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REFERENCES

Barnicoat, C. R., Logan, A. G. & Grant, A. I. (1949). *J. agric. Sci.* **39**, 44.
Bloch, K. (1947). *Physiol. Rev.* **27**, 574.
Bloch, K. (1948). *Cold Spr. Harb. Sym. quant. Biol.* **13**, 29.
Bloch, K. & Kramer, W. (1948). *J. biol. Chem.* **173**, 811.
Dickens, F. & Šimer, F. (1931). *Biochem. J.* **25**, 973.
Elliott, K. A C., Benoy, M. P. & Baker, Z. (1935). *Biochem. J.* **29**, 1937.
Elsden, S. R., Hitchcock, M. W. S., Marshall, R. A. & Phillipson, A. T. (1946). *J. exp. Biol.* **22**, 191.
Elsden, S. R. & Phillipson, A. T. (1948). *Ann. Rev. Biochem.* **17**, 705.
Folley, S. J. (1940). *Biol. Rev.* **15**, 421.
Folley, S. J. (1945). In Marshall's *Physiology of Reproduction*, 3rd ed. chap. 20 (ed. Parkes, A. S.). London: Longmans, Green.
Folley, S. J. (1949). *Biol. Rev.* **24**, 316.
Folley, S. J. & French, T. H. (1948*a*). *Nature, Lond.*, **161**, 933.
Folley, S. J. & French, T. H. (1948*b*). *Biochem. J.* **42**, xlvii.
Folley, S. J. & French, T. H. (1948*c*). *Biochem. J.* **43**, lv.
Folley, S. J. & French, T. H. (1949*a*). *Nature, Lond.*, **163**, 174.
Folley, S. J. & French, T. H. (1949*b*). *Biochem. J.* **44**, xlv.
Folley, S. J. & French, T. H. (1949*c*). *Biochem. J.* **45**, 117.
Folley, S. J. & French, T. H. (1949*d*). *Biochem. J.* **45**, 270.
Graham, W. R. Jun., Houchin, O. B., Peterson, V. E. & Turner, C. W. (1938). *Amer. J. Physiol.* **122**, 150.
Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. London: Chapman and Hall.
Kleinzeller, A. (1943). *Biochem. J.* **37**, 674.
Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
McClymont, G. L. (1949). *Biochem. J.* **45**, i.
Popják, G. & Beeckmans, M.-L. (1949*a*). *Biochem. J.* **44**, xxxvi.
Popják, G. & Beeckmans, M.-L. (1949*b*). *Biochem. J.* **44**, xxxvii.
Popják, G., Folley, S. J. & French, T. H. (1949). *Arch. Biochem.* **83**, 508.
Reid, R. L. (1950). *Nature, Lond.*, **165**, 448.
Reineke, E. P., Stonecipher, W. D. & Turner, C. W. (1941). *Amer. J. Physiol.* **132**, 535.
Rittenberg, D. & Bloch, K. (1945). *J. biol. Chem.* **160**, 417.
Turner, C. W. (1939). *Sex and Internal Secretions*, 2nd ed. chap. 11 (ed. Allen, E.). London: Baillière, Tindall & Cox.

A Chemical Study of Rabbit Antiovalbumin

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There are three main theories purporting to explain the formation of antibodies in an animal in response to the injection of an antigen. All accept the principle that combination of antibody with antigen depends on the configuration of parts of the antibody molecule being complementary to parts of the antigen molecule. The theory due to Breinl & Haurowitz (1930) and Mudd (1932) postulated that the specific configuration of the antibody arose because the antigen acted as template around which the protein-synthesizing system made new antibody. Burnett (1941) offered criticism of the theory and suggested that the protein-synthesizing system itself, was modified by contact with antigen. Pauling (1940) brought forward a different theory, in which the formation of antibody was regarded as due to refolding of preformed γ-globulin, in the presence of antigen, into a new configuration complementary to that of the antigen. An apparently successful attempt to demonstrate this mode of formation *in vitro* (Pauling & Campbell, 1942) has not been confirmed (Campbell, 1948); moreover, the theory fails to explain many characteristics of immunity (Burnett, 1941) such as: (1) the persistence of antibodies in plasma for considerable periods after the last known contact with antigen; (2) the difference between primary and secondary response to injection of antigen; (3) the increase in antibody titre after bleeding of the animal; (4) the increase in concentration of non-specific serum globulin during immunization; (5) the evidence that the site of antibody formation is localized; (6) the demonstration that antibody can retain its specific action after denaturation (Erickson & Neurath, 1945).

