DIFFERENTIAL LOCALIZATION OF CELL SURFACE AND SECRETORY COMPONENTS IN RAT INTESTINAL EPITHELIUM BY USE OF LECTINS

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ABSTRACT

Sections through various levels of small intestine from adult male rats were examined by fluorescence microscopy after treatment with fluorescein isothiocyanate-labeled lectins from *Dolichos biflorus*, *Lotus tetragonolobus*, *Ricinus communis*, and *Triticum vulgare* (wheat germ). The latter three lectins reacted with the microvillar portion of the epithelial cells lining the crypts and villi in sections of intestine adjacent to the pylorus. This pattern of reactivity was sharply altered along the first 15 cm of intestine so that in sections distal to this point the luminal surfaces of only those epithelial cells in the crypts and at the base of the villi reacted with the *L. tetragonolobus* and *R. communis* lectins, whereas the wheat germ lectin reacted with the surfaces of the cells lining the villi. In sections from the distal end of the small intestine, all three lectins reacted with the surfaces of cells only at the base of the villi and in the crypts.

These results show a difference in surface components in cells at various portions on the villi and the dependence of these differences on the region of intestine. The *D. biflorus* lectin reacted with approximately 25% of the goblet cells at each level of intestine studied whereas the reactivities of the goblet cells with the other three lectins were dependent upon the region of intestine.

INTRODUCTION

The differentiation of a cell is dependent to a large extent upon its environment and, therefore, upon its cell surface through which the effects of the environment must be mediated. A deeper insight into the control of cell differentiation may thus be provided by a study of the development of cell surface components. The intestinal epithelium is an ideal system for studying such differentiative events because of the spatial segregation of the epithelial cells according to their degree of differentiation.

The villi of the intestine are covered by a single layer of epithelial cells which undergo mitosis in the crypts at the base of the villi and then move up the villi until they are extruded from the tips of the villi (17, 22). This progression of cells from the crypts to the villi tips occurs in adult rats within 48 h (3, 11, 17) and is accompanied by a number of differentiative events which include a change in morphology from cuboidal to columnar shape, the acquisition and changes in length of microvilli (22, 23, 29), and the appearance of or changes in

various enzymes of the microvilli and other cellular enzymes (4, 11, 12, 36). Some of the epithelial cells in the crypts differentiate into the secretory goblet cells which are interspersed among the columnar epithelial cells (22, 23).

The microvillar membranes are associated with a layer of carbohydrate-rich material which appears to be synthesized by the columnar cells and incorporated as an integral part of the membrane structure (13). In the rat, changes in this cell surface material with differentiation have been indicated by differences in sugar incorporation and transferase activities of cells from various regions of the villi (38, 39) as well as by a difference in susceptibility among isolated cells to react with concanavalin A (30, 37), a plant lectin with specificity for α-D-mannosyl, α-D-glucosyl, and α-*N*-acetyl-D-glucosaminyl residues (9).

This paper describes the differential localization of some cell surface and secretory components in the rat intestinal epithelium by fluorescence microscopy using fluorescein isothiocyanate derivatives of lectins from *Dolichos biflorus*, *Ricinus communis*, *Lotus tetragonolobus*, and *Triticum vulgare* (wheat germ). These lectins have specificities for terminal nonreducing α-*N*-acetyl-D-galactosaminyl (7), β-D-galactosyl (27, 28), α-L-fucosyl (25, 33), and β-*N*-acetyl-D-glucosaminyl (2) residues, respectively. Recently, wheat germ lectin has also been reported to bind to *N*-acetyl-neuraminic acid (10). The differential association of some of these lectin receptors with cells at different portions of the villi has been found to be dependent on the region of intestine in which the villi are located.

MATERIALS AND METHODS

Sources

N-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, and β-methyl-D-galactoside were obtained from Pfanstiehl Chemical Corp., Waukegan, Ill. L-Fucose was purchased from Sigma Chemical Co., St. Louis, Mo. *Dolichos biflorus* seeds were obtained from S. B. Penick and Co., New York, and *Lotus tetragonolobus* seeds were from Thompson and Morgan, Ltd., Ipswich, England. *Ricinus communis* seeds were a gift from Dr. Garth Nicolson, The Salk Institute, La Jolla, California, and wheat germ was kindly supplied by Dr. Paul K. Stumpf, University of California, Davis, Calif.

