Polypeptide Chain Structure of Rabbit Immunoglobulins. III. Secretory γA-Immunoglobulin from Colostrum*

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ABSTRACT: Purified secretory γA-immunoglobulin, obtained from rabbit colostrum, was found to have a molecular weight of approximately 370,000. The heavy (α) chain obtained after total reduction and alkylation of the molecule was easily distinguishable from heavy chains of other immunoglobulins (γ and μ) by the "fingerprint" of its tryptic peptides and by its higher mobility toward the anode in acrylamide gel relative to γ chain. The molecular weight of the α chain, determined in 5 M guanidine·HCl, was 64,000 ± 3000. Besides α and light chains, a third kind of chain, "transport" (T) chain, was identified by acrylamide electrophoresis in urea of the totally reduced and alkylated molecule. T chains were isolated along with light chains by gel filtration of the totally reduced molecule in 5 M guanidine·HCl. However, dialysis of the unreduced secretory protein *vs.* 5 M guanidine, 0.01 M in iodoacetamide, led to the dissociation of 10% of the molecule as a fraction rich in T chains and to the decrease in the $s_{20,w}$ of some of the α- and light-chain units to 7.2 S. The components in the fraction rich in T chains had a molecular weight of about 50,000, a value which decreased upon reduction. A model was proposed for the secretory γA-immunoglobulin. According to this, the molecule is assembled of four pairs of α and light chains and one or two T chains.

Structural studies of human γA-immunoglobulins have established some general characteristics of these proteins. First, serum proteins of the γ A class have been shown to be made up of heavy and light chains. The light chains of either normal or myeloma γA-immunoglobulins are similar to those of γM- and γG-immunoglobulins but their heavy chains are different from those present in other classes when examined by electrophoresis or by fingerprinting (Carbonara and Heremans, 1963; Cohen and Porter, 1964b; Bernier *et al.*, 1965; Vaerman *et al.*, 1965). Second, both human and mouse *serum* γA-immunoglobulins have been shown to be heterogeneous by sedimentation analysis (Vaerman *et al.*, 1965; Fahey, 1961). Furthermore, Tomasi *et al.* (1965) have shown that the sedimentation coefficient of the principle serum component is 7 S while that of the corresponding protein from colostrum or saliva is 11 S. Third, Tomasi *et al.* (1965) further demonstrated that human colostral or salivary γA-immunoglobulin had extra antigenic sites when compared with the corresponding serum protein. At least a part of the secretory γA-immunolgobulin was shown to be synthesized locally in the salivary glands. Subsequent work by South *et al.* (1966) has indicated that these antigenic sites are associated with a *transport piece* which is probably synthesized in salivary glands either in the presence or absence of the immunoglobulin itself and that this component is excreted either by itself or associated with γA protein.

This present paper is concerned with the size, number, and mode of association of the component polypeptide chains of a secretory γA-immunoglobulin. The aim of this study was to generalize the known structural characteristics of the γA class of immunoglobulins and to compare these molecules with those of other classes of immunoglobulins. The rabbit was chosen as a source for the secretory γA-immunoglobulin because: (1) the structural studies on the γM- and γG-immunoglobulins of this species would allow comparisons (Cohen and Porter, 1964a; Small and Lamm, 1966; Lamm and Small, 1966) and, (2) the recen -lopment of a method to isolate secretory γA-immunoglobulin from rabbit colostrum (Cebra and Robbins, 1966) made available sufficient amounts of material.

Materials and Methods

Procedures which were developed for the study of the polypeptide chains of rabbit γG- and γM-immunoglobulins (Small and Lamm, 1966; Lamm and Small, 1966) were applied to the colostral γA-immunoglobulin. The secretory γA-immunoglobulin was prepared by the method of Cebra and Robbins (1966), which consists of gel filtration of clarified rabbit colostrum on Sephadex G-200 and then DEAE-cellulose chromatography of the appropriate fraction. One preparation, used in expt 4, was recycled through

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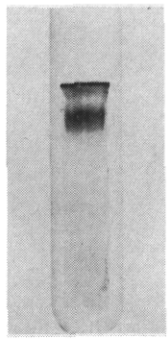

FIGURE 1: Disc electrophoresis pattern of purified colostral γA-immunoglobulin in buffer.

a column of Sephadex G-200 at pH 6.8 before being dissociated in guanidine.

Separation of Component Chains. The secretory immunoglobulin was extensively reduced with 0.1 M dithiothreitol in the presence of 7 M guanidine (Small and Lamm, 1966) and then was alkylated by the addition of a 1.2-fold molar excess of iodoacetamide relative to the sulfhydryl concentration. Solid Tris was added to maintain the pH at 8.2. The solution was then diluted to a density equivalent to that of 5.2 M guanidine·HCl and was applied to an upward flowing column of Sephadex G-200 equilibrated with 5.0 M guanidine·HCl. The sample was followed by 10–20 ml of approximately 5.5 M guanidine·HCl. The effluent was monitored by measurement of absorption at 280 mμ. Alternately, the intact secretory γA-immunoglobulin was equilibrated against a solution which was 5 M in guanidine·HCl, 0.01 M in iodoacetamide, and 0.1 M in Tris, pH 8.0. The dissociated protein was fractionated by gel filtration on the upward flow column of Sephadex G-200. The major component, which was eluted first, was then subjected to reduction and alkylation as described above.

