## Immunoglobulin A: Localization in Rectal Mucosal Epithelial Cells

Abstract. *Immunofluorescence studies with monospecific antiserums to human serum immunoglobulins consistently revealed immunoglobulin A within the apical portion of the cytoplasm of rectal mucosal epithelial cells in normal subjects, as well as in patients with various bowel diseases. Immunoglobulins M, G, and D were not demonstrated within mucosal epithelial cells. The predominance of lymphoid cells containing immunoglobulin A in the lamina propria of intestinal tissues was confirmed.*

Although immunoglobulin A (IgA) is a minor component of the serum immunoglobulins, it is the major immunoglobulin contained in lymphoid cells of the lamina propria of the gastrointestinal tract (*1*) and in certain external secretions in man (*2*) and rabbits (*3*). In the parotid gland, nasal and bronchial mucosa, and nasopharyngeal tonsils (*4*), lymphoid cells containing IgA also have been shown to predominate over cells containing immunoglobulin M (IgM) or immunoglobulin G (IgG). Tomasi and co-workers described an additional antigenic determinant characteristic of the IgA of external secretions, demonstrated this "secretory piece" in the cytoplasm of parotid acinar cells, and speculated regarding the possible biologic significance of this unique antibody system (*2*). The piece becomes bound to IgA possibly to stabilize and protect this locally produced antibody (*5*). We regularly have found IgA within human intestinal epithelial cells, which may represent a transepithelial cell pathway for IgA between the lamina propria and the luminal surface.

We used a direct immunofluorescent technique with goat antiserums (labeled with fluorescein isothiocyanate) to human serum IgA, IgG, and IgM, and sheep antiserum to rabbit $\gamma$-globulin (*6*). Indirect fluorescent antibody studies were done with rabbit antiserum to human immunoglobulin D (IgD) (*7*). Each antiserum was monospecific as tested by immunoelectrophoresis and double diffusion in agar gel. Protein concentrations and ratios of fluorescein to protein were determined for each conjugated antiserum (*8*); dilutions were used to achieve a final protein concentration of approximately 2.0 mg/ml (*9*); and two absorptions with lyophilized mouse-liver powder resulted in the virtual absence of nonimmunospecific staining.

Control staining experiments to establish specificity included: incubation with unlabeled goat antiserum to the corresponding human immunoglobulin prior to the application of the appropriate fluorescent antibody; prior incubation with other unlabeled antiserums; staining with labeled antiserum to heterologous $\gamma$-globulin; and preliminary absorption of the conjugates with (i) hypergammaglobulinemic human serum or (ii) mixed A, B, and O human erythrocytes washed ten times.

Fluorescent antibody studies of 30 subjects revealed immunospecific staining for IgA within the apical portion of the cytoplasm of mucosal epithelial cells of normal and abnormal human rectal biopsies. Similar findings were noted in a smaller number of colonic, small intestinal, and appendiceal tissues. The IgA was not seen near the nuclei at the basal aspect of the cells or in the mucus-containing portion of the goblet cells (Figs. 1 and 2). The cellular fluorescence was distributed along the entire epithelial layer lining the glandular duct (Fig. 2), occasionally within the ducts, and in the particulate matter of surface mucus. This intraepithelial IgA was observed in all patients regardless of serum IgA concentration, except in an individual without detectable serum IgA where no IgA was demonstrable within the rectal or small intestinal mucosa. Epithelial cells did not contain detectable IgM, IgG, or IgD. In one case, parotid and rectal tissues were obtained at autopsy 2 hours after death. Apical cytoplasmic IgA occurred within colonic mucosal epithelial cells, and immunospecific IgA also was demonstrated in stromal lymphoid cells of the parotid gland as well as in the cytoplasm of occasional acinar cells. Our studies also confirmed the predominance, in the lamina propria of intestinal tissues (*10*), of lymphoid cells containing IgA.