Isolation of Lectins

The *D. biflorus* lectin was isolated as previously described (5, 7) by absorption on to a column of hog A + H blood group substance insolubilized by copolymerization with the *N*-carboxyanhydride of L-leucine (16). The lectin was specifically eluted from the column by 0.01 M *N*-acetyl-D-galactosamine, and the hapten was removed from the lectin by chromatography on Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.).

L. tetragonolobus lectin was prepared according to the method of Yariv et al. (40), by application of a 10% wt/vol seed extract in 0.01 M phosphate-buffered saline (PBS) pH 7.2, to a column of agarose-epsilon-aminocaproyl-fucosamine (Miles-Yeda, Ltd., Miles Laboratories, Inc., Kankakee, Ill.). The lectin was specifically eluted from the column by 0.04 M L-fucose.

R. communis seeds contain two lectins (RCA_I and RCA_{II}) that have a specificity for terminal, nonreducing, galactose-like residues, but differ in their molecular weights and abilities to be inhibited by different sugars (27, 28, 34). The 120,000-mol wt lectin (RCA_I) is more specific for β-D-galactosyl residues and was the lectin employed in this study. The supernate from a 10% wt/vol extract of *R. communis* seeds in 0.01 M PBS, pH 7.2, was applied to a Sepharose-6-B column. After extensive washing of the column with PBS, the RCA_{II} lectin was eluted with 0.01 M *N*-acetyl-D-galactosamine. The column was then specifically eluted with 0.01 M D-galactose to obtain the RCA_I lectin (6). The hapten was removed from the lectin by chromatography on BioGel P-10.

The wheat germ lectin was isolated by affinity chromatography on ovomucoid-Sepharose (18, 20). The lectin was eluted from the column with 0.1 N acetic acid and then dialyzed against 0.01 M PBS, pH 7.2.

Each of the lectins was coupled with fluorescein isothiocyanate (isomer I, Sigma Chemical Co.) in 0.05 M carbonate buffer, pH 8.5 (31). Approximately 1-3 mg of fluorescein isothiocyanate was added per mg of lectin. In the case of *L. tetragonolobus*, 0.04 M L-fucose was present in the reaction mixture. After mixing for 10 min at room temperature, the conjugated lectin was separated from free dye by chromatography on BioGel P-10. The fluorescein isothiocyanate (FITC) content of the FITC-conjugated lectin preparations was determined from the absorbance at 492 nm and the protein concentration was estimated by absorbance at 280 nm and from nitrogen content determined by the ninhydrin procedure (5). The average number of moles of FITC bound per mole of lectin (Table I) was calculated using molecular weights of 110,000 for *D. biflorus* lectin,[1] 120,000 for RCA_I (27, 28), 120,000 for *L. tetragonolobus* lectin (40), and 23,000 for wheat germ agglutinin (18).

The FITC-conjugated lectins were tested for activity by hemagglutination with a Takatsy microtitrator using 0.025-ml loops and a 2% suspension of erythrocytes.

Fluorescence Microscopy

Adult male Sprague-Dawley rats were used in this investigation. The animals were sacrificed by decapita-

[1] Carter, W. G., and M. E. Etzler, unpublished observations.

TABLE I

Hemagglutination Titers of FITC-Conjugated Lectins in the Presence and Absence of Specific Inhibitors

FITC-conjugated lectin	Average no. moles FITC/mole lectin*	Inhibitor	Type erythrocyte	Titer‡
Dolichos biflorus	0.45	—	A_1	16
		0.15 M *N*-acetyl-D-galactosamine	A_1	0
Ricinus communis	0.88	—	0	128
		0.15 M β-methyl-D-galactoside	0	0
Lotus tetragonolobus	0.44	—	0	16
		0.15 M L-fucose	0	0
Wheat germ	0.39	—	A_1	8
		0.2 M *N*-acetyl-D-glucosamine	A_1	0

* Dilutions of each FITC-lectin solution with unlabeled lectin changed only the intensity of fluoresence and not its pattern of localization.

‡ Titer represents final dilution of lectin capable of agglutinating a 2% erythrocyte suspension. The fluorescence pattern for each lectin was not changed by use of solutions with higher titers.

