Biochem. 17, 425.
Bray, G. A. (1960), *Anal. Biochem. 1*, 279.
Brecher, P. I., Vigersky, R., Wotiz, H. S., and Wotiz, H. H. (1967), *Steroids 10*, 635.
Burton, K. (1956), *Biochem. J. 62*, 315.
Chauveau, J., Moulé, Y., and Rouiller, Ch. (1956), *Exp. Cell Res. 11*, 317.
Jensen, E. V., Hurst, D. J., DeSombre, E. R., and Jungblut, P. W. (1967), *Science 158*, 385.
Jensen, E. V., Suzuki, Y., Kawashima, T., Stumpf, W., Jungblut, P., and DeSombre, E. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 632.
Jungblut, P. W., Hughes-McCann, S., Hughes, A., Little, M., Rosenfeld, G. C., and Wagner, R. K. (1971), *Advan. Biosci. Berlin* (in press).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Milgrom, E., and Baulieu, E. E. (1969), *Biochim. Biophys. Acta 194*, 602.
Puca, G. A., and Bresciani, F. (1969), *Nature (London) 223*, 745.
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51*, 660.
Shyamala, G., and Gorski, J. (1968), *J. Biol. Chem. 244*, 1097.
Talwar, G. P., Segal, S. J., Evans, A., and Davidson, O. W. (1965), *Proc. Nat. Acad. Sci. U. S. 52*, 1059.
Toft, D., and Gorski, J. (1966), *Proc. Nat. Acad. Sci. U. S. 55*, 1574.
Toft, D., Shyamala, G., and Gorski, J. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1740.
Truong, H., and Baulieu, E. E. (1971), *Biochim. Biophys. Acta 237*, 167.
Venderly, R. (1955), *in* The Nucleic Acids, Vol. 2, Chargaff, E., and Davidson, J. N., Ed., New York, N. Y., Academic Press, p 155.
Wang, T. Y. (1966), *J. Biol. Chem. 241*, 2913.
Wang, T. Y. (1967), *in* Enzymology, Vol. XII, Part A, Colowick, S. P., and Kaplan, N. O., Ed., New York, N. Y., Academic Press, p 417.

Chemical and Physicochemical Studies of the Component Polypeptide Chains of Rabbit Secretory Immunoglobulin A*

Jose A. O'Daly† and John J. Cebra‡

ABSTRACT: The polypeptide chains of rabbit colostral secretory immunoglobulin A—α, light, secretory component, and fast component—are characterized in terms of chemical and physicochemical parameters. Procedures are given to isolate secretory α chain and F component to homogeneity. Molecular weights and amino acid compositions of the component polypeptide chains are discussed. A revised model for the rabbit secretory immunoglobulin A is presented which includes four α chains (54,000 each), four light chains (22,000 each), one secretory component (60,000), and one F component (15,000) to account for 379,000 molecular units.

A salient characteristic of the sIgA[1] molecules found in various mammalian secretions is their sedimentation coefficient of about 11 S (Tomasi *et al.*, 1965; Cebra and Robbins, 1966; Porter and Allen, 1969; Vaerman and Heremanns, 1970; Pahud and Mach, 1970) reflecting a molecular weight of 385,000 (Cebra and Small, 1967; Newcomb *et al.*, 1968). A particular feature that sets the sIgA apart from all other immunoglobulins is its content of "extra-antigenic sites" (Tomasi *et al.*, 1965) which have been taken to define a third polypeptide—the secretory component different from heavy and light chains. Taking advantage of the finding that secretory component dissociates from rabbit sIgA in 5 M guanidine·HCl (Cebra and Small, 1967) this unique polypeptide has been isolated from the parent molecule (O'Daly and Cebra, 1971a) as well as from a fraction of colostrum not containing immunogloublins (O'Daly and Cebra, 1971b). Values for the molecular weight of this polypeptide range between 76,000 (Newcomb *et al.*, 1968) and 58,000 (Tomasi and Bienenstock, 1968) and considerable ambiguity persists regarding molecular characteristics which could be used to clearly distinguish secretory component from other polypeptide chains of the sIgA molecule. Using analytical and preparative zone electrophoresis in polyacrylamide gels a correlation has been found between the antigenic activities used to define component polypeptide chains of sIgA and their electrophoretic mobilities (O'Daly and Cebra, 1971a). Such an approach has led us to confirm the presence of a fast-migrating component of rabbit sIgA (Cebra and Small, 1967). Our analysis also supports the conclusions of Halpern and Koshland (1970) that this component is different from α, light, and secretory component (O'Daly and Cebra, 1971a) and may represent a fourth kind of polypeptide chain of the parent molecule. In the present paper the isolation of secretory α chain, secretory light chain, and fast component[2] (F component) is described. These three polypeptides, as well as the secretory component dissociated from sIgA and the secretory component found free in colostrum, are char-

* From the Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218. *Received April 5, 1971.* Supported by Grant No. AI-09652 from the National Institute of Allergy and Infectious Diseases.

† Fellow of the Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

[1] WHO meeting on the Nomenclature of Immunoglobulins Šterzl and Riha (1970).

acterized on the basis of their molecular weights and amino acid compositions. The data presented are interpreted in terms of a model for the sIgA having four pairs of α and light chains, one secretory component chain, and a maximum of one F component.[2]

Materials and Methods

Rabbit Colostrum. The methods for obtaining colostrum and the isolation of sIgA have been outlined previously (Cebra and Robbins, 1966; O'Daly and Cebra, 1971a).

Complete Reduction and Radioalkylation of sIgA. sIgA (50 mg) was dissolved in 5 ml of 6.7 M guanidine·HCl containing 0.1 M Tris–acetate buffer (pH 8.0). Dithiothreitol was added to a final concentration of 0.02 M and the protein solution was held for 2 hr at room temperature (Cebra and Small, 1967). Radioalkylation was achieved by adding 0.025 μCi of [^{14}C]-iodoacetic acid (13.9 mCi/mmole) followed several minutes later by unlabeled iodoacetic acid (2.1 moles/1.0 mole of dithiothreitol in 2 M Tris. Immediately after alkylation (30 min), excess reagents were removed by gel filtration through Sephadex G-25 equilibrated in 0.1 M Tris–acetate buffer (pH 8.0). This procedure resulted in the reduction and carboxymethylation of all half-cystines.

