Disulphide Bridges of the Heavy Chain of Human Immunoglobulin G2

By C. MILSTEIN AND B. FRANGIONE*

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, U.K.

(Received 17 August 1970)

Amino acid sequences around the disulphide bridges of the heavy chain of an immunoglobulin of the γ_2 subclass have been studied. The protein was digested with pepsin and the digest fractionated by Sephadex. Screening of the eluate by one-dimensional electrophoresis of oxidized and unoxidized samples was used as an assay and pools of fractions were prepared. Identification by diagonal electrophoresis of several inter- and intra-chain disulphide bridges was done on the pooled fractions. The inter-heavy-chain bridged peptide included four cystine residues. Comparison with proteins of other human subclasses indicated that the intrachain bridges identified are the bridges of the invariable section of γ_2 heavy chains. The amino acid sequence of one cysteine acid peptide that may have been derived from the variable part of the molecule was determined. Partial reduction followed by carboxymethylation with radioactive iodoacetate of two proteins of the γ_2 class showed a number of labelled peptides that could be identified as being related to the inter-chain bonded cystine residues.

The human immunoglobulin G class comprises four subclasses that differ in the structure of their heavy chains. The subclasses (IgG1, IgG2, IgG3 and IgG4†) have been shown to contain a large degree of homology in selected regions of the heavy chains (Milstein, Frangione & Pink, 1967; Prahl, 1967; Frangione, Milstein & Pink, 1969; Pink, Buttery, de Vries & Milstein, 1970) and particularly around the intrachain disulphide bridges.

In contrast, the interchain disulphide bridges that stabilize the tetrameric structure differ markedly in all subclasses (Frangione *et al.* 1969) not only in the number and distribution of the disulphide bonds but also in the sequence of the section that includes the interchain heavy-heavy bridges ('hinge region').

The IgG1 subclass is the major subclass and IgG2 is the second: its most distinct functional characteristic seems to be its inability, unique in the IgG class, to give positive skin-fixation reactions (Terry, 1965).

In this paper we describe the characterization of the disulphide bridges believed to be characteristic of the γ_2 heavy chains with special emphasis on the interchain bonds. Most of the experiments described were performed on one myeloma protein Sa which is an IgG2 molecule containing a λ light chain.

* Present address: New York University School of Medicine, New York, N.Y. 10016, U.S.A.

† Abbreviations: IgG, immunoglobulin G; Cya, cysteine acid.

A preliminary report containing part of the results obtained in this work has been published (Frangione *et al.* 1969). Results obtained with another IgG2 protein are also described.

MATERIALS AND METHODS

Disulphide bridges of protein Sa. This protein was prepared from serum by chromatography on DEAE-cellulose, with 50 mM-sodium phosphate buffer, pH 7, and a gradient to 0.2 M. Experiments with proteins isolated by use of a Pevikon block at pH 8.2 raised the suspicion of some disulphide interchange (Frangione, 1968). The purified protein (10 mg/ml) was dissolved in 20 mM-HCl and titrated to pH 2.0 with 0.1 M-HCl. Pepsin (enzyme/substrate ratio 1:40) was added and the pH kept at 2 for about 4 h at room temperature. It was then left for a further 16 h at 37°C and freeze-dried. The digest was applied to a Sephadex G-50 column equilibrated with 5% (v/v) formic acid and fractions were collected in a fraction collector.

Location of cystine peptides. Two samples from each fraction were dried in tubes. One sample was oxidized with performic acid and the other was used as such. Oxidation with performic acid was done by dissolving the sample in one drop of formic acid, adding one drop of performic acid and incubating the contents at room temperature for 30 min. The tubes were dried in a desiccator over NaOH, one drop of water was added and the samples were dried again to remove all traces of performic acid. The oxidized and unoxidized samples were applied side by side in 2 cm bands along a sheet of Whatman no. 1 paper. The paper was subjected to electrophoresis at pH 6.5 for 1 h and developed with cadmium-ninhydrin

reagent [100ml of 1% (w/v) ninhydrin in acetone and 15ml of 5% (w/v) cadmium acetate in 25% (v/v) acetic acid]. The presence of cysteine acid peptides was indicated by spots present in the oxidized samples and absent in the control and the cystine peptides by spots in the unoxidized sample that were not present in the oxidized one. This preliminary analysis was used to select individual fractions for further studies. Fractions were pooled accordingly and identification of the cystine peptides was done on

each pool by the diagonal technique (Brown & Hartley, 1963) as described by Milstein (1966).

