Tanford, C. (1955), *J. Phys. Chem. 59,* 798.
Tanford, C. (1968), *Adv. Protein Chem. 23,* 121.
Tanford, C. (1970), *Adv. Protein Chem. 24,* 1.
Tanford, C., Kawahara, K., and Lapanje, S. (1967a), *J. Am. Chem. Soc. 89,* 729.
Tanford, C., Kawahara, K., Lapanje, S., Hooker, T. M., Jr., Zarlengo, M. H., Salahuddin, A., Aune, K. C., and Takagi (1967b), *J. Am. Chem. Soc. 89,* 5023.
Waissbluth, M. D., and Grieger, R. A. (1974), *Biochemistry 13,* 1285.
Warren, J. R., and Gordon, J. A. (1971), *Biochim. Biophys. Acta 229,* 216.

Ultracentrifuge Studies of the Binding of IgG of Different Subclasses to the Clq Subunit of the First Component of Complement†

Verne N. Schumaker,* Mary Ann Calcott, Hans L. Spiegelberg, and Hans J. Müller-Eberhard‡

ABSTRACT: Normal IgG and myeloma proteins of the IgG1, 2, 3, and 4 subclasses were mixed with human Clq and studied in the analytical ultracentrifuge for complex formation. Binding of IgG to Clq is apparent both from the enlargement of area and from the increase in sedimentation rate of the well-separated schlieren peak of Clq. The accurate determination of binding parameters requires that sedimentation rates be corrected for hydrodynamic interaction, and area measurements corrected for the Johnston–Ogston effect. At the highest immunoglobulin concentrations employed in these studies, more than ten IgG molecules are bound to each Clq. If we assume that the number of binding sites must be an integral multiple of 6, then the data best support a 12 binding site model, although an 18 site model cannot be ruled out. Myeloma IgG proteins of all subclasses bind to Clq, with affinities decreasing in the order G3 > G1 > G2 > G4. No binding of IgA to Clq could be detected.

The first component of complement (Cl) is a protein–calcium complex composed of at least three subunits, Clq, Clr and Cls (Lepow et al., 1963; Naff et al., 1964). A fourth subunit, Clt, has been reported (Assimeh and Painter, 1975). Clq has a number of sites specific for the binding of IgM and IgG. The location of the binding site on IgG has been reported to be within the C_H2 domain of the F_c subunit (Kehoe and Fougereau, 1970; Yasmeen et al., 1976), but the C_H3 domain may also be involved in the binding of Clq (Allan and Isliker, 1974; Ovary et al., 1976). The binding of antibody to Clq at multiple sites appears to trigger the activation of Clr (Naff and Ratnoff, 1968; Valet and Cooper, 1974b; Ziccardi and Cooper, 1976) which in turn results in proteolytic cleavage and activation of Cls (Valet and Cooper, 1974a; Sakai and Stroud, 1974). This initiates the classical pathway since Cls possesses proteolytic and esteratic activity for C4, C2, and synthetic substrates. Some recent reviews are by Müller-Eberhard (1975), Cooper (1973), Lepow (1972), Kinsky (1972), and Ruddy et al. (1972).

Isolation and purification of Clq has been performed by several different procedures (Müller-Eberhard and Kunkel, 1961; Agnello et al., 1970; Calcott and Müller-Eberhard, 1972; Volanakis and Stroud, 1972; Sledge and Bing, 1973b), and the molecule has been examined with the electron microscope (Svehag et al., 1972; Shelton et al., 1972; Knobel et al., 1975). It is a remarkable object, composed of a central body from which radiate six fibrillar arms ending in six peripheral subunits. The molecule can be dissociated to yield noncovalently bonded subunits (Calcott and Müller-Eberhard, 1972; Volanakis and Stroud, 1972; Reid et al., 1972; Yonemasu and Stroud, 1972; Heusser et al., 1975). Upon reduction and alkylation, three polypeptide chains are produced (Reid et al., 1972; Yonemasu and Stroud, 1972; Heusser et al., 1975; Bhattacharyya et al., 1974; Lowe and Reid, 1974), all of which appear to have a collagen-like region (Calcott and Müller-Eberhard, 1972; Reid et al., 1972; Bhattacharyya et al., 1974; Lowe and Reid, 1974).

The binding of immunoglobulins to Clq has been studied by ultracentrifugation (Müller-Eberhard and Calcott, 1966), affinity chromatography (Sledge and Bing, 1973a), and by a "$C\bar{1}$ binding assay" (Augener et al., 1971). Digestion studies indicate that the peripheral subunits are sites of attachment of the Clq to IgG (Knobel et al., 1974).

