

LOCALIZATION OF FREE AND BOUND SECRETORY COMPONENT  
IN HUMAN INTESTINAL EPITHELIAL CELLS

A MODEL FOR THE ASSEMBLY OF SECRETORY IgA\*

BY MARSHALL E. POGER AND MICHAEL E. LAMM†

(From the Department of Pathology, New York University School of Medicine,  
New York 10016)

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The predominant immunoglobulin in external secretions of humans and various other mammals is a special form of IgA known as secretory IgA (sIgA),<sup>1</sup> a molecule composed of a dimer of serum-type IgA plus a J chain (in common with IgM) and a distinctive polypeptide chain known as secretory component (SC) (1-4). Not only does SC occur in a bound form (BSC) as part of sIgA, but a portion is also present in a free form (FSC). Recently, FSC and BSC have been isolated from human and rabbit colostrum, and the two forms compared by a number of criteria. They are antigenically cross-reactive, indistinguishable by amino acid composition and size, yield similar tryptic peptide maps, and have identical *N*-terminal amino acid sequences (references 5-7 and footnote 2). However, we and others (8-10) have observed that in addition to common antigenic determinants, FSC has determinants not found in BSC, and vice versa. These antigenic differences, which are thought to be due to differences in the conformation of SC, have been utilized to quantitate FSC in the presence of BSC by immunoprecipitation (11).

Histological localization of SC (2, 12-19) and  $\alpha$ -chain, the heavy chain of IgA (2, 12, 13, 15, 19, 20), in organs which elaborate external secretions has been studied by immunofluorescence. For such work, antibody to SC has usually been obtained by absorbing antibodies to sIgA with serum-type IgA and normal human serum (NHS). By this means SC has been identified in the cytoplasm of epithelial cells in mucous membranes, spaces between these cells, and secretions.

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<sup>1</sup> *Abbreviations used in this paper:* APB-cellulose, (para-aminophenylbutyryl aminoethyl)-cellulose; BSC, bound secretory component; FITC, fluorescein isothiocyanate; FSC, free secretory component; H & E, hematoxylin and eosin; NCS, normal umbilical cord serum; NHS, normal human serum; PBS, phosphate-buffered saline; SC, secretory component; sIgA, secretory IgA.

<sup>2</sup> Cunningham-Rundles, C., M. E. Lamm, and E. C. Franklin. 1974. Human secretory component. *N*-terminal amino acid sequences and peptide maps of the form occurring in exocrine immunoglobulin A and the free form. Manuscript submitted for publication.

Also by immunofluorescence,  $\alpha$ -chains have been described in plasma cells adjacent to mucosal glands, in epithelial cells of some mucous membranes, in spaces between these cells, and in luminal secretions. Staining for J chain follows the same distribution as  $\alpha$ -chain (18).

Current hypotheses on synthesis, secretion, and assembly of sIgA (12, 13) are based on immunofluorescence data and radioactive labeling experiments (2, 21-25). Serum-type IgA plus J chain is thought to be synthesized and secreted locally by mucosal plasma cells, and then to diffuse across the epithelial basement membrane into spaces between overlying epithelial cells. In contrast, SC is thought to be produced by mucosal epithelial cells from which it is secreted into the spaces between these cells and the lumen of mucosal glands. However, the exact locus where the products of the two types of cells (plasma cell and epithelial cell) are combined to yield the fully assembled sIgA molecule has not been established. There are several possibilities. First, assembly could occur primarily extracellularly, either in the spaces between mucosal epithelial cells or in the lumen, which would be consistent with the assembly *in vitro* of sIgA from dimeric IgA and FSC (26, 27). Because the tight junctions between the apical portions of mucosal epithelial cells do not allow passage of large molecules into glandular lumens (28), it has been suggested that sIgA, previously assembled in the spaces between epithelial cells, could reach the lumen by traversing the apical portion of mucosal epithelial cells (13). Second, assembly could occur at the epithelial cell membrane (29). A third possibility is that the plasma cell products first enter the epithelial cell, in which complexing with SC takes place, to be followed by secretion of sIgA into the lumen.

Previous immunofluorescence studies have not utilized antisera capable of distinguishing between FSC and BSC. Consequently, we decided to study the cytological and histological distribution of FSC and BSC in human tissues with fluorescent antibodies rendered specific for each of the two forms of SC. The results support a model in which final assembly occurs within the epithelial cell.

#### *Materials and Methods*

*Antigens.*—FSC (6) and sIgA (7) were isolated from human colostrum, and were pure according to several immunologic and zone electrophoretic criteria. A human IgA1, K-myeloma protein (i.e., serum-type IgA) was isolated from ascitic fluid (provided by Dr. E. C. Franklin, New York University School of Medicine) by starch block electrophoresis (30). Normal human serum from several persons was pooled. Pooled normal cord serum (NCS) was obtained from fresh umbilical cord sera individually shown to lack IgA on Ouchterlony analysis. Human IgG was obtained from Schwarz/Mann Div., Becton, Dickinson & Co, Orangeburg, N. Y., and a purified human IgM myeloma protein was obtained from Dr. E. C. Franklin.