Dissociable Secretory Component and Free Secretory Component. The dissociable secretory component was separated from sIgA by gel filtration on a column of Sephadex G-200 in 5 M guanidine·HCl (Cebra and Small, 1967), and purified by recycling through the same column followed by chromatography on SE-Sephadex (O'Daly and Cebra, 1971a). The secretory component found free in the colostrum was isolated as previously described (O'Daly and Cebra, 1971b).

Isolation of Secretory Light Chain and F Component. A mixture of L chain and F component was obtained by gel filtration of completely reduced and alkylated sIgA on a column of Sephadex G-200 equilibrated in 5 M guanidine·HCl (see below). This mixture (30 mg) was dialyzed against 0.1 M Tris–acetate buffer (pH 8.0) for 24 hr and then against water for another 24 hr with frequent changes of the dialysis solution. After dialysis the protein solution was freeze-dried, dissolved in 8 M urea which was 0.01 M in Tris–acetate (pH 8.6), and then chromatographed on a column (10-ml packed wet ion exchanger) of DE-52 cellulose equilibrated with the same solvent. Stepwise elution was performed with increasing concentrations of Tris–acetate buffer.

Analytical Gel Acrylamide Electrophoresis. Disc electrophoresis in 10 M urea was performed following the method of Reisfeld and Small (1966) using 6.5% acrylamide monomer concentration in the lower gel. Electrophoresis was carried out for 3 hr at 2.5 mA/gel. The resulting protein bands were stained with 0.05% buffalo black in 7.5% aqueous acetic acid and destained electrophoretically in the same solvent. In order to visualize carbohydrate-containing components, duplicate gels were stained with the periodic acid–Schiff reagent (Zacharias *et al.*, 1969). Ordinarily sIgA and its component chains were totally reduced and carboxymethylated with [^{14}C]iodoacetic acid before analysis by gel electrophoresis. The *S*-carboxymethylcysteine content of each of the polypeptide chains separated in the gel was calculated after measuring the radioactivity of successive 0.83-mm fractions. These fractions were eluted from the gel with an autogel divider (Savant, Hicksville, N. Y.) in 0.1 M Tris–acetate (pH 8.0), collected directly into scintillation vials, and counted in a liquid scintillation counter (Packard).

Preparative Acrylamide Electrophoresis. The technique for analytical disc electrophoresis (Reisfeld and Small, 1966) was adapted for use in the preparative gel electrophoresis apparatus (Poly-Prep, Büchler Instruments). The lower gel was composed of 15 ml of 6.5% acrylamide solution, 15 ml of Tris·HCl–tetramethylethylenediamine solution, and 30 ml of ammonium persulfate solution. The upper gel was made as described by the above authors but only 5 ml was used. Electrophoresis was carried out for 20 hours using a current of 80 mA. Fractions (5 ml) were collected with an LKB fraction collector. The flow rate of the effluent buffer, 0.1 M Tris–acetate (pH 8.0), was maintained at 21 ml/hr with a peristaltic pump (Büchler Instruments).

Ultracentrifugation. Analytical ultracentrifugation was performed in a Spinco Model E ultracentrifuge. High-speed short-column sedimentation equilibrium measurements were made (Yphantis, 1964). The samples of secretory component unreduced and/or completely reduced and alkylated were dialyzed at different concentrations against a 5 M Ultra Pure guanidine·HCl solution (Mann Research Laboratories) until complete equilibrium between solvent and solution was attained as judged from refractive index measurements (Kielley and Harrington, 1960). Alternative runs with the native secretory component were made using 0.1 M Tris–acetate (pH 8.0) as solvent. The density of the Tris–acetate solution was measured in a 25-ml Gay Lussac picnometer at 20°. α chain and fast component (see below), after complete reduction and alkylation, were analyzed in 5 M guanidine·HCl in the same way as for secretory component. The partial specific volume ($\bar{v}$) of secretory component was assumed to be 0.732, the same as that of an α-chain–secretory component mixture (4:1, mole/mole) (Cebra and Small, 1967), and of fast component to be 0.703, the same as for light chains (Small and Lamm, 1966). The calculations were done with the aid of a computer using the Yphantis program (Roark and Yphantis, 1969). The $\bar{v}$ of secretory component and F component were also calculated from the amino acid compositions (Schachman, 1957), and the values of 0.723 and 0.716 were obtained for the secretory component (dissociable and/or free) and for the F component, respectively. No significant differences were seen in the molecular weight values calculated from either set of $\bar{v}$.

Amino Acid Analysis. Samples were hydrolyzed for 20 hr at 110° in evacuated (10 μ), sealed tubes, containing 0.5 ml of constant-boiling HCl, 10 μl of 0.1 M phenol, and 10 μl of 0.1 M dithiothreitol. Analyses were performed on a Beckman Model 120B amino acid analyzer. The color yield value for *S*-carboxymethylcysteine, including a correction for degradation, was obtained by carboxymethylating reduced glutathione (Mann Research Laboratories) and hydrolyzing this derivative under the same conditions used for hydrolysis of samples. Overall color yields for *S*-carboxymethylcysteine were 0.946 and 0.943 with respect to glutamic acid and glycine, respectively.

Sugar Analysis. The protein samples were hydrolyzed in 4 N HCl, for 6 hr at 100° in evacuated sealed tubes. Analysis for glucosamine and galactosamine were carried out in a Beckman amino acid analyzer using a second buffer of 0.2 M sodium citrate (pH 4.14).

Peptide Maps. Purified secretory component and α chain (3-mg amounts) were each digested with 100 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone treated trypsin at

[2] This F component would more properly be designated J chain (Halpern and Koshland, 1970) should it prove not to be a fragment of one of the other three chains.

