Clin. exp. Immunol. (1980) **39**, 297–306.

Analysis of immune complexes in synovial effusions of patients with rheumatoid arthritis*

D. MALE, I. M. ROITT & F. C. HAY *The Department of Immunology, Middlesex Hospital Medical School, London*

(*Accepted for publication* 13 *August* 1979)

SUMMARY

Immune complexes were isolated from the synovial effusions of patients with seropositive definite or classical rheumatoid arthritis by centrifugation over a sucrose–polyethylene glycol gradient. Physicochemical and immunochemical analysis showed IgG and IgM to be the predominant molecular species with lesser amounts of C1q and moderate amounts of IgA and activated C4 and C3. Very low concentrations of $C\bar{1}r$, $C\bar{1}s$, factor B and β_2-microglobulin were detected. Trace amounts of four other components totalling less than 4% of the total protein, were seen and their molecular weights established. Reasons were advanced for thinking that fibrinogen, human serum albumin and α_2-macroglobulin were only secondarily associated with the complexes. The data are consistent with the hypothesis that IgG is the main, if not the only antigen, responsible for provoking and maintaining the pathological changes in rheumatoid arthritis.

INTRODUCTION

The inflamed synovium in rheumatoid arthritis is a site of active antibody synthesis. These antibodies contribute to the local formation of immune complexes, which are regularly found in the synovial fluids of these patients (Munthe & Natvig, 1971), and which have been correlated with local complement depletion and degree of disease activity (Winchester, Agnello & Kunkel, 1970).

The fixation of complement by the complexes has been inferred from the reduction of CH50 and decreased levels of specific components in the synovial fluids of seropositive rheumatoid arthritis patients as compared with subjects suffering from degenerative joint diseases (Winchester *et al.*, 1970; Ruddy & Austen, 1970). These results suggest that complexes are a major factor in the pathogenesis of the disease.

Numerous antibody specificities have been reported in synovial fluid complexes and cryoprecipitates, including IgG and IgM antiglobulins, antibodies to $F(ab')_2$, nuclear antigens and viral components, activated C3 and collagen (Cracchiolo *et al.*, 1971; Natvig & Munthe, 1975; Mellbye & Munthe, 1971; Cremer *et al.*, 1974; Mellbye & Natvig, 1971; Steffen, 1975); however, the relative contribution of each antibody specificity has not been assessed previously nor have antigens other than IgG itself been identified in association with the antibodies. We have, therefore, undertaken an analysis of the composition of these complexes in an attempt to identify the antigens concerned.

Immune complexes and aggregates from synovial fluid have been stabilized and precipitated in a single step procedure involving sequential density gradient ultracentrifugation and PEG precipitation. The precipitates consisted of fast sedimenting material of low PEG solubility found in varying amounts

* A preliminary report of this work has been presented in the 26th Colloquium 'Protides of the Biological Fluids'. Brussels, 1978.

Correspondence: Professor I.M. Roitt, Department of Immunology, Middlesex Hospital Medical School, Arthur Stanley House, 40–50 Tottenham Street, London W1P 9PG, UK.

0099-9104/80/0200-0297$02.00

in all the fluids investigated. The proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the identity of all the major components determined by combining information from a number of techniques developed specifically for this purpose (Male & Roitt, 1979).

MATERIALS AND METHODS

Chemicals. Tris (hydroxymethyl) aminomethane (Trizma base), n,n′-methylene-bis-acrylamide, and polyethylene glycol (PEG) av. mol. wt 6000 were purchased from Sigma biochemicals. All other chemicals were Analar grade.