*Antisera.*—Anti-FSC, anti-sIgA and antimyeloma IgA were raised in rabbits (7). Animals being immunized with sIgA received injections of rabbit antiserum to human serum-type IgA to suppress the antibody response to  $\alpha$ - and L chains, and enhance the antibody response to BSC (31). On immunoelectrophoresis vs. whole colostrum, both anti-FSC (unabsorbed) and anti-sIgA (after absorption with serum-type IgA and NHS) showed only precipitin lines due to FSC and BSC; these displayed partial identity. No other reactions with whole colostrum (or with purified lactoferrin, a protein which tends to contaminate preparations of SC and which often reacts with anti-SC antisera according to the literature) were detected.

*Absorption of Antisera.*—Antisera were made specific for FSC, BSC, or  $\alpha$ -chains by the use of solid immunoadsorbents. (Para-aminophenylbutyrylaminoethyl)cellulose (APB-cellulose) was prepared and linked to the various protein antigens by diazotization (32). Absorptions were accomplished by mixing the appropriate immunoadsorbent with antiserum (1 ml packed cellulose/4 ml whole serum) for 20 min at room temperature, followed by removal of the adsorbent by centrifugation. The supernate was then tested for completeness of absorption by Ouchterlony analysis. Absorptions were repeated until unwanted precipitation lines were no longer identified, and then once more. From 2 to 4 absorptions were required for each antiserum. The final preparations retained strong precipitating activity for the antigenic determinants being selected. To obtain antiserum specific for FSC, the original anti-FSC antiserum was absorbed with sIgA-APB-cellulose. To obtain specific anti-BSC, anti-sIgA antiserum was absorbed with a mixture of FSC-APB-cellulose, myeloma IgA-APB-cellulose, and NHS-APB-cellulose. Anti- $\alpha$ -chain antiserum was prepared by absorbing antimyeloma IgA serum with IgG-APB-cellulose and NCS-APB-cellulose. Further absorptions, indicated by the results of preliminary immunofluorescence studies were done in order to enhance the specificity of staining; these were accomplished by adding minute amounts of appropriate purified soluble antigens to the solid-absorbed immunoglobulins after coupling with fluorescein (see below).

*Conjugation of Antibody and Fluorescein.*—After absorption of antisera with solid immunoadsorbents, the IgG fraction was isolated by DEAE-cellulose chromatography (0.01 M sodium phosphate buffer, pH 8.0), shown to be pure by immunoelectrophoresis, and concentrated to about 10 mg/ml by ultrafiltration. Conjugation to fluorescein isothiocyanate (FITC) was carried out using FITC adsorbed to Celite (Calbiochem, La Jolla, Calif.) (33). FITC on Celite (20 mg/100 mg IgG) was suspended in 0.5 M sodium carbonate buffer, pH 9.0 (volume equivalent to that of the IgG solution). The IgG was immediately added dropwise with stirring at room temperature. The pH remained constant, and after 20 min the Celite was removed by centrifugation, and the supernate was passed through Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) equilibrated in 0.0175 M sodium phosphate buffer, pH 6.3. The fluoresceinated IgG was then chromatographed on a DEAE-cellulose column equilibrated with the same buffer. Peaks were eluted stepwise, starting with the equilibrating buffer, followed by the addition of 0.15 M sodium chloride in the original buffer, and then 0.25 M sodium chloride in the original buffer (34). The major fraction of conjugated IgG was eluted in the 0.15 M sodium chloride fraction and had a molecular fluorescein/protein ratio (35) of approximately 2. The purified conjugate was then ultrafiltered to 1 mg/ml for immunofluorescent staining. Fluoresceinated anti-FSC, anti-BSC, anti- $\alpha$ -chain, and normal rabbit IgG were prepared.

*Tissue Preparation.*—Frozen sections cut from tissue blocks taken from grossly normal areas of freshly removed surgical specimens were initially examined after staining with hematoxylin and eosin (H & E). Blocks showing normal histology were used for cutting multiple frozen sections, approximately 4  $\mu$ m thick, which were mounted on glass slides and kept at  $-10^{\circ}\text{C}$  before fixation. 7 small intestines, 5 large intestines, 3 gallbladders, 4 salivary glands, and 10 bronchi were examined.

For most of the studies, sections were fixed in acetone for 10 min before staining with fluorescent antisera. Unfixed and more lightly fixed sections were examined on occasion. Some sections were fixed in absolute ethanol rather than acetone. Some tissue blocks were fixed in 10% neutral buffered formalin for 4 h at  $2-5^{\circ}\text{C}$  followed by washing with 30% sucrose for 12 h before sectioning (13).

*Immunofluorescence.*—The routine staining procedure consisted of wetting the sections in phosphate-buffered saline (PBS) (0.15 M sodium chloride—0.01 M sodium phosphate, pH 7.5) followed by staining with a drop of fluorescent antibody for 30 min in a moist chamber. Sections were then dipped twice (5 min each time) in PBS and mounted in a glycerol-PBS mixture. Fluorescent antibody preparations employed included anti-FSC, anti-BSC, and anti- $\alpha$ -chain reagents.