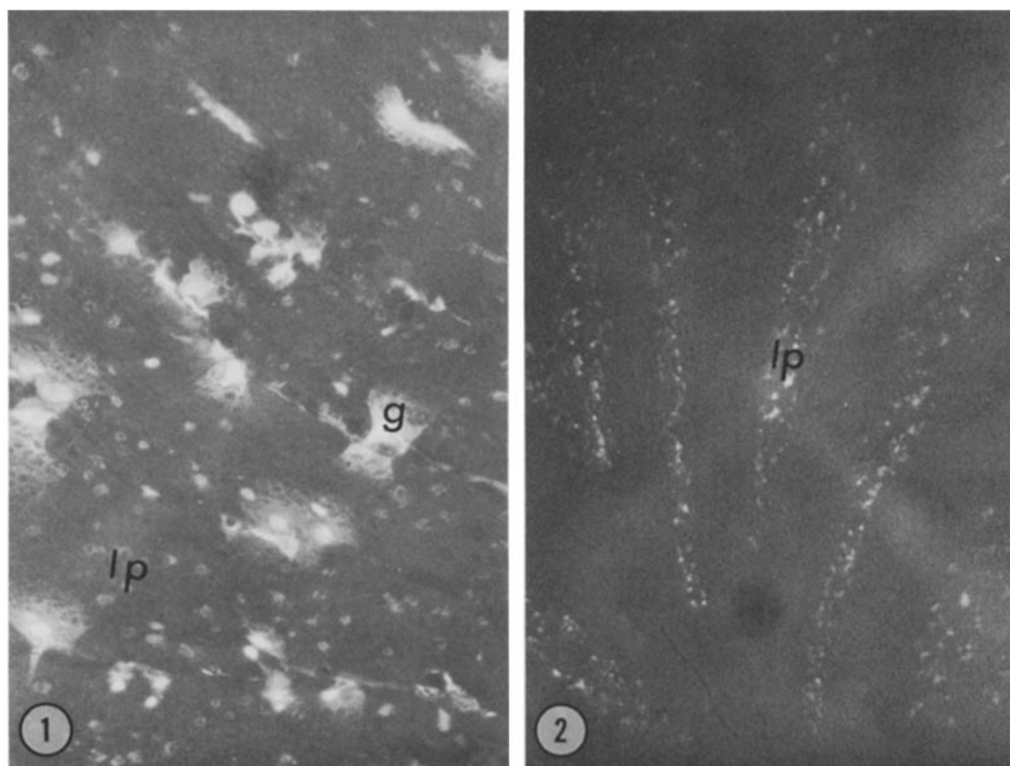


FIGURE 1 Section of intestine 20 cm from pylorus treated with FITC-*Dolichos biflorus* lectin. Bright fluorescence is in some goblet cells (*g*) and their exuded secretory material along sides of villi which in this view extend downward diagonally from left to right. Weak fluorescence in cells in lamina propria (*lp*) is nonspecific and is also seen in controls (Fig. 2). × 180.

FIGURE 2 Control section of intestine 20 cm from pylorus treated with FITC-*Dolichos biflorus* lectin in presence of 0.15 M *N*-acetyl-D-galactosamine. Nonspecific fluorescence is in cells in lamina propria (*lp*). Villi extend downward. No fluorescence is in goblet cells. × 180.

tion, and segments of the intestine were removed next to the pylorus, and at 2-cm, 5-cm, 10-cm, 20-cm, and 25-cm distances from the pylorus. In some experiments, segments were also removed at the 50- and 75-cm levels, as well as 5-cm proximal to the caecum. The tissue was frozen immediately on blocks with liquid carbon dioxide and 8-μm sections were cut on a cryostat. The sections were mounted on slides, fixed for 1 min in cold 95% ethanol, and then briefly dried at room temperature.

The slides were washed in 0.01 M PBS, blotted dry, and then treated with approximately 100 μl of the appropriate FITC-conjugated lectin at concentrations of 0.5–2 mg/ml. Control sections were treated with a solution of the same concentration of lectin but containing sufficient hapten to inhibit the lectin activity. The hemagglutination titers of the FITC-conjugated lectins and their controls are shown in Table I. The slides were placed in a humid atmosphere for 30 min, then washed, and mounted in 0.01 M PBS.