Partial reduction and carboxymethylation of proteins Sa and Til. This was done as described by Frangione & Milstein (1968) by using [^{14}C]iodoacetic acid (The Radiochemical Centre, Amersham, Bucks., U.K.). The labelled proteins were digested with pepsin (enzyme/substrate ratio 1:20) in 5% (v/v) formic acid for 14 h at 37°C. The digest was freeze-dried and when indicated subjected to tryptic digestion, (enzyme/substrate ratio 1:50) in 1% NH_4HCO_3 adjusted to pH 8.0 with NH_3 for 6 h at 37°C. The digest was subjected to electrophoresis at pH 3.5 for 1 h at 3 kV and purified after oxidation by electrophoresis at pH 3.5 and pH 6.5. Band 3 of Fig. 8 was further purified at pH 8.9.

High-voltage electrophoresis of peptides was carried out as described by Ambler (1963) or Milstein (1966). Mobilities (m) at pH 6.5 are expressed as fractions of the distance between ϵ -DNP-lysine and aspartic acid.

N-Terminal residues were allowed to react with dansyl chloride (Gray, 1967) and derivatives were identified as described by Woods & Wang (1967). Serine and threonine or glutamic acid and aspartic acid were identified with solvent IV of Crowshaw, Jessup & Ramwell (1967). The results of dansyl-Edman procedure are shown with arrows over the peptide on which the experiments were performed.

RESULTS

Plate 1 shows the one-dimensional screening of the peptic digest after ultrafiltration on a Sephadex G-50 column. It clearly shows that the higher-molecular-weight fractions, emerging immediately after the breakthrough of the column (fractions 93-95), were mainly composed of cystine peptides. Clearly visible are one nearly neutral (Sa1 α) and two basic peptides before oxidation (Sa1 β and Sa1 γ). The column fractions were combined as shown in Plate 1 and the pools were studied separately.

Fig. 1. Diagram of the diagonal electrophoresis of a sample of pooled fraction Sa1 of Plate 1. Electrophoresis in both dimensions was at pH 6.5. The double arrow shows the position of the origin. The rest of the ionogram showed no visible spots.

Table 1. *Amino acid composition of peptides derived from a peptic digest of protein Sa*

Peptide	Sa1a2	Sa1a3	Sa1b1	Sa1b2	Sa2A1	Sa2A2	Sa2N1*	Sa2N2	Sa2N4	Sa2a5	Sa2a6
Lysine	1.0	3.0		2.6				+			1.1
Histidine											1.0
Arginine	0.95	0.9	1.1			1.2			1.1		
Cysteic acid	3.4	3.6	0.7	0.8	1.0	1.0	1.1	0.8	0.9	0.8	1.0
Aspartic acid		1.0		1.0			1.0				3.1
Threonine		0.9	1.8		1.0	1.8		1.7	1.9		2.0
Serine			1.0	1.0	1.0	4.0		1.1	3.8		1.1
Glutamic acid	1.0	2.1	1.0	1.0	1.0	1.1		1.0	1.2		
Proline	4.8	4.7	1.1	2.0	1.0	2.1		1.1	2.1		1.1
Glycine	1.0	1.1		1.1						1.1	
Alanine	2.0	2.0		1.0		1.0		0.9	1.0	1.9	
Valine	1.9	3.9	1.9	1.1			3.0	0.9			1.0
Isoleucine			0.8	1.0							
Leucine				1.0		1.0			1.0	1.8	
Tyrosine				1.0							0.7
<i>N</i> -Terminus	Arg	Lys	Ile	Tyr	Pro	Pro	Cya		Pro	Ala	

* 90h hydrolysis.