Physical Measurements. The extinction coefficient for the whole colostral γA molecule in 5 M guanidine was determined by diluting measured volumes of a globulin solution into 5 M guanidine and 0.1 M NaOH. From the previously established (Cebra and Robbins, 1966) value for the extinction coefficient in base the corresponding value for the other solvent was calcuable using optical density measurements. The extinction coefficient for the α chain was obtained by exhaustive dialysis of the protein against water, addition of measured volumes of the soluble chain to guanidine solution for optical density measurements in 5 M guanidine, and drying of other measured volumes of the α chain in water to constant weight as described for the whole molecule (Cebra and Robbins, 1966).

Partial specific volumes ($\bar{V}$) were obtained on dialyzed samples as suggested by Casassa and Eisenberg (1960) to avoid the necessity of having to measure the amount of bound salt. The density was measured in density gradient columns saturated with either 5 M guanidine or dilute KCl solutions for guanidine solutions of the α chain and γA-immunoglobulin or for saline solutions of the whole protein, respectively (Small and Lamm, 1966). The protein concentration was obtained by diluting measured volumes and making optical density measurements before the protein solutions came into contact with organic solvents.

Sedimentation velocity experiments were performed in the Spinco Model E analytical ultracentrifuge and the s (observed) in 3.1 M guanidine·HCl was corrected to $s_{20,w}$ by assuming a $\bar{V}$ of 0.692 in 3.1 M guanidine and of 0.703 in aqueous solution. Guanidine densities were taken from the work of Kielley and Harrington (1960) and the viscosity correction used was 1.0804 (E. Mihalyi, personal communication). High-speed, short-column sedimentation equilibrium studies were performed by the method of Yphantis (1964) and the calculations were done with the aid of a computer using a program developed for this purpose.[1]

Analytical Techniques. Disc electrophoresis was performed in acrylamide gel in the absence of urea by the method of Davis (1964) and in the presence of urea by the method of Reisfeld and Small (1966). Goat antisera were prepared and gel diffusion experiments were performed as previously described (Cebra and Robbins, 1966). The technique of fingerprinting as applied to immunoglobulin chains has been described previously (Small *et al.*, 1966).

Results

Purity of Colostral γA-Immunoglobulin. The purity of the different preparations of colostral γA-immunoglobulin used in this study was similar to that of the protein characterized previously (Cebra and Robbins, 1966). Immunoelectrophoretic analysis using the antiserum to colostrum showed only one precipitin line. No immunoglobulin of other classes could be detected using specific antiheavy chain reagents. Sedimentation velocity experiments showed no evidence of material which sedimented more slowly than the major component but did show the presence of small amounts of faster sedimenting material, presumably aggregates of γA-immunoglobulin (Cebra and Robbins, 1966). Figure 1 depicts a disc electrophoretic pattern of γA-immunoglobulin which had been recycled through a column of Sephadex G-200. Only one migrating component was visible in the stained gel.

Molecular Weight of Colostral γA-Immunoglobulin. Table I gives the extinction coefficients and partial specific volumes ($\bar{V}$) of the whole molecule and some of its component polypeptide chains. These values of $\bar{V}$ were used in the calculations of molecular weights.

Figure 2 shows the ln C *vs.* (radius^2) plot for one of the six experiments designed to determine the molecular weight of intact γA-immunoglobulin. There is slight upward curvature of the most radial two or three points, as would be expected from the presence

[1] Program available upon request to P. A. S. or to Beckman Instrument Co., Palo Alto, Calif.

TABLE I: Extinction Coefficients and Partial Specific Volumes.

	$E^{1\%}_{280\ m\mu}$ 1 cm	$\bar{V}$
γA-Immunoglobulin		
in nonguanidine solvents	13.5[a]	0.703[b]
in 5 M guanidine·HCl	12.8	0.685
α chain		
in 5 M guanidine·HCl	10.6	0.732
Light chain		
in 5 M guanidine·HCl	11.4[c]	0.703[c]

[a] In 0.1 N NaOH (Cebra and Robbins, 1966). [b] In 0.14 M NaCl–0.01 M phosphate buffer, pH 7.4. [c] From Small and Lamm (1966).

TABLE II: Molecular Weights × 10^{-3} of γA-Immunoglobulin from Colostrum.

Speed (rpm)	Starting Concentration (mg/ml)[a] 0.25	0.5	0.75	Av ± Std Dev
	Weight Average			
10,589	370	381	370	385 ± 14
8,225	389	405	381	
10,589[b]	363	375	369	369 ± 6
	z Average			
10,589	404	430	390	415 ± 15
8,255	415	420	430	

[a] Solvent is that given in legend to Figure 2. [b] Calculated disregarding the few most centrifugal points.

of small amounts of higher molecular weight material already shown by the sedimentation velocity method (see Cebra and Robbins, 1966). The straight line through all the points corresponds to a molecular weight of 370,000, but if the last few points are disregarded the molecular weight becomes 363,000.

The weight-average molecular weights obtained at three different initial protein concentrations and at two different speeds of centrifugation are summarized in Table II. The average of the values given in the top two rows gives a molecular weight of 385,000 for secretory γA-immunoglobulin. If the values corresponding to the most centrifugal points are disregarded for the higher speed runs, an average molecular weight of 369,000 is obtained (third row of figures). The higher *z*-average molecular weight, giving an average value of 415,000, illustrates the effect of the small amount of contamination with aggregated material.