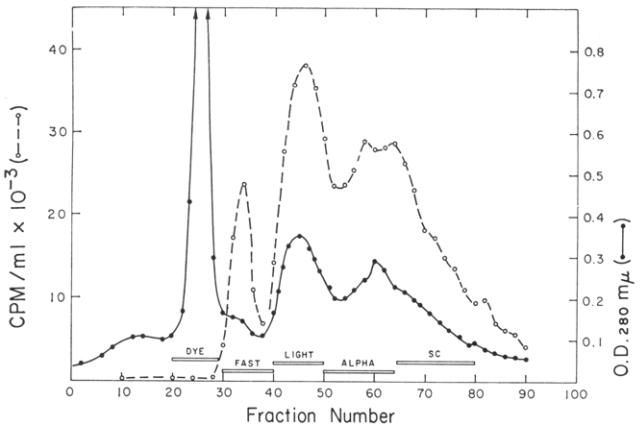

FIGURE 1: Separation of the polypeptide chains of rabbit sIgA (~50 mg) on a preparative acrylamide column. Details of the procedure are found in the text. The dye used was Evans blue. The horizontal bars in the figure indicate the main polypeptide components present in the fractions (5 ml) as judged by their position of migration when individually analyzed by analytical gel acrylamide electrophoresis.

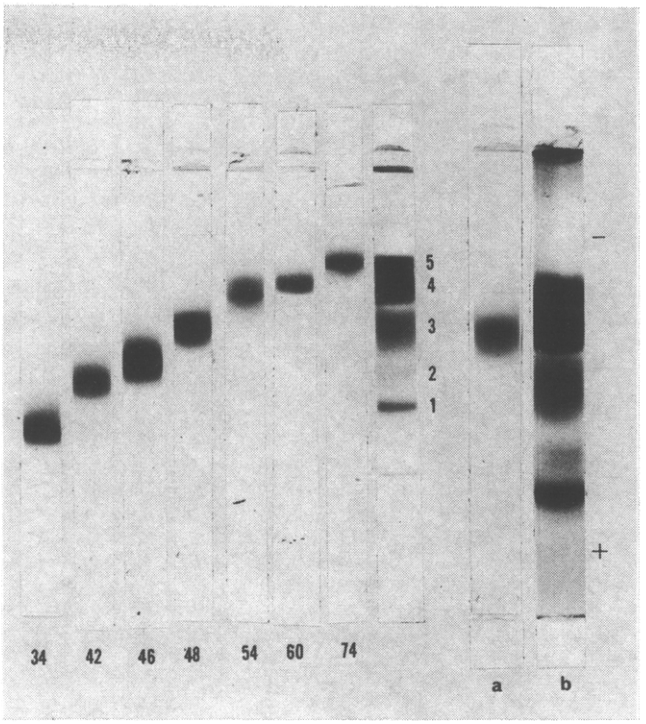

FIGURE 2: Polyacrylamide disc gel electrophoresis in 10 M urea at pH 8.6. All samples (300–500 μg) were totally reduced and alkylated. A current of 2.5 mA/gel was applied for 3 hr. The numbers on the horizontal row indicate the fractions obtained from the preparative acrylamide electrophoresis (Figure 1). The numbers in the vertical column indicate the components of sIgA preparations which may be distinguished in these gels (O'Daly and Cebra, 1971a): 5 is secretory chain; 4 is α chain; 3 is light chain; 2 is unidentified; 1 is fast component; (a) completely reduced and alkylated rabbit secretory IgA, stained with the PAS–Schiff reaction; (b) same amount of sIgA as in part a, electrophoresed parallel to it and stained with buffalo black.

pH 8.1 for 12 hr at 37°. The digests (1.5 mg) were first subjected to descending chromatography for 12 hr using a mixture of pyridine, *tert*-butyl alcohol, and water in the ratio of 3:3:3.5 (v/v). High-voltage electrophoresis was then performed at right angles to the direction of the chromatography at 3000 V for 45 min in pyridine–acetate buffer (pH 3.6). The peptide maps were stained with a cadmium acetate–ninhydrin solution (Heilman *et al.*, 1957).

Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Gel electrophoresis was performed after total reduction and alkylation following the method of Weber and Osborn (1969).

Results

Preparation of α Chain and Fast Component. Resolution of the different polypeptide chains of the totally reduced and carboxymethylated sIgA molecule by preparative acrylamide gel electrophoresis is depicted in Figure 1. The horizontal bars indicate those fractions containing the bulk of each of the different polypeptides as judged by analytical acrylamide electrophoresis (Figure 2). The fractions 50–64, containing mostly α chain, were pooled, dialyzed against water, and freeze-dried. The protein was taken up in 5 M guanidine·HCl solution and applied to a reverse flowing column of Sephadex G-200. Figure 3 shows the elution profile of this gel filtration. When the major protein fractions (pool A, Figure 3) were analyzed by gel acrylamide electrophoresis, protein bands migrating at the position of the α chain were seen (Figure 4a). The polypeptide isolated in this manner could further be identified as α chain and differentiated from secretory component by its high content of carbohydrate as shown with the PAS–Schiff reaction (Figure 4c). It should be noted that when the sIgA is electrophoresed on the urea gels and stained with PAS–Schiff reagent only the α chain is stained (Figure 2a). The F component could be isolated directly by preparative acrylamide electrophoresis. The fractions 30–40 (Figure 1) were pooled and analyzed on gel. Only a single component migrating with the electrophoretic mobility of F component (O'Daly and Cebra, 1971a) was found in these fractions (Figure 2).

An alternate method for the preparation of large amounts of F component was developed. A mixture of totally reduced and alkylated sIgA and radiolabeled F component, obtained by means of a preparative acrylamide electrophoresis as described above, was first chromatographed on Sephadex G-200 in 5 M guanidine·HCl. The second protein fraction to be eluted from the column (pool B, Figure 5) was a mixture of light

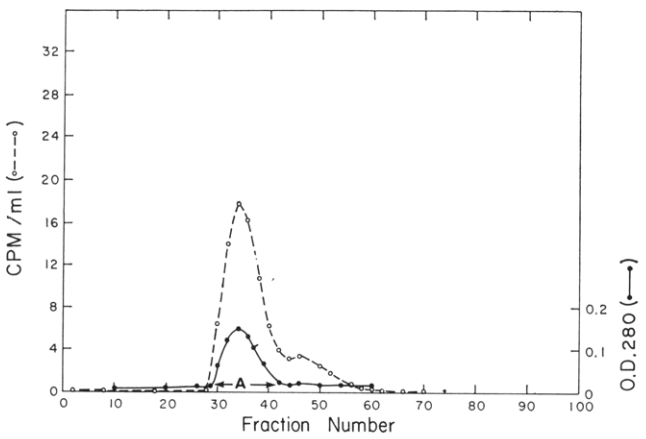

FIGURE 3: Purification of α chain. The fractions (5 ml) 50–60 (~10 mg) from the preparative gel acrylamide separation depicted in Figure 1 were applied to a column (2.5 × 100 cm) of Sephadex G-200 equilibrated in 5 M guanidine·HCl. The major protein fraction (pool A) corresponds to α chain.

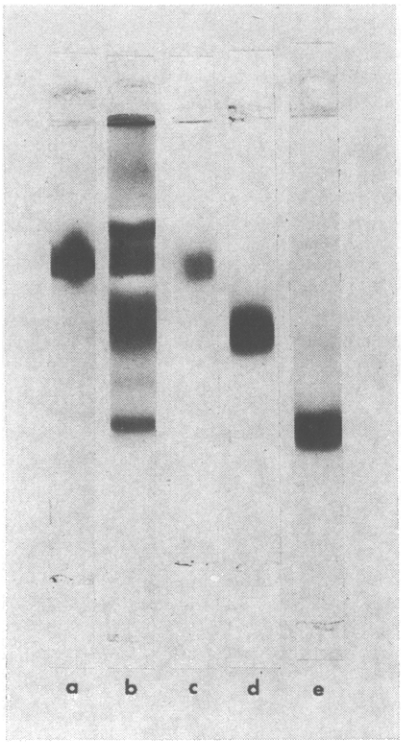

FIGURE 4: Polyacrylamide disc electrophoresis in 10 M urea at pH 8.6. The gels were electrophoresed under the same conditions as given in Figure 2. (a) α chain from pool A (Figure 3); (b) sIgA; (c) α chain stained with PAS–Schiff reaction; (d) 0.2 M fraction from Figure 6 (light chain); (e) 0.4 M fraction from Figure 6 (F component).

chains and F component. The protein from such a fraction (~30 mg) was chromatographed on a column of DE-52 cellulose. The elution profile of this column is shown in Figure 6. The protein eluted with 0.4 M Tris–acetate contained most of the radioactivity and migrated as F component in analytical acrylamide gels (Figure 4e). The protein eluted with 0.2 and 0.25 M Tris–acetate buffer migrated as light chains (Figure 4d). The fraction eluted with 0.3 M Tris–acetate buffer showed a mixture of both molecular species.

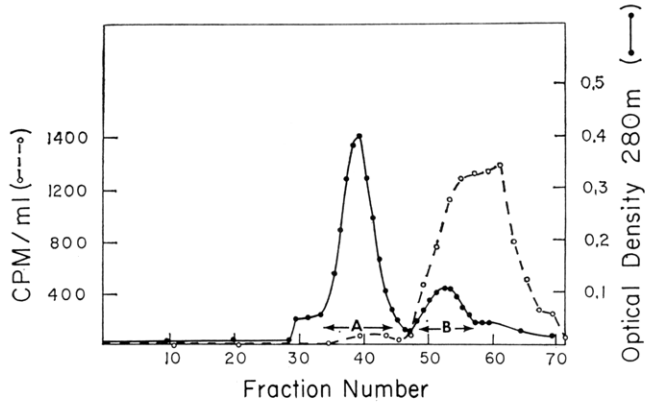

FIGURE 5: Fractionation of totally reduced and alkylated sIgA (~50 mg) on a column (2.5 × 100 cm) of Sephadex G-200 equilibrated in 5 M guanidine·HCl. The protein solution was mixed with ~1 mg of totally reduced and radioalkylated F component obtained from the preparative acrylamide electrophoresis. Pool A is a mixture of α chain and secretory component; pool B contains light chain and F component. Fractions (5 ml) were collected.

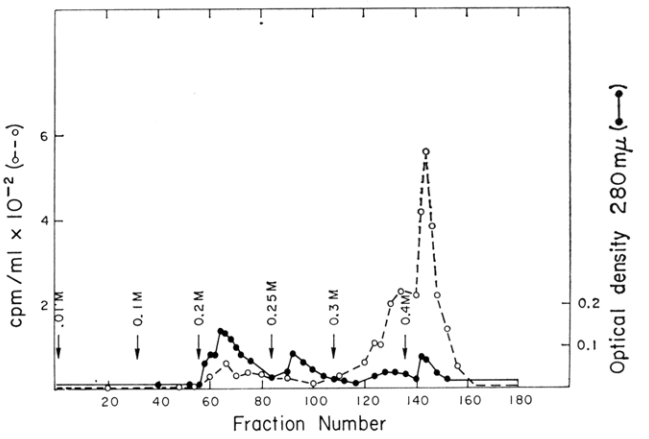

FIGURE 6: Separation of light chains from F component. (The sample, pool B, Figure 5 (~30 mg), was chromatographed on a column (0.5 × 10 cm) of DE-52 cellulose equilibrated in 8 M urea containing 0.01 M Tris–acetate buffer (pH 8.6).) Fractions of 5 ml were collected. The stepwise elution with increasing concentrations of Tris-acetate buffer are indicated by the vertical arrows.

Molecular Weight Studies. Molecular weights for purified secretory component and F component were approximated from calculations based on electrophoretic mobility in acrylamide gels containing urea and sodium dodecyl sulfate. The purified, dissociable secretory component, and free secretory component had estimated molecular weights of 80,000. The purified F component had an estimated molecular weight of 22,500. The molecular weight for α chain was also approximated by mixing totally reduced and alkylated γ chain with ^{14}C-labeled α chain obtained after total reduction and radioalkylation. Figure 7 shows the profile of the chromatography of both molecular species in Sephadex G-200 equilibrated in 5 M guanidine·HCl. The superposition of radioactivity and OD_{280} units points to the similarity in size of both molecular species.