EXPLANATION OF PLATE I

Fractionation of the peptic digest of protein Sa by Sephadex G-50. Samples of numbered fractions and performic acid-oxidized fractions (Ox in bottom part of the figure) were compared by paper electrophoresis at pH6.5. Markers were added to the edge of the paper. Neutral indicates the position of neutral amino acids and XCF the position of Cyanol FF.

Disulphide bridges of pool Sa1. The diagonal electrophoresis of pool Sa1 (Fig. 1) indicated the presence of two sets of cysteic acid peptides. The amino acid composition of the purified peptides is shown in Table 1. Further characterization of the peptides was as follows.

Peptide Sala2 and Sala3. Peptide Sala2 was very resistant to further digestion with other proteolytic enzymes (thermolysin and pronase). It was subjected to the dansyl–Edman procedure. The degradation was unambiguous up to the twelfth step, but after that a mixture of dansyl-amino acids was obtained. The sequence of peptide Sala2 is therefore uncertain in its *C*-terminal stretch as shown:

The presence of glutamic acid rather than glutamine was based on the mobility of the peptide (0.5 at pH 6.5) which indicates a net charge of –3.

The amino acid composition of peptide Sala3 is shown in Table 1. The dansyl-Edman procedure gave the sequence of the first eight residues.

The partial sequence of peptide Sala3 was:

The net charge of the peptide was deduced from its mobility (0.4 at pH 6.5) to be –3 indicating that no amide groups were present. It was concluded that peptide Sala3 included Sala2, which was produced by a further peptic split at the Glu-Arg bond. These two peptides were disulphide bridged

Fig. 2. Diagram of the diagonal electrophoresis of a sample of pooled fraction Sa1 of Plate 1. Hatched areas indicate ninhydrin-negative isatin-positive spots. Other details are as in Fig. 1.

Fig. 3. Sequence of peptide Sa2A2 (see also Table 2).

Fig. 4. Disulphide-bridge peptides defining the heavy-light interchain bridge. The arrows indicate the cysteic acid peptides produced by oxidation.

Table 2. Peptides isolated from peptide Sa2A2 after digestion with trypsin

The products were fractionated by paper electrophoresis at pH 6.5 (mobilities, *m*, relative to aspartic acid are given) and further purified at the pH indicated. Peptide Sa2A2TN was ninhydrin-negative and isatin-positive.

Peptide	<i>m</i>	Purification pH	Composition	Dansyl–Edman results
Sa2A2T1	0.50	—	Thr 1.0; Ser 2.0; Glu 1.0	Ser-
Sa2A2T2	0.42	—	Thr 2.0; Ser 3.0; Glu 1.1	Ser-Thr-Ser-Glx-Ser-Thr
Sa2A2TN	0	3.5	Arg 0.9; Cya 0.9; Ser 1.1; Pro 2.0; Ala 1.0; Leu 1.0	Pro-Leu-Ala-Pro-Cya-

Table 3. *Peptides isolated from peptide Sa2b2 after digestion with trypsin*

The products were fractionated by paper electrophoresis at pH 6.5 (mobilities, *m*, relative to aspartic acid are given) and further purified by electrophoresis at the pH indicated.

Peptide	<i>m</i>	Purification pH	Composition	Dansyl-Edman Results
Sa2b2T1	0.30	—	Glu 1.0; Pro 2.0; Gly 0.9; Ala 1.0; Ile 1.0; Leu 1.0	Gly-Leu-Pro-Ala-Pro-Ile-Glu
Sa2b2T2	0.20	—	Lys 1.0; Cya 0.9	
Sa2b2T3	-0.35	2	Lys 2.0; Cya 1.0; Tyr 1.0	Tyr-
Sa2b2T4	-0.44	2	Lys 0.9; Asp 1.1; Ser 1.0; Val 1.0	Val-Ser-Asx-
Sa2b2T5	-0.5	2	Lys 1.0; Tyr 1.0

Fig. 5. Sequence of peptide Sa2b2 (considered identical with Sa2N3 and Sa1b2) as deduced from the results of Table 3.

Fig. 6. Peptides Sa2b1, Sa1b1 and Sa2N1.

before oxidation (see Fig. 1) and define the presence of the inter-heavy-chain bridges.

Other minor spots associated with these two peptides were too faint to allow of definite characterization.

Peptides Sa1b1 and Sa1b2. These two peptides are clearly bridged to each other before oxidation (see Fig. 1). The bulk of the peptides, however, was obtained in the second pooled fraction of the Sephadex column as Sa2b1 and Sa2b2. The sequence of these peptides is discussed below.

Pool Sa2. The diagonal 'map' of this pool is shown in Fig. 2. This fraction contained three spots (Sa2N4, Sa2A1 and Sa2A2) which were ninhydrin-negative and isatin-positive. The amino acid composition of the isolated peptides is shown in Table 1.

Peptides Sa2N4 and Sa2A2. From their composition, staining characteristics and mobility at pH 6.5 they are considered to be the same peptide departing from the diagonal at different points

Fig. 7. Sequence of peptide Sa2a6. The subtilisin derivatives and the dansyl-Edman results are also shown.

because of a difference in the peptide to which they are bridged (Sa2N2 and Sa2A1 respectively). Sa2A2 was digested with trypsin and the results are shown in Table 2. The sequence deduced is shown in Fig. 3.

Peptides Sa2A1 and Sa2N2. These two peptides are the peptic digestion products of the C-terminal section of λ chains (Milstein, 1965) as shown in Fig. 4. The two proposed disulphide-bridge peptides which after oxidation give rise to Sa2N2, Sa2N4, Sa2A1 and Sa2A2 are shown in Fig. 4.

Peptides Sa2b2 and Sa2N3. The disulphide-bridged partners of Sa2b1 and Sa2N1 are Sa2b2 and Sa2N3. These two peptides and peptide Sa1b2 appeared to be identical by composition, mobility and N-terminal residue (tyrosine). Peptide Sa2b2 was subjected to tryptic digestion (Table 3). The sequence deduced is shown in Fig. 5.

Peptides Sa2N1 and Sa2b1. Peptide Sa2b1 had the same composition, mobility and staining characteristics (it reacts very slowly with ninhydrin because it contains isoleucine in the N-terminal position) as peptide Sa1b1. Its N-terminal sequence established by the dansyl-Edman technique is as shown in Fig. 6.

Peptide Sa2N1 had the composition shown in Table 1. It appears to be related to peptide Sa2b1 as shown in Fig. 6.

Peptide Sa2a5. The sequence of this peptide was established by the dansyl-Edman procedure as Ala-Ala-Leu-Gly-Cya-Leu. A peptide (Sa2a2) related to it gave the sequence Gly-Cya-Leu.

Peptide Sa2a6. The sequence of this peptide was established by the dansyl-Edman procedure as

Table 4. *Peptides derived by subtilisin digestion of peptide Sa2a6*

The products were fractionated by paper electrophoresis at pH 6.5. Mobilities (*m*) at pH 6.5 are shown.

Peptide	<i>m</i>	Composition
Sa2a6S1	-0.23	Lys 0.9; His 0.9; Asp 0.9; Ser 1.1; Pro 1.2; Val 0.9
Sa2a6S2	0.09	Asp 1.0; Thr 0.9
Sa2a6S3	0.43	Cys 1.0; Asp 1.0; Thr 1.0; Tyr 0.9

Table 5. *Other peptides isolated from protein Sa*

m indicates mobility at pH 6.5 relative to aspartic acid taken as 1.0.