Separation of Polypeptide Chain Components. After extensive reduction and alkylation of the γA-immunoglobulin it was passed through a column of Sephadex G-200 in the presence of 5 M guanidine·HCl. Figure 3 shows a typical elution pattern. The material comprising the first major fraction to be eluted will be referred to as heavy or α chain and the material represented by the second component to emerge as light

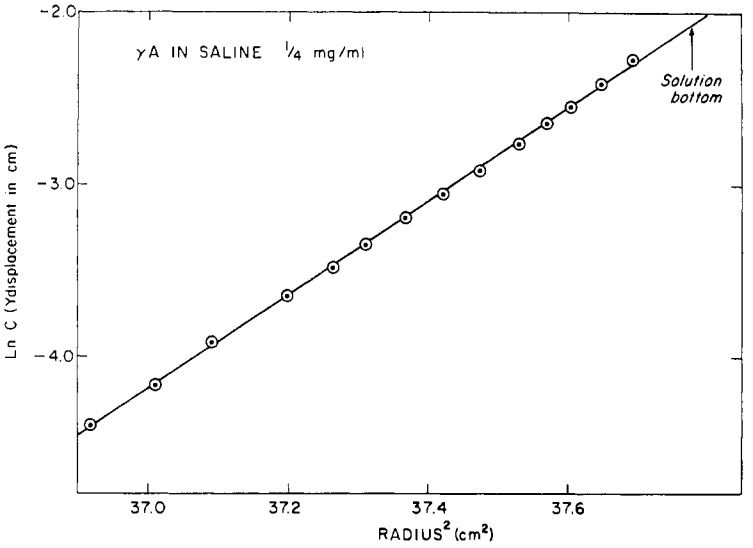

FIGURE 2: Plot of the logarithm of the vertical displacement from the base line of a fringe measured in centimeters (ln *C*, *Y* displacement in centimeters) *vs.* distance from center of rotation squared (radius²) from the sedimentation equilibrium experiment using 0.25-mg/ml solution of colostral γA-immunolobulin in 0.14 M NaCl–0.01 M phosphate buffer, pH 7.4, run for 48 hr at 20° and 10,589 rpm. The straight line is the least-squares fit of the points.

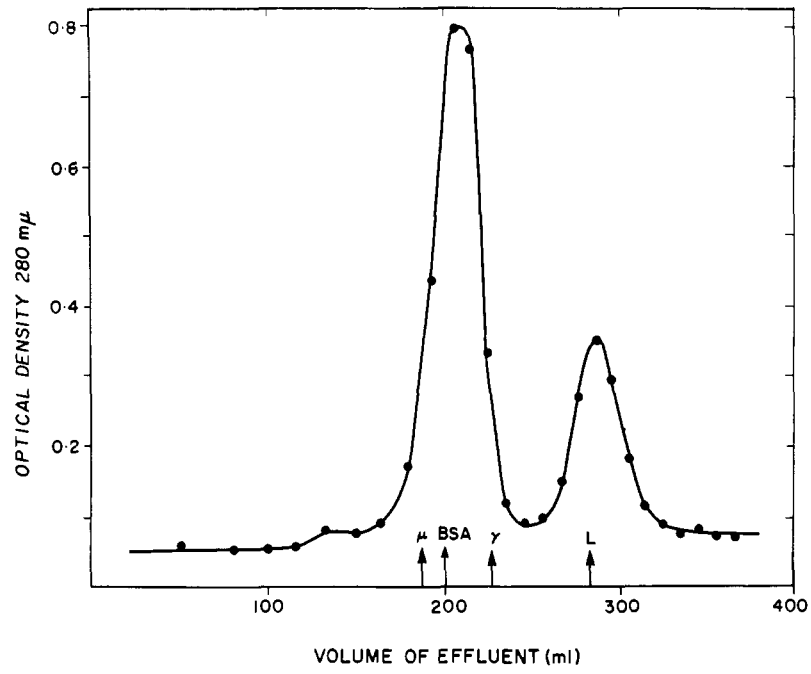

FIGURE 3: Gel filtration in the presence of 5.0 M guanidine·HCl on a column of Sephadex G-200 (120 × 2.5 cm) of colostral γA-immunoglobulin (expt 1 of Table III). The arrows show the position of the peak of the elution pattern for the following extensively reduced and alkylated materials when they were passed through this same column: (1) heavy chain (μ) of rabbit γM-immunoglobulin; (2) bovine serum albumin (BSA); (3) heavy chains (γ) of rabbit γG-immunoglobulin; and (4) light chains (L) from either rabbit γM- or γG-immunoglobulin.

chainlike" material. There was a third major fraction which is not shown but which emerged at approximately 470 ml and contained the reducing and alkylating agents.

The elution diagram shows that α chain was eluted at a position intermediate between that required for the μ and γ heavy chains of the two other classes of immunoglobulin. Bovine serum albumin was eluted from the calibrated column at about the same position as α chain. Table III summarizes the recoveries and elution positions for this and other gel filtration experiments.