Antisera. Antisera to C4, $C\bar{1}r$ and $C\bar{1}s$ were kindly supplied by Dr K. Reid of the Biochemistry Department, Oxford. Antiserum to IgA was a generous gift from Dr G. Bull. Antisera to $F(ab')_2$ and to fibrinogen were raised in our own laboratories by immunization of sheep and rabbits respectively with purified antigens. Anti-fibrinogen produced a single precipitin line in immunodiffusion against normal human serum, while anti-$F(ab')_2$ reacted to produce at least three lines. Antisera to C3 and Factor B were obtained from OTEA Behringwerke. Antibodies to β_2-microglobulin and α_2-macroglobulin were purchased from Dakoimmunoglobulins and an antiserum to IgG Fc from Seward Immunostics.

Synovial fluids. These were obtained following therapeutic drainage of knee joints of patients with seropositive definite or classical rheumatoid arthritis, attending the Middlesex Hospital. The samples were spun immediately at 400 *g* for 20 min to remove cells and larger debris and were then either used immediately or stored at −20°C until required.

Separation and characterization of complexes. The details of the methods used for the isolation and characterization of the immune complexes are described fully elsewhere (Male & Roitt, 1979). Briefly, immune complexes were isolated from synovial fluids by centrifugation through a stepped sucrose gradient (18–26%) containing a PEG gradient (2–8%) in the lower half of the tube. The tubes were spun at 30,000 rpm (top 60,000 *g*; bottom 105,000 *g*) for 16 hr and at 20°C to preclude non-specific cryoprecipitation.

SDS-PAGE in one dimension was performed according to the method of Laemmli (1970). Complexes obtained from the sucrose/PEG gradients were reduced and separated in 8% gels with a 3% stacking gel in a discontinuous Tris/HCl buffer system.

SDS-PAGE in two dimensions was used to separate associated peptides of individual molecules. Unreduced molecules were separated in a 5% gel in the first dimension. After reduction with 2-mercaptoethanol the constituent peptides were separated in an 8·5% gel in the second dimension. Gels were fixed in 30% methanol/10% acetic acid, and the peptides visualized by staining with 0·1% Coomassie Brilliant Blue. Autoradiography of labelled gels was performed by exposure to Kodirex film

Analysis of individual components was carried out by immunoprecipitation of the individual molecules of the ^{125}I-labelled complex with specific antisera in agar gels using immunoelectrophoresis and/or immunodiffusion or counter current electrophoresis. After extensive washing the precipitin arcs were excised and analysed by 1-dimensional SDS-PAGE. Labelled peptides of the individual molecules specifically precipitated from the complexes were visualized by autoradiography.

RESULTS

Immune complexes were prepared by sedimentation through a combined sucrose/PEG gradient designed so that only material of S value >8·5 entered the PEG portion of the gradient. This system, whose detailed characteristics are described elsewhere (Male & Roitt, 1979), allows substantial enrichment of immune complexes and aggregates, separating them from monomer immunoglobulins. The precipitates from the gradients were analysed both by conventional SDS-PAGE, and in a 2-dimensional SDS-PAGE system which identifies the constituent peptides derived from each parent molecule, and allows separation of peptides of identical molecular weights originating from different parent molecules which cannot be resolved in 1-dimensional SDS-PAGE.

Fig. 1 illustrates the 2-dimensional SDS-PAGE analyses of the precipitates from three different synovial fluids. Separation in the first dimension of unreduced molecules is from left to right. After reduction, separation of their constituent peptides in the second dimension is from top to bottom. In this system peptides from one molecule are aligned in vertical groups, which are listed in column 1 of Table 1. The three gels in Fig. 1 illustrate the extent of variation between the samples. The molecular weights of the peptides are derived from standards (IgG, phosphorylase a, bovine serum albumin, aldolase, DNAse, and chymotrypsinogen). 1-Dimensional gels were used for the estimation of the peptide molecular weights, since the band mobility on 1-dimensional gels can be measured more accurately than spot mobility on 2-dimensional gels. The molecular weights of the peptides and their letter designations (Fig. 2) are listed in Table 1 together with the molecular weights of their parent molecules which are derived from the mobility in the first dimension of 2-dimensional SDS-PAGE,

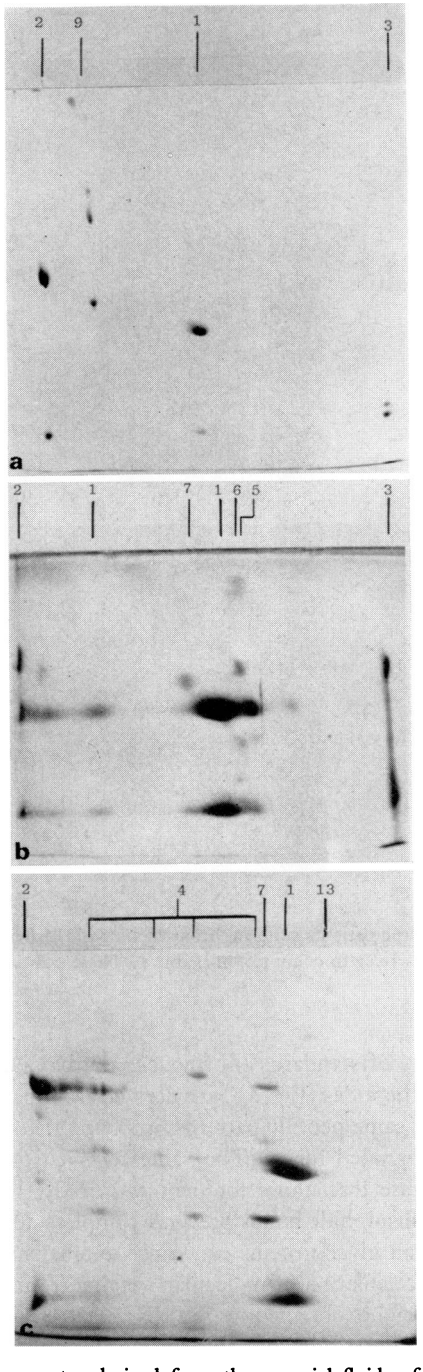

FIG. 1. Immune complexes/aggregates derived from the synovial fluids of three patients suffering from rheumatoid arthritis, were analysed by 2-dimensional SDS-PAGE. Unreduced molecules were separated from left to right in the first dimension, in 5% acrylamide. After reduction the peptides of each molecule were separated from top to bottom in 8·5% acrylamide. Molecular groups correspond to column 1 of Tables 1 and 2. The three patients shown illustrate the extent of variation between samples. The simplest complexes/aggregates contained only IgG, IgM, C1q frequently with the presence of some partially aggregated fibrinogen (a). In some cases, IgG aggregates in the complex were incompletely dissociated in the first dimension (b).

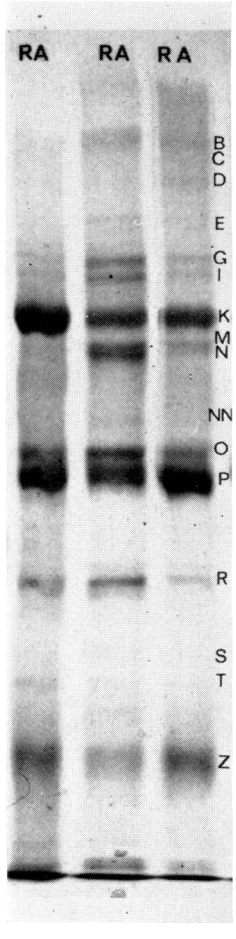

FIG. 2. Immune complexes of three patients suffering from rheumatoid arthritis, analysed by 1-dimensional SDS-PAGE. Letter designations refer to column 2 of Table 1. These gels were used to confirm molecular weights of peptides.

by interpolation from another set of standards (fibrinogen, thyroglobulin, IgG, phosphorylase a and bovine serum albumin). Group 4 peptides (Fig. 1b) are derived from parent molecules of several molecular weights, all giving rise to the same peptide patterns, implying that this molecular species is present in several polymerized and/or degraded forms. The values derived for the molecular weights of unreduced molecules are less accurate than those for peptides, firstly, for the reason noted above, and secondly because the presence of disulphide bonds prevents complete denaturation of the peptide chains. The presence of sugar residues on glycoproteins can cause anomalous electrophoretic behaviour and may introduce an error into the calculation of molecular weights from this data.

In many cases the molecular weights obtained by SDS-PAGE techniques gave a strong indication of the molecular identity of the peptide group, but in addition, immunochemical methods were used to establish or confirm these identities. Complexes were pre-labelled with ^{125}I and specific antigenic species precipitated by countercurrent electrophoresis or double diffusion in agar gels using monovalent antisera; the precipitin arcs were then excised and analysed by 1-dimensional SDS-PAGE under reducing conditions. Autoradiography of the fixed gel allowed visualization of the peptides from each molecule isolated according to its reaction with the specific antiserum. The autoradiograph patterns were then compared with the peptide groups from the 2-dimensional gels, to assign identities to each group. Fig. 3 shows the peptides from the precipitin arcs using the different antisera indicated. Anti-fibrinogen

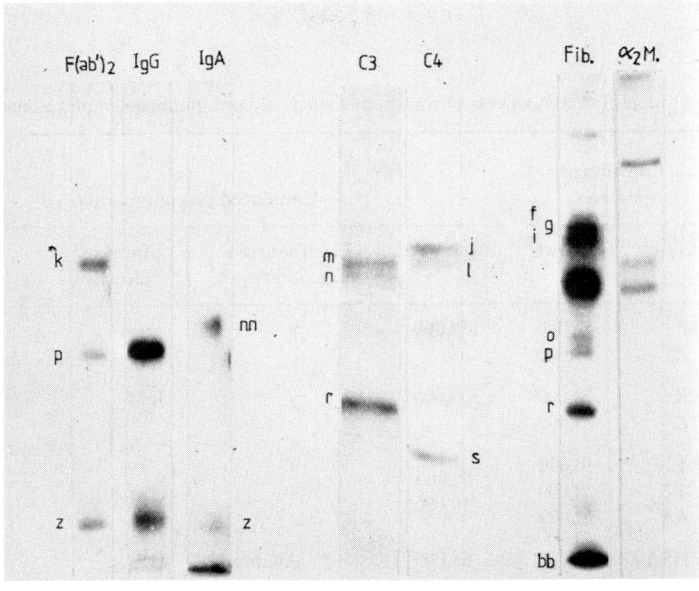

FIG. 3. Identification of the peptides from a single antigenic species by immunoprecipitation SDS-PAGE. Autoradiographs of the peptides from single molecules of immune complexes/aggregates isolated by precipitation with monovalent antisera in agar gels. Antisera used, and band identities are indicated. These were used to confirm or identify the antigenic identities of peptide groups derived from 2-dimensional SDS-PAGE.

also precipitates a band (mol. wt approx. 70,000) which is not present on the 2-dimensional gels. Where band patterns can be unequivocably correlated with bands on the SDS gels the letter designation is indicated. The electrophoretic mobility of certain molecules was ascertained by conventional immunoelectrophoresis (2% agar; barbitone pH 8·4) prior to SDS-PAGE analysis. Table 1 gives the identity of the parent molecule in each group based upon peptide structure, molecular weights and electrophoretic mobility and antigenic characteristics where available. The final column shows the relative abundance of each molecule in the synovial fluid complexes analysed, assessed by observation of more than forty samples. IgG, IgM and C1q were the most abundant components, followed by fibrinogen (variable), IgA, C3, C4, α_2-macroglobulin and serum albumin. In addition to the components identified there are several molecules present to a variable extent but in trace amounts only on the two dimensional gels and for these, only molecular weight data are available (Table 2). Low levels of $C\bar{1}r$, $C\bar{1}s$, factor B and β_2-microglobulin have been identified by immunoprecipitation only but we cannot yet say whether these are identical with any of the trace molecules listed in Table 2. Some gels were also stained for carbohydrate by periodic acid Schiff according to the technique of Glossman & Neville (1971). Positive staining was observed coincident with bands K (IgM) and to a lesser extent R (C3/fibrinogen) and P (IgG). Therefore, there would appear to be no component in the precipitates containing as much carbohydrate as is present in these glycoproteins. Complexes at a concentration of 0·5 mg protein/ml were tested for DNA by the diphenylamine method of Giles & Myers (1965) after digestion of protein components with 2% w/w pronase at 37°C for 16 hr. This method has a sensitivity of 10 μg DNA/mg original protein; DNA was detected in the complexes isolated from 6/16 synovial fluids but in only one case were values in excess of 17·5 μg/mg protein observed (Table 3). (A separate study on six seronegative patients revealed a high DNA content in just one.) In none of the positive patients was it possible to demonstrate significant levels of antibodies to DNA in the 7S fraction.