Sections were examined with a Leitz Dialux fluorescence microscope equipped with an

Osram HBO 200 high pressure mercury vapor light source, BG 12 excitation filter, BG 38 suppression filter, and a K 530 barrier filter (E. Leitz, Inc., Rockleigh, N. J.). Controls included: blocking, in which the section was treated with unconjugated antiserum before staining with the same antibody in conjugated form; absorption of antibody reagents with the purified homologous antigen in soluble form after antibody conjugation with FITC; and staining with fluoresceinated normal rabbit IgG.

*Specificity of Fluorescent Antibodies.*—Before conjugation with FITC, anti-FSC and anti-BSC had been absorbed with solid immunoadsorbents until anti-FSC no longer precipitated sIgA and anti-BSC no longer precipitated FSC or serum-type IgA or Ouchterlony analysis. To evaluate whether this specificity persisted on immunofluorescence analysis, frozen sections of small bowel were treated with solid-absorbed fluoresceinated anti-FSC and anti-BSC. Both preparations stained the cytoplasm of columnar epithelial cells of mucosal glands, most prominently in the apical region and the supranuclear Golgi zone. Anti-BSC also stained plasma cells in the lamina propria. The qualitative similarity in the staining of epithelial cells by these two antibody preparations did not distinguish between the following two possibilities: an overlap in the distribution of FSC and BSC, or a lack of specificity. However, the staining of plasma cells by the anti-BSC preparation was evidence that the specificity obtained, using Ouchterlony analysis as a criterion, was not necessarily valid for immunofluorescence. Therefore, further absorptions of solid-absorbed fluoresceinated anti-FSC and anti-BSC were done with purified soluble antigens to increase the specificity of the fluorescence.

*Anti-FSC:* In case solid-absorbed anti-FSC still cross-reacted with BSC, a minute amount of soluble sIgA was added. Immunofluorescence staining of small bowel sections then showed loss of the prominent apical cytoplasmic staining of epithelial cells but intense staining of the Golgi area persisted. This reagent was considered specific for FSC and was used in the ensuing studies.

*Anti-BSC:* Anti-BSC, treated with solid immunoadsorbent, still reacted on immunofluorescence with serum-type IgA (i.e., plasma cells) and might also have reacted slightly with FSC because it stained the Golgi zone weakly. The addition of soluble IgG and myeloma IgA to this antibody preparation abolished the fluorescence of plasma cells; however, prominent apical cytoplasmic staining persisted, as did weak Golgi zone staining. Because this reagent stained the apical cytoplasm of columnar gland cells strongly, but stained the Golgi zone (shown by the anti-FSC reagent to be the principal locus of FSC) only weakly, it was used in the ensuing immunofluorescence studies to detect BSC.<sup>3</sup>

*Anti- $\alpha$ -chain:* Antimyeloma IgA was treated with solid immunoadsorbents to be specific for  $\alpha$ -chains by double diffusion analysis. To evaluate specificity, frozen sections of small bowel were examined. Specific staining was limited to mucosal plasma cells and the apical cytoplasm of columnar cells of mucosal glands. Further absorptions carried out by adding minute amounts of pure soluble IgG and IgM to the anti- $\alpha$ -chain preparation produced only a slight decrease in staining intensity and no change of pattern. This latter reagent was considered to be specific for  $\alpha$ -chains on immunofluorescence analysis, and was used in the following studies.

## RESULTS

*Small Intestine.*—The various specimens showed almost identical staining patterns. Anti-FSC stained the cytoplasm of mucosal columnar cells (both crypts and villi) most prominently in the supranuclear Golgi zone (Fig. 1). Both anti- $\alpha$  and anti-BSC prominently stained the luminal border of columnar cells

<sup>3</sup> It was not possible to improve the specificity of the anti-BSC reagent by adding both soluble-myeloma IgA and soluble FSC. When this was attempted all staining, including the apical cytoplasm where BSC predominates, disappeared, probably because the soluble FSC and IgA had combined (26, 27) to produce effectively sIgA, and hence BSC.

in mucosal crypts (Figs. 2 and 3); no staining of villous epithelial cells was seen. The staining for FSC and  $\alpha$ -chains, which sometimes showed linear, radially oriented striations, was identical and had a variable depth measuring from 3 to 5  $\mu$ m. This distribution correlates partially with the location of microvilli on the luminal surface of crypt epithelial cells (36), but since small intestinal microvilli measure only about 1  $\mu$ m in height (reference 37 and footnote 4), sIgA is most probably located not only in the region of the microvilli but also in a zone of underlying apical cytoplasm.

No staining of goblet cells for FSC, BSC, or  $\alpha$ -chains was seen, a result which can be attributed to the high degree of specificity of our antisera. The interepithelial cell spaces, which could be identified above the nucleus, consistently did not stain for FSC, BSC, or  $\alpha$ -chains.  $\alpha$ -chains, without SC, were prominent in many lamina propria plasma cells, both in villous cores and about crypts (Fig. 3).