Peptide	SaP2a	SaP2b1	SaP2b2	Sa3b2b
Cysteic acid	1.0	1.1	1.0	1.0
Methionine sulphone	0.7		1.0	
Threonine		0.9		1.9
Serine	1.7		2.0	2.0
Glutamic acid				1.0
Glycine				3.1
Valine	1.0		1.1	1.0
Isoleucine				1.0
Leucine		1.0		
Phenylalanine			0.8	
<i>m</i>	0.45	0.60	0.35	0.50

shown in Fig. 7. The peptide is slightly acidic (unlike the homologous peptide of $\gamma 1$ heavy chains which is basic) indicating the presence of one aspartic acid residue. To define which of the residues occur as aspartic acid a subtilisin digest was prepared and analysed. The results are shown in Table 4. From the mobilities of the subtilisin derivatives it is clear that the aspartic acid occurs in Sa2a6S1 (Table 4). It is clear from the diagonal maps that Sa2a6S1 and Sa2a6 form a disulphide bridge.

Other peptides isolated from protein Sa. The fractions of the Sephadex column that were more retarded included other disulphide-bridge peptides. Some were present in small amounts and were not further characterized. Two of them were studied.

Disulphide-bridged peptide SaP. A set of peptides was isolated by diagonal electrophoresis at pH 3.5. Their mobility before oxidation was 0.7 with respect to a glycylalanine marker. After oxidation they gave rise to three cysteic acid peptides which were further purified by electrophoresis at pH 6.5. The three derivatives are shown in Table 5. Their composition, mobility and staining characteristics were identical with those of peptides obtained from proteins of other subclasses and corresponding to the inter-chain disulphide bridge nearest to the carboxy-terminal end (Frangione, Milstein & Franklin, 1968). They were not studied further.

Peptide Sa3b2b. The composition of this peptide

is shown in Table 5 and its sequence was established by the dansyl-Edman procedure as:

Thr-Cys-Thr-Val-Ser-Gly-Gly-Ser-Ile-Gly-Glu

The peptide was obtained from the basic region of the first dimension (mobility before oxidation was 0.15 relative to aspartic acid). However, after oxidation it became very acidic. It did not contain any basic residue and because of its size and mobility it must include a glutamic acid residue. Therefore it must have been bridged to a basic peptide. Such a peptide was observed but was not sufficiently separated from the bulk of the peptides present in the diagonal line and could not be purified in the same experiment. No further attempts to isolate it were made. It probably derives from the 'variable' section of the molecule.

Identification of the carboxymethylcysteine residues obtained by partial reduction and alkylation of proteins Sa and T₁l. Fig. 8 shows the main radioactive bands obtained from digests of partially reduced and carboxymethylated proteins Sa and T₁l. The main radioactive bands were purified as described in the Materials and Methods section and their amino acid compositions are shown in Tables 6, 7 and 8. Some very weak bands present in the radioautographs (shown in broken lines) could not be analysed. From their mobilities and compositions they are all derived from the bridges involved in interchain

Fig. 8. Diagram showing the radioactive bands obtained after electrophoresis at pH 3.5 of a peptic (P) and peptic-tryptic digest (PT) of partially reduced and carboxymethylated myeloma proteins Sa and Til.

binding. The only discrepancy is the presence in SaP1b and SaP1c of an extra serine and an extra phenylalanine that must occur due to differences in the conditions of pepsin digestion. These two peptides were present together with SaP1a in the radioactive band labelled 1 in Fig. 8, and could be separated during the purification procedure. Serine and phenylalanine were also present in TilPT2 and in SaPT2. The interpretation of these results is synthesized in Fig. 9. The differences between protein Til and Sa are due to their L chains being of the κ and λ types respectively.

DISCUSSION

Like other immunoglobulin chains it is almost certain that $\gamma 2$ chains are made up of a variable and an invariable section and the bridges summarized in Fig. 10 are bridges of the invariable part of the molecule. This is indicated by the homology of the sequences with those of other molecules. The sequences around the half-cystine that bind light chains (residue 131 in Fig. 10) and residues 144 and 200 that form the Fd C disulphide bridge (the bridge present in the constant part of the Fd fragment) are in fact completely identical in $\gamma 2$ and $\gamma 4$ molecules but differ by several residues from $\gamma 1$ and $\gamma 3$ chains. Almost identical sequences are also found around the bridges that are present in the Fc fragment of $\gamma 1$, $\gamma 3$, $\gamma 4$ and the homologous bridges of $\gamma 2$ (for which no Fc can be prepared owing to the known resistance of $\gamma 2$ subclass to papain digestion) (Frangione, Franklin, Fudenberg & Koshland, 1966; Grey & Kunkel, 1967).

