Distribution of Blood Group A Antigen in the Epithelial Mucins of the Digestive Tract of Sprague-Dawley Rats[1] (34279)

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Water-soluble blood group A, B, and/or O(H) antigens have been demonstrated in most epithelial mucin-containing cells of human secretors (1–4). Similar antigenic determinants in secretions or tissues from a variety of species have been reported (5). The tissue distribution of these antigenic determinants in species other than man have not been described or conflicting data has not been reported. Hammarström *et al.* (6), employing a hemagglutination inhibition technique found large amounts of A antigen in the colon, small intestine, and stomach of rats. Halpern *et al.* (7) using the fluorescent antibody technique noted A antigen in the colon but not the stomach or salivary glands of rats.

In the following study the fluorescent antibody technique was used to demonstrate A antigenic determinants in tissue taken from several levels of the digestive tract of Sprague-Dawley rats. The distribution of histochemically demonstrable sulfomucins, sialomucins, and neutral epithelial mucins in these tissues was related to the location of A antigen.

Methods and Materials. Immediately after sacrifice blocks of fresh tissue were taken from various portions of the digestive tract of 10 adult Sprague-Dawley rats, four male and six females (Table I). The tissue labeled tongue was from the base of the tongue. The small intestine (pylorus to ileocecal valve) varied from 120 to 130 cm in length. The tissue labeled jejunum and ileum were taken 30 and 120 cm from the pylorus, respectively. The large intestine (ileocecal valve to the anal orifice) measured 25–30 cm. Complete cross sections were taken from the cecum, ascending colon (5 cm from the ileocecal valve), transverse colon (12 cm from the ileocecal valve) and rectum (1 cm above the anal orifice).

Blocks of tissue were fixed in 10% neutral buffered formalin and processed into paraffin. Care was taken to keep the paraffin baths at or below 60°. On one occasion the temperature of the baths went to 75°. The intensity of specific fluorescence in the tissues was greatly reduced. Sections were cut at 6 μ and stained by the hematoxylin and eosin, periodic acid-Schiff's (PAS), diastase periodic acid-Schiff's (D-PAS), alcian blue (AB) at pH 2.5 and AB at pH 0.5 methods (8).

Blood group A antigenic determinants were demonstrated using the indirect fluorescent antibody technique. That is, sections were incubated with human anti-A typing serum (Hyland Laboratories, Los Angeles, California) for 30 min, washed $\times 2$ in phosphate buffered (pH 7.2; 0.01 *M*) saline (PBS) for 15 min, incubated with the fluorescein labeled rabbit antihuman IgG (Antibodies, Inc., Davis, California) for 30 min, washed in PBS for 15 min, and mounted in phosphate buffered glycerin (1:10), pH 7.2. The rabbit antihuman IgG-F was absorbed with 100 mg of bovine liver powder/ml and diluted 1:10 with PBS prior to use.

Controls included substituting human AB serum or human anti-A absorbed with 10 mg of A antigen/ml (Charles Pfizer and Co., New York, New York) for the human anti-A in the above sequence. Also, tissues were incubated with antihuman IgG-F without prior exposure to human anti-A. Sections of duodenum from a known human blood group A secretor and a blood group O secretor were also used (4).

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The immunofluorescence preparations were examined with a small Zeiss fluorescence unit which included a HBO-200 light source, a UG-1 excitor filter and a Wratten 2-A barrier filter. Photographs were made with Kodak 135 high speed Ektachrome film. Black and white prints were made from the color transparencies using a Kodak 58 green filter.

Results. Epithelial mucins as demonstrated by the PAS reaction were found in the acini or goblet cells of all the tissues examined. Diastase digestion which removes glycogen did not reduce the subsequent PAS staining. The intensity of staining was estimated from 0 to 4+ (Table I).

Salivary glands. The mucous acini in the tongue and sublingual glands stained intensely and uniformly with PAS and AB at pH 2.5. There was little variation from animal to animal. The sublingual glands did not bind AB at pH 0.5 and gave a variable reaction for A antigen. A few cells in seven sublingual glands contained A antigen. These usually were isolated cells but occasionally two or three cells in an acinus showed specific fluorescence (Fig. 1D). The number of cells containing A antigen in a given section was less than 1% of the total. In one of these glands only 3 cells reacted and in another only 4 cells. On the other hand, the mucous acini in the tongue stained intensely and uniformly with AB at pH 0.5 and were rich in A antigen.

The submaxillary gland acini and the seromucous acini in the tongue stained less intensely with the PAS. The acinar cells and mucus in the lumen of ducts in both were rich in A antigen (Fig. 1C). There was minimal staining of submaxillary gland acini with AB at pH 2.5 and no staining of the seromucous acini in the tongue (Table I).

The serous acini in parotid glands stained moderately with the PAS but did not bind AB at pH 2.5 or 0.5. The A antigen was demonstrated in nine animals. The percentage of cells reacting varied from 1 to 5% in seven animals. Approximately 50% of the cells in two glands showed specific fluorescence. The intensity of fluorescence was usu-

TABLE I. Immunofluorescence for A Antigen and Histochemical Staining in the Digestive Tract of Sprague-Dawley Rats.

Tissue	Animal no.:[b]	D-PAS[a] 1–10	AB (pH 2.5)[a] 1–10	AB (pH 0.5)[a] 1–10	Immunofluorescence 1	2	3	4	5	6	7	8	9	10
Tongue, seromucous acini		++-+++	0	0	+++	+++	+++	+++	+++	+++	+++	+++	++	++
mucous acini		+++-+++	+++++	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Parotid gland		++-+++	0	0	—[c]	—[c]	—[c]	—[c]	0	—[c]	—[c]	—[c]	—[c]	—[c]
Submaxillary gland		++-+++	±	0	+++++	+++++	+++++	+++++	+++++	+++	+++	+++	+++	+++
Sublingual gland		++-+++	+++++	0	0	—[c]	—[c]	—[c]	0	0	—[c]	—[c]	—[c]	—[c]
Stomach, pylorus		+++	+++	+++-++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Duodenum, goblet cells		+++++	+++++	0-+++ (2)[d]	0	0	0	—[c]	0	0	0	0	—[c]	0
Brunner's glands		+++++	0	0	+++	+++	+++	++	++	++	+++	+++	+++	+++
Jejunum		+++++	+++	0-+++ (2)[d]	0	0	0	—[c]	0	0	0	—[c]	0	0
Ileum		+++++	+++	0-+++ (2)[d]	0	0	0	—[c]	0	0	0	—[c]	—[c]	0
Cecum		+++++	+++	++-+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Colon, ascending		+++++	+++	++-+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
transverse		+++++	+++	++-+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Rectum		+++++	+++	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

[a] Diastase removes glycogen but epithelial mucins remain and colors red with the PAS; AB binds acid groups at pH 2.5 but binds only sulfate groups at pH 0.5.
[b] Animals 5–10 were females.
[c] Many of the mucin-containing cells did not show specific fluorescence; the ones that did were +–+++ (parotid) or ++++–+++ (small intestine and sublingual gland).
[d] Indicates the total number of animals in which no staining was noted.

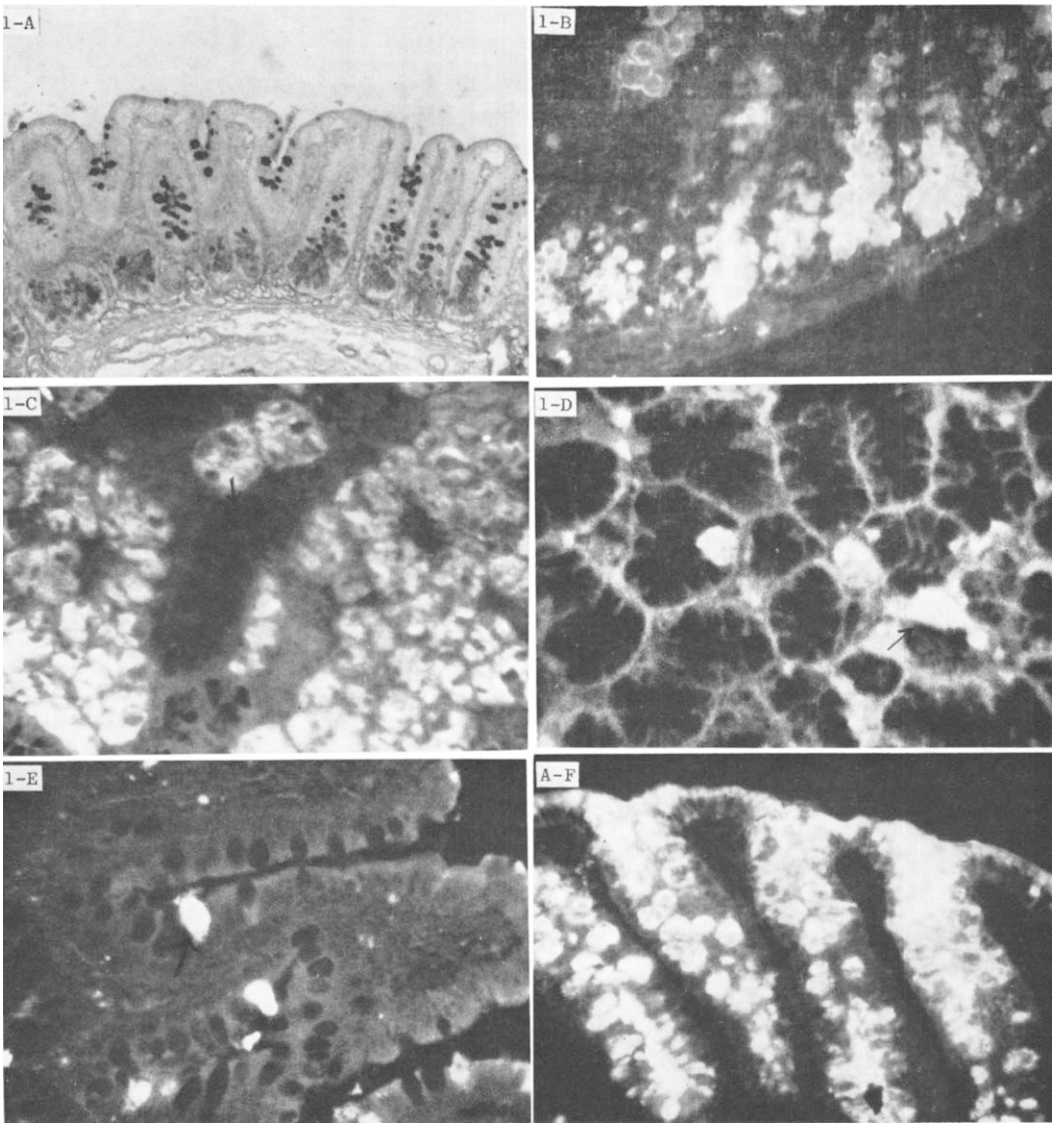

FIG. 1A. PAS stain of cecum; many columnar cells did not stain. The intensity of staining of the deep portion of the crypts is less than the goblet cells near the lumen; ×470. (B–F) Indirect immunofluorescence for A antigen; bright areas were yellow-green; ×1408. (B) Cecum; the deep portion of the crypts (bottom) fluoresced more intensely than the goblet cells near the surface. (C) Submaxillary gland; duct epithelium (arrow) did not fluoresce. (D) Sublingual gland; an occasional cell (arrow) reacted for A antigen. (E) Jejunum; occasional goblet cells showed specific fluorescence (arrow). (F) Rectum; specific fluorescence was seen from the luminal surface (top) to the base of the gland.

ally + to ++ but occasionally was +++ (Table I).

Stomach. In the pylorus, PAS positive material was found throughout the mucosa, although the intensity of staining was greater in the luminal one-third. The cytoplasm of all the glandular cells did not stain. The distribution of AB staining at pH 2.5 and 0.5 was comparable to the PAS. However, the intensity of pH 0.5 staining in the inner two-thirds of the mucosa were slightly less. The A antigen was found throughout the mu-

cosa and the intensity of specific fluorescence correlated well with the intensity of PAS staining.

Small intestine. The goblet cells throughout the small intestine stained intensely with the PAS and AB at pH 2.5. The AB reaction at pH 0.5 was variable. In two animals no staining was noted. In the other eight animals a mottled pattern was seen. Most goblet cells did not react; others stained well and there were gradations in between. A similar mottled pattern was seen in the immunofluorescence preparations for A antigen. The A antigen was not demonstrated in the mucosa of seven animals. Approximately half the goblet cells in the ileum of one animal had A antigen (Table I). In two animals some of the goblet cells at all levels contained A antigen (Fig. 1E). There was no correlation between the AB pH 0.5 staining and the presence of the A antigen.

Brunner's glands stained intensely with the PAS method. No reaction with AB at pH 2.5 or 0.5 was observed. The A antigen was demonstrated in the Brunner's glands of all animals.

Large intestine. The cytoplasm of many of the columnar cells particularly on the luminal surface did not stain with the PAS or AB, and did not contain A antigen. Goblet cells throughout the large intestine stained well with the PAS. The deep portion of crypts in the cecum and ascending colon did not stain as intensely as the goblet cells in the luminal portion of the mucosa (Fig. 1A). The AB staining at pH 2.5 was intense throughout. The AB staining at pH 0.5 in the proximal large intestine was less than in the distal segment largely due to the failure of the deep portion of the crypts in the cecum and the ascending colon to stain. Large intestinal goblet cells were rich in A antigen. The deep portion of the crypts in the cecum and ascending colon fluoresced more intensely than the goblet cells in the luminal one-third of the mucosa (Fig. 1B). In the distal segment the intensity of specific fluorescence was the same throughout the mucosa (Fig. 1F).

Immunofluorescence controls. When human AB serum or anti-A absorbed with A antigen were substituted for anti-A in the indirect fluorescent antibody sequence no specific fluorescence was observed. Tissues exposed to rabbit antihuman IgG-F without prior incubation with human anti-A failed to show specific fluorescence.

Discussion. The presence of A antigen in the salivary gland duct secretions and the lumen of the intestine suggest that Sprague-Dawley rats are secretors of A antigen. The tissue distribution of A antigen in the digestive tract of the rats described here is similar to that reported in human blood group A secretors (1–4). The mosaic pattern of A antigen in parotid glands and goblet cells of the small intestine and the abundance of A antigen in the terminal large intestine of the rat are notable exceptions.

The abundance of A antigen throughout the mucosa of the rat large intestine is of particular interest. This is similar to the pattern described in the human fetus (3). However, by adult life the terminal large intestine is said to be devoid of blood group antigens although it continues to secrete an abundance of epithelial mucin (2–4, 9, 10). This loss of blood group antigenic determinants has been attributed to hydrolysis by bacterial enzymes known to be present in the large intestine of man (9, 12). Hoskins and Zamcheck recently showed that similar bacterial enzymes are present in the large intestine of conventional rats (11, 12). Further, the amount of blood group substance in the lumen of the rat large intestine was less in conventional than germ free rats. Despite the presence of bacterial enzymes, the mucosal glands throughout the rat large intestine are rich in A antigen as demonstrated in our study and by others (6, 7). Hence, the paucity of blood group antigens in the mucosal glands of the adult human large intestine must be on some other basis.

Mucins in the lumen of the small intestine at all levels contained A antigenic determinants. Most of this probably came from glands higher in the digestive tract as small intestinal goblet cells were usually devoid of A antigen. In the three animals where A antigen was demonstrated in goblet cells a mosaic pattern similar to that previously described in Brunner's glands and salivary

glands of blood type A individuals was noted (4). That is, some goblet cells contained A antigen while adjacent histochemically identical goblet cells did not react. The rat sublingual glands and parotid glands showed a similar mosaic pattern. Presumably all of the mucin-secreting cells have the same genes. This suggests that the gene-controlled enzymes necessary for the addition of A antigenic determinants are not functioning in some cells. Whether this is a temporary or permanent characteristic of these cells is not known.

Cells that contained a great deal of mucin, *i.e.*, goblet cells and the acinar cells in the sublingual glands can be recongized readily in the immunofluorescence preparations. Therefore when some of these cells showed specific fluorescence and others did not, the mosaic pattern was evident. Other cell types, *i.e.*, columnar cells in the intestine, salivary gland duct epithelium, and some cells in gastric glands contained small quantities of mucin or no mucin. In the immunofluorescence preparations we could not distinguish the mucin-containing cells from the nonmucin-containing cells. Therefore, when these cells did not show specific fluorescence we could not be sure whether this was due to mucin not being present or to the presence of mucin which did not have A antigenic determinants. For this reason, our failure to identify a mosaic pattern in other tissues does not necessarily mean that some degree of mosaicism was not present.

Some correlation between the presence of A antigen and the histochemical staining characteristics of the epithelial mucin was evident. All cells and secretions containing water-soluble A antigen reacted with the PAS stain. This would be expected as A antigen contains carbohydrate which the PAS stains. On the other hand, all mucins giving a strong PAS reaction did not contain A antigen. Presumably these mucins have other antigenic determinants. Most epithelial mucins which contain A antigen have acid groups. Either sulfate groups, sulfomucins and/or carboxyl groups, sialomucins, are present. However, these acid groups are not a part of the A antigenic determinants. The absence of acid groups from chemically purified A antigen and the brilliant specific fluorescence for A antigen shown here in the seromucous glands of the tongue and Brunner's glands of the duodenum, both of which fail to stain with AB, support this concept (5, 13). On the other hand, the presence of abundant carboxyl or sulfate groups in the mucin did not appear to interfere with the reactivity of A antigenic determinants with specific antibody, *i.e.*, the terminal rat colon.

As the specific area of the stomach and specific salivary gland studied by Halpern *et al.* (7) was not described, their failure to find A antigen in these tissues may be due to tissue selection. The rat forestomach is lined by squamous epithlium and does not secrete mucin. Unlike the submaxillary and the lingual glands which were rich in A antigen, we found some parotid and sublingual glands to be devoid of water soluble A antigen. Others contained so few reacting cells that they could be overlooked.

Summary. Using the indirect fluorescent antibody technique water-soluble blood group A antigen was demonstrated throughout the digestive tract of Sprague-Dawley rats. Water-soluble A antigen was found only in mucin-containing cells or secretions as identified with the periodic acid-Schiff's stain. However, many mucin containing cells were devoid of A antigen. A mosaic pattern of A antigen distribution was noted in the sublingual glands, parotid glands and goblet cells of the small intestine similar to that previously described in some human tissue. With the exception of the abundance of A antigen throughout the rat large intestine, the mosaic pattern in the parotid glands and small intestine, the distribution of this antigen was comparable to that previously described in human blood group A secretors.

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