In this communication we report the results of binding studies between Clq and myeloma proteins of the IgG1, 2, 3, and 4 heavy chain subclasses, as well as normal IgG. These studies have all been performed with the ultracentrifuge. Since high concentrations of immunoglobulins must be employed to saturate the binding sites on Clq, some sedimentation anomalies occur and must be corrected to allow quantitative interpretation of binding data. These anomalies cause an apparent reduction of the amount of immunoglobulin bound to Clq. Therefore, a minimum number of binding sites can be estimated from the uncorrected sedimentation data. It ranges from

† From the Department of Chemistry (Contribution No. 3554) and the Molecular Biology Institute, University of California, Los Angeles, California, 90024 (V.N.S.), and the Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037 (M.A.C., H.L.S., H.J.M.-E.). *Received March 8, 1976.* This work was supported by Research Grants GM 13914, AI 10734, and AI 07007 and Research Fellowship 1 F03 AI 51187 from the National Institutes of Health. Computing was funded through an intramural grant from the University of California, Los Angeles, for the use of the facilities of the Campus Computing Network.

‡ Cecil H. and Ida M. Green Investigator in Medical Research, Scripps Clinic and Research Foundation.

four to six binding sites per Clq. When the data are corrected, however, larger estimates are obtained, and our best estimate indicates that more than ten binding sites are present on the Clq molecule.

Materials and Methods

Isolation of Human Clq. Over the course of these experiments, 12 different preparations of Clq have been used. The typical isolation procedure is described in detail by Calcott and Müller-Eberhard (1972). The euglobulins, including Clq, are precipitated by adding 3 volumes of distilled water to fresh serum at pH 7. This is followed by chromatography on carboxymethylcellulose. Since Clq is highly basic, it is separated from 80% of the euglobulins in this step. Next, advantage is taken of its high molecular weight to further purify the Clq on Sephadex G-200. The final purification is then accomplished using Pevikon block electrophoresis, yielding up to 8 mg of Clq from 800 ml of serum.

Myeloma Proteins. Some of the myeloma proteins used in this study were generously provided by Dr. Howard Grey. The IgG myeloma proteins were isolated by DEAE[1]-cellulose chromatography or Pevikon block electrophoresis (Spiegelberg et al., 1968). Monomeric 7S IgA proteins were isolated by Pevikon electrophoresis and Sephadex G-200 gel filtration. Traces of contaminating IgG were removed by passage through a Sepharose 4B column to which rabbit anti-human IgG antibodies were coupled.

Ultracentrifugation. Immediately prior to mixing the immunoglobulin and the Clq, the immunoglobulin was centrifuged at 45 000 rpm for 30 min in a swinging bucket rotor to remove any large aggregates formed during storage. The IgG and Clq were mixed together and immediately examined in the analytical ultracentrifuge.

The schlieren optical system was used to follow the migrating boundaries of IgG and IgG–Clq complex. Usually, two cells were run simultaneously, one with wedge window. In one cell was placed a mixture of Clq and IgG myeloma protein in 0.05 M sodium phosphate buffer, pH 7.0. When centrifuged, two sedimenting peaks were observed: the slower boundary of IgG and the faster moving IgG–Clq complex. In the other cell was placed a solution of Clq at the same concentration to serve as a control. Thus, the difference in areas between the Clq complex peak of the mixture run and the Clq peak of the control run should yield the amount of IgG bound in the complex. A correction must be made for a well-understood centrifuge artifact, the Johnston–Ogston effect, which distorts the area measurements in the mixture run. This correction is described in an appendix to this paper (see paragraph at the end of this paper concerning supplementary material).

Alternatively, binding can be measured from the increased sedimentation velocity of the complex, as described later.

Sedimentation coefficients wer determined by measuring the radial position, r, of the maximum ordinant, and plotting $\log r$ vs. t and taking the slope of the least-squares line. Values were then corrected for the density (1.0043 g/ml) and the relative viscosity (1.0318) of 0.05 M sodium phosphate buffer, pH 7.0, 20.0 °C, assuming a partial specific volume of 0.73 ml/g for all proteins.

Peak positions on the schlieren photographs were determined using a microcomparator equipped with a double micrometer stage allowing precision measurements in both the X and Y directions on the photographic film. Areas were determined by tracing an enlargement on graph paper, cutting, and weighing.

The Johnston–Ogston effect is associated with a convective process which occurs in the region between the fast and slow boundaries. When the Johnston–Ogston effect is large, this results in some distortion in peak shape, and the baseline never really returns to zero in the region between the two boundaries. In a few cases, some subjectivity is required in the decision of where to separate the two areas.

All analytical ultracentrifugation was performed using a black anodized rotor. Rotor temperature was initially adjusted to 20.0 °C and was maintained at this temperature during the course of the run, using the "Rotor Temperature Indicator Control" system supplied by the manufacturer, Beckman Instruments, Spinco Division, Palo Alto, Calif. Double sector, aluminum-filled Epon centerpieces were used in all experiments, providing each schlieren peak with a baseline for convenient area measurements. A photometer was used to measure illumination of the view screen at the beginning of each experiment, allowing an exposure time to be selected for best contrast of the schlieren image.

All centrifuge runs were performed at 52 640 rpm. Ten photographs were taken at 8-min intervals after reaching speed. The 32- and 48-min pictures were used for area analysis, although occasional late pictures were selected when the complex peak was unusually sharp.

Theoretical Sedimentation Rates of Clq–IgG Complexes. In order to compute the sedimentation rates of Clq and its complexes with IgG, eq 23 of Bloomfield et al. (1967) was employed to calculate the translational frictional coefficient. The model selected for Clq was composed of six subunits arranged at the vertices of a regular octahedron and a seventh subunit at the center. Subunit shape was assumed to be spherical. Molecular weights of 51 250 and 102 500 were selected for the peripheral and central subunits, respectively, which correspond to radii of 24.5 and 30.8 Å, assuming a partial specific volume of 0.73 cm^3/g. These radii were then increased by 5% to represent a reasonable hydration value of about 16%. The hydrated radii used were 25.7 and 32.3 Å, respectively. The center of each peripheral subunit was chosen a distance of 135.5 Å from the central subunit. Thus, the span between two opposite peripheral subunits is 320 Å, reasonably close to the value of 330 Å reported by Svehag et al. (1972). This distance was selected because it yields the observed sedimentation coefficient for Clq.

The IgG is represented by a hydrodynamically-equivalent sphere having the same translational frictional coefficient as the real molecule. This is a sphere having a radius of 57.2 Å. Upon binding of the IgG to a peripheral subunit on the Clq, the subunit is replaced by a larger sphere of radius 58.9 Å ($58.9^3 = 25.7^3 + 57.2^3$). When two IgG are bound, the subunit radius was taken as 71.4 Å.

To determine the average sedimentation coefficient for a complex composed of 1 Clq and n IgG, eq 23 is applied separately to each nonequivalent isomeric arrangement of the n IgG at 12 binding sites, to yield a frictional coefficient. Using the frictional coefficient and the molecular weight of a particular arrangement, the sedimentation rate is calculated. The appropriate average is then taken, and the results are listed in Table I.

If the logarithm of the molecular weight is plotted as a function of the logarithm of the calculated sedimentation coefficients listed in Table I, a gently curving line is obtained. We have approximated this gentle curve by a straight line,

[1] Abbreviations used: DEAE, diethylaminoethyl.

TABLE I: Calculated Sedimentation Rates of Clq Binding 0 to 12 Molecules of IgG.

No. of Bound IgG	Mol Wt of Complex	Sedimentation Coeff[a]
0	410 000	10.15
1	570 000	12.93
2	730 000	15.10
3	890 000	17.11
4	1 050 000	19.06
5	1 210 000	20.97
6	1 370 000	22.87
7	1 530 000	25.17
8	1 690 000	27.13
9	1 850 000	29.64
10	2 010 000	31.83
11	2 170 000	33.98
12	2 330 000	36.11

[a] $s = M(1 - \bar{v}\rho)/Nf$, where $\bar{v} = 0.73$ cm^3/g, $\rho = 1.00$ g/cm^3, and f is calculated by eq 23 of Bloomfield et al. (1967), using $\eta = 0.01$ P.

yielding an expression good to within 2% over the range of 0 to 6 bound IgG:

$$\frac{M}{M_0} = \left(\frac{s}{s_0}\right)^{\alpha} \quad (1)$$

where the exponent, α, equals 1.47.

Results

A detailed tabulation of most of the data analyzed in this paper is available in the microfiche edition of this journal (see paragraph at the end of this paper concerning supplementary material). Five ultracentrifuge runs were made using IgG isolated from pooled, normal serum. Thirteen runs were made using IgG1 myeloma immunoglobulins from six donors. Sixteen runs were made using IgG2 myeloma immunoglobulins from eight donors. Eight runs were made using IgG4 myeloma immunoglobulins obtained from three donors.

Figure 1 is an example of experimental data analyzed in this study. The lower schlieren tracing is the control of Clq sedimenting by itself at a concentration of 1.36 mg/ml. The upper tracing is a mixture of normal IgG (4.15 mg/ml) and Clq (1.36 mg/ml). From an inspection of the pattern, it is obvious that the area of the Clq peak is markedly enhanced in the mixture. Measurement of the area shows that it has more than doubled (2.82 vs. 1.36 mg/ml). We assume that this increase in area is caused by the binding of the IgG to Clq. An uncorrected estimate of the amount bound is given by $(2.82 - 1.36)/1.36 = 1.07$ mg of IgG/mg of Clq. Multiplication of this number by the ratio of molecular weights yields $1.07 \times (410\,000/160\,000) = 2.75$ molecules of IgG bound to one molecule of Clq. (In making this computation, we have assumed the refractive increments of Clq and IgG are equal. Preliminary measurements indicate that the refractive increments for these two proteins are similar or equal.)

In Figure 2A are plotted the uncorrected binding data for all runs, computed as just described, as a function of the logarithm of the IgG concentration. Through the experimental points are drawn the best-fitting least-squares curves for a model composed of six independent binding sites, according to the formula (Tanford, 1961):

$$\bar{\nu} = \frac{nK_a c}{1 + K_a c} \quad (2)$$

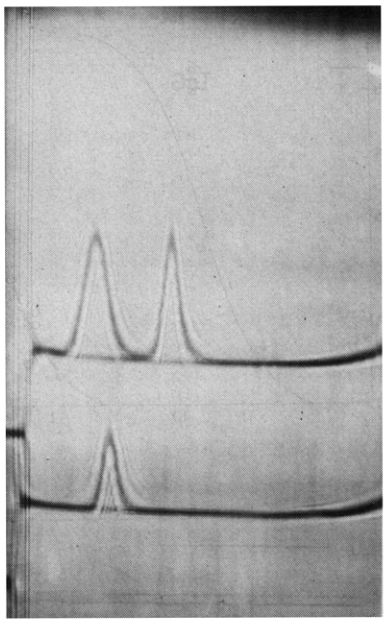

FIGURE 1: Schlieren patterns used for analysis of run 318. The lower schlieren tracing is the control of Clq sedimenting by itself at a concentration of 1.36 mg/ml. The upper tracing is a mixture of IgG (4.15 mg/ml) and Clq (1.36 mg/ml).

where $\bar{\nu}$ is the average number of IgG bound to Clq, n is the number of sites, c is the concentration of IgG, and K_a is the association constant giving the best least-squares fit of the curve through the points.

Several features are apparent from this plot of the uncorrected data: (a) the six binding site model fits the uncorrected IgG and G1 data fairly well; (b) the G2, G3, and G4 data are too scattered to permit selection of models on the basis of curve shape; (c) two of the G3 points lie above five bound immunoglobulin molecules, and the steepness of rise suggests that saturation has not been obtained and there may actually be more than six binding sites present on each Clq molecule.

In order to correct the binding data for hydrodynamic interaction, it is necessary to measure the various "s vs. c" interaction parameters: k_{ss}, k_{ff}, k_{sf}, and k_{fs}. See the appendices (supplementary material) for the mathematical definition of these quantities. The subscripts f and s refer to the fast (Clq) and slow (IgG) components, respectively. It is not possible to measure the last two of these interaction parameters in mixtures of Clq and IgG, of course, because of the binding of IgG to Clq. Instead, we have used a IgA myeloma protein, which apparently does not bind to Clq. The IgA was depleted of traces of IgG using an affinity column of anti-IgG. To obtain k_{fs}, mixtures of Clq and IgA were studied over a wide range of IgA concentrations from 4 to 32 mg/ml. To obtain k_{sf}, a synthetic boundary of IgA was formed by layering Clq over a mixture containing Clq and IgA. The Clq concentration was 1.4 mg/ml. A paired control contained IgA alone. A decrease in the sedimentation rate of the IgA boundary of about 0.2 S was measured. This experiment was repeated three times. Values of k_{ff} and k_{ss} were measured for the Clq and IgA by boundary sedimentation of a dilution series. The various values of k obtained in these experiments are $k_{ff} = 0.058 \pm 0.009$ ml/mg, $k_{ss} = 0.017 \pm 0.002$ ml/mg, $k_{fs} = 0.026 \pm 0.002$ ml/mg, and $k_{sf} = 0.026 \pm 0.003$ ml/mg, where the listed errors are standard errors of the slope.

Binding data obtained from area measurements corrected for the Johnston–Ogston effect are presented in Figure 2B.