Equilibrium Ultracentrifugation Studies. The values calculated from the plots of ln C/(radius)$^2/_2$ as well as the extrapolated values for the number-average, weight-average, and Z-average molecular weights obtained by use of the computer program are given in Table I.

The various estimations indicate a molecular weight of about 60,000 for both free and dissociable secretory component, 54,000 for α chain, and 15,000 for F component.

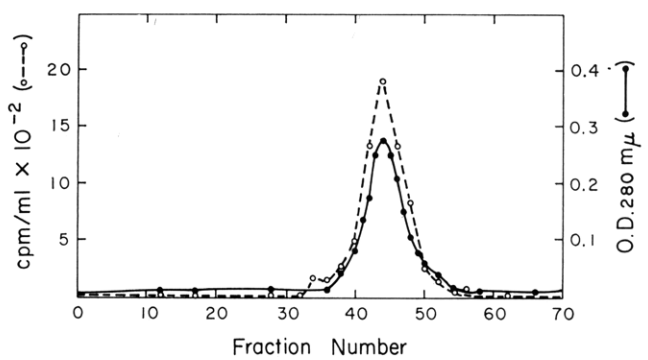

FIGURE 7: Cochromatography of α chain and γ chain. A mixture of 1 mg of ^{14}C-labeled purified α chain (see Figure 3) and 5 mg of totally reduced and alkylated γ chain was applied to a column (2.5 × 100 cm) of Sephadex G-200 equilibrated in 5 M guanidine·HCl. Fractions (5 ml) were collected.

TABLE I: Values for the Molecular Weights of the Component Polypeptides of sIgA Estimated from Equilibrium Ultracentrifugation Data.

Polypeptide:	Secretory Component from IgA						Free Secretory Component	α Chain		F Component
Solvent:	0.1 M Tris–Acetate		5 M Guanidine Unreduced		5 M Guanidine Totally Reduced		5 M Guanidine Unreduced	5 M Guanidine Totally Reduced	5 M Guanidine Totally Reduced	5 M Guanidine Totally Reduced
Concentration (mg/ml):	~1	~0.5	~1	~0.5	~1	~0.5	~1	~1	~0.5	~1
ln C *vs.* $r^2/_2$	73,630	60,923	63,114	63,564	57,331	62,622	61,230	54,443	49,436	14,457
Number average	66,777	67,667	62,744	60,320	55,979	57,968	60,056	47,321	45,820	14,635
Weight average	67,769	68,526	63,076	61,736	56,704	60,737	61,202	55,553	46,746	15,070
Z average	69,324	69,604	63,525		57,875				50,260	21,164
Speed (rpm)	21,740	21,740	32,000	32,000	32,000	32,000	39,460	39,460	39,460	44,770

TABLE III: Amino Acid and Amino Sugar Content of Component Polypeptides of sIgA.

	Amino Acid Residues % (Moles %)							Amino Acid Residues per Mole[a]						
	Secretory Component		α Chain	α Chain	F Component Preparative[b]	F Component DE-52[c]	Light Chain	Secretory Component		α Chain	α Chain	F Component Preparative[b]	F Component DE-52[c]	Light Chains
	Dissociating	Free						Dissociating	Free					
Lysine	5.67	5.62	3.54	4.08	4.95	4.34	4.15	31	31	18	20	7	6	9
Histidine	1.26	2.05	1.82	1.92	1.46	1.38	0.77	7	11	9	10	2	2	2
Arginine	3.73	3.74	3.00	3.07	6.65	5.66	1.34	20	21	15	15	9	8	3
Aspartic acid	9.66	9.48	8.66	8.88	15.93	14.41	9.44	52	52	43	45	21	19	20
Threonine	6.84	6.09	8.66	7.84	8.92	9.79	12.76	37	34	43	40	12	13	26
Serine	8.25	9.25	9.02	8.77	7.44	8.45	8.97	45	51	45	44	10	11	19
Glutamic acid	12.13	11.95	10.47	10.57	10.42	9.98	10.84	65	66	52	53	14	13	22
Proline	6.11	6.67	8.21	9.31	7.39	7.05	7.00	33	37	41	47	10	10	14
Glycine	9.10	9.75	8.95	8.39	3.68	4.77	7.78	49	54	44	43	5	6	16
Alanine	5.95	5.16	5.66	6.03	3.00	4.45	7.10	32	28	28	30	4	6	15
Valine	9.27	8.35	8.71	8.90	6.76	5.80	8.97	50	46	43	45	9	8	19
Methionine	0.58	0.42	0.27	0.62	0.41	0.74	0.62	3	2	2	3	0.5	1	1
Isoleucine	2.86	2.97	2.89	2.86	5.75	5.34	3.47	15	16	14	14	8	7	7
Leucine	8.49	8.13	9.55	8.62	6.76	7.04	5.65	46	45	47	44	9	9	12
Tyrosine	3.36	2.75	3.33	2.99	3.55	4.06	4.77	18	15	17	16	5	5	9
Phenylalanine	3.66	3.81	3.79	4.31	2.04	2.27	3.37	20	21	19	21	3	3	7
SCM[d]	3.00	3.74	3.69	4.85	4.85	4.71	2.94	20	21	18	14	6	6	6
Glucosamine								4.3	4.3	21.8			4.7	
Galactosamine								3.6	3.7	16.3			1.9	2.6[e]

[a] Calculated taking molecular weights of 60,000 for secretory component, 54,000 for α chain, 15,000 for F component, and 22,000 for light chain. [b] F component obtained after preparative acrylamide electrophoresis. [c] F component obtained after DEAE-52 chromatography. [d] *S*-Carboxymethylcysteine. [e] Total amino sugars.

TABLE II: Quantitation of F Component in sIgA.

Expt	sIgA (μg)	Counts/min		Counts/min per SCMC Residue[a]		Moles of F Component/4 Moles of L Chain[b]
		L Chain	F Component	L Chain	F Component	
1	200	5,419	1270	903	211	0.93
2	300	8,114	1545	1352	257	0.76
3	400	15,088	2671	2514	445	0.70

[a] Relative specific activities obtained by dividing counts per minute by number of residues of *S*-carboxymethylcysteine per mole of each polypeptide. [b] Obtained from relative specific activities of *S*-carboxymethylcysteines of L chain and F component assuming four L chains per molecule of sIgA.

Stochiometry of F Component vs. Light Chains in sIgA. In order to determine how many fast-component polypeptides occur in an sIgA molecule, different amounts of totally reduced and radioalkylated sIgA were subjected to analytical acrylamide gel electrophoresis until the tracking dye (Evans blue) migrated out of the gel. Immediately after this, the gel, inside its special Plexiglass tube was fragmented with an autogel divider (Savant) at a constant speed of 1 mm/12 sec, using 0.1 M Tris–acetate buffer (pH 8.0), as eluting solvent. Figure 8 shows one of three similar analyses. The stained gel schematized in the figure was electrophoresced at the same time, stained with buffalo black, and the positions of the bands were located as shown. Thus fraction 20 contains secretory component; fractions 22–28 contain light chain and fractions 42–48 contain the fast component. The relative areas under the tracing representing the light chain and the F component were then determined and taken as a measure of the relative concentrations of *S*-carboxymethylcysteine occurring in the two polypeptides in the whole sIgA molecule. Based on the assumption that there are four light chains in every sIgA molecule (Cebra and Small, 1967) and on the known molecular weights and amino acid compositions of both light chain and F component, it was calculated that no more than one F component could occur in each sIgA molecule (Table II).

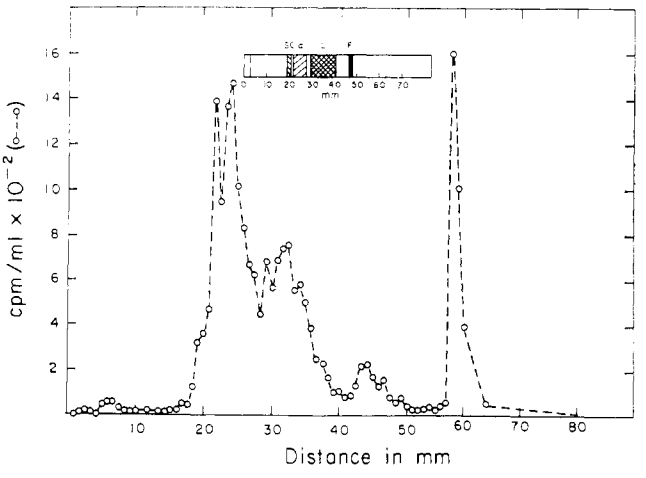

FIGURE 8: Quantitation of *S*-carboxymethylcysteine content of component chains of sIgA separated by analytic disc gel electrophoresis. The sample, 200 μg of sIgA, totally reduced and radioalkylated, was electrophoresed in a polyacrylamide gel and scanned with an autogel divider. For details, see text.

Amino Acid Composition. Table III shows the residues (per cent) and the total number of residues of each amino acid per mole of purified dissociable secretory component, purified free secretory component, α chain, and the fast component purified either by preparative acrylamide electrophoresis or by DE-52 chromatography. Similar data for the light chains purified from the sIgA are also included for comparison.

Peptide Maps. Because of the similarities between α chain and secretory component with respect to molecular weights and amino acid composition, further characteristics differentiating these two polypeptides were sought. Tryptic digests of each polypeptide were analyzed by two-dimensional chromatography and electrophoresis. In Figure 9A, the secretory component peptide map shows more peptides which stain intensely compared to the α-chain map (Figure 9B). On the other hand, the α-chain digest shows many more less intense spots, probably due to the presence of a "variable" region in this polypeptide. Overall the maps of the digests of the two polypeptides appear quite different.

Discussion

The overall molecular weight of sIgA was estimated to be about 385,000 from physical measurements of the rabbit protein (Cebra and Small, 1967) and later of the human protein (Newcomb *et al.*, 1968; Hurlimann *et al.*, 1969). Assuming that immunogloublin molecules were comprised of pairs of heavy and light chains, the data for the size and proportions of components in sIgA were interpreted as indicating four pairs of α and light chains in the parent molecule (Cebra and Small, 1967). Analysis of sIgA in dissociating solvents indicated that the molecule could be broken up into two subunits of two pairs each of light and heavy chains with the loss of a polypeptide (Cebra and Small, 1967). The two subunits were found to reassociate again when the dissociating solute was removed, even in the absence of the smaller component (Lawton and Mage, 1969).

Evidence for the presence of a polypeptide in sIgA in addition to the heavy and light chains associated with immunoglobulins has accumulated since the observation of Tomasi *et al.* (1965) that "extra antigenic sites" were present in this molecule compared to serum IgA. The evidence for this third polypeptide, called secretory component, being distinct from any subclass of α chain, is as follows. (1) It seems antigenically distinct from α chain of serum IgA and from light chain