The slides were examined with a Zeiss fluorescence microscope using a UG 1 exciter filter and a number 41 barrier filter which filters light below 410 nm. Pictures were taken with a Zeiss 35-mm automatic camera (Carl Zeiss Inc., New York).

At levels of the intestine in which differences were obtained in fluorescence patterns with the various lectins, tissue sections were treated using various dilutions of FITC-conjugated lectins with unlabeled lectins. In each case, dilution of the FITC-lectin with unlabeled lectin caused a change only in intensity of fluorescence. For each dilution at which fluorescence was detected, no difference occurred in the pattern of localization of the fluorescence.

RESULTS

Immunofluorescence with FITC-D. biflorus Lectin

Fluorescence microscopy of each of the intestinal sections treated with the FITC-labeled *D. biflorus* lectin showed the fluorescence was con-

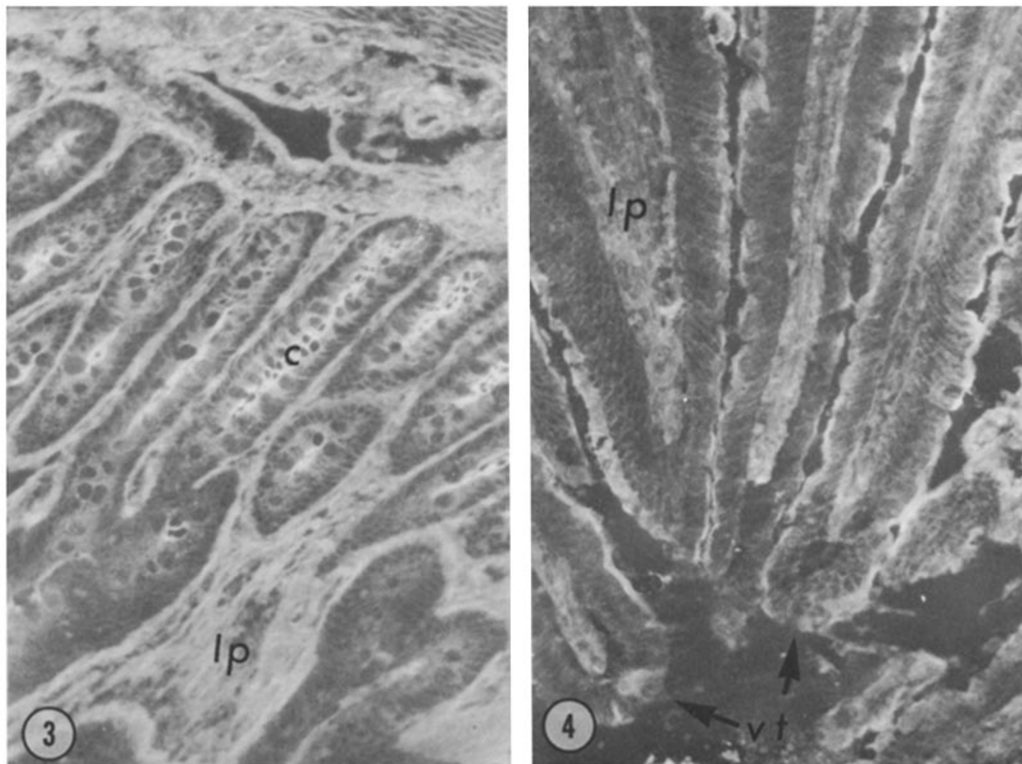


FIGURE 3 Section of intestine adjacent to the pylorus treated with FITC-RCA$_I$ lectin. This micrograph is a view of the crypt region showing bright fluorescence on luminal surfaces of cells in crypts (*c*). Weak fluorescence is in lamina propria region (*lp*). $\times$ 180.

