plex because of the lesser specificity of chymotrypsin, but here also, with few exceptions, the two peptide maps are clearly dissimilar. Admittedly, in both pairs of maps the complexity is such that it would be difficult to claim noncoincidence for some of the spots, purely because there are so many of them. Therefore, the data presented here cannot completely eliminate the possibility of very restricted local homology at some points within the polypeptide chains (perhaps at the nucleotide-binding site), but the overall makeup of the two polypeptide chains is clearly different.

Thus actin and tubulin from *Pecten irradians* striated muscle and gill cilia microtubule doublets, respectively, have significantly different molecular weights that agree with values for their homologs from other species. Comparative peptide mapping, with tryptic or chymotryptic digests, shows that most of the resulting peptides from the two proteins are dissimilar. Association or interaction with an adenosine triphosphatase and the binding of molar ratios of nucleotides are the only remaining similarities between actin and tubulin. In terms of comparative mechanochemistry, a common ground for muscle contraction and ciliary beat must now be sought at the enzymatic level. In this regard, the microtubule protein of brain tissue, in a manner quite parallel to actin, significantly increases the magnesium-activated adenosine triphosphatase of myosin; and the protein complex thus formed undergoes characteristic viscosity drops on addition of ATP (*14*). It remains to be demonstrated, however, that these properties are held by other tubulin fractions of more certain purity.

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Ultrastructure of Secretory and High-Polymer Serum Immunoglobulin A of Human and Rabbit Origin

Abstract. *Electron micrographs of immunoglobulins A from human and rabbit colostrum, which were purified on tall agarose columns, revealed Y-shaped molecules (125 by 140 angstroms). The linear dimensions of the arms were 55 to 75 by 25 to 30 angstroms. A molecular model is postulated in which two immunoglobulin A monomers are superimposed on each other in a close-packed state with the secretory piece inserted in the constant region of the α-chains. High-polymer (11 or 13S) immunoglobulin A molecules (total span 100 to 110 angstroms) from human serum were composed of four arms (50 to 55 by 20 angstroms) joined at a contrast-rich center.*

Immunoglobulin A (IgA) is the predominant globulin in the secretions on mucous membranes. Secretory IgA (s-IgA) has a higher sedimentation coefficient (approximately 11*S*) than the major serum IgA component (*1*) and possesses a unique protein, referred to as the secretory piece (SP) (*1*) or transport piece (*2, 3*). This protein has been demonstrated in free form in secretions of agammaglobulinemic patients (*3*) or individuals lacking IgA (*4*). Cebra and Small (*5*) concluded that the rabbit s-IgA molecule was composed of four heavy and four light peptide chains plus one or two so-called T chains which make up the SP. In studies of human s-IgA Tomasi and Czerwinski (*6*) and Newcomb *et al.* (*7*) reached the same conclusions.

Essentially no information concerning the quaternary structure of s-IgA or high-polymer serum IgA is available. There are basically three different approaches applicable to analyses of the overall layout of protein molecules and the spatial arrangement of their subunits—hydrodynamic studies, x-ray diffraction studies, and electron microscopy. We now report on comparative studies of the size and conformation of colostrum IgA and high-polymer, serum IgA molecules by the use of electron microscopy.

Nine human and five rabbit s-IgA preparations were partially purified by centrifugation and gel filtration (*8*). The first eluted peak on Bio-Gel A-15m (Fig. 1a) contained chylomicrons by lipid staining and phase contrast microscopy; IgA was distributed throughout the second major elution peak. The ascending part of this peak contained also immunoglobulin M (IgM) and the descending part contained two, occasionally three, unidentified colostrum proteins. These contaminating proteins were detected by immunodiffusion only in fractions concentrated eight to ten times.

Only s-IgA was demonstrable within a restricted central area of the peak. Three human s-IgA preparations (3 to 5 mg of protein per milliliter) were subjected to analytical ultracentrifugation. The $s_{20,w}$ values were 11.6, 11.4, and 10.7.

A relatively weak poliovirus-neutralizing antibody activity, which coincided with the distribution of s-IgA in the A-15m fractions, was noted when IgA from human colostrum was assayed (Fig. 1a). No IgM or IgG was detected by immunodiffusion in the fractions having most antibody activity, and absorption with antiserum to IgM caused no reduction in activity.

Immunodiffusion and immunoelectrophoretic analyses of concentrated fractions from the central part of the major elution peak of A-15m curves indicated the presence of complexes of s-IgA and albumin in three preparations of human whey (Fig. 1a). Since s-IgA is rather resistant to reduction (*1, 9*), these complexes could be dissociated by mild reduction (0.1*M* 2-mercaptoethanol) and alkylation of the whey prior to gel filtration. In addition, this treatment eliminated the IgM contamination in the major s-IgA peak, the IgM subunits being recovered in the IgG elution region (Fig. 1b).

High-polymer IgA from human serum was partly purified by a combi-

nation of fractionation on tall agarose columns (Fig. 1c) and zone electrophoresis on Pevicon blocks. But the yields were low, and it could not be determined with certainty whether the major IgA component was of 11*S* or 13*S* type.

Specimens for electron microscopy were prepared by placing a drop of buffer solution containing the purified immunoglobulins on a 400-mesh carbon-coated grid. The grid was washed with the negative straining material, and excess fluid was withdrawn with a filter paper. The grids were dried in the air and immediately examined in a Philips EM 200 electron microscope at a magnification of 46,000; double condensed illumination was used. The instrument was equipped with an anticontamination device cooled by liquid nitrogen. Three different negative stains were used: 2 percent sodium tungstosilicate (*p*H 6.0) and 1 percent solutions of uranyl formate or uranyl acetate, both at *p*H 4.6.

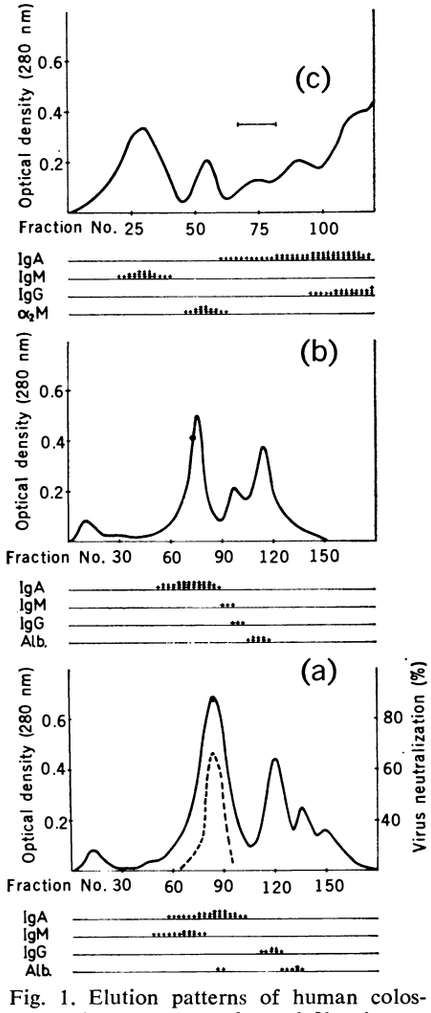

Fig. 1. Elution patterns of human colostrum whey or serum after gel filtration on tall agarose (Bio-Gel A-15m) columns (*8*). (a) Elution pattern of 4 ml of whey. The broken line indicates antibody activity; the filled circle denotes fractions examined by electron microscopy. (b) Elution pattern of 3 ml of reduced (0.1*M* 2-mercaptoethanol) and alkylated (0.15*M* iodoacetate) whey. The encircled fraction was examined by electron microscopy. (c) Elution pattern of 20 ml of serum on two columns, 2.5 by 400 cm, coupled in series. Horizontal bar indicates fractions which were pooled and further fractionated.