The complexes from two patients were labelled with ^{125}I and run on 2-dimensional SDS-PAGE. More than 96% of the activity was present in the peptides of identified molecules (Table 1); on the somewhat

TABLE 1. Identified components of rheumatoid synovial fluid immune complexes/aggregates

	Constituent peptides		Unreduced parent molecule			
Group*	Code†	Mol. wt	Mol. wt‡	Electrophoretic mobility§	Antigenic identity	Relative abundance¶
1	P Z	52,000 25,000	150,000	γ	IgG	+++
2	K Z	78,000 25,000	> 500,000	γ	IgM	+++
3a	T1 T2 AA	30,000 28,000 22,000	66,000 410,000	—	C1q	++
3b	HSA	70,000	68,000	Albumin	HSA	(+)
4	F G I O P R BB	96,000 94,000 89,000 58,000 52,000 40,000 20,000	164,000 235,000 295,000 385,000	β–γ	Fibrinogen	0 to ++
5	(D) M N R	112,000 75,000 72,000 40,000	135,000	β	C3	+
6	J L S	82,000 78,000 33,500	150,000	β	C4	+
7	NN Z	62,000 25,000	166,000	β	IgA	+
8	—	127,000 90,000 85,000	—	α	α_2-Macroglobulin	+
9	—	125,000 108,000 58,000	350,000	—	Fibrinogen	0 to ++

* Defined by 2-dimensional SDS-PAGE.

† Band designation from 1-dimensional SDS-PAGE.

‡ Determined by mobility of unreduced molecules in the first dimension of 2-dimensional SDS-PAGE.

§ Electrophoretic mobility in agar, pH 8·4.

¶ Based on staining.

TABLE 2. Trace components of synovial fluid complexes not yet identified

Group	Molecular weight	
	Constituent peptides	Unreduced parent molecule
10	99,000 77,000	195,000
11	58,000 38,000	85,000
12	80,000	85,000
13	130,000	130,000

Table gives data on four molecules, derived from 2-dimensional SDS-PAGE giving peptide molecular weights and molecular weight of parent molecules. They are present in trace quantities and are unidentified.

TABLE 3. Comparison between DNA content of the sucrose/PEG precipitation and the presence of anti-DNA in the 7S fraction of synovial fluid

Patient	Sucrose/PEG precipitate	Synovial fluid 7S fraction	
	μg DNA/mg protein*	IgG (μg/ml) in test†	Anti-dsDNA per cent binding (mean$\pm$s.d.)†
R.R.	11	n.a.	n.a.
R.A.	17·5	1509	4·7$\pm$1·5
C.S.	16	1374	3·1$\pm$0·7
V.C.	13	1089	4·2$\pm$2·3
E.J.	34	1311	3·5$\pm$0·4
J.G.	15	1767	3·3$\pm$0·8

* Determined by diphenylamine assay.

† Values determined by Farr assay using tritiated sonicated dsDNA from *E. coli* (Miles Laboratories). Limit of normality for five normal sera run concurrently (mean+2 s.d.) was 5·3%, the IgG concentration of the test samples ranging from 800–1800 μg/ml.

uncertain assumption that all proteins are labelling in a comparable fashion, the unidentified components (groups 10–13) may be assumed to represent no more than 4% of the total protein in the complex.