Pauling's theory postulates only a change in the physical state during transformation of γ-globulin

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into antibody, whereas other theories permit a difference in chemical composition between inert γ-globulin and antibody globulin. A comparison of the chemical structure of antibody with that of associated globulin should provide some evidence by which to judge these theories. In the past, a variety of amino-acid analyses of antibodies and various globulin fractions from normal and immune sera have been carried out (Calvery, 1935; Smith, 1946; Smith, Greene & Bartner, 1946; Velluz, 1934). Though no doubt most of these could be criticized on the grounds of analytical technique or purity of material, the general conclusion seems justified that few, if any, differences exist in overall amino-acid composition between the active and inactive material. It is, however, possible that proteins of very similar amino-acid content could differ greatly in general structure, and advantage has therefore been taken of other techniques to investigate this problem further. In particular, end-group assay has been used to estimate the number of open polypeptide chains and the identity of the amino-acids in the terminal sequence has been established. Although only the nature of the terminal pentapeptides was established, the possibilities of variation in this fragment are so great that some inference as to comparative structure of the rest of the inert γ-globulin and antibody globulin may be drawn.

Electrophoretically pure γ-globulin was prepared from sera of rabbits strongly immunized with ovalbumin, and the chemical properties of the fraction (about one-third) combining with antigen were compared with those of the immunologically inert fraction. This seemed preferable to comparing the properties of pure antibody prepared by dissociation of specific precipitate (Heidelberger & Kendall, 1936; Haurowitz, Tekman, Bilen & Schwerin, 1947) with the γ-globulin from normal rabbits.

METHODS

Immunization of rabbits. The animals were given, twice weekly, intravenous injections of 0·5 ml. of a 5% (w/v) solution of crystalline ovalbumin in isotonic saline, for 6 weeks. After 10 days' rest the animals were bled, and the serum tested for activity. The most responsive animals were kept and given a second course of injections, the rest being discarded. In this way was acquired a stock of some twelve responsive animals which have been injected and bled regularly for more than 12 months.

Preparation of γ-globulin. It was found that the simple Na_2SO_4 fractionation of serum described by Kekwick (1940) for the preparation of human γ-globulin was equally successful in the preparation of γ-globulin from the sera of normal and immune rabbits. As would be expected, the yield was higher using immune sera, but in both cases electrophoretic examination showed the γ-globulin preparations to contain only traces of contaminants. Some loss of antibody occurred during the preparation, but attempts to avoid this by modifying the procedure led to electrophoretic impurity and were not pursued.

Flocculation tests. Tests were carried out under standard conditions in a 45° bath with indirect lighting, the tubes being only three-quarters submerged to stimulate convection.

Reduction of S–S *bonds.* Reduction was effected by dissolving Na thioglycollate in approximately 5% γ-globulin solution to give a neutral solution, 1·1 M with respect to thioglycollate.

End-group assay. Estimation of unreactive amino and iminazole groups, and determination of the terminal peptide sequence was carried out using a technique previously described (Sanger, 1945; Porter & Sanger, 1948; Porter, 1948; Sanger, 1949; Porter, 1950*a*). A collective description of these methods has been given by Porter (1950*c*).

Ovalbumin. This was a five-times recrystallized specimen given by Prof. R. K. Cannan.

RESULTS

Ovalbumin

(The abbreviations DNP and FDNB are used in this paper for dinitrophenyl and fluorodinitrobenzene.) As a preliminary to work with the specific precipitate, the free amino groups of ovalbumin were estimated. By no method of hydrolysis could a significant amount of N^1-dinitrophenyl amino-acid be obtained from ovalbumin after reaction of the protein with 1:2:4-fluorodinitrobenzene. It is therefore concluded that ovalbumin is a cyclic molecule or alternatively, that the carbohydrate, known to be present, is condensed on the N^1-amino groups. As the carbohydrate is firmly bound (Neuberger, 1938) and the N^5-amino groups of lysine react quantitatively with FDNB, the second alternative is possible.

All the iminazole groups of the histidine residues reacted with FDNB whether the protein was native or denatured (Porter, 1950*a*).

γ-Globulin

End-group assay was carried out on γ-globulin prepared from normal and immune sera when native and denatured. The specific precipitate and the inert γ-globulin, left in solution after precipitation of the antiovalbumin were also examined. In working with specific precipitate the sodium bicarbonate concentration during reaction with FDNB was lowered to 4%, as this was sufficient to maintain the pH at 8·5 and lessened the risk of dissociating the complex. Unfortunately no decisive evidence could be obtained as to whether splitting of the complex occurred during the reaction; however, the complex was insoluble in the sodium bicarbonate solution and remained so on addition of the ethanolic solution of FDNB. It is therefore unlikely that dissociation of the precipitate occurred. In calculating the result in terms of γ-globulin, allowance was made for the weight of ovalbumin present. This was estimated as described (Porter, 1950*b*). The results of these estimations are summarized in Table 1.

Table 1. *End-group assay on rabbit γ-globulin by reaction with FDNB*

Protein	Terminal amino-acid	Mol. of terminal amino-acid/protein unit of mol. wt. 160,000		No. of reactive lysine N^6-amino groups/protein unit of mol. wt. 160,000	
γ-Globulin from normal rabbit	Alanine	1·1	1·2	66	70
γ-Globulin after acid denaturation	Alanine	1·2	—	77	—
γ-Globulin from immune rabbit	Alanine	1·0	0·9	68	71
Immune γ-globulin after absorption of antibody	Alanine	1·0	1·1	73	69
Immune γ-globulin in 6M-guanidine	Alanine	1·0	—	70	—
Specific precipitate antibody	Alanine	1·0	1·0	70	75
Specific precipitate in 6M-guanidine	Alanine	1·1	—	70	—

It would seem that all the γ-globulin preparations and fractions examined, whether immunologically active or not, are identical in having one terminal alanyl residue per molecule. This presumably means that γ-globulin, if homogeneous, is a single polypeptide chain of some 1500 amino-acid residues. The possibility of cyclic structures being present cannot be ignored, but seems improbable in view of the results obtained with papain-HCN hydrolysis (Porter, 1950*b*). This treatment split the molecule, to give an active fragment having one alanyl terminal residue per unit of mol. wt. 40,000 and other fragments, none of which had this terminal residue. The active fragment was presumably the terminal quarter of the polypeptide chain and as papain-HCN produces quarter molecules from γ-globulin (Petermann, 1946) it seems unlikely that a cyclic configuration is included in the structure.

It is evident from the results given in Table 1 that the combination of antibody and antigen in the specific percipitate did not result in the blocking of the N^1-amino group or of any significant quantity of N^6-amino groups.

The normal and immune γ-globulins and specific precipitate were tested for unreactive iminazole groups with the results shown in Table 2. Comparison of these results with those obtained with

Table 2. *Reactivity of the iminazole group of γ-globulin with FDNB*

γ-globulin	Histidine content (g. histidine/100 g. protein)	Unreactive histidine content (g. histidine/100 g. protein)	Unreactive histidine as percentage total histidine
Normal	2·56	0·05	2
Immune	2·56	0·07	2·5
Specific precipitate	2·56	0·17	7

other proteins (Porter, 1950*a*) shows that in normal and immune γ-globulin the iminazole rings are in a reactive state. The specific precipitate failed to react with FDNB to the same extent when parallel experiments were run, and the difference is probably significant. Such a result indicates that one iminazole group is blocked per molecule of γ-globulin. Alternatively, since the molecular ratio of antibody to ovalbumin ~~and~~ the specific precipitate is 3:1, three iminazole groups in the ovalbumin might be blocked.

Terminal peptide sequence

The terminal peptide sequence was determined by the method of Sanger (1949). Both inert γ-globulin, left after the absorption of antiovalbumin, and specific precipitate were principally used, though other fractions were also examined. As ovalbumin has no N^1-amino groups, the specific precipitate could be regarded as pure antibody if allowance was made for the weight of antigen. A weighed amount (about 0·8 g.) of air-dried DNP-γ-globulin fraction was dissolved in A.R. 12N-hydrochloric acid and incubated at 37° for 7 days.

Fractionation of partial hydrolysate. The black solution was diluted with several volumes of water and extracted four times with ethyl acetate, the extracts being washed with small volumes of water. Colour may continue to be extracted by further amounts of ethyl acetate, but it contains no N^1-DNP peptides. The ethyl acetate solution was extracted three times with 1% $NaHCO_3$, and each $NaHCO_3$ extract washed with fresh ethyl acetate. The $NaHCO_3$ solutions were combined and acidified with HCl, extracted with ethyl acetate, and the extracts washed with N-HCl, combined and concentrated. With DNP-insulin this procedure separated N^6-DNP-lysyl peptides from N^1-DNP peptides, but with DNP-γ-globulin, where the ratio of lysyl to alanyl peptides is about 100:1, complete separation was not effected and some DNP-lysyl peptides reached the final ethyl acetate extract.

Chromatographic separation of DNP peptides. The combined ethyl acetate extracts containing N^1-DNP peptides were taken to dryness, redissolved in wet 15% butanol chloroform (*B*15) and put on a *B*15 wet silica column. The fast band was taken to dryness and re-run on a *B*5 column, the fast band was again taken to dryness and re-run on a chloroform column. The fast moving band from the *B*15 column, when run on a *B*5 frequently tailed badly, but again moved fast or with $R=1$ (*R* has been defined by Martin & Synge, 1941). The slow moving bands from the *B*15 columns, which were lysyl peptides, were discarded. On the *B*5 column the slow moving band was shown to contain N^6-DNP-lysine and was also discarded. On the chloroform column there was a trace of fast moving coloured

material, a band moving at a rate of about $R0{\cdot}5$ (A_1) another $R0{\cdot}2$ (A_2), and slower moving colour which on development with $B1$ resolved into three distinct bands, $R0{\cdot}3$ (A_3), $R0{\cdot}15$ (A_4) and $R0{\cdot}1$ (A_5). In addition, there was a little colour which moved very slowly on $B1$ columns, and was stationary on chloroform columns. This colour was present on complete hydrolysis, when DNP specific precipitate or when the DNP derivative of inert γ-globulin was used. It could not be identified as a DNP amino-acid and on hydrolysis did not give rise to an amino-acid. This suggests that it is not a peptide and is probably an artefact.

Identification of terminal peptide sequence. The different bands were collected and estimated colorimetrically (Porter, 1950*c*). The yield of each peptide was estimated by reference to a standard curve of DNP-alanine and the results are expressed as mol. of peptide per mol. of protein (Table 3).

Table 3. *Yields of terminal DNP peptides from partial hydrolysis of DNP-γ-globulin and DNP specific precipitate*

	Mol./unit of mol. wt. 160,000	
Peptide	Inert globulin	Antibody globulin of specific precipitate
DNP alanine	0·37	0·40
DNP A_1	0·29	0·25
DNP A_2	0·22	0·23
DNP A_3	0·15	0·17
DNP A_4	0·09	0·11
Total	1·12	1·16

The material from each band was extracted into organic solvent, taken to dryness, redissolved in a very small volume of 6N-HCl and the solution sealed in a capillary tube for 24 hr. hydrolysis at 105°. Identification of the amino-acids in the hydrolysate was by partition chromatography on paper, using phenol-water in a coal gas NH_3 atmosphere as the principal solvent system. Butanol-acetic acid (Partridge, 1948) and butanol-benzyl alcohol (Consden, Gordon & Martin, 1944) were also found to be useful solvent systems. The DNP amino-acid present was identified by extracting the hydrolysate with a small volume of ether and running the ether-soluble material on silica-gel columns in the usual manner.