FIGURE 4 Section of intestine 2 cm from pylorus treated with FITC-RCA$_I$ lectin. Fluorescence is in brush border (microvilli) of epithelial cells lining the villi. The villi extend downwards in this view. Note that fluorescence in cells at tips of the villi (*vt*) is dimmer than that of cells along sides of villi. Weak fluorescence is in lamina propria (*lp*). $\times$ 180.

fined to the goblet cells on the villi and the secretory cells in the crypt region (Fig. 1). Some cells in the lamina propria region also showed fluorescence, however, this fluorescence was determined to be nonspecific since it was also found in the controls (Fig. 2). A combination of phase and fluorescence microscopy indicated that not all of the goblet cells reacted with the lectin. A comparison of adjacent sections treated either with FITC-labeled *D. biflorus* lectin or with alcian blue showed that approximately 20–25% of the goblet cells reacted with *D. biflorus* lectin. The goblet cells reactive with *D. biflorus* lectin appeared to be randomly distributed on the villi; this distribution was found at each level of the intestine examined.

Immunofluorescence with FITC-Ricinus communis Lectin (RCA_I)

Sections of duodenum adjacent to the pylorus and 2 cm from the pylorus treated with FITC-labeled RCA_I lectin showed strong fluorescence in the microvillar region of the epithelial cells lining the crypts and the sides of the villi (Figs. 3 and 4). The fluorescence in those cells at the tips of the villi was weaker than that of the cells at the base of the villi. There was also weak fluorescence in the cells of the lamina propria region; this fluorescence was specific and did not occur in the control sections treated in the presence of β-methyl-D-galactoside. No staining was observed in the goblet cells.

The RCA_I lectin reacted with the lamina propria of all levels of the intestine studied (Figs. 3–10), however, there was a marked difference in the pattern of reactivity of this lectin with the brush border of the epithelial cells at the various regions of intestine. In the section of duodenum 5 cm from the pylorus only those epithelial cells toward the lower portion of the villi and in the crypts showed fluorescence (Fig. 5). By the 10-cm level, very little fluorescence was seen on the surface of the epithelial cells lining the villi (Figs. 6 and 7); at this level and at the 15-, 20-, 25-, 50-, and 75-cm levels, the epithelial cell fluorescence was confined to the luminal surface of the cells at the bases of the villi and in the crypts (Figs. 8 and 9). In sections 5-cm proximal to the caecum, very little if any fluorescence is obtained with the epithelial cells (Fig. 10).

Beginning at the 10-cm level, the RCA_I lectin reacted weakly with some of the goblet cells (Fig. 6). In sections through lower levels, most of the goblet cells reacted with the lectin (Figs. 8 and 9)

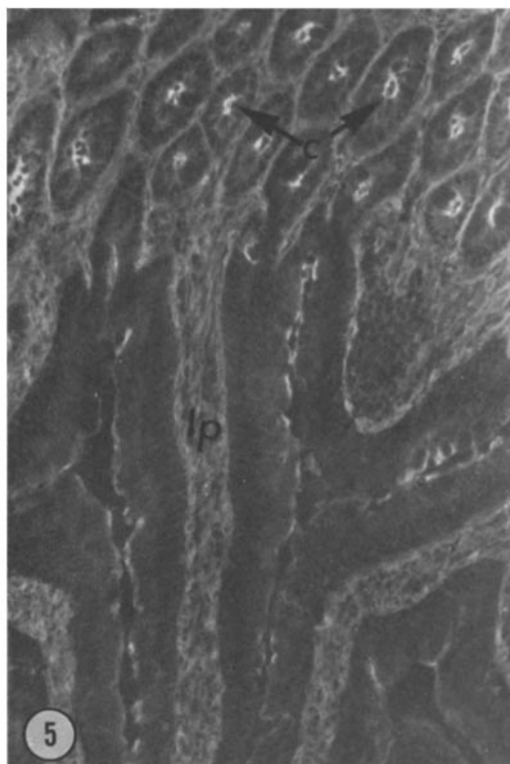


FIGURE 5 Section of intestine 5 cm from pylorus treated with FITC-RCA_I lectin. Bright fluorescence is on luminal surfaces of the epithelial cells in the crypts (*c*) and along the lower portions of the villi which extend downward in this view. Weak fluorescence is in lamina propria (*lp*). × 180.

whereas at the distal end of the intestine this reaction was much weaker and not all of the goblet cells reacted (Fig. 10).

Immunofluorescence with FITC-L. tetragonolobus Lectin

Sections of duodenum adjacent to the pylorus treated with FITC-labeled *L. tetragonolobus* lectin showed strong fluorescