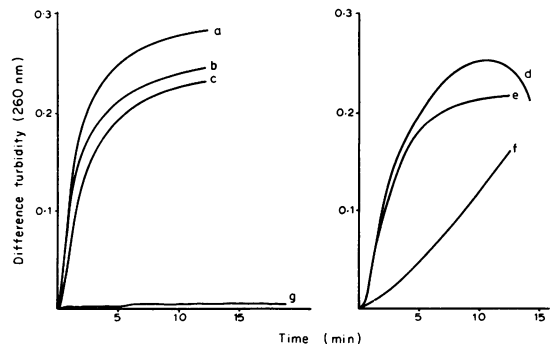
Figure 1. Difference turbidity analysis of the reaction of the antibody combination G7c and F7c with immunoglobulin G; (a) human; (b) baboon; (c) gorilla; (d) bovine $\gamma 2$; (e) sheep $\gamma 1$; (f) bovine $\gamma 1$; (g) new world monkey.

and a fall off from a transitory maximum consistent with an apparent clearing of the solution due to flocculation.

DISCUSSION

Most studies of cross-reactions between antibody elicited to human IgG and the immunoglobulins of other species have employed precipitation techniques (Bauer, 1970, 1971b; Neoh *et al.*, 1974). Such techniques may not be appropriate to monoclonal antibodies and in the present study haemagglutination has been used since the antibody-producing clones were originally identified using this assay. When a polyclonal sheep anti-Fcγ antiserum was used the titres obtained (Table 1) reflect a phylogenetic relationship between the species studied similar to that previously reported. By contrast the monoclonal antibodies exhibit a highly individual reaction profile with no apparent overlap between twenty-three distinct antibody-producing clones. The antibodies designated WC2 and Wa12(b) were initially treated as separate clones isolated from the same well of the culture plate. Subsequently identity of behaviour in all test systems including isoelectric focusing lead us to conclude that they represent a single clone. The agreement in the results obtained with these separately cloned samples demonstrates the precision and reproducibility of the assay system.

The individuality of the reaction profiles suggest that twenty-three distinct epitopes are being recognized and defined by the panel of antibodies studied. This implies that the total number of structurally distinct epitopes on human Fcγ recognized by the BALB/c mouse is very large and a considerable additional number of clones would have to be assayed before different clones recognizing identical epitopes would be encountered. Further analysis suggests that fourteen distinct epitopes expressed by the Cγ2 homology region are identified with the potential for the definition of many more. Each Cγ2 homology region is composed of approximately 110 amino acid residues and exhibits an independent tertiary structure within the Fcγ region. It might thus be compared with other proteins of similar size *viz.* lysozyme, sperm whale myoglobin and cytochrome C. Lysozyme and sperm whale myoglobin have been shown to express three and four immunogenic epitopes respectively and their main structural feature is that they constitute projections from the surface of the molecule, e.g. at bends (Atassi & Habeeb, 1977; Atassi 1978). This model is consistent with the demonstration that the combining site of an antibody molecule may be a relatively deep cleft (Poljak, Amzel & Phizacherley, 1976). However the combining site may also be a shallow groove which could accommodate and bind architecturally relatively bland topographical features which might be more numerous. The argument that antigenic structures may not be restricted to bends is supported by studies of the antigenicity of Cytochrome C (Jemmerson & Margoliash 1979) which has the structure of a tightly folded oblate spheroid with no sharp bends.

The heterogeneity of a polyclonal antibody response is in part accounted for by the production of structurally distinct molecules having differing binding affinities for the same epitope. Differences in affinity between monoclonal antibodies directed against the same epitope may be reflected in the haemagglutination titre, e.g. G7c and F1d which are present in the ascitic fluid at similar concentrations. The affinity of binding to non-homologous epitopes—cross-reactive epitopes expressed on the IgG of other species—would be less than for the homologous system so these antibodies would exhibit differing profiles of cross-reactivity. However such an explanation does not substantially reduce the number of epitopes delineated since this argument would not appear to be relevant to the sixteen high titre antibodies ($\log_2$ 10 or more) which have unique reaction profiles. In future studies the cross-reactivity profiles will be further investigated using a simple binding assay.

Individual antibodies suggest themselves for studies of the correlation of amino acid substitutions, with tertiary structural changes and epitope expression. Thus antibodies 3e7 and C4 have a very high titre against epitopes that are restricted in their expression across the species studied. The antibodies E1g and F7c are of particular interest as they represent autoantibodies in the mouse and are directed against epitopes expressed by a wide variety of species IgG and must recognize a very conserved structural feature of the IgG molecule. The specificity of these antibodies has superficial parallels with human rheumatoid factors (Jefferis, 1980a) and such antibodies might be explored to probe and delineate this autoantigenic site of human IgG. However Jemmerson & Margoliash (1979) have suggested that autoantibodies of clinical (pathological) relevance may be directed against species specific rather than species common determinants.

In the absence of primary structural data and an

observed lack of cross-reactivity of antisera raised to human IgG with the predominant 7S immunoglobulin present in chicken serum Leslie & Clem (1969) proposed that it be designated IgY rather than IgG. However, in the present study the anti-Fcγ antibodies F7c and K1d gave titres $\log_2 6$ (1/64) and $\log_2 9$ (1/512) with chicken immunoglobulin, respectively. This provides definitive characterization of the predominant chicken serum immunoglobulin as a structural homologue of human IgG. Using carp and rabbit anti-chicken 'IgY' antisera Hadge, Feibig & Ambrosius (1980) have demonstrated a weak cross-reactivity with human IgG. However, since whole 'IgY' was used as immunogen it is not possible to conclude that antigenic cross-reactivity between epitopes expressed within the constant domain of the heavy chains have been detected. Indeed, the authors suggest that the 7S immunoglobulin of all non-mammalian species should be designated IgY. An interesting study of the cross-reactivity of a mouse monoclonal anti-mouse IgG1'a' allotype antibody (Parsons & Herzenberg, 1981) showed a similar degree of reactivity for IgG2 proteins and a component of chicken serum, this providing somewhat indirect evidence for the existence of IgG in the chicken.

Of practical interest and importance is the demonstration that antibodies We10, Wc2 and A4L, A55 show specificity for bovine γ1 and γ2 IgG subclasses, respectively. There are important functional differences between bovine γ1 and γ2 antibodies and the availability of reliable reagents would be of interest to veterinary immunologists. At present only research reagents have been reported (Butler, Canterero & McGiven 1980) which are extremely difficult and tedious to prepare and are not reproducible. A radial immunodiffusion assay specific for bovine γ1 has been developed using the A55 antibody. Antibodies G7c and F7c react with both bovine γ1 and γ2 and can be applied to turbidimetric difference analysis (Fig. 1). The characteristics of the bovine γ2 reaction indicate that this antibody combination could be used for the quantification of bovine IgG in the centrifugal analyser (Jefferis, Deverill, Ling & Reeves, 1980b) as well as by the Mancini technique. The same combination, G7c and F7c, should also be applicable to studies of primate and sheep IgG. Other antibodies or combinations of antibodies from the present panel should similarly be of value in the study of the immunoglobulins of other selected species. The specificity of the difference turbidimetry system is interestingly illustrated by the lack of development of turbidity with the

IgG of the new world monkey. From Table 1 it can be seen that whilst antibody F7c reacts with this IgG the antibody G7c does not.

These studies suggest new perspectives for the standardization of qualitative and quantitative assay procedures for immunoglobulin classes and subclasses across the vertebrate order.

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REFERENCES

ARNON R. & GEIGER B. (1977) In: Immunochemistry—An Advanced Textbook (Ed. by L. E. Glynn and M. W. Steward), p. 307–364. Wiley & Sons, Chichester.

ATASSI M.Z. (1978) Precise determination of the entire antigenic structure of lysozyme. Immunochemistry, 15, 909.

ATASSI M.Z. & HABEEB A.F.S.A. (1977) In: Immunochemistry of Proteins, Vol. 2. (Ed. by M. Z. Atassi), p. 177. Plenum Press, New York.

BAUER K. (1970) Cross-reactions between human and animal plasma proteins. I. The immunological evolution groups (IEG) I and II. Humangenetik. 8, 325.

BAUER K. (1971a) The different phylogenetic age of antigenic determinants on human L chains. Humangenetik, 12, 261.

BAUER K. (1971b) Cross-reactions between human and animal plasma proteins. IV. Non-eutheria mammalia. Humangenetik. 13, 49.

BAUER K. (1974) Cross-reactions between human and animal plasma proteins. V. The role of antiserum and immunological specificity in relation to the phenomenon of cross-reactions. Humangenetik, 21, 179.

BUTLER J.E., CANTARERO L.A. & McGIVEN P.L. (1980) Measurement of bovine sub-class antibodies (IgG1 and IgG2) using the amplified enzyme-linked immunosorbent assay. Molec. Immunol. 17, 645.

COOMBS R.R.A., WILSON A.B., EREMIN O., GURNER B.W., HAEGERT D.G., LAWSON Y.A., BRIGHT S. & MUNRO A.J. (1977) Comparison of the direct antiglobulin rosetting reaction with the mixed antiglobulin rosetting reaction for the detection of immunoglobulin of lymphocytes. J. immunol. Meth. 18, 45.

ESTEVES M.-B. & BINAGHI R.A. (1972) Antigenic similarities among mammalian immunoglobulins. Immunology, 23, 137.

HADGE D., FEIBIG H. & AMBROSIUS H. (1980) Evolution of low molecular weight immunoglobulins. I. Relationship of 7S immunoglobulins of various vertebrates to chicken IgY. Develop. Comp. Immunol. 4, 501.

JEFFERIS R. (1980a) The activity and possible significance of anti-immunoglobulins. In: Recent Advances in Clinical

Immunology, No. 2 (Ed. by R. A. Thompson) p. 45. Churchill Livingstone, London.

JEFFERIS R., DEVERILL I., LING N.R. & REEVES W.G. (1980b) Quantitation of human total IgG, kappa IgG and lambda IgG in serum using monoclonal antibodies. *J. immunol. Meth.* **39**, 355.

JEMMERSON R. & MARGOLIASH E. (1979) Specificity of antibody response to self-antigens. *Nature (Lond.)*, **282**, 468.

KOHLER G. & MILSTEIN C. (1975) Continuous culture of fused cells secreting antibody of predefined specificity. *Nature (Lond.)*, **256**, 495.

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

LESLIE G.A. & MARTIN L.N. (1978) Structure and function of serum and membrane IgD. In: *Contemporary Topics in Molecular Immunology*, Vol. 7 (Ed. by F. P. Inman), p. 1. Plenum Publishing Corporation, New York.

LING N.R., BISHOP S. & JEFFERIS R. (1977) Use of antibody coated red cells for the sensitive detection of antigen and in rosette tests for cells bearing surface immunoglobulins. *J. immunol. Meth.* **15**, 279.

LOWE J., HARDIE D., JEFFERIS R., LING N.R., DRYSDALE P., RICHARDSON P., RAYUNDALIA C., CATTY D., APPLEBY P., DREW R. & MACLENNAN I.C.M. (1981) Properties of monoclonal antibodies to human immunoglobulin kappa and lambda chains. *Immunology*, **42**, 649.

NEOH S.H., JAHODA D.M., ROWE D.S. & VOLLER A. (1974) Immunoglobulin classes in mammalian species identified by cross-reactivity with antisera to human immunoglobulins. *Immunochemistry*, **10**, 805.

PARSONS M. & HERZENBERG L.A. (1981) A monoclonal mouse antiallotype antibody reacts with certain human and other vertebrate immunoglobulins: genetic and phylogenetic findings. *Immunogenetics*, (In press.)

POLJAK R.J., AMZEL L.M. & PHIZACHERLEY R.P. 1976. Studies on the three-dimensional structure of immunoglobulins. *Prog. Biophys. molec. Biol.* **31**, 67.

PORTER P. (1971) Immunoglobulin A in bovine mammary secretion and serum of neonatal calf. *Biochem. biophys. Acta.* **236**, 664.

PORTER P. (1979) Structural and functional characteristics of immunoglobulins of common domestic species. In: *Advances in veterinary Science and comparative Medicine*, Vol. 23 (Ed. by B. I. Osburn and R. O. Schultz), p. 1 Academic Press, New York.

PORTER P. & PARRY S.H. (1976) Further characterisation of chicken IgA in serum and secretions with evidence of a possible analogue of mammalian secretory component. *Immunology*, **31**, 407.

SAKTA S. & ATASSI M.Z. (1980) Immunochemistry of serum albumin. X. Five major antigenic sites of human serum albumin are extrapolated from bovine albumin and confirmed by synthetic peptides. *Molec. Immunol.* **17**, 139.

STEENSGAARD J., JACOBSEN C., LOWE J., HARDIE D., LING N.R. & JEFFERIS R. (1980) The development of difference turbidimetric analysis for monoclonal antibodies to human IgG. *Molec. Immunol.* **17**, 1315.

URBANSKI G.J. & MARGOLIASH E. (1977) Topographic determinants on cytochrome C. 1. The complete antigenic structure of rabbit, mouse and guanaco cytochromes C in rabbits and mice. *J. Immunol.* **118**, 1170.

VAERMAN J.P., HEREMANS J.F. & VAN KERCKHOVEN G. (1969) Identification of IgA in several mammalian species. *J. Immunol.* **103**, 1421.