Observations by others have suggested the presence of IgA within epithelial cells. Crandall *et al.* reported that antiserum to $\alpha$-chain reagent stained the proximal portion of rabbit intestinal glandular crypt cells slightly (*3*). Rossen *et al.* reported both 7*S* and 11*S* IgA in serous acinar cells of

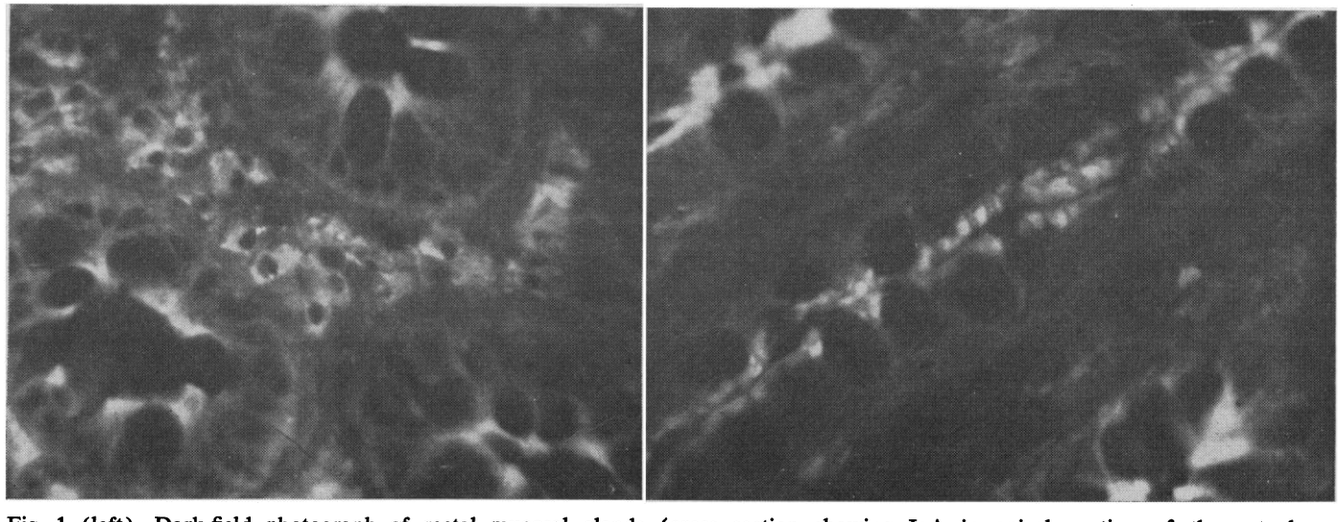

Fig. 1 (left). Dark-field photograph of rectal mucosal glands (cross section showing IgA in apical portion of the cytoplasm of epithelial cells. Lymphoid cells containing IgA are in close relation to the glands (original magnification, × 400). Fig. 2 (right). Longitudinal section of a rectal gland (center) showing apical cytoplasmic IgA lining the ductular lumen. There is an absence of staining of the mucus portion of goblet cells, nuclei, and basal cytoplasm (original magnification, × 400).

human nasal and bronchial submucosa and submaxillary salivary gland (*11*). Crabbé *et al.* reported that fluorescein-labeled antiserums against human IgA and IgG produced staining of the mucus of intestinal goblet cells (*1*). They considered these findings nonspecific because they could be extinguished after absorption of the conjugated antiserum with washed, mixed A, B, and O erythrocytes. We have not observed staining of the mucus of intestinal goblet cells. Our control staining experiments established the immunospecificity of the intraepithelial IgA. Further, the findings on IgA were identical when goat antiserum to human IgA from a different source was used (*12*).

Recently, IgA was demonstrated within human nasopharyngeal tonsillar epithelium. This immunofluorescence was attributed to the presence of lymphoid cells (containing IgA) within the epithelial cells (*4*). We occasionally observed IgA cells in the overlying surface mucus, but not within intestinal mucosal epithelial cells. There is good evidence for the presence of lymphocytes within intestinal epithelial cells, although the exact source, nature, function, and fate of these lymphocytes remain uncertain (*13*).