The results obtained are given in Table 4. In addition to the amino-acids listed in Table 4 much smaller quantities of alanine were also frequently found and are believed to have arisen from the partial decomposition of DNP-alanine during hydrolysis. The presence of two distinct peptides A_4 and A_5 with the same constituent amino-acids must arise, in view of the composition of the smaller peptides, from the presence of an amide group on the aspartyl residue in one of them. It is highly probable therefore that the terminal tetrapeptide is alanyl-leucyl-valyl-asparaginyl. In order to confirm this, it was desirable to isolate sufficient quantities of the tetrapeptides, to enable the result of their partial hydrolysis under similar conditions to be studied. Attempts to achieve this by using shorter times of hydrolysis (4–6 days) were not successful; the only result was the production of smaller quantities of all these peptides, together with higher peptides. However, two pentapeptides moving very slowly on a $B3$ column, or at nearly $R = 1{\cdot}0$ on a $B5$ column, were isolated and examined. Both contained, in addition to the amino-acids previously found, glutamic acid, and it is probable therefore that this is the fifth amino-acid in the sequence.

Table 4. *Amino-acid composition of DNP peptides from DNP-γ-globulin*

Peptide	
A_1	DNP-alanine, leucine
A_2	DNP-alanine, leucine, valine
A_3	DNP-alanine, leucine, valine, aspartic acid
A_4	DNP-alanine, leucine, valine, aspartic acid

Throughout this work no distinction could be found between the products of partial hydrolysis of the inert DNP-γ-globulin and the DNP specific precipitate.

Reduction of antibody globulin with sodium thioglycollate

From the previous work it seemed probable that there was considerable similarity, in the amino-acid sequence of the polypeptide chain in all fractions of γ-globulin. The effect of reduction of S–S bridges with thioglycollate was therefore examined, as in the absence of other stable intrachain bonds, this might be expected to have a pronounced effect on the molecular configuration.

Blumenthal (1936) showed that reduction of ovalbumin with M-thioglycollate had no influence on its antigenic behaviour. This was confirmed and it was also noted that oxidation of the SH groups present in this protein, by titration with iodobenzoate (Bailey & Perry, 1947) had no influence on its combination with antibody, though complete removal of SH groups was confirmed by the absence of a nitroprusside reaction in guanidine solution.

The effect of sodium thioglycollate on the immune γ-globulin was examined qualitatively as follows.

Immune γ-globulin solution (1 ml. of approx. 10%) was mixed with 1 ml. of a neutral 2·2M-solution of Na thioglycollate at room temperature. After different time intervals 0·1 ml. samples of the mixture were removed and diluted to 5 ml. with saline, thus reducing the thioglycollate concentration to 0·022M. Previous work had shown that this concentration had little or no effect on the flocculation of antibody and antigen, though it should be sufficient to prevent reoxidation during the period of the test. The titre and time of flocculation of the reduced immune globulin was now found by the α procedure (Boyd, 1946) using serial (50%) dilution of ovalbumin solution.

After a few minutes contact with 1·1 M-thioglycollate, the time of flocculation was increased fivefold, though the optimal proportion was unaffected. The flocculation time increased with increase in time of contact with 1·1 M-thioglycollate at a decreasing rate to reach a maximum at about 10–15 times the original value (2 hr. compared with an original flocculation time of 10 min.). This was reached after several hours' contact with reducing agent. Precipitation with antigen still occurred even after the immune globulin had been in contact with the thioglycollate for 27 hr. at room temperature. SH estimations were carried out using the ferrocyanide method (Anson, 1942), but were unsatisfactory, as, after 20 hr. reduction, they gave values exceeding the theoretical maximum for the known cystine content of globulin. If globulin, reduced for 2 hr., was dialysed free of thioglycollate (against running tap water for 24 hr.) reoxidation occurred and the increased time of flocculation was completely reversed. This reversal on dialysis was obvious though not complete after 27 hr. reduction.

DISCUSSION

The time of flocculation of different sera may vary considerably, even when the antibody concentration is the same. The factors which cause this variation are unknown. The effect of reduction with thioglycollate suggests that the time of flocculation may be influenced by the structure of the antibody. As no SH groups were detectable in the native protein, nor were demonstrable after denaturation, it seems likely that the effect of the thioglycollate consists of a reduction of S–S bonds to SH groups. It seems that the combining power of the antibody had only been slightly affected by the breaking of the S–S bridges and that reoxidation can almost completely reverse the effect. This could be explained if other stable intrachain bonds existed which held the molecule in the original configuration; alternatively the combining sites of the antibody may be so small and relatively distant from the cystine residues that they are little influenced by the rupture of the S–S bonds. It is particularly interesting to note that combining power can be kept relatively constant while the rate of flocculation is greatly altered. Apparently the connexion between combination and precipitation is not so direct as has been suggested in the lattice theory (Marrack, 1938) of the precipitin reaction.