Characterization of Polypeptide Chain Components. In order to approximate the mass of each of the components eluted from the Sephadex column a plot of $\sqrt{K_D}$ *vs.* $\sqrt{MW}$ was made based on the method of Andrews (1964) and this is shown in Figure 4. Since all the components were extensively reduced and alyklated and subjected to sieving in the presence of 5 M guanidine·HCl, their configurations should approach those of a random coil. Since shape factors should, therefore, be minimized, the elution position should permit a fair estimate of mass. In Figure 4 it can be seen that, by position of elution, the light-chain-

TABLE III: Summary of Gel Filtration Experiments with Extensively Reduced and Alkylated Material.

Expt	Material Applied	Amt (mg)	% Recov	H[a]/(H + "L")	K_D of H[b]	L /(H + L)	K_D of L
I	γA	33.5	89	71.5	0.235	28.5	0.471
II	γA	14.4	96	70.5	0.210	29.5	0.449
III	Pool 1	22.4	101	79.5	0.224	20.5	0.468
IV	Pool 1	7.5	72	79.0	0.222	21.0	0.446
					0.223 ± 0.010		0.457 ± 0.013

[a] Based on optical density at 280 mμ. [b] $K_D = V_E - V_0/V_i$, where V_E is the peak elution volume, V_0 the excluded volume (130 ml for this column as measured with macroglobulin), and V_i the included volume. $V_0 + V_i$ = the elution volume for small molecule (~470 for this column). See text and Andrews (1964).

like material coincides in size with light chains isolated from rabbit γG- or γM-immunoglobulin and hence would appear to have a similar mass (~23,000). However, the α chain falls between γ and μ chains and its position of elution on the graph corresponds to a molecular weight of 63,500 ± 3000.

Short-column, high-speed sedimentation equilibrium studies were performed on the α chain in the presence of 5 M guanidine·HCl. Figure 5 shows a typical ln *C vs.* (radius²) plot, and unlike the plot for intact γA-immunoglobulin (Figure 2) there was no indication of heterogeneity. The values obtained for the molecular weights are summarized in Table IV and these

TABLE IV: Molecular Weights × 10^{-3} of α Chain in 5 M Guanidine·HCl.[a]

Speed (rpm)	Starting Concn (mg/ml) 0.2	0.4	0.6	Av (±) Std Dev
Sample from Expt I of Table III				
Weight averages				
35,600	61.3	63.2	63.8	63.7 ± 1.4
27,690	64.1	65.7	63.9	
z averages				
35,600	63.6	65.1	57.6	62.2 ± 3.2
27,690	65.8	60.8	60.2	
Sample from Expt III of Table III				
Weight averages				
35,600	66.3	62.4	60.9	65.2 ± 3.3
27,690	69.4	68.0	64.0	
z averages				
35,600	66.7	60.4	60.9	63.8 ± 3.7
27,690	68.5	65.1	61.0	

[a] Taking $\bar{V} = 0.732$.

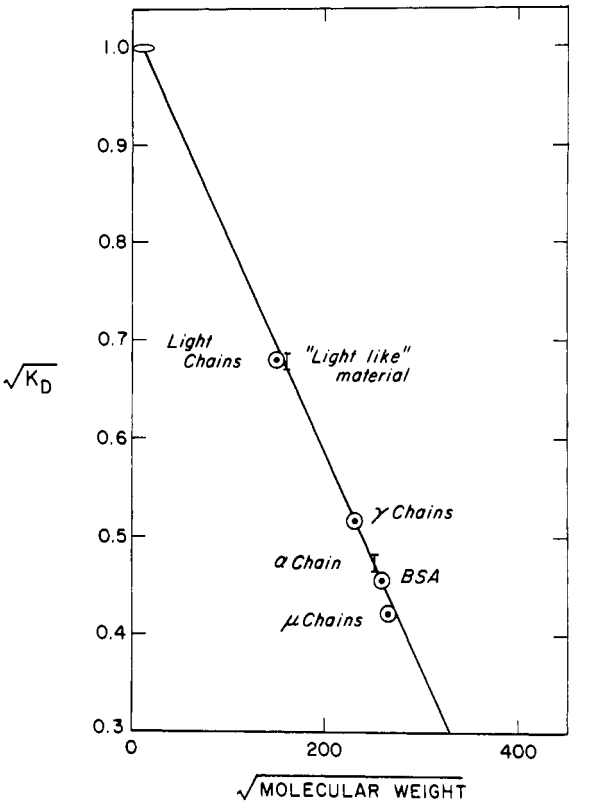

FIGURE 4: Plot of $\sqrt{K_D}$ (see footnote to Table III for definition) *vs.* $\sqrt{MW}$ for extensively reduced and alkylated materials filtered through the standard column of Sephadex G-200 in the presence of 5 M guanidine·HCl. The molecular weights of the marker materials used in this plot were 22,500 for the light chains and 53,000 for γ chains (Small and Lamm, 1966), 70,000 for μ chains (Lamm and Small, 1966), and 67,000 for BSA (Phelps and Putnam, 1960). The vertical bars represent the range of values corresponding to the standard deviations of the values for K_D given in Table III.

show no apparent dependence on initial concentration or speed of centrifugation. The close agreement between the mean value for the weight-average molecular weights, and the mean for the *z*-average molecular weights, further affirms the homogeneity of the α chain. Since there was no apparent concentration dependence, the molecular weight was obtained by averaging all 12 determinations of weight-average molecular weight obtained for both samples of α chain to yield the figure of 64,500 ± 2500. This value is in good agreement with the value of 63,500 ± 3000 estimated by gel filtration.