DISCUSSION

In the present studies we have analysed complexes isolated by ultracentrifugation through a combined sucrose/PEG gradient. Although this system leads to precipitation of only 10–20% of the low molecular weight complexes in the range 9–11S, the larger complexes known to be present in rheumatoid synovial fluids (Winchester & Kunkel, 1968) are preferentially recovered. Since the larger complexes may be particularly active in complement fixation and cellular binding due to clustering of Fc regions (Gergely

et al., 1978) this may not be a disadvantage. The sucrose/PEG gradient was constructed to ensure relatively low non-specific precipitation of monomeric IgG but large molecules of low PEG solubility may also be precipitated, although only traces of IgM and C1q were recovered when normal human serum was processed by this procedure. It is debatable, therefore, whether the quantities of material reacting with anti-fibrinogen represent complexed molecules or non-specific precipitation of fibrin degradation products formed as a consequence of an acute inflammatory reaction in the joint. Since amounts of this material recovered in the synovial fluid precipitates from different patients varied widely, being totally absent in some cases, the latter explanation seems the most probable. The presence of several molecular forms of the protein would be consistent with the occurrence of partial activation and/or degradation and this would fit with observations of the failure of normal coagulation and plasminolytic mechanisms in joint fluids of animal models (Harrold, 1973).

We are also inclined to think that the modest amounts of α_2-macroglobulin recovered in the sucrose/PEG precipitates result from non-specific interactions related to its ability to bind to and inhibit proteolytic enzymes. The α_2-macroglobulin peptide bands of 90,000 and lower are characteristic of the cleaved form produced following tryptic digestion of the molecule associated with irreversible inactivation of the enzyme (Hall & Roberts, 1978). In addition to the native uncleaved chains which barely enter the 8% gels, there is also a major band of molecular weight 127,000 which may be an artefact of reduction (Roberts, Riesen & Hall, 1973). The α_2-macroglobulin could be complexed with proteolytic enzymes released from neutrophils or macrophages involved in the joint inflammatory processes or might bind to activated complement components on the immune complex itself. Similarly, the presence of human serum albumin in the precipitate probably reflects a non-specific association with molecules of the complexes since albumin could only be recovered from serum or osteoarthritic synovial effusions by sucrose/PEG centrifugation if precipitable material such as aggregated IgG were added.

Without question the dominant components of the complex were IgG and IgM with lesser amounts of C1q. More modest contributions were made by IgA, C4 and C3. The presence of C1q and C4 in its activated form following cleavage of the α-chain strongly implicates these complexes as the major trigger for the complement sequence in the rheumatoid synovial effusion, and furthermore suggests that the classical pathway is predominant; indeed, only traces of factor B were demonstrable immunochemically. Trace amounts of $C\bar{1}r$, $C\bar{1}s$ and β_2-microglobulin (cf. Böhm *et al.*, 1978) could also be detected. This leaves only the four unidentified components listed in Table 2 which between them are estimated to make up less than 4% of the total protein of the complexes: of these, the group 11 peptides have molecular weights consistent with $C\bar{1}r$. It is possible that one of these trace components may be a joint or an exogenous microbial antigen primarily responsible for complex formation. Alternatively, we may be dealing with an unknown antigen which is particularly susceptible to proteolytic enzymes and which has undergone degradation prior to sampling the synovial fluid for analysis, or which is insoluble (e.g. collagen) and has remained undetected by the techniques we employ. Further studies are clearly indicated and certain rather obvious candidates such as the nuclear antigen associated with transformed cells should be checked.

However, it could equally well be argued that since the components which we have characterized constitute 96% of the material in the complexes, they already represent the predominant antigen–antibody system with IgG itself as the essential autoantigen. It is relevant to note that immunofluorescent studies on the synovial tissue of rheumatoid patients have shown that up to 50% or more of the plasma cells are making antibody to IgG (Munthe & Natvig, 1975) and that up to 50% of the IgG in the synovial fluid complexes binds to Fcγ columns (Winchester, 1975). The contribution made by cells synthesizing anti-idiotypic antibodies has yet to be assessed. The demonstration of elevated serum anti-globulins in nearly all patients with either 'seropositive' or 'seronegative' rheumatoid arthritis (Nineham, Hay & Roitt, 1976) further emphasizes the central importance of IgG autosensitization in this disorder. The crucial feature may be the unique ability of the IgG anti-IgG molecules to self-associate (Pope *et al.*, 1975) and form complexes which can be stabilized by polyvalent binders of the IgG Fc moiety such as IgM anti-IgG (classical rheumatoid factor) and C1q. This then leads to activation of the classical complement pathway and induction of an acute inflammatory reaction, while combination with surface

receptors of macrophage-like cells in the synovium may stimulate them to form the pannus (as may lymphokines released from IgG sensitized T cells). The release of enzymes such as collagenase and neutral proteinases from the cells involved at the inflammatory foci leads to breakdown of cartilage through degradation of collagen as suggested by others (Woolley, Crossley & Evanson, 1977; Wahl *et al.*, 1975).

Local production of IgG anti-globulins within the joint is presumably required for significant complex formation to occur as a result of high concentrations of the self-associating molecules; in the circulation, an excess of competing irrelevant IgG will tend to inhibit self-association and keep down the size of the complexes. Thus, although a high proportion of patients with rheumatoid arthritis have circulating complexes, only a minority have significant extra-articular vasculitis and complications such as nodule formation would seem to arise from local anti-globulin production (Nowoslawski & Brzosko, 1967). Notwithstanding the lack of information on the origin of IgG autosensitization in the joints, our present data do not rebut the hypothesis that IgG is the main, if not the only, antigen responsible for provoking the pathological changes in rheumatoid arthritis.

We thank the Medical Research Council for research support and for a studentship to David Male. We are grateful to Dr K. Reid (Oxford) for providing antisera to C4, $C\bar{1}r$ and $C\bar{1}s$, and to Dr G. Bull (Southampton) for anti-IgA. We particularly thank Dr M. Corbett and her clinical colleagues, and Sister C. Hall of the Middlesex Hospital for generous help in providing synovial fluid samples from their patients.

REFERENCES

BÖHM, U., FALUS, A., MERETEY, K. & BOZSODY, S. (1978) High Beta-2-microglobulin ratio synovial fluid/serum in rheumatoid arthritis. Parallelism with hidden rheumatoid factor and inverse relation to free rheumatoid factor. *4th European Immunology Meeting, Budapest*, p. 128, (abstract).

CRACCHIOLO, A., GOLDBERG, L., BARNETT, E.V. & BLUESTONE, R. (1971) Studies of cryoprecipitates from synovial fluids of rheumatoid patients. *Immunology*, **20**, 1067.

CREMER, N.E., HURWITZ, D., QUISMORIO, F.P., LENNETS, E.H. & FRIOU, G.J. (1974) Antiviral antibodies in rheumatoid synovial fluid and cryoprecipitates. *Clin. exp. Immunol.* **18**, 27.

GERGELY, J., MEDGYESI, G.A., FÜST, G. & RAJNAVOLGYI, E. (1978) Constitution of immune complexes and their interaction with the complement system. *4th European Immunology Meeting, Budapest*, p. 8, (abstract).

GILES, K.W. & MYERS, A. (1965) An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature*, **206**, 93.

GLOSSMAN, H. & NEVILLE, D.M. (1971) Glycoprotein of cell surfaces. *J. biol. Chem.* **246**, 6339.

HALL, P.K. & ROBERTS, F.C. (1978) Physical and chemical properties of human plasma α_2-macroglobulin. *Biochem. J.* **171**, 27.