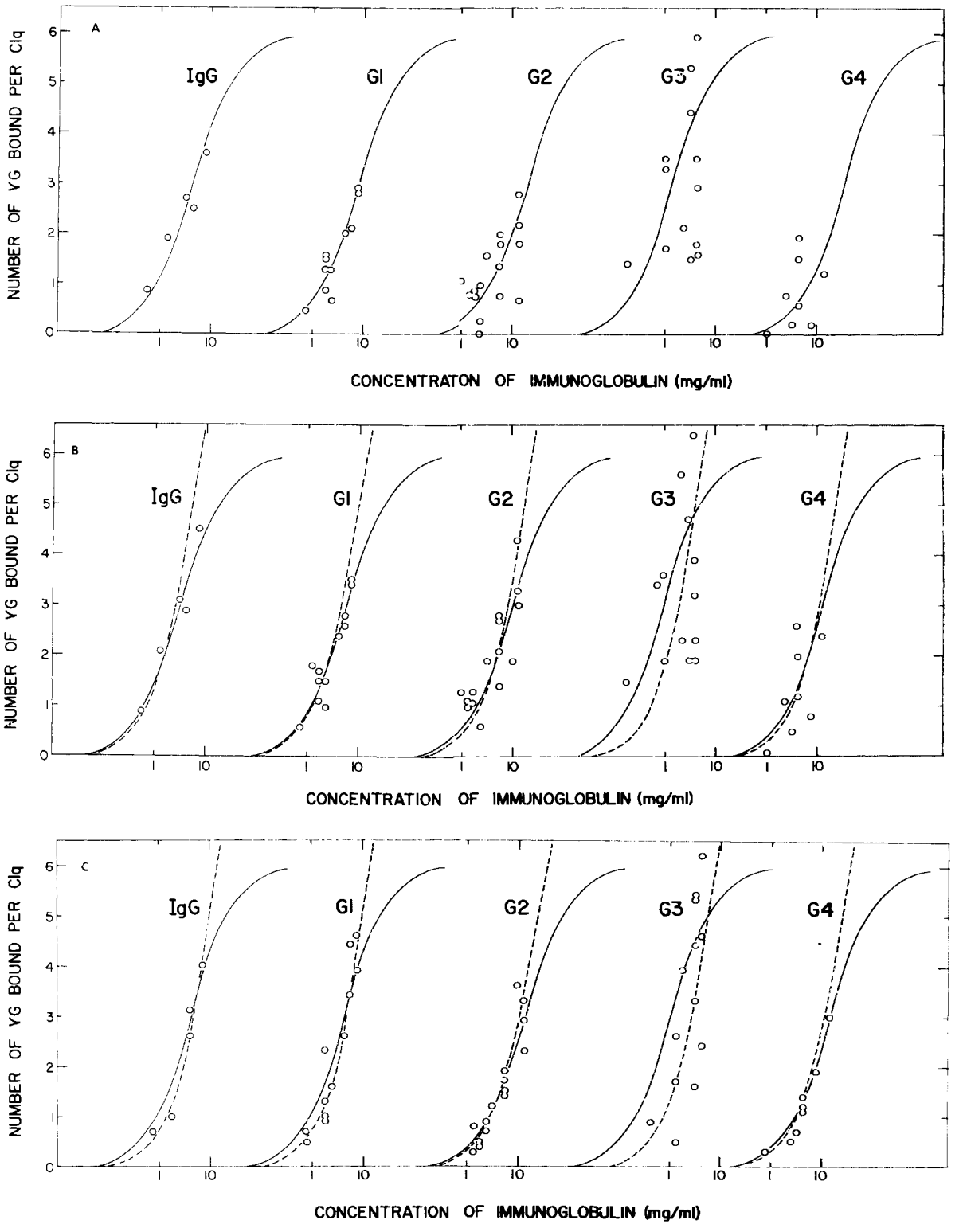

FIGURE 2: The binding of immunoglobulins to Clq is shown in this illustration. The vertical axes give the number of immunoglobulin molecules bound per Clq. The horizontal axes are logarithmic scales of immunoglobulin concentration. Points at 1 and 10 mg/ml are shown for each set of experimental data. The smooth curves drawn through the data points are the theoretical curves for 6 (solid lines) and 12 (dashed lines) independent binding sites which represent a best fit to the experimental data. (A) Uncorrected binding data calculated directly from the increase in area of the schlieren peak of Clq when the immunoglobulin is present. (B) Binding data corrected for the Johnston–Ogston effect, using eq 5, 6, and 7 of Appendix I (see paragraph concerning supplementary material at the end of this paper) and the measured s vs. c interaction parameters. (C) Binding data obtained from the sedimentation rates of the complex peaks, as described by eq 1 of the text. The observed sedimentation rates were corrected for concentration dependence using eq 1a and 9 of Appendix I.

These data were corrected using eq 5, 6, and 7 of Appendix I and the measured s vs. c interaction parameters. Through the experimental points are drawn the "best" curves for 6 and 12 independent binding sites according to eq 2.

An entirely independent estimate of the binding of IgG to Clq may be obtained from the sedimentation rates of the complex peaks, as described by eq 1. The observed sedimentation rats must be first corrected for the s vs. c effect using eq

1a and 9 of Appendix I. These binding data are presented in Figure 2C. Through the experimental points are drawn the "best" curves for 6 and 12 binding sites according to eq 2.

From an inspection of the data presented in Figures 2B and 2C, it is apparent that both the 6 and 12 binding site models fit the data almost equally well.

In an attempt to distinguish between the 6 and the 12 binding site model, we performed another series of experiments employing high concentrations of one IgG1 myeloma protein, obtained from a single donor. In order to use high concentrations of IgG1, we could only add a small volume of the Clq solution. As a result, the Clq concentration employed in this study was low, 0.2 mg/ml. Area measurements on these tiny schlieren peaks were not possible. Nevertheless, the Clq did form clear, sharp peaks. Sedimentation rates could be accurately measured, and the binding of IgG1 estimated from them. The "s vs. c" correction was employed. The number of bound IgG was estimated from a plot of the data presented in Table I.

The results of this high-concentration study are shown in Figure 3. Four of the six points indicate binding in excess of six IgG1 molecules for each Clq. The highest binding ratio seen is 10.8 IgG1/Clq. Evidently, each peripheral subunit can bind two or three molecules of IgG, making a total of 12 or 18 binding sites on the Clq. In an attempt to distinguish between these two models, we have used a binding equation of the Hill type (Van Holde, 1971):

$$\bar{\nu} = \frac{n(K_a c)^p}{1 + (K_a c)^p} \tag{3}$$

where p represents the degree of cooperativity. The best value of p may be found by plotting log $[\bar{\nu}/(n - \bar{\nu})]$ as a function of log c. The slope of the straight line which may be drawn through the points plotted in this fashion yields p directly. The intercept gives K_a These values of p and K_a may be reused in eq 3 to calculate the theoretical binding curve which best fits the experimental data assuming 12 or 18 binding sites. Both of these curves have been plotted in Figure 3. The 12-binding-site curve, which is the solid line, appears to fit the experimental points a little better than the 18-site curve, drawn as the broken line. Neither model may be eliminated, however, as both appear to provide an adequate fit to the experimental data.