A tryptic peptide fingerprint was obtained for the α chain and it is compared with fingerprints of γ and μ chain in Figure 6. The γ, μ, and α chains were all derived from different rabbits, but difference in origin probably accounts for very few, if any, of the many differences observed in the fingerprints. The fingerprints of γ chains vary only very slightly between animals of different allotype and, as far as they have been studied, not at all among animals of the same allotype (Small *et al.*, 1966). Thus, the many differences observed between the heavy chains depicted in Figure 6 probably are indicative of differences in the amino acid sequence of each of the three different classes of heavy chains. It is important to recall, however, that highly variable regions of sequence will not be revealed by fingerprinting (Small *et al.*, 1965). Thus, the fingerprints demonstrate clear sequence differences between heavy chains but do not exclude the presence of similar runs of sequence in the so-called variable regions of all three heavy chains.

Disc electrophoresis patterns, obtained in the presence of urea, are shown in Figure 7 for α chain, light-chainlike material, and other fractions. As might be expected, the α chain (Figure 7 (2)) had a greater mobility toward the anode than the γ chain, also

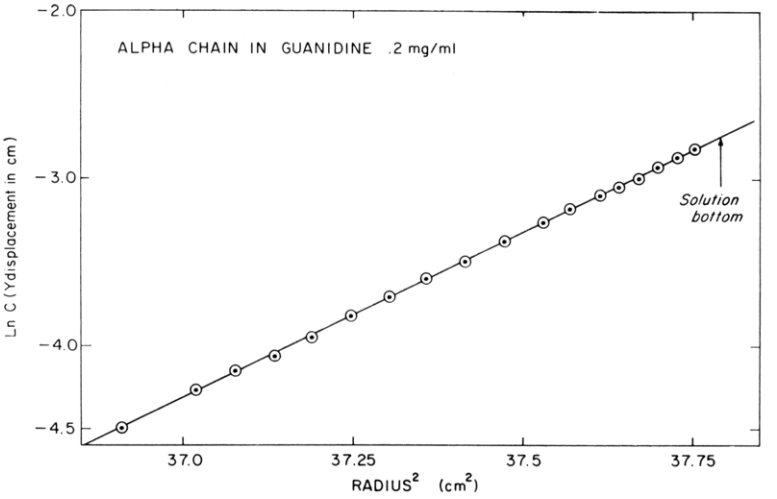

FIGURE 5: Plot of ln C *vs.* (radius^2) for α chain (0.2-mg/ml initial concentration) in 5 M guanidine·HCl (density = 1.121) after 48 hr at 27,690 rpm and 20°.

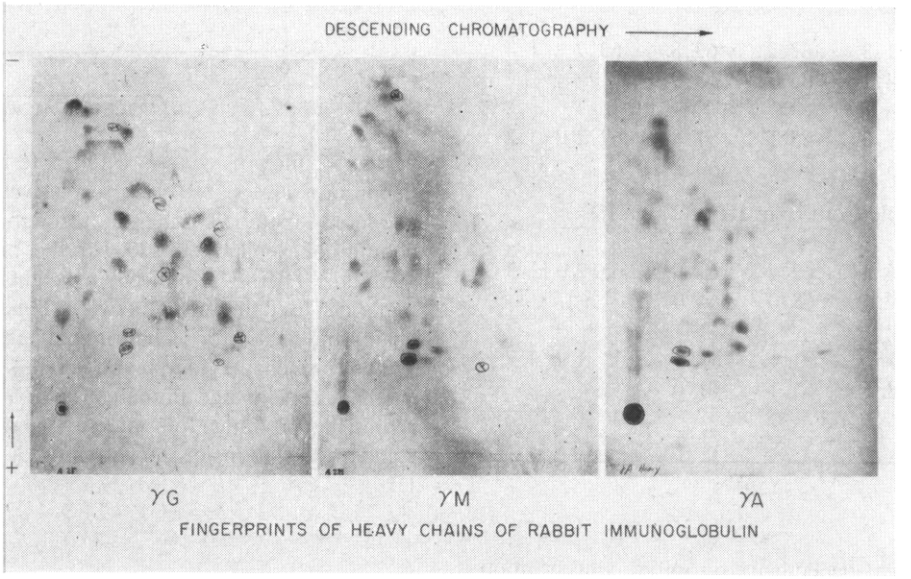

FIGURE 6: Fingerprints of tryptic peptides from the heavy chains of γA-, γG-, and γG-immunoglobulins. The fingerprints of the μ and γ chains were obtained in an earlier study (Lamm and Small, 1966).

shown for comparison (Figure 7 (1)). Both heavy chains exhibited multiple and regular banding and it is, therefore, likely that α and γ chains, like light chains, have common and variable regions. Presumably the variable regions account for the electrophoretic heterogeneity. The pattern given by the mixture of the two heavy chains demonstrates the absence of any overlap in mobility between them (Figure 7 (3)).

Figure 7 also shows the disc electrophoresis patterns of light chains obtained from γG-immunoglobulin and light-chainlike material obtained from secretory γA-immunoglobulin. The average mobility and multiplicity of bands seen in the two preparations was quite similar except for a faster migrating band seen in the light-chainlike material from the γA-immunoglobulin. When the light-chain fractions from the two immunoglobulins were mixed the disc electrophoresis pattern showed approximately the same multiplicity of bands spaced at the same intervals as seen for either of the preparations alone. Again, the faster migrating component, derived from the γA-immunoglobulin, was

apparent. The significance of this distinct component will be considered below. Fingerprints of the light-chainlike material of γA-immunoglobulin gave many spots which were similar in position to those of light chains from γG-immunoglobulin. However, there were, in addition, other spots not seen in fingerprints of light chain alone.