*Colon.*—The colonic mucosal crypts are composed mainly of mucous-filled goblet cells between which may be columnar cells, either isolated or in groups. All specimens of colon showed identical staining. FSC was found predominantly in the supranuclear Golgi zone of columnar crypt cells (Fig. 4 *a*), similar to its location in the small bowel. sIgA, identified by the simultaneous presence of BSC and  $\alpha$ -chains, was seen throughout the apical half of the columnar crypt cell cytoplasm in a granular distribution which was most dense at the luminal surface and decreased in density deeper into the cytoplasm (Figs. 4 *b* and 5).  $\alpha$ -chains were also found in numerous plasma cells of the lamina propria (Fig. 5). No goblet cell staining was seen.

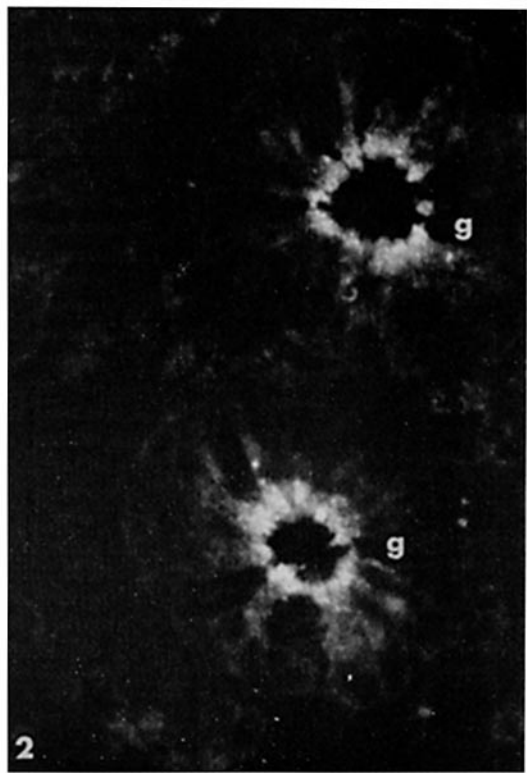
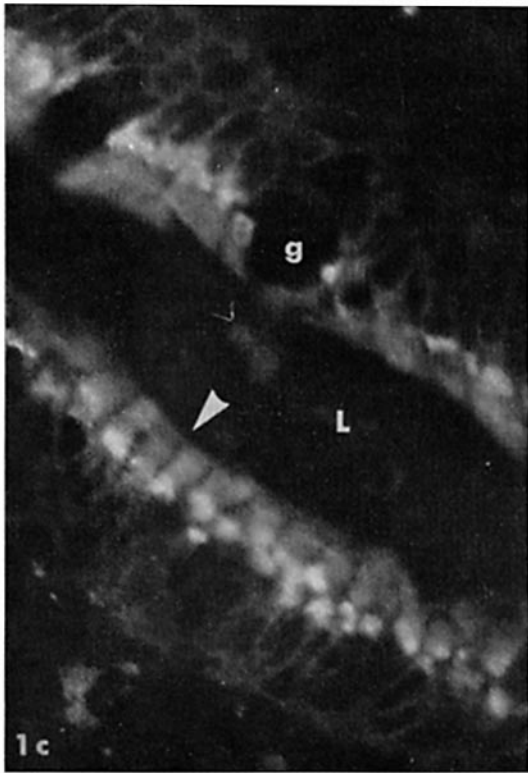
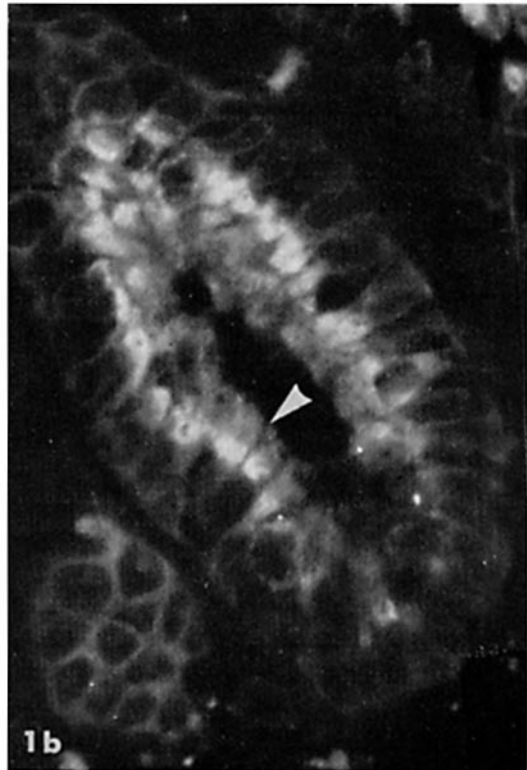
To identify interepithelial cell spaces in colonic mucosa is more difficult than in small bowel because of the large number of goblet cells among which there may be isolated columnar cells. Thin frozen sections are required since staining of such individual columnar cells can easily be mistaken for staining of an intercellular space (Fig. 6). Our interpretations, accordingly, are based on areas containing groups of two or more columnar cells between goblet cells. As in small bowel, intercellular spaces could only be identified above the nucleus. We were unable to identify FSC, BSC and  $\alpha$ -chains in intercellular spaces.

*Other Organs.*—In the gallbladder mucosa, FSC was present in the cytoplasm of the columnar surface epithelial cells in a finely granular pattern (Fig. 7). sIgA could not be identified since neither anti-BSC nor anti- $\alpha$ -chain reagent stained the mucosa, which is consistent with the rare occurrence of plasma cells in H & E-stained sections. Interepithelial cell spaces did not stain for FSC.

The bronchial glands and the salivary glands contain acini which may be serous, mucous, or mixed. The serous cells consistently stained for SC whereas the mucous cells were uniformly negative (Fig. 8).

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<sup>4</sup> The height of the microvilli within small intestinal crypts in our specimens was confirmed in electron micrographs, which were kindly done by Dr. D. Sabatini, New York University School of Medicine.



## DISCUSSION

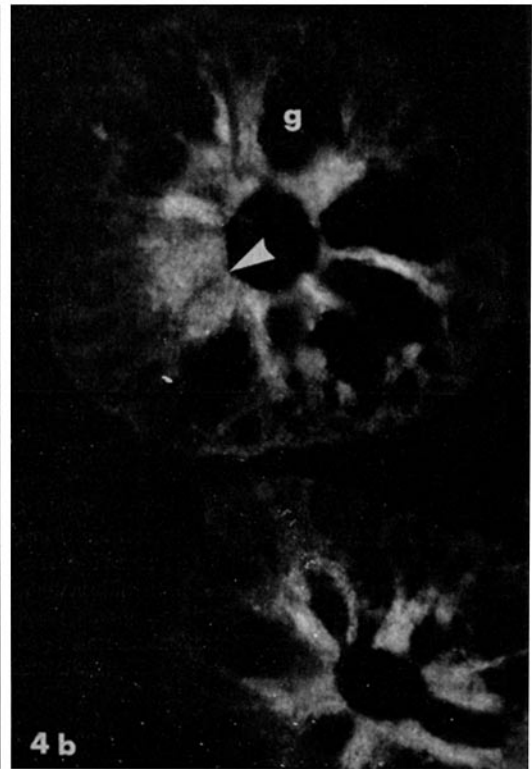
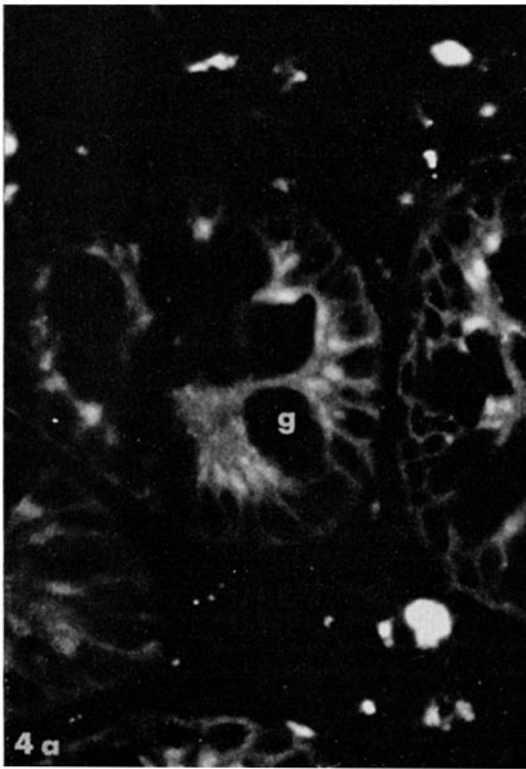
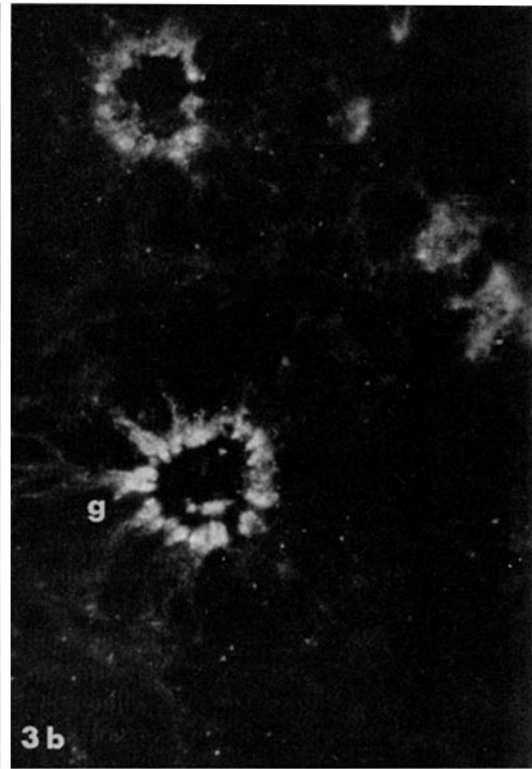
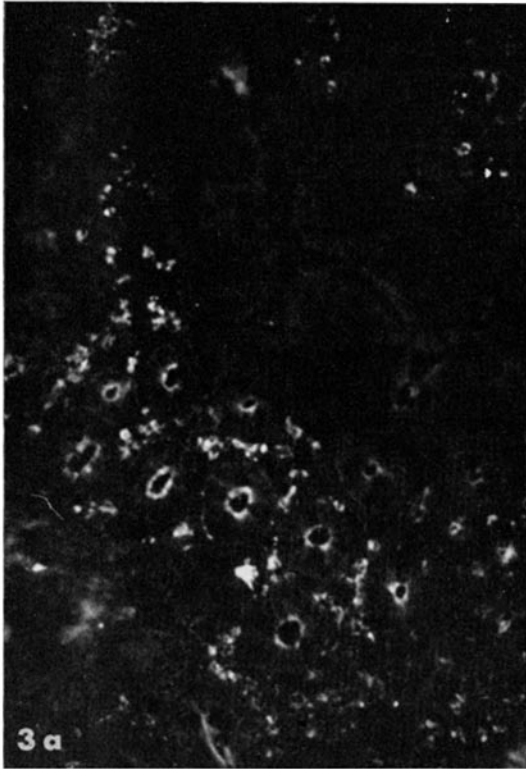
The object of these studies was to investigate the histological and cytological distribution of BSC vs. FSC, which necessitated reagents which were specific for SC, and able to discriminate between the bound and free forms. The antisera to be used to prepare the fluorescent reagents were initially absorbed with solid immunoadsorbents, which rendered them specific by the criterion of Ouchterlony analysis but not completely specific by the more sensitive criterion of immunofluorescence. A possible explanation for this result is that the antigen is joined to the adsorbent by a diazonium bond, which utilizes principally tyrosine residues in the antigen, thus blocking certain antigenic sites and making it impossible to achieve complete absorption with a solid adsorbent alone. Whatever the reason, the subsequent addition of a small amount of pure soluble antigen afforded a more complete absorption such that anti-FSC and anti-BSC stained different regions of the cytoplasm of the columnar cells of the large and small bowel. Even without absorption, the anti-FSC and anti-BSC reagents did not stain mucosal goblet cells in the intestines. The lack of mucous cell staining was not limited to the intestines since the bronchial glands and salivary glands, which consistently showed SC in the cytoplasm of the epithelial cells lining serous acini, did not show staining in mucous acini.

The prominent Golgi zone staining for FSC that we observed in mucosal columnar epithelial cells of large and small bowel has not been reported previously. The reason for this is not clear. One possibility is related to the method of fixation. For example, we found that with formaldehyde or absolute ethanol Golgi staining was not apparent. It is also likely that our antisera are somewhat different from those used by others. Most preceding immunofluorescence studies, in which SC was observed predominantly in the apical portions of epithelial cells, have utilized antisera to sIgA rather than to FSC. These antisera have been absorbed to react only with SC, both bound and free, but probably more with bound. In contrast, our antisera vs. sIgA were obtained from rabbits which also received antiserum to serum-type IgA (31); after absorption with serum-type IgA and IgG they stained the Golgi zone as well as the apical cytoplasm of columnar cells. In addition to antiserum raised against

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FIG. 1. Human small intestinal mucosa treated with fluorescent anti-FSC. (*a*) At low magnification ( $\times 160$ ) FSC can be seen in the cytoplasm of columnar epithelial cells of crypts (below) and villi (above). Staining is most intense in the supranuclear Golgi zone (see also 1 *b* and 1 *c*). (*b* and *c*) Higher magnification ( $\times 760$ ) of epithelium in crypts and villi, respectively, showing that FSC is most concentrated in the supranuclear Golgi zone. Goblet cells (*g*) and intercellular spaces (arrowheads), where identifiable, do not contain FSC. The letter *L* in 1 *c* denotes the intestinal lumen between adjacent villi. Staining in the lamina propria (blue in the original) is nonspecific.

FIG. 2. Human small intestinal mucosa treated with fluorescent anti-BSC. BSC is present in the apical cytoplasm of columnar epithelial cells lining the crypts, and is absent from the Golgi zone and goblet cells (*g*).  $\times 760$ .





sIgA, we also employed antiserum raised against purified FSC, and this latter yielded the most prominent Golgi staining.

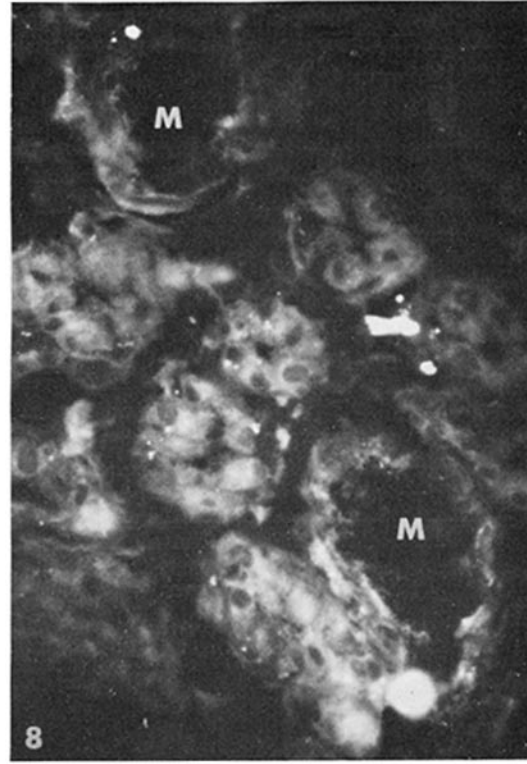
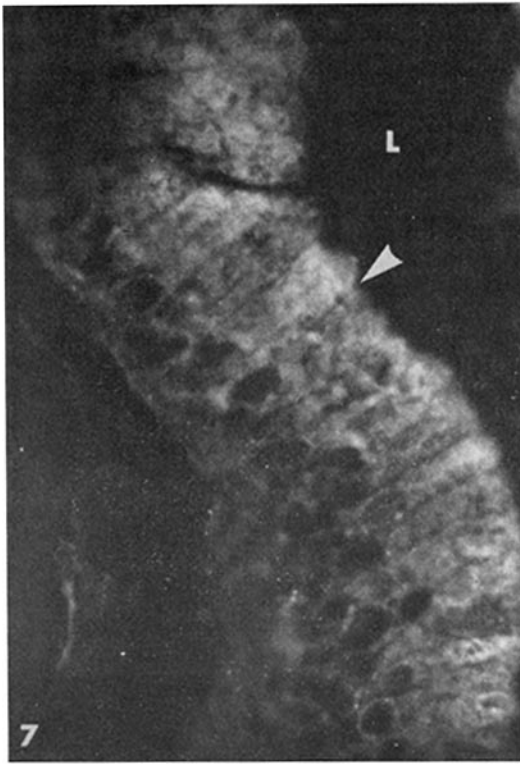
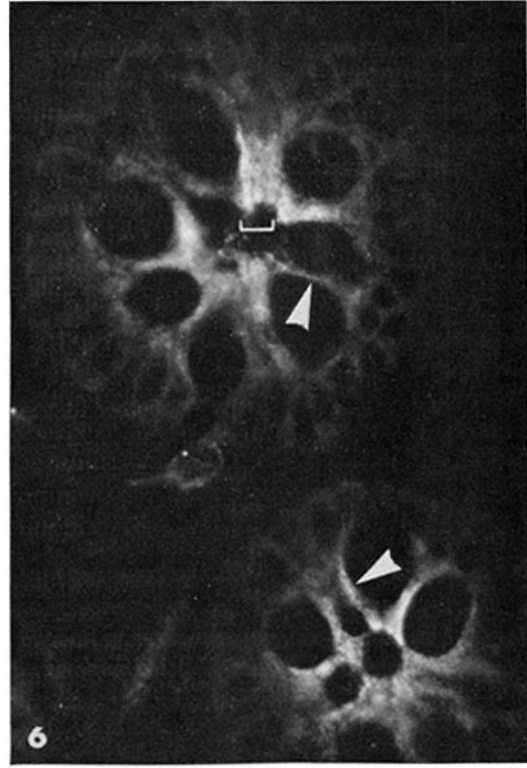
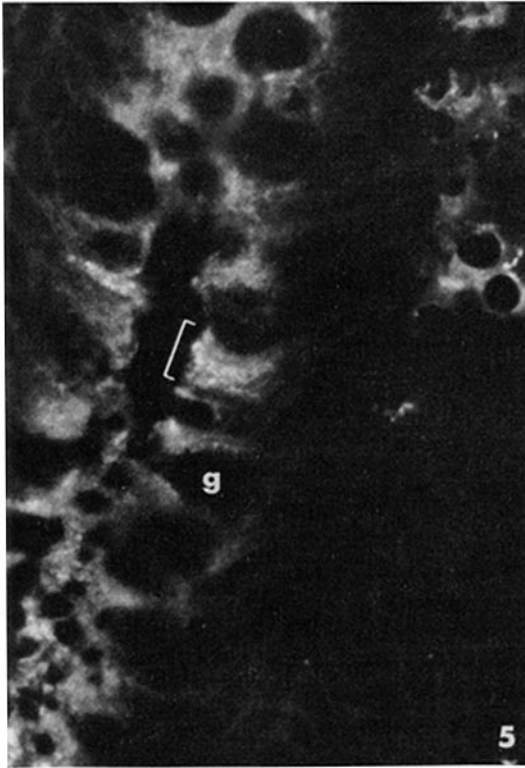
The Golgi apparatus is known to participate in the synthesis and packaging for secretion of complex mucoproteins after protein synthesis has taken place in the adjacent rough surfaced endoplasmic reticulum (38, 39). Thus, in the small and large bowel the presence of FSC principally in the Golgi zone offers additional evidence beyond the mere presence of SC in epithelial cells that SC is in fact synthesized *de novo* in columnar epithelial cells.

The IgA portion of sIgA is synthesized by local plasma cells. Although the formation of sIgA therefore requires the synthesis of appropriate subunits by two distinct types of cell, it is probable that the production of SC by epithelial cells and the local production of IgA by plasma cells are not directly interdependent. For example, FSC can be produced by epithelial cells without associated IgA production in plasma cells as seen in the mucosa of the gallbladder and also in nonlactating young adult female breast (M. E. Poger, unpublished data). Furthermore, agammaglobulinemic persons and newborn infants synthesize and secrete FSC (21).