HARROLD, A.J. (1973) Elimination of fibrinogen from synovial joints. *Ann. Rheum. Dis.* **32**, 29.

LAEMMLI, U.K. (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T.4. *Nature*, **227**, 681.

MALE, D.K. & ROITT, I.M. (1979) Analysis of the components of immune complexes. *Mol. Immunol.* **16**, 197.

MELLBYE, O.J. & MUNTHE, E. (1971) Specific binding of immunoconglutinin in tissues and synovial fluids from patients with rheumatoid arthritis. *Clin. exp. Immunol.* **8**, 713.

MELLBYE, O.J. & NATVIG, J.B. (1971) Evidence for immune complexes containing antibody to the pepsin site of IgG in rheumatoid synovial fluids. *Clin. exp. Immunol.* **8**, 889.

MUNTHE, E. & NATVIG, J.B. (1971) Characterization of IgG complexes in eluates from rheumatoid tissue. *Clin. exp. Immunol.* **8**, 249.

MUNTHE, E. & NATVIG, J.B. (1975) Immunoglobulin classes, subclasses and complexes of IgG rheumatoid factor in rheumatoid plasma cells. *Clin. exp. Immunol.* **12**, 55.

NATVIG, J.B. & MUNTHE, E. (1975) Self-associating IgG rheumatoid factor represents a major response of plasma cells in rheumatoid inflammatory tissue. *Ann. N.Y. Acad. Sci.* **256**, 88.

NINEHAM, L.J., HAY, F.C. & ROITT, I.M. (1976) Laboratory diagnosis of rheumatoid arthritis: a solid phase radioassay for IgG and IgM antiglobulin. *J. clin. Pathol.* **29**, 1121.

NOWOSLAWSKI, A. & BRZOSKO, W.J. (1967) Immunopathology of rheumatoid arthritis. II. The rheumatoid nodule (the rheumatoid granuloma). *Pathologia Europaea*, **2**, 302.

POPE, R.M., MANNIK, M., GILLILAND, B.C. & TELLER, D.C. (1975) The hyperviscosity syndrome in rheumatoid arthritis due to intermediate complexes formed by self-association of IgG-rheumatoid factors. *Arthritis Rheum.* **18**, 97.

ROBERTS, R.C., RIESEN, W.A. & HALL, P.K. (1973) Studies on the quarternary structure of human serum α_2-macroglobulin. *Proteinase Inhibitors* (ed. by H. Fritz, H. Tschesche, L.J. Greene and E. Truscheit) pp. 63–71. Springer-Verlag, Berlin.

RUDDY, S. & AUSTEN, K.F. (1970) The complement system in rheumatoid synovitis. I. An analysis of complement component activities in rheumatoid synovial fluids. *Arthritis Rheum.* **13**, 713.

STEFFEN, C. (1975) Collagen antibodies and collagen–anticollagen immune complexes in rheumatoid arthritis. *Z. Rheumatol.* **34**, 391.

WAHL, L.M., WAHL, S.M., MERGENHAGEN, S.E. & MARTIN, G.R. (1975) Collagenase production by lymphokine activated macrophages. *Science*, **187**, 261.

WINCHESTER, R. (1975) Characterization of IgG complexes in patients with rheumatoid arthritis. *Ann. N.Y. Acad. Sci.* **256**, 73.

WINCHESTER, R.J., AGNELLO, V. & KUNKEL, H.G. (1970) Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterization and relationship to lowered complement levels. *Clin. exp. Immunol.* **6**, 689.

WINCHESTER, R. & KUNKEL, H.G. (1968) High molecular weight complexes involving γ-globulin in joint fluids from rheumatoid arthritis patients. *Arthritis Rheum.* **11**, 849.

WOOLLEY, D.E., CROSSLEY, M.J. & EVANSON, J.M. (1977) Collagenase at sites of cartilage erosion in the rheumatoid joint. *Arthritis Rheum.* **20**, 1231.