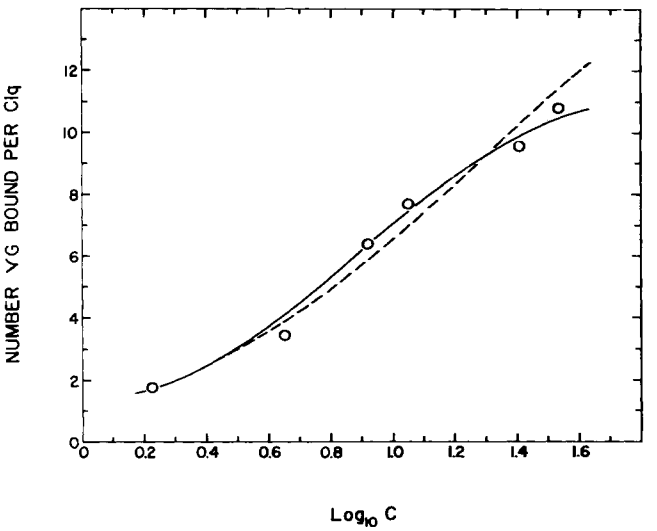

FIGURE 3: Corrected data for binding of G1 immunoglobulins to Clq. These data are taken from the high concentration study described in the text. The binding was estimated from a plot of the values listed in Table I, and the observed increase in sedimentation rates, corrected for s vs. c dependence. The smooth curves drawn through the experimental points are theoretical curves for 12 (—) and for 18 (- - -) binding sites. These curves were computed from eq 3. The cooperativity parameter and binding constant were adjusted to provide a best fit. For 12 binding sites, a cooperativity of 1.24 was found. For 18 sites, the best fit cooperativity was 0.93.

Discussion

It is apparent that there is considerable scatter among the experimental points presented in Figures 2A, 2B, and 2C, particularly for the myeloma proteins. Although some error is undoubtedly due to experimental technique, we believe that most of the scatter is caused by variations in binding affinities among the myeloma proteins isolated from the same subclass. Thus, the binding sites may vary slightly, "probably influenced by the variable region of the light and heavy polypeptide chains where antibodies differ the most strikingly." (See Spiegelberg, 1974, particularly Tables IV and VI, and Fgures 4 and 5). Much less scatter is seen for the IgG data shown in Figures 2A, 2B, and 2C, which constituted a dilution series of the same protein preparation; similarly, the scatter is less for the data presented in Figure 3 in which the same myeloma protein is used for all the points.

The Clq macromolecule has a substantial number of binding sites for IgG, according to the data presented above. Even the uncorrected results (Figure 2A) show as many as six bound immunoglobulin molecules for the IgG3 subclass. This is consistent with the model of Clq as determined by electron microscopy, which shows six peripheral subunits, and with fractionation studies, which indicate that the binding sites are located on these peripheral subunits.

The electron microscope model of Clq would also be compatible with a molecule which possessed 12 or 18 binding sites. If 12 sites were present on the Clq, then each peripheral subunit would presumably have 2. Since the subunits are composed of portions of three polypeptide chains, which are probably not identical, though they are similar in amino acid composition, one, two, or three sites for each subunit would seem possible.

Both of the corrections which we have applied to the binding data, the Johnston–Ogston correction, and the s vs. c correction are in the direction of causing an increase in the amount of IgG apparently bound to Clq. But even so, the corrected data in the main study did not permit a clear choice between the 6 and the 12 site models, as shown in Figures 2B and 2C. It was evident from these results that studies at higher concentrations were required.

The high concentration studies which are presented in Figure 3 clearly indicate that more than 6 binding sites are present. Either the 12 or 18 binding sites models provide an adequate fit to experimental data. Moreover, strong cooperativity between the binding sites does not appear to be present. "Best fit" cooperativities of 1.24 and 0.93 were found for the 12- and 18-site models.

Regardless of which of the two models is selected, the relative binding affinities are strongest for the G3 subclass, followed by G1, then by G2, and then by G4. This ordering is the same as that of Augener (Augener et al., 1971), who used the C1 binding assay to detect the binding of immunoglobulin to $C\bar{1}$, the enzymatically active form of the first component. However, those workers found no binding at all to G4 myeloma immunoglobulin. Likewise, Ishizaka et al. (1967) found that aggregates of G1, G2, and G3 fix complement, whereas G4 did not. On the other hand, we find that G4 does bind to Clq, with a binding affinity only a little less than that of G2. This finding

might be related to the recent report indicating that the F_c fragment of IgG4 is far better in fixing C1 than the intact IgG4 molecule (Isenman et al., 1975).