Investigation of Noncovalently Bound Components of the Secretory γA-Molecule. Attempts to determine the molecular weight of intact secretory γA-immunoglobulin in 5 M guanidine·HCl by sedimentation equilibrium indicated that the molecule dissociated in this solvent to a mixture of components. The secretory γA-immunoglobulin was, therefore, dissolved in 5 M guanidine·HCl, made 0.01 M in iodoacetamide to eliminate disulfide interchange, and after dialysis *vs.* this solvent it was passed through the column of Sephadex G-200. Figure 8 depicts the elution pattern of one of three very similar experiments. In each case, the material in pool 2 accounted for about 10% of the total optical density units of the dissociated secretory protein. It should be emphasized that the three separable components were all derived from the γA molecule, since in normal aqueous buffer there is no evidence of any smaller components analogous to pools 2 and 3 after gel filtration. Apparently the components in pools 2 and 3 were not covalently linked in the molecule and were dissociated in guanidine.

Pool 1 material was shown to be heterogeneous by sedimentation velocity experiments but it contained a major component with a sedimentation coefficient of 7.2 S and a minor component of 10.6 S (Figure 9). Analysis by immunodiffusion showed that this pool contained all the detectable α chains as well as light chain. In an attempt to evaluate the mass of the pool material, portions of the leading, central, and trailing fractions of pool 1 (see circled points of Figure 8) were studied by sedimentation equilibrium methods. All three samples were heterogeneous, but the material eluted first gave molecular weights around 300–350,000 while the material eluted last in pool 1 gave values around 200,000.

Pool 1 material, when extensively reduced and alkylated, gave an elution pattern in 5 M guanidine from a column of G-200 reminiscent of that of the whole molecule, except that the amount of light-chain-like material was in decreased amounts. Table III indicates that the light-chainlike material accounted for only about 20.5% of the whole instead of the 29% gotten from the intact molecule. Thus it appears that the material in pools 2 and 3 (Figure 8) contributed to the light-chainlike material from the whole molecule.

The average mass of the components in pool 2 was approximately 50,000 as judged by its elution position from the Sephadex column. Sedimentation equilibrium studies of pool 2 were performed in 5 M guanidine·HCl at concentrations of 1/3, 2/3, and 1 mg/ml, and speeds of 35,600 and 24,630 rpm. The ln C *vs.* (radius^2) plots were linear, there was no obvious speed or concentration dependence of the molecular weights, and the z averages were similar to weight averages. Hence, the material was felt to be relatively homogeneous. The average of six weight-average molecular weight determinations was 52,500 ± 5700. A $\bar{V}$ of 0.703, *i.e.*, that of light chain from γG-immunoglobulin, was assumed for these calculations. When β-mercaptoethanol was added to the 5 M guanidine solutions of pool 2, the molecular weight fell, but accurate estimates were not obtainable owing to heterogeneity of the product.

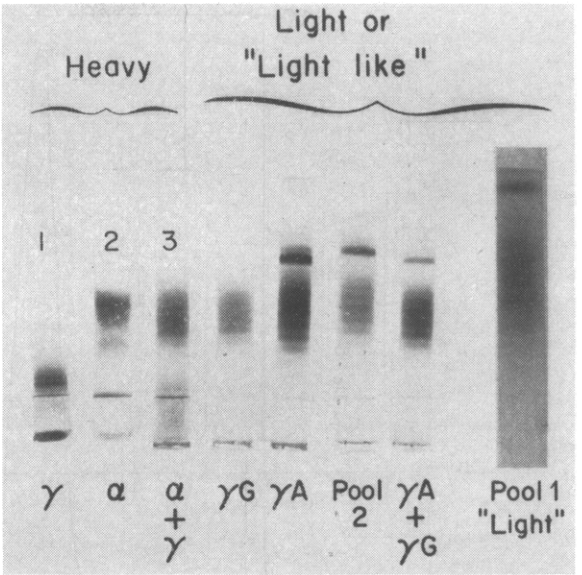

FIGURE 7: Disc electrophoresis in urea of isolated heavy chains (γ and α) from γG- and γM-immunoglobulins and of light chains and light-chain-containing fractions from these proteins. All specimens were reduced and alkylated.

Analysis of pool 2 material by immunodiffusion methods (not shown) indicated that it contained no detectable α chain but some light chain. Disc electrophoresis of the reduced and alkylated pool 2 material (Figure 7) also showed the presence of the light chain but in addition demonstrated that this fraction contained a component in high concentration which corresponded in mobility to the fast-migrating component seen in the totally reduced whole molecule and in the light-chainlike material. Thus, it appeared that in guanidine, a component which was neither α nor light chain dissociated from the secretory γA-immunoglobulin. This component (T chain) had the mobility of a γ_1-globulin and could be further purified by chromatography on DEAE-cellulose. For this purpose pool 2 material was dialyzed *vs.* 0.01 M phosphate buffer, pH 7.5, and applied to a DEAE-column equilibrated with the same buffer. After washing with two to three column volumes of this buffer, purified T chain was eluted with 0.3 M NaCl–0.01 M phosphate, pH 7.5. This purified-chain was shown to be essentially devoid of light-chain allotypic sites by the method of R. G. Mage, G. O. Young, and S. Dray (in prepara-