The identification of BSC in the apical cytoplasm of columnar crypt cells of colon and small bowel is consistent with the staining of the same regions with anti- $\alpha$ -chain reagents. Other studies have shown a similar intracellular staining pattern for  $\alpha$ -chains (12-14, 18, 40). Thus, BSC and  $\alpha$ -chain (i.e., sIgA) are present in the apical cytoplasm of the same cell which synthesizes SC. These observations favor a model in which the assembly of sIgA occurs intracellularly after FSC is concentrated in the Golgi area. The granular distribution of BSC which increases in prominence in the apical cytoplasm toward the luminal border (especially in the colon) is consistent with the current model for the elaboration of numerous external secretions in which the secretion product, after leaving the Golgi zone, is increasingly concentrated in granules which move to-

FIG. 3. Human small intestinal mucosa treated with fluorescent anti- $\alpha$ -chain. (a) Low magnification ( $\times 160$ ) showing villi (upper right) and crypts (lower left).  $\alpha$ -chains are seen at the luminal borders of epithelial cells lining the crypts; they are absent from the villous epithelium.  $\alpha$ -chains also are present in numerous plasma cells within the lamina propria of both villi and crypts. The staining pattern with anti-BSC is identical except that there is no fluorescence due to plasma cells in the lamina propria. (b) Higher magnification ( $\times 760$ ) of crypts.  $\alpha$ -chains are prominent in the apical cytoplasm of columnar epithelial cells (compare with the location of BSC in Fig. 2). Goblet cells (g) do not contain  $\alpha$ -chains. The fluorescence in the lamina propria is due to plasma cells.

FIG. 4. Crypts in human colonic mucosa. (a) Treated with fluorescent anti-FSC. FSC is located in the cytoplasm of columnar epithelial cells and is absent from goblet cells (g). As in the small intestine, FSC is most concentrated in the supranuclear Golgi zone. The fluorescence in the lamina propria is nonspecific, ( $\times 760$ ). (b) Treated with fluorescent anti-BSC. BSC is most prominent in the apical half of the cytoplasm of columnar epithelial cells where it is distributed in a granular fashion; there is no accentuation of the Golgi zone. BSC is absent from goblet cells (g). The intercellular spaces (arrowhead) do not fluoresce.  $\times 860$ .



ward the luminal surface where they are discharged (41, 42). The point of entry of  $\alpha$ -chains into the epithelial cell and the exact intracellular location where assembly of sIgA occurs have still to be determined. With immunofluorescence we are probably only identifying zones where FSC, BSC, and  $\alpha$ -chains are concentrated. The most direct interpretation of our data is that assembly occurs in the supranuclear Golgi zone or in the adjacent apical cytoplasm.

#### SUMMARY

Antibody reagents were made specific for each of the two forms of human SC, FSC and BSC, which is an integral part of the sIgA molecule. With the fluorescent antibody method the cytological and histological localization of FSC, BSC, and  $\alpha$ -chains has been studied in various human mucous membranes. SC was present in columnar epithelial cells of the intestines and in the cells of serous acini of bronchial and salivary glands. In contrast, SC was not found in intestinal goblet cells or cells of mucous acini of bronchial and salivary glands. In the columnar epithelial cells of the small and large bowel, FSC was present most prominently in the Golgi zone, and much less prominently in the apical cytoplasm. On the other hand, BSC and  $\alpha$ -chains were located only in the apical cytoplasm in an overlapping manner. The results favor a model in which sIgA is assembled inside epithelial cells from SC, which was synthesized in the same cell, and IgA, which entered the epithelial cell after synthesis in and secretion by a plasma cell.

We wish to thank Dr. Gloria Gallo for her interest and for the use of her fluorescence microscope.

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FIG. 5. Crypt in human colonic mucosa treated with fluorescent anti- $\alpha$ -chain.  $\alpha$ -chains fluoresce in a granular fashion in the apical cytoplasm, similar to the distribution of BSC in Fig. 4 b. The bracket encloses several columnar cells. Goblet cells (g) are negative. Numerous plasma cells in the lamina propria stain for  $\alpha$ -chains.  $\times 760$ .

FIG. 6. Crypts in colonic mucosa treated with fluorescent anti-BSC. Columnar epithelial cells in groups can be identified between goblet cells (e.g., the three bracketed cells). Single columnar cells between goblet cells (arrowheads), on the other hand, can be confused with intercellular spaces.  $\times 860$ .

FIG. 7. Human gallbladder mucosa treated with fluorescent anti-FSC. FSC is located in the cytoplasm of the surface columnar epithelial cells; intercellular spaces (arrowhead) are negative. L denotes lumen.  $\times 760$ .

FIG. 8. Human bronchial glands treated with fluorescent anti-SC. Serous acini contain SC in the cytoplasm of the epithelial cells. SC is not present in mucous acini (M).  $\times 400$ .

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