The strength of binding may be estimated from the single site molar dissociation constants, K_d (= $1/K_a$), for the interactions between Clq and IgG or the various myeloma proteins. These may be determined directly from Figures 2A, 2B, and 2C as the abscissal values of the midpoints of the binding curves, converted to a molar scale (by dividing by 160 000 mmol/ml). Thus, from the data presented in Figure 2C and assuming the 12 independent binding site model is valid, we obtain for IgG and G1, G2, G3, and G4, respectively, values of K_d of 47, 85, 157, 34, and 229 μM. Or, converting to a free energy scale, $\Delta G° = R(298.15) \ln K_a$ yields 5.9, 5.6, 5.2, 6.1, and 5.0 kcal/mol. These numbers may be compared with the inhibition constant of 110 μM found by Sledge and Bing (1973a) for IgG. In their experiments, the IgG was used to inhibit the binding of ^{125}I-labeled Clq to Sepharose-coupled IgM.

It has been shown that moderate pressures generated in the ultracentrifuge may affect the equilibrium constants for associating systems (Josephs and Harrington, 1967; Kegeles et al., 1967). To estimate the importance of pressure effects, we may calculate the change in the free energy of association between a peripheral subunit of Clq and IgG. We will assume that the molecules are located at a depth of 1 cm below the meniscus and 6.7 cm from the axis of rotation, at a rotor speed of 52 640 (approximately 200 atm). Taking the combined molecular weight of IgG plus a peripheral subunit to be 211 250, and assuming a change in partial specific volume of 0.0006 ml/g (which has been observed by Josephs and Harrington (1967)), we calculate a free energy change of 0.6 kcal/mol.

Some remarks on the uncertainties involved in the calculation of hydrodynamic parameters seem warranted. In the first place, the equation of Bloomfield et al. (1967) was adjusted to give the observed frictional coefficient for Clq, by varying the distance between the peripheral and central subunits until the calculated and observed sedimentation coefficients were the same. We are less certain of the remaining values listed in Table I since the geometry of binding of the immunoglobulins to Clq is not known at this time. The value of the exponent in eq 1 will reflect both changes in molecular weight and frictional coefficient. If the immunoglobulin penetrates the hydrodynamic sphere of the Clq upon binding, then the frictional coefficient will not increase as much as it would if the binding were purely external. For the model we have chosen, the immunoglobulin replaces a peripheral subunit; therefore, a partial penetration occurs. The exponent value of α = 1.47 seems reasonable for this case. A small error in α would not be expected to change the results significantly. For example, if s/s_0 = 1.5, the ν values computed for α = 1.4, 1.5, and 1.6 are 1.96, 2.14, and 2.34 respectively. Thus, the interpretation of the binding results would be insensitive to a small variation in the exponent of eq 1.

An experimental estimate of the exponent may be made by adjusting α until the binding data determined by sedimentation give a best fit to the binding data determined from the area measurements. We find the best fit to occur at α = 1.56. Nevertheless, we have used the value of 1.47 in our calculations because we wish to obtain entirely independent estimates of binding from area and sedimentation data.

The Johnston–Ogston correction affects the estimation of binding as determined from area measurements. It depends primarily upon the magnitude of the interaction parameter k_{fs}. The interaction parameters were determined from the sedimentation of mixtures of IgA and Clq. IgA may lack the segmental flexibility of some IgG, which would lead to an underestimation of the interaction parameters. Moreover, we are assuming that the interaction parameters are the same for Clq and for the complexes of Clq and IgG. These estimations and assumptions would not seriously affect the IgG3, normal IgG, or Igl binding data, however, since the Johnston–Ogston correction is fairly small for mixtures of these proteins and Clq, averaging 10, 14, and 20% corrections to the amount of immunoglobulin bound, respectively. The Johnston–Ogston correction is larger for those proteins which bind weakly to Clq because the fast and slow peaks are closer together The average correction to the binding data is 49 and 75% for the IgG2 and IgG4 data points, respectively.

The s vs. c correction affects the estimation of binding as determined from sedimentation velocity measurements. It depends primarily upon the magnitude of the interaction parameter k_{fs}. Here again, this quantity has been measured using mixtures of IgA and Clq. It may be underestimated because of the lack of segmental flexibility in IgA. Again, we are assuming that the interaction parameters are the same for the complex and for Clq. Fairly substantial adjustments in the binding data arise when the s vs. c corrections are applied to the observed sedimentation velocities. In almost all cases the new values represent a marked improvement when compared with the binding values determined from the area measurements. Since this improvement may be seen for many cases where the Johnston–Ogston effect is small, we may conclude that a useful estimate of k_{fs} has been measured with the mixtures of Clq and IgA.

Supplementary Material Available

Appendices I (the Johnston–Ogston and s vs. c corrections employed in this study) and II (tabulated numerical data for Figures 2A, 2B, and 2C) as noted in the text (10 pages). Ordering information is given on any current masthead page.

References

Agnello, V., Winchester, R. J., and Kunkel, H. G. (1970), *Immunology 19,* 909.

Allan, R., and Isliker, H. (1974), *Immunochemistry 11,* 243–248.

Assimeh, S. N., and Painter, R. (1975), *J. Immunol. 115,* 482.