The results which have been obtained from end-group assay suggest that antiovalbumin and the inert γ-globulin with which it is associated are both single long chain polypeptides with the terminal sequence, alanyl-leucyl-valyl-aspartyl-glutamyl. There is some evidence that an amide group may be on the β-carboxyl of the aspartic acid. No distinction could be found between the two γ-globulin fractions, nor was any evidence of heterogeneity of either fraction found. This is surprising in view of the known inhomogeneity of γ-globulin fractions from various species: thus electrophoretic (Deutsch, Alberty & Gostling, 1946), ultracentrifuge (Pedersen, 1945) and salting-out studies (Derrien, 1947) have all demonstrated the presence of more than one component. It is however, worth noting that inhomogeneity could exist even if all the materials consisted of the same amino-acids arranged in the same sequence in the polypeptide chain. It has been shown that a number of polar groups in a protein may or may not be capable of ionization, or be available to different reagents, including solvents (cf. Anson, 1945). It is clear that the failure of phenolic groups to ionize, as occurs in ovalbumin (Crammer & Neuberger, 1943), or the unavailability of amino groups, as occurs in some serum globulin (Porter, 1948), will have a marked effect on the electrophoretic mobility and solubility of the protein. The molecular weights of several proteins have been shown to depend, over a wide range, on the concentration of protein and salt in a solution. Presumably the measured molecular weight is the resultant of association and dissociation of the smallest unit (cf. Adair, Bailey & Tsao, 1949). The recent work of Northrop & Goebel (1949) on crystalline horse pneumococcus type I antibody, where solubility studies showed the crystalline material to be quite heterogeneous and also very unstable, suggests that the breaking of labile bonds such as are responsible for the unreactivity of polar groups and the association of molecules in solution, may be responsible for the apparent heterogeneity. The several components of such a mixture might even in some cases be the result of the preparative procedure. It is possible, therefore, that various changes of labile bonding of the molecule may account for the many components of γ-globulin.

In the particular case of antibody and other γ-globulin the limitations of the methods used in this work should be emphasized. Thus, with the material available, it is possible that a component representing 10 % of the total which had a different amino-acid in the fourth position would have been missed, particularly if the adjacent peptide linkage was unusually labile to the type of hydrolysis used. This might be overcome by the use of more DNP-globulin per estimation and by varying the methods of partial hydrolysis, but owing to the necessity of immunizing large numbers of rabbits, sufficient material was not available.

From the finding that few if any amino groups and only one or two iminazole groups were bound in the floccule, and that breaking the S–S bonds had little effect on combining power, it seems probable that the combining site of the antibody is small. How far the identity of the terminal amino-acid sequence in the

antibody and inert γ-globulin may be used to infer identity of sequence throughout the remainder of the molecule, is debatable. As 19^5 pentapeptides could theoretically occupy the terminal position it is clear that the similarity between the biologically active and inert fractions cannot be coincidental. It therefore seems possible that this similarity will extend to a considerable part of the whole molecules, but the combining sites which appear to be small may well have quite different composition from the equivalent section of the inert material.

The results described are in agreement with Pauling's theory of antibody formation in that no chemical distinction between the fractions could be found. According to this theory the rupture of the S–S bonds would have been expected to alter the configuration and therefore to destroy the antibody activity, but it is possible that the configuration of part of the molecule was unaffected by the reaction.

Final conclusions as to the validity of this and other theories of antibody formation must await more detailed work, but it is clear that the chemical evidence described here, contrary to the physiological evidence discussed in the introduction, is in accordance with Pauling's theory.

SUMMARY

1. End-group assay has shown that the immunologically active and inactive fractions of γ-globulin from rabbit antiovalbumin sera are alike in having one terminal alanyl residue per molecule.