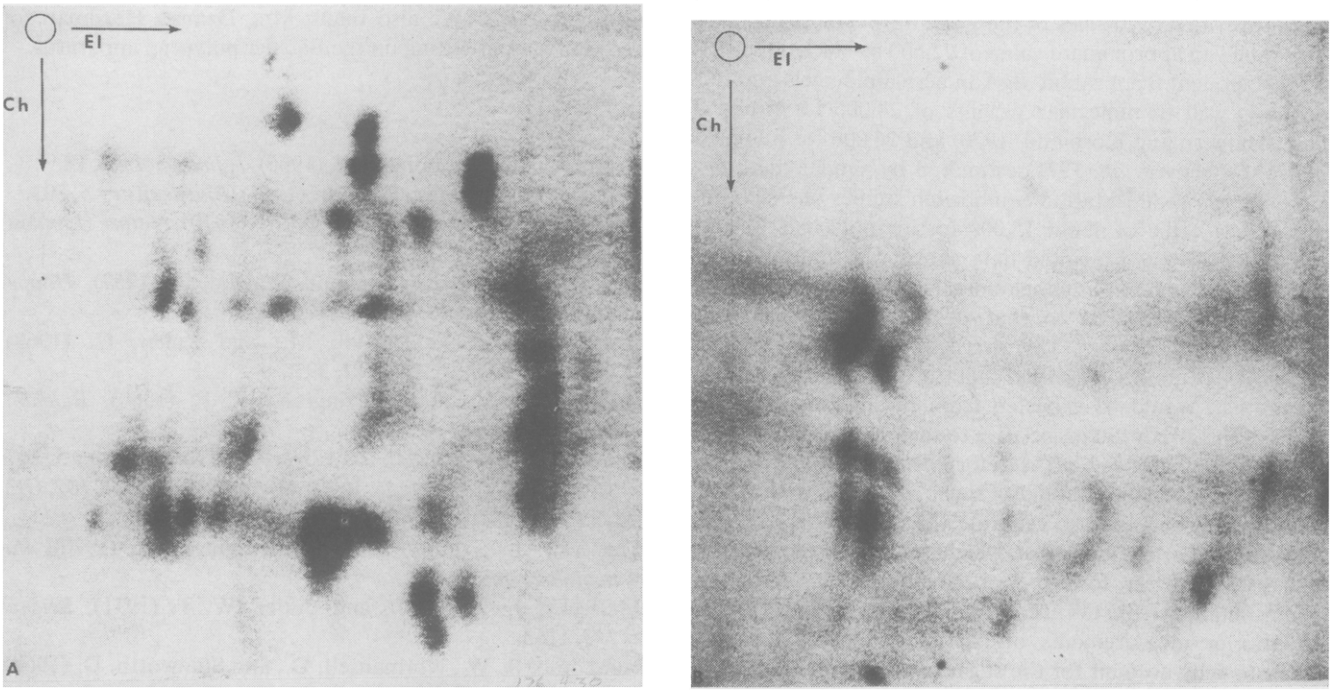

FIGURE 9: Fingerprints of tryptic peptides from secretory component (A) and α chain (B). Directions of chromatography (Ch), electrophoresis (El), and the point of sample application (O) are indicated.

(Tomasi *et al.*, 1965; O'Daly and Cebra, 1968). Fluorochrome coupled anti-secretory component does not stain plasma cells in proximity to secretory gland ducts or in the intestinal lamina propria of humans or rabbits (Tomasi *et al.*, 1965; O'Daly and Cebra, 1968) although these cells contain abundant quantities of IgA. The fluorochrome coupled anti-secretory component does stain epithelial cells lining secretory gland ducts (Tourville *et al.*, 1969) and the crypts of Lieberkuhn of the intestine (O'Daly *et al.*, 1971). (2) The secretory component was found to occur free in the saliva of dys-γ-globulinemic humans who produced only trace amounts of IgA (South *et al.*, 1966). Since then, free secretory component has been isolated from the secretions of many species (Pahud and Mach, 1970; Porter and Allen, 1964; O'Daly and Cebra, 1971b), including cow's milk, which ordinarily has little IgA. Since free intact heavy chains of immunoglobulins are not known to occur naturally, it seems unlikely that secretory component is a kind of α chain. (3) The α chain and secretory component described here from rabbit sIgA differ markedly in amino sugar content which is high in the former and low in the latter polypeptide. (4) The peptide maps of α chain and secretory component presented here suggest these two chains differ considerably in primary structure. Thus the two polypeptides, secretory α chain and secretory component, appear to be distinct although they have similar molecular weights, amino acid compositions, and blocked α-amino groups at their N terminii (J. A. O'Daly and J. J. Cebra, unpublished data) and despite the absence of a direct comparison of their antigenic characteristics.

More recently, Halpern and Koshland (1970) have advocated the presence of even a fourth different polypeptide chain in sIgA. This component, which they called "J chain" and which is called F component here, had been observed previously (Rejnek *et al.*, 1966; Cebra and Small, 1967) upon gel electrophoresis of completely reduced sIgA. It had been thought to represent secretory component, but this possibility could not be tested by antigenic analysis until native secretory component and native F component were isolated (O'Daly and Cebra, 1971a). Finally, each of the individual components of sIgA, defined by their antigenic characteristics, were correlated with components migrating differently in acrylamide gels upon electrophoresis (O'Daly and Cebra, 1971a). The fastest migrating polypeptide, F component, was only released from the sIgA molecule after reduction and was found to be distinct from secretory component (Halpern and Koshland, 1970; O'Daly and Cebra, 1971a). The cellular localization of F component was found to be the same as that of α chain (O'Daly and Cebra, 1971a). At present, the F component or J chain has not been proved distinct from a fragment of one of the other component polypeptides of sIgA. Recently, however, Mestecky *et al.* (1971) have described a J chain from human serum immunoglobulin M which had properties similar to those from human secretory IgA.

There is some variation among the molecular weight values reported for the component polypeptides of sIgA. Those values approximated using molecular sieves, either of polyacrylamide or Sephadex in the presence of solvents which promote unfolding of proteins, are uniformly higher than values estimated for the same polypeptides by sedimentation equilibrium methods. For instance, values for free secretory component of 85,000 (human) and 75,000 (bovine) were estimated by electrophoresis in acrylamide sieves (Mach, 1970) and for human secretory component dissociated from sIgA of 70,000 and 76,000 by filtration through Sephadex G-200 in sodium decyl sulfate or 5 M guanidine, respectively (Halpern and Koshland, 1970; Newcomb *et al.*, 1968). Our value of 80,000 for secretory component dissociated from rabbit sIgA obtained by acrylamide electrophoresis is comparable to these but the molecular weight of 60,000 which we found for this same protein by sedimentation equilibrium studies is the same as Tomasi and Calvanico (1968) calculated for human secretory component using similar ultracentrifuge data.