While we have used an antiserum to 7*S* IgA, positive staining in the surface mucus within the gastrointestinal lumen suggests that this antiserum also is reactive against secretory IgA. Supplementary investigations of the intraepithelial IgA are in progress to define further the immunochemical form of this immunoglobulin. It is unknown whether IgA enters the epithelial cells (i) derived from local lymphoid cells, (ii) by intraepithelial synthesis, or (iii) by absorption from the overlying surface mucus. It is probable that IgA enters epithelial cells from the adjacent lymphoid cells (containing IgA) of the lamina propria (*5*), but the mechanism by which this takes place remains unclear.

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7. Kindly provided by Dr. John L. Fahey, National Cancer Institute, Bethesda, Maryland.
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## Ornithine-Urea Cycle Activity in Xenopus laevis: Adaptation in Saline

Abstract. *The concentration of urea in the blood and the rate of urea excretion were markedly elevated in* Xenopus *maintained in hypertonic saline for 2 to 3 weeks. These changes were accompanied by a twofold increase in the activity of the ornithine-urea cycle as measured in liver slices. The activity of carbamoyl phosphate synthetase rose threefold in frogs adapted to saline. These results suggest that changes in activities of urea cycle enzymes may be important in the adaptation of aquatic organisms to environments of varying salinities.*

The South African frog, *Xenopus laevis*, is normally aquatic and, like many water-dwelling animals, excretes the major fraction of its waste nitrogen as ammonia (*1*). *Xenopus*, however, can live for extended periods out of water or in hypertonic (0.9 percent) saline (*2*). In such situations, where the availability of water is restricted, the animals accumulate urea, but not ammonia, in their tissues (*2, 3*), thus simultaneously avoiding toxic amounts of ammonia and improving their osmotic status with respect to the environment. Accumulation of urea in response to water deprivation has been recorded in a number of other amphibia (*4, 5*). For example, the concentration of urea in the plasma of *Rana cancrivora* increased nearly tenfold when these frogs were transferred from freshwater to 80 percent seawater (800 mosmole/liter, *4*). An elevated concentration of urea in the tissues appears to serve the same purpose in these animals as it does in elasmobranchs (*6*), that is, to decrease water loss by reducing the osmotic gradient between the animal and its environment.

The manner in which the urea concentration of the tissues is elevated in amphibia placed in hypertonic media is not fully understood. One contributing factor is a lowered rate of urine flow such as that observed in *R. cancrivora* (*7*) and *X. laevis* (*8*) when the ambient osmolarity is increased. Janssens (*3*), however, calculated that the rate of urea production increased in *Xenopus* after its transfer to saline. The nature of this adaptation in urea synthesis is unknown. We have examined the possibility that the activity of the ornithine-urea cycle is elevated in *Xenopus* kept in hypertonic saline.

Male frogs (*Xenopus laevis*) weighing about 35 g (*9*) were maintained in tap water and were not fed for 18 days before use. At the beginning of the experiment, each frog was placed in 0.5 liter of either tap water or a saline (300 mosmole/liter) solution ($Na^+$, 150 meq/liter; $K^+$, 3.4 meq/liter; $Ca^{2+}$, 6.8 meq/liter; $Cl^-$, 160 meq/liter; $HCO_3^-$, 0.6 meq/liter). This saline solution is hypertonic to normal *Xenopus* plasma, which was estimated on the basis of plasma electrolyte content to have an osmolarity of 235 mosmole/liter. Solutions were changed daily. The water temperature was held at 24° to 26°C. On the 14th or 21st day of the experiment, we measured the nitrogen excretion by replacing the solutions with 500 ml of either sodium phosphate buffer (*p*H 6.5, 0.01*M*; freshwater frogs) or similarly buffered saline (saline-adapted frogs) and analyzing samples of the media taken at