Augener, W., Grey, H. M., Cooper, N. R., and Müller-Eberhard, H. J. (1971), *Immunochemistry 8,* 1011.

Bhattacharyya, S. N., Passero, M. A., and Lynn, W. S. (1974), *Biochim. Biophys. Acta 342,* 343.

Bloomfield, V., Van Holde, K. E., and Dalton, W. O. (1967), *Biopolymers 5,* 149.

Calcott, M. A., and Müller-Eberhard, H. J. (1972), *Biochemistry 11,* 3443.

Cooper, N. R. (1973), *Contemp. Top. Mol. Immunol. 2,* 155.

Ellerson, J. R. Yasmeen, D., Painter, R. H., and Dorrington, K. J. (1972), *FEBS Lett. 24,* 318.

Heusser, C. H., Boesman, M., Knobel, H. R., Jacot-Guillarmod, H., and Isliker, H. (1975), *Immunochemistry 12,* 213.

Isenman, D. E., Dorrington, K. J., and Painter, R. H. (1975), *J. Immunol. 114,* 1726.

Ishizaka, T., Ishizaka, K., Salmon, S., and Fudenberg, H. (1967), *J. Immunol. 99,* 82.

Josephs, R., and Harrington, W. F. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 1587.
Kegeles, G., Rhodes, L., and Bethune, J. L. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 45.
Kehoe, J. M., and Fougereau, M. (1970), *Nature (London) 224,* 1212.
Kinsky, S. C. (1972), *Biochim. Biophys. Acta 265,* 1.
Knobel, H. R., Heusser, C., Rodrick, M. L., and Isliker, H. (1974), *J. Immunol. 112,* 2094.
Knobel, H. R., Villiger, W., and Isliker, H. (1975), *Eur. J. Immunol. 5,* 78.
Lepow, I. H. (1972), *Prog. Immunol., Int. Congr. Immunol., 1st,* 579–593.
Lepow, I. H., Naff, G. B., Todd, W. D., Pensky, J., and Hinz, C. F. (1963), *J. Exp. Med. 117,* 983.
Lowe, D. M., and Reid, K. B. M. (1974), *Biochem. J. 143,* 265.
Müller-Eberhard, H. J. (1975), *Annu. Rev. Biochem. 44,* 697.
Müller-Eberhard, H. J., and Calcott, M. A. (1966), *Immunochemistry 3,* 500.
Müller-Eberhard, H. J., and Kunkel, H. G. (1961), *Proc. Soc. Exp. Biol. Med. 106,* 291.
Naff, G. B., Pensky, J., and Lepow, I. H. (1964), *J. Exp. Med. 119,* 593.
Naff, G. B., and Ratnoff, O. D. (1968), *J. Exp. Med. 128,* 571.
Ovary, Z., Saluk, P. H., Quijada, L., and Lamm, M. E. (1976), *J. Immunol. 116,* 1265–1271.
Reid, K. B. M. (1974), *Biochem. J. 141,* 189.
Reid, K. B. M., Lowe, D. M., and Porter, R. R. (1972), *Biochem. J. 130,* 749–763.
Ruddy, S., Gigli, I., and Austen, K. F. (1972), *N. Engl. J. Med. 287,* 489, 545, 592, 642.
Sakai, K., and Stroud, R. M. (1974), *Immunochemistry 11,* 191.
Shelton, E., Yonemasu, K., and Stroud, R. M. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 65.
Sledge, C. R., and Bing, D.H. (1973a), *J. Biol. Chem. 248,* 2818.
Sledge, C. R., and Bing, D. H. (1973b), *J. Immunol. 111,* 661.
Spiegelberg, H. L. (1974), *Adv. Immunol. 19,* 259.
Spiegelberg, H. L. (1975), *J. Clin. Invest. 56,* 588–594.
Spiegelberg, H. L., Fishkin, B. G., and Grey, H. M. (1968), *J. Clin. Invest. 47,* 2323.
Svehag, S. E., Manhem, L., and Bloth, B. (1972), *Nature (London), New Biol. 238,* 117.
Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N.Y., Wiley, p 533.
Tanford, C. (1973), The Hydrophobic Effect, Wiley, New York, N.Y., p 4.
Trautman, R., Schumaker, V. N., Harrington, W. F., and Schachman, H. K. (1954), *J. Chem. Phys. 5,* 135.
Valet, G., and Cooper, N. R. (1974a), *J. Immunol. 112,* 339.
Valet, G., and Cooper, N. R. (1974b), *J. Immunol. 112,* 1667.
Van Holde, K. E. (1971), Physical Biochemistry, Englewood Cliffs, N.J., Prentice-Hall, p 62.
Volanakis, J. E., and Stroud, R. M. (1972), *J. Immunol. Methods 2,* 25.
Yasmeen, D., Ellerson, J. R., Dorrington, K. D., and Painter, R. H. (1976), *J. Immunol. 116,* 518.
Yonemasu, K., and Stroud, R. M. (1972), *Immunochemistry 9,* 545.
Ziccardi, R. J., and Cooper, N. R. (1976), *J. Immunol. 116,* 504–509.