The experiments described in this paper refer

Table 6. Carboxymethylcysteine peptides isolated from a peptic digest of partially reduced and carboxymethylated $\gamma G2$ myeloma protein Sa (see also Figs. 8 and 9)

Mobilities are given relative to aspartic acid as 1.0. Abbreviation: Cms, carboxymethylcysteine sulphone.

Peptide	P1a	P1b	P1c	P0	P3	P2
Lys	1.0	1.0	0.8			0.8
Arg	0.8	0.8	*		0.9	
Cms	1.4	1.3	1.0	0.4	0.4	0.4
Thr				1.0	1.7	1.7
Ser	0.2	1.2	1.0	1.0	3.5	1.2
Glu	0.9	1.0	1.0	1.0	1.0	1.0
Pro	5.0	5.0	5.0	1.0	2.0	1.0
Gly	1.1	1.2	1.0			
Ala	2.0	2.0	1.7		1.0	1.1
Val	1.9	1.5	2.1			1.1
Leu					1.0	
Phe			0.8			
Mobility at pH 6.5	0.52	0.43	0.47	0.80	0.23	0.28

* Could not be calculated because of problems in the base line.

Table 7. Carboxymethylcysteine peptides isolated from a peptic-tryptic digest of partially reduced and carboxymethylated γ G2 myeloma protein Sa (see also Figs. 8 and 9)

Mobilities are given relative to aspartic acid as 1.0.

Peptide	PTI	PTII	PTIII	PT3	PT4
Lys		0.8	1.0		1.0
Arg		1.0		1.0	
Cms	2.2	1.0	1.9	0.4	0.5
Thr					1.6
Ser		0.8		1.0	1.0
Glu	1.2	1.2	1.1		1.1
Pro	4.4	4.0	4.4	2.0	1.0
Gly	1.1	1.2	1.2		
Ala	1.8	2.0	1.9	1.0	0.9
Val	2.0	1.9	2.2		1.5
Leu				1.0	
Mobility at pH6.5	0.84	0.43	0.73	0	0.27

Table 8. Carboxymethylcysteine peptides isolated from a peptic-tryptic digest of partially reduced and carboxymethylated γ G2 myeloma protein Til (see also Figs. 8 and 9)

Mobilities are given relative to aspartic acid as 1.0.

Peptide	PT1	PT2	PT3	PT4
Arg			0.9	
Cms	1.9	1.3	0.5	0.5
Ser		1.1	1.0	
Glu	1.1	1.0		0.9
Pro	4.6	4.4	1.8	
Gly	1.2	1.2		1.2
Ala	2.0	1.8	1.1	
Val	1.8	2.1		
Leu			1.1	
Phe		0.8		
Mobility at pH6.5	0.85	0.83	Neutral	1.0

Fig. 9. Carboxymethylated cysteine peptides derived from proteins Sa and Til. (a) Peptides shown to be involved in the heavy-light chains binding in protein Sa. Sequences of κ and λ C-terminal peptides were taken from Milstein (1965). (b) Peptides involved in heavy-heavy chain binding in protein Sa. Cmc, carboxymethylcysteine.