Another example of higher estimates of molecular weight from sieving data relative to those obtained in the ultracentri-

fuge is provided by studies of the J chain (F component). We determined an approximate value of 22,500 by electrophoresis of F component from rabbit sIgA in acrylamide gels and this compares well to molecular weights of 23,000 for rabbit J chain (Halpern and Koshland, 1970) and 26,000 for human J chain (Mestecky *et al.*, 1971) estimated by similar methods. However, our sedimentation equilibrium studies of F component gave a value of about 15,000 for its molecular weight. Shubert (1970) has presented data showing a retardation of migration of various immunoglobulin heavy chains containing carbohydrate compared to that of the same polypeptide without oligosaccharides. This decrease in mobility in acrylamide gels containing dodecyl sulfate and urea was much greater than would be expected from the higher molecular weight of the glycoproteins relative to the polypeptides devoid of carbohydrate. Since both secretory component and J chain contain an appreciable amount of sugar compared to the usual "marker" proteins used to calibrate the acrylamide sieves, it seems possible that molecular weight estimates made using these gels may err toward higher values. The molecular weights obtained from ultracentrifuge data depend also on the estimates or measurements of partial specific volume ($\bar{v}$). Failure to fully account for the sugar content of the polypeptides would result in higher estimates of $\bar{v}$ and hence in too high and not too low an approximation of the molecular weight. Thus these considerations suggest that the molecular weights based on sedimentation equilibrium data are likely to be the most accurate.

There is little data concerning the molecular weight of α chain from sIgA. Our previous estimate of 64,000 (Cebra and Small, 1967) was obtained using what has now been shown to be a mixture of secretory component and α chain. The present value of 54,000 has been obtained using similar sedimentation–equilibrium methods with purified α chain. Using the same procedures we obtained a molecular weight of 55,000 for rabbit gamma chain, the heavy chain of IgG, and this is in good agreement with the accepted value of 53,000 (Lamm and Small, 1966). Coelution of the completely reduced and carboxymethylated α and γ chains (Figure 7) from a Sephadex G-200 column in 5 M guanidine·HCl also supported the determination of similar molecular weights for the two classes of heavy chains.

Thus, we would like to propose a revised model of the structure of sIgA (Cebra and Small, 1967) consisting of four α chains ($4 \times 54{,}000$), four light chains ($4 \times 22{,}000$), one secretory component (60,000) and, if it is a unique component, one F component or J chain (15,000) to account for 379,000, a value close to that of 385,000 found for whole sIgA. This suggested model is based on the hypothesis that secretory component and J chain are integral and unique parts of the whole sIgA molecule. The secretory component appears to occur at least once in most or all sIgA molecules (O'Daly and Cebra, 1968; Mestecky *et al.*, 1970) and the F component (J chain) seems to be present in slightly less than one-quarter of the molar amount of light chains. Primary structural studies should indicate whether F component is a distinct polypeptide and should indeed be designated "J chain."

Acknowledgments

We thank Miss Kelly Baumgartner for her excellent technical assistance. We also thank Mrs. Deanna Hardman for her kind help in setting up the Büchler polyprep apparatus.

References

Cebra, J. J., and Robbins, J. B. (1966), *J. Immunol. 97*, 12.
Cebra, J. J., and Small, P. A., Jr. (1967), *Biochemistry 6*, 503.
Halpern, M. S., and Koshland, M. E. (1970), *Nature (London) 228*, 1276.
Heilman, J., Barrolier, J., and Watzke, E. (1957), *Hoppe-Zeyler's Z. Physiol. Chem. 309*, 219.
Hurlimann, J., Waldesbuhl, M., and Zuker, C. (1969), *Biochim. Biophys. Acta 181*, 393.
Kielley, W. W., and Harrington, W. F. (1960), *Biochim. Biophys. Acta 41*, 401.
Lamm, M. E., and Small, P. A., Jr. (1966), *Biochemistry 5*, 267.
Lawton, A. R., and Mage, R. G. (1969), *J. Immunol. 102*, 693.
Mach, J. P. (1970), *Nature (London) 228*, 1278.
Mestecky, J., Kraus, F. W., and Voight, S. A. (1970), *Immunology 18*, 237.
Mestecky, J., Zikan, J., and Butler, W. T. (1971), *Science 171*, 1163.
Newcomb, R. W., Normansell, D., and Stanworth, D. (1968), *J. Immunol. 101*, 905.
O'Daly, J. A., and Cebra, J. J. (1968), *Protides Biol. Fluids Colloq.*, 205.
O'Daly, J. A., and Cebra, J. J. (1971a), *J. Immunol. 107*, 436.
O'Daly, J. A., and Cebra, J. J. (1971b), *J. Immunol. 107*, 449.
O'Daly, J. A., Craig, S. W., and Cebra, J. J. (1971), *J. Immunol. 106*, 286.
Pahud, J. J., and Mach, J. P. (1970), *Immunochemistry 7*, 679.
Porter, P., and Allen, W. D. (1969), *Immunology 17*, 789.
Reisfeld, R. A., and Small, P. A., Jr. (1966), *Science 152*, 1253.
Rejnek, J., Kosta, J., and Kotyneko, O. (1966), *Nature (London) 209*, 926.
Roark, D. E., and Yphantis, D. A. (1969), *Ann. N. Y. Acad. Sci. 164*, 245.
Schachman, H. K. (1957), *Methods Enzymol. 4*, 32.
Shubert, D. (1970), *J. Mol. Biol. 51*, 287.
Small, P. A., Jr., and Lamm, M. E. (1966), *Biochemistry 5*, 259.
South, M. A., Cooper, M. D., Wollheim, F. A., Hong, R., and Good, R. A. (1966), *J. Exp. Med. 123*, 615.
Šterzl, J., and Riha, I., Ed. (1970), Developmental Aspects of Antibody Formation and Structure, Prague, Academic Press, p 469.
Tomasi, T., and Calvanico, N. (1968), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 27*, 617.
Tomasi, T. B., Jr., and Bienenstock, J. (1968), *Advan. Immunol. 9*, 2.
Tomasi, T. B., Tan, E. M., Soloman, A., and Prendergast, R. A. (1965), *J. Exp. Med. 121*, 101.
Tourville, D. R., Adler, R. H., Bienenstock, J., and Tomasi, T. B. (1969), *J. Exp. Med. 129*, 411.
Vaerman, J. P., and Heremans, J. F. (1970), *Immunology 18*, 27.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.
Zacharias, R. M., Zell, T. E., Morrison, J. H., and Woodlock, J. J. (1969), *Anal. Biochem. 30*, 148.