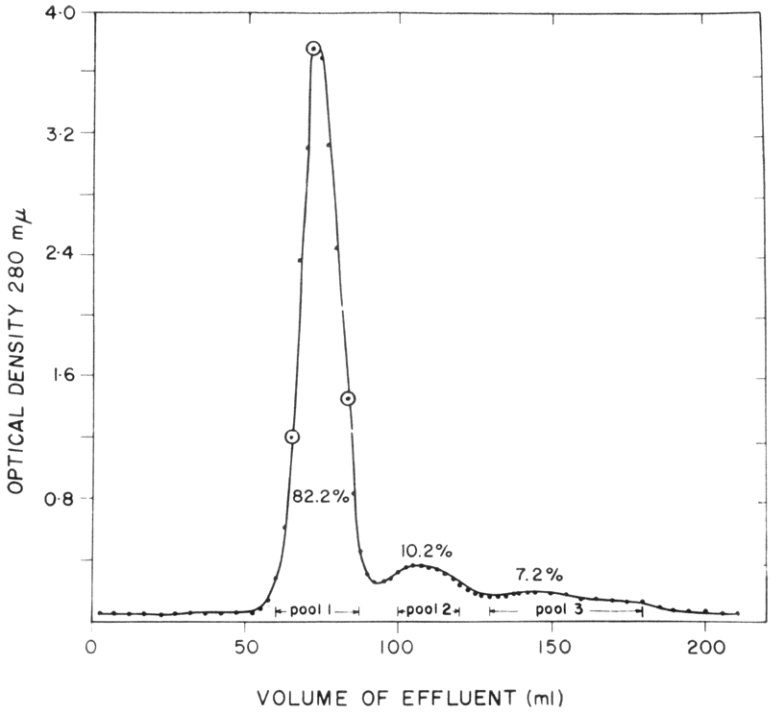

FIGURE 8: Gel filtration pattern of colostral γA-immunoglobulin dialyzed *vs.* 5 M guanidine·HCl, 0.01 M in iodoacetamide, and passed through a column of Sephadex G-200 (2.5 × 56.5 cm) equilibrated with 5 M guanidine·HCl. A total of 58 optical density units was applied in 3.0 ml.

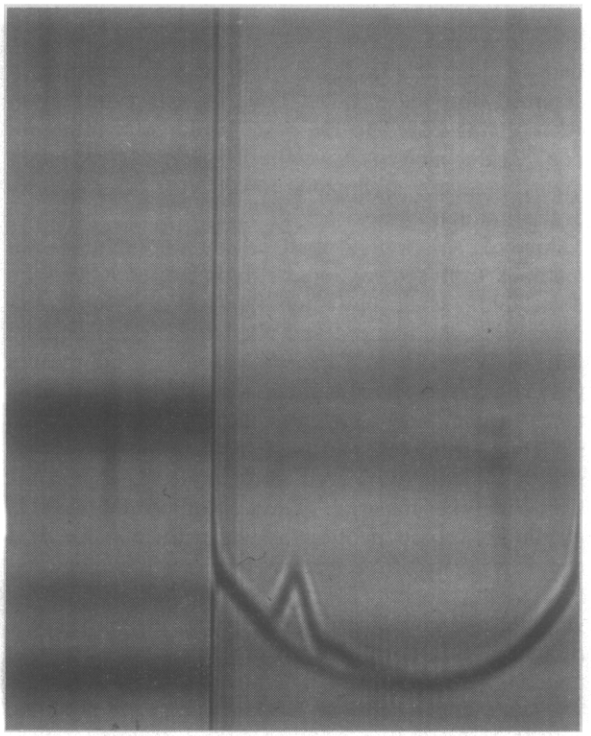

FIGURE 9: Sedimentation velocity pattern of pool 1 (see Figure 8) in 3.1 M guanidine·HCl at 20°. The sedimentation coefficients (observed) were 5.72 and 8.37 S and when corrected for density and viscosity the $s_{20,w}$ were 7.2 and 10.6 S.

tion).[2] Disc electrophoresis gave no evidence of light-chain contamination and fingerprints of the limited amount of material available, although technically unsatisfactory, definitely showed some spots different from those of light chains and some others apparently similar. Thus, the T chain seems to be definitely not just electrophoretically fast light chain(s) although it is of similar size and may even have limited segments of amino acid sequence similar to those of light chains.

Pool 3, shown in Figure 7, contained material which was eluted at a volume corresponding to monomeric light chains. Analysis by gel diffusion showed that its major component was, in fact, light chain.

Discussion

The data concerning the number, proportions, and molecular weights of the polypeptide chains of secretory γA-immunoglobulin make it possible to propose the following model for this protein: (a) that it consists of three distinct kinds of polypeptide chains, α chain,

[2] Dr. Rose Mage of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., kindly estimated the amount of b4- and b5-containing material in the T-chain sample by the method of radial diffusion in agar-containing antisera. No b5-reacting material was detectable but 2–3% of the sample did react with anti-b4 antisera. This figure may be slightly low since the sample was probably dimer while the standards were monomeric light chain.

light chain, and transport (T) chain; (b) that it is assembled out of four pairs of α and light chains and one or two transport chains; (c) that the transport piece acts to hold together two halves of the molecule, each consisting of two pairs of α and light chains.