Fig. 10. Comparison of the disulphide bridges in the invariable section of $\gamma 2$ and $\gamma 4$ heavy chains. When $\gamma 2$ and $\gamma 4$ sequences differ both variants are shown one above the other. The sequence of Vin($\gamma 4$) is taken from Pink *et al.* (1970). Numbering of residues is based on the sequence of protein Eu (Edelman *et al.* 1969). Between 220 and 226 a gap of three residues is required to preserve homology of $\gamma 2$ and $\gamma 4$ with $\gamma 1$. The arrows show the main peptides discussed in the text.

mainly to protein Sa. Experiments performed on two other proteins of the $\gamma 2$ subclass (proteins Dav and Til) (Frangione, 1968) gave disulphide bridge diagonal 'maps' that were very similar to the ones obtained with protein Sa. The only significant differences could be traced to differences in the light-chain type.

The inter-heavy-chain bridges of protein Sa occur through half-cystine residues of peptides Sa1a2 and Sa1a3. This segment includes four half-cystine residues and the diagonal 'map' experiments cannot distinguish a situation in which all four are involved in the interchain binding from one in which two of them form an intrapeptide bridge. This second possibility seems, however, unlikely. The partial reduction and carboxymethylation experiment did not disclose anomalous peptides containing intrachain bridges not reduced by the mild conditions used. If such an intrachain bridge was present it would have to be much more sensitive to reduction

than any other intrachain bridge. Sensitive intrachain bridges in IgG have been described (O'Donnell, Frangione & Porter, 1970) but they were not quantitatively reduced as they would have to be to explain our results. The experiments do not throw light on the parallel or antiparallel nature of the bridges.

For a direct attack on these problems it is necessary to split the disulphide-bridged peptide to separate the different cystine derivatives. We attempted treatment with pronase and thermolysin but with no success. Partial acid hydrolysis would seem to be the only possibility of a direct answer. The results obtained so far indicate that the half-cystine in the sequence Lys-Cys forms a parallel interchain bridge (M. B. Perry & C. Milstein, unpublished work).

The technical assistance of Mr J. M. Jarvis is gratefully acknowledged. B.F. is a senior investigator of the Arthritis Foundation, U.S.A.

REFERENCES

- Ambler, R. P. (1963). *Biochem. J.* **89**, 349.
- Brown, J. R. & Hartley, B. S. (1963). *Biochem. J.* **89**, 59p.
- Crowshaw, K., Jessup, S. J. & Ramwell, P. W. (1967). *Biochem. J.* **103**, 79.
- Edelman, G. M., Cunningham, B. A., Gall, E. W., Gottlieb, P. D., Rutishauser, U. & Waxdal, M. J. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 78.
- Frangione, B. (1968). Ph.D. Thesis: University of Cambridge.
- Frangione, B., Franklin, E. C., Fudenberg, H. H. & Koshland, M. E. (1966). *J. exp. Med.* **124**, 715.
- Frangione, B. & Milstein, C. (1968). *J. molec. Biol.* **33**, 893.
- Frangione, B., Milstein, C. & Franklin, E. C. (1968). *Biochem. J.* **106**, 15.
- Frangione, B., Milstein, C. & Pink, J. R. L. (1969). *Nature, Lond.*, **221**, 145.
- Gray, W. R. (1967). In *Methods in Enzymology*, vol. 11, p. 469. Ed. by Hirs, C. H. W. New York and London: Academic Press.
- Grey, H. M. & Kunkel, H. G. (1967). *Biochemistry, Easton*, **6**, 2326.
- Milstein, C. (1965). *Nature, Lond.*, **205**, 1171.
- Milstein, C. (1966). *Biochem. J.* **101**, 338.
- Milstein, C., Frangione, B. & Pink, J. R. L. (1967). *Cold Spring Harb. Symp. quant. Biol.* **32**, 31.
- O'Donnell, I. J., Frangione, B. & Porter, R. R. (1970). *Biochem. J.* **116**, 261.
- Pink, J. R. L., Buttery, S. H., de Vries, G. M. & Milstein, C. (1970). *Biochem. J.* **117**, 33.
- Prahl, J. W. (1967). *Biochem. J.* **105**, 1019.
- Terry, W. D. (1965). *J. Immun.* **95**, 1041.
- Woods, K. R. & Wang, K. T. (1967). *Biochim. biophys. Acta*, **133**, 369.