2. In none of the fractions was a significant number of unreactive amino or iminazole groups detected, and they occurred only in small amounts in the specific precipitate.

3. All globulin fractions were alike in having the terminal tetrapeptide alanyl-leucyl-valyl-aspartyl with glutamic acid probably occupying the fifth position.

4. Rupture of the disulphide linkages by reduction with thioglycollate had little effect on the combining power of the antibody, but greatly increased the time of flocculation.

5. The significance of the results is discussed in terms of γ-globulin structure and theories of antibody formation.

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REFERENCES

Adair, G. S., Bailey, K. & Tsao, T. C. (1949). *Biochem. J.* **45**, v.

Anson, M. L. (1942). *J. gen. Physiol.* **25**, 355.

Anson, M. L. (1945). *Advanc. prot. Chem.* **2**, 361.

Bailey, K. & Perry, S. V. (1947). *Biochim. Biophys. Acta*, **1**, 506.

Blumenthal, D. (1936). *J. biol. Chem.* **113**, 433.

Boyd, W. C. (1946). *Fundamentals of Immunology*. London: Staples Press.

Breinl, F. & Haurowitz, F. (1930). *Hoppe-Seyl. Z.* **192**, 45.

Burnett, F. M. (1941). *The Production of Antibodies*. Melbourne: Macmillan.

Calvery, H. O. (1935). *J. biol. Chem.* **112**, 167.

Campbell, D. H. (1948). *Ann. Rev. Microbiol.* **2**, 269.

Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.

Crammer, J. L. & Neuberger, A. (1943). *Biochem. J.* **37**, 302.

Derrien, Y. (1947). *Svensk. kem. Tidskr.* **59**, 139.

Deutsch, H. F., Alberty, R. A. & Gostling, L. J. (1946). *J. biol. Chem.* **165**, 21.

Erickson, J. O. & Neurath, H. (1945). *J. gen. Physiol.* **28**, 421.

Haurowitz, F., Tekman, Sh., Bilen, M. & Schwerin, P. (1947). *Biochem. J.* **41**, 304.

Heidelberger, M. & Kendall, F. E. (1936). *J. exp. Med.* **64**, 161.

Kekwick, R. A. (1940). *Biochem. J.* **34**, 1248.

Marrack, J. R. (1938). *Chemistry of Antigens and Antibodies*. London: H.M. Stationery Office.

Martin, A. J. P. & Synge, R. L. M. (1941). *Biochem. J.* **35**, 1358.

Mudd, S. (1932). *J. Immunol.* **23**, 423.

Neuberger, A. (1938). *Biochem. J.* **32**, 1435.

Northrop, J. H. & Goebel, W. F. (1949). *J. gen. Physiol.* **32**, 705.

Partridge, S. M. (1948). *Biochem. J.* **42**, 238.

Pauling, L. (1940). *J. Amer. chem. Soc.* **62**, 2643.

Pauling, L. & Campbell, D. H. (1942). *J. exp. Med.* **76**, 211.

Pedersen, K. O. (1945). *Ultracentrifuge Studies on Serum and Serum Fractions*. Upsala, Sweden: Almquist and Wiksel.

Petermann, M. L. (1946). *J. Amer. chem. Soc.* **68**, 106.

Porter, R. R. (1948). *Biochim. Biophys. Acta*, **1**, 105.

Porter, R. R. (1950*a*). *Biochem. J.* **46**, 304.

Porter, R. R. (1950*b*). *Biochem. J.* **46**, 479.

Porter, R. R. (1950*c*). *Methods in Medical Research*, **3**. (In the Press.)

Porter, R. R. & Sanger, F. (1948). *Biochem. J.* **42**, 287.

Sanger, F. (1945). *Biochem. J.* **39**, 507.

Sanger, F. (1949). *Biochem. J.* **45**, 563.

Smith, E. L. (1946). *J. biol. Chem.* **164**, 345.

Smith, E. L., Greene, R. D. & Bartner, E. (1946). *J. biol. Chem.* **164**, 359.

Velluz, L. (1934). *C.R. Soc. Biol., Paris*, **116**, 981.