Electron micrographs of human and rabbit s-IgA revealed an abundance of flexible particles with a structure resembling the letter Y or a wishbone (Fig. 2, top). The overall dimension of these molecules was 125 Å (mean value) and 140 Å (maximum value). The width of the molecule was 25 to 30 Å. The peripheral ends of the two most adjacent arms of the Y-shaped molecule often showed an increased breadth (35 Å). The latter two arms were 65 to 75 Å in length, while the third arm measured 50 to 55 Å. The angle between the longer arms usually varied between 30 and 60 degrees. Bending was commonly observed in this part of the molecule, suggesting a certain degree of intramolecular flexibility. When the arms of the molecule were assumed to be cylinders with circular cross sections, its molecular weight was calculated to be 140,000 to 150,000. This unexpected low value and the fact that the width of the arms (30 to 35 Å) is compatible with a single heavy-light chain pair have led us to postulate a model for the molecule in which two IgA monomers, as viewed in the microscope, are superimposed on each other in a close-packed state with the SP inserted in the constant region of the four α-chains. The molecular weight similarly calculated for such a dimer molecule plus the SP would be around 350,000—in better agreement with the reported molecular weight of 380,000 to 390,000 (*7*, *10*).

The high-polymer IgA from human serum, examined in the electron microscope, was eluted from the agarose columns in a position intermediate to that of 18 to 19*S* α_2M (macroglobulin) and 7*S* IgA. The predominant structure in such IgA preparations, further purified by zone electrophoresis, was composed of four filamentous subunits joined at a central point of high contrast (Fig. 2, bottom). The total span of the molecule was 100 to 110 Å, and the linear dimensions of the subunits were 55 by 20 Å. The size of the subunits is approximately the same as that of monomeric Fab fragments of IgG. A possible explanation for the limited length of the subunits is that a close-packed Fc region, responsible for polymerization, is discernible only when the molecule is embedded sideways, which is a rare event.

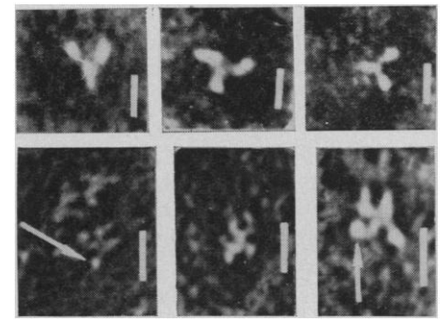

Fig. 2. Electron micrographs of purified human and rabbit IgA molecules. (Top) Variable forms of Y-shaped, single secretory IgA molecules. There was no observable difference in size and conformation between human and rabbit s-IgA molecules. (Bottom) Single human high-polymer serum IgA molecules each with four visible appendages. One appendage is indicated by arrows. The scale lines represent 10 nm.

Many of the techniques described earlier for the purification of colostrum IgA either have been rather time-consuming or would seem to yield IgA preparations of relatively low purity. The technique used in our study involved only two (or three) steps: centrifugation, (reduction-alkylation), and gel filtration on exceptionally tall Bio-Gel A-15m columns. Analyses by immunodiffusion, Mancini technique, and electron microscopy indicated that the s-IgA samples prepared were of high purity. A minor contamination of albumin, apparently complexed with the IgA, was, however, noted in some s-IgA preparations. Complexes of serum IgA and albumin have been observed (*11*) but complexes formed by s-IgA and albumin have not been described. The s-IgA–albumin complexes could be dissociated by mild reduction in accordance with studies of complexes of albumin with serum IgA or IgM (*12*).

The fine architecture of IgM, IgG, α_2M, and lipoprotein molecules has recently been clarified with the aid of electron microscopy (*13*). The degree of contamination of these molecules in purified IgA preparations could therefore be ascertained by screening the preparations in the microscope. When examining 14 human and rabbit s-IgA

preparations, devoid of most of these contaminants, the presence of wishbone or Y-shaped molecules was a characteristic recurrent phenomenon. The gross morphology of the s-IgA molecule resembled serum IgG, but the linear dimension of the s-IgA molecule was larger and it was less electron-transparent than IgG, indicating a greater thickness, compatible with the molecular model for s-IgA postulated above. Tomasi has published optical rotary dispersion data (*14*) suggesting a close similarity between serum IgG and serum IgA as well as s-IgA.

Normal, as well as paraproteinemic, serums contain IgA with sedimentation coefficients ranging from 7 to 17*S* (*12*, *15*). The excellent resolving capacity of the tall agarose columns used in our study permitted a partial separation of high-polymer serum IgA from 7*S* IgA. The predominant structure in the high-polymer IgA (11 to 13*S*) preparations was made up of four filamentous subunits joined at a central point of high contrast; Ballieux *et al.* (*16*) suggested that the monomers in high-polymer myeloma IgA were joined at their Fc parts. Others (*6*, *17*) have been unable to recover any significant amount of intact Fc or F'c material after proteolytic degradation of 7*S* serum IgA and 11*S* myeloma IgA. The two-dimensional profiles of the subunits in the high-polymer IgA molecules examined in the electron microscope revealed no contribution of Fc parts. It is possible, however, that the contrast-rich, rather compact, center of the molecule represents a close-packed Fc region visible only when the molecule is embedded sideways.

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L-Dopa: Effect on Concentrations of Dopamine, Norepinephrine, and Serotonin in Brains of Mice

Abstract. *Large doses of* L-*dopa given to mice produced marked increases in brain dopamine, no change in norepinephrine, and a remarkable decrease in brain serotonin. This reduction apparently results from a release or displacement, or both, of serotonin from its storage sites.*

Large daily doses (2 to 8 g) of L-dopa (dihydroxyphenylalanine) are used for the relief of akinesia, rigidity, and tremor in Parkinson patients (*1*). We investigated the problem of how these large amounts of the precursor of dopamine (DA) affect the concentrations of norepinephrine (NE) and serotonin (5HT) in the brain.

White male mice (ICR) were given varying doses (100, 200, and 400 mg per kilogram of body weight) of a warmed L-dopa solution (10 mg/ml). Concentrations of the amines in the brain were determined at peak action time (30 minutes after administration of L-dopa, intraperitoneally). At peak action time, the behavioral effects are proportional to the doses of L-dopa, and symptoms include increasing alertness and irritability; at very high doses, fighting and jumping are noted. There was an increase in the concentration of dopamine proportional to the dose of L-dopa, but no significant effect on norepinephrine concentrations occurred (Table 1). Apparently the step from dopamine to stored norepinephrine is not easily achieved. The concentrations of brain serotonin decreased markedly in proportion to the dose of L-dopa administered. These data suggest that dopa and dopamine may cause changes in the metabolism and storage of serotonin in the brain.

We also determined the concentrations of indoleacetic acid (HIAA) in the brains of normal mice and mice treated with L-dopa (400 mg/kg; intraperitoneally) 1 hour before they were killed. The concentration of HIAA in controls was 0.37 ± 0.01 μg per gram

Table 1. Effect of L-dopa on concentrations of biogenic amines in the brains of male mice (ICR). The mice were killed at peak action time (30 minutes after the intraperitoneal administration of L-dopa).

L-Dopa (mg/kg)	No. of determinations	DA (μg/g of brain)	Percentage of change	NE (μg/g of brain)	Percentage of change	5HT (μg/g of brain)	Percentage of change
Controls	17	1.01 ± 0.02	0	0.45 ± 0.01	0	0.54 ± 0.01	0
100	12	3.34 ± 0.37	332	0.48 ± 0.01	7	0.44 ± 0.01	−18
200	12	6.22 ± 0.74	617	0.47 ± 0.01	5	0.30 ± 0.02	−45
400	15	11.85 ± 0.90	1177	0.43 ± 0.01	−5	0.20 ± 0.01	−63