The first point (a) is supported by the isolation of α chain and the demonstration of its mass homogeneity and uniqueness among immunoglobulin heavy chains as judged by fingerprinting and by immunologic criteria. Light chains, common to all immunoglobulins, were present by many criteria. There remained a third component, present in a mixture with light chains after fractionation of the totally reduced and alkylated secretory protein, which was distinguished by its high mobility in disc gel electrophoresis. Rejnek *et al.* (1966) have recently observed a very similar, if not identical, entity in S-sulfonated human colostral globulin after starch gel electrophoresis. They were unable to find it in serum γG-immunoglobulin, but they did not isolate and investigate serum γA-immunoglobulin. Presumably this third chain could correspond to the piece of human salivary γA-immunoglobulin with unique antigenic sites compared with the serum protein (Tomasi *et al.*, 1965). South *et al.* (1966) have further investigated these unique antigenic sites and found them both on the secretory γA-immunoglobulin and free in the saliva of patients unable to make this γA protein. They named this component transport piece. Because we were unable to identify any additional component from the colostral protein and because those components which were isolated accounted for the mass of the whole molecule, we have taken the name transport (T) chain for its third unique polypeptide chain.

The molecular weight of the intact secretory protein was found to be approximtely 370,000 while that of the α chain was about 64,000. The mass of the light chain has previously been determined at 22,500 (Small and Lamm, 1966). The structural pattern found for all other immunoglobulins so far studied has been the occurrence of heavy and light chains in pairs. Thus the mass of the secretory protein could accommodate four pairs of α and light chains ($(4 \times 64{,}000) + (4 \times 22{,}500) = 346{,}000$) and still have 24,000 units unaccounted for.[3] The uncertainty in this number of 24,000 is obviously fairly high, since any errors in heavy- and light-chain molecular weights are multiplied by four and the difference itself is only 7% of the molecular weight of the whole molecule. However, this difference is close to the proportion of the total optical density units (10%) contained in pool 2, rich in T chains dissociable from the secretory protein in guanidine. These T chains appear to have a mass close to that of light chains, since they are isolated together after reduction and alkylation. However, the T chains appear to be dissociated by guanidine from the molecule as disulfide-linked dimers and to have a mass of about 50,000. Since over one-half of pool 2 material is apparently T chain, some, if not all, of these dimers must be T–T and not T–light. Thus it seems likely that each secretory γA-immunoglobulin contains at least one and probably two T chains. Until the light and T chains can be separated from each other after total reduction and alkylation of the whole molecule and their relative proportions and masses are evaluated, it will not be possible to conclusively decide this point.

Dialysis of the secretory protein against 5 M guanidine, as well as releasing T chain and some monomeric light chains, also resulted in the decrease in mass of the major α- and light-chain units to about one-half. The sedimentation coefficient of the majority of these units became 7.2 S. Thus it seems likely that, with release of T chains, the secretory molecule was dissociated into units consisting, on the average, of two pairs of α and light chains and these would have a mass ($(2 \times 64{,}000) + (2 \times 22{,}500)$) of 173,000. These presumably could resemble the 7 S monomeric unit found for serum γA-immunoglobulin (Vaerman *et al.*, 1965; Tomasi *et al.*, 1965). The role of T chain in preserving the integrity of the secretory protein can only be assessed by its isolation and reconstitution with the 7.2 S units; however, it seems possible that it causes or stabilizes the dimerization of these units to yield the 10.8S secretory protein.

The bulk of the T chain apparently is noncovalently associated with the α–light chain units since it can be dissociated from the secretory protein with guanidine in the presence of iodoacetamide. However, some T chain persists in the α–light chain units after guanidine treatment (see Figure 7, right) and all these latter are not dispersed to 7.2 S units (see Figure 9). Reduction seems to liberate all T chain from α chain, so it may be possible that some T chain is bound to the molecule through interchain disulfide bonds. The interaction between heavy (γ) and light chains to form an interchain disulfide bond is very specific (Olins and Edelman, 1964) and involves one particular half-cystine on the light chain (Milstein, 1966). Further, it seems likely that the α and light chains are synthesized together in lymphoid cells (Bernier and Cebra, 1965) while the T chains are synthesized separately in acinar cells adjacent to collecting ducts in the case of salivary glands (Tomasi *et al.*, 1965). Thus, it would seem that any covalent bonding of the T chain to the rest of the protein through formation of interchain disulfide bonds would occur secondarily, after the assembly together of α and light chains, and would perhaps arise by disulfide interchange reactions. Since T chains seem peculiar to γA-immunoglobulin one might hypothesize that its noncovalent and covalent associations take place with the α chain. However, further work is required to determine the exact number of T chains and their specificity of association in the secretory

[3] It is possible to make a rough estimate of the number of α chains per molecule on the basis of the data in Tables I–IV by using the formula: molecular weight of γA $\times$ extinction coefficient of γA $\times$ % of optical density in α-chain fraction/molecular weight of α $\times$ extinction coefficient of α. Using 70.5% for the per cent of α chain one gets a figure of 4.9 α chains. Since five pairs of α and light chains cannot be accommodated in the mass of the intact molecule we think that a number of 4 is the most likely.

γA-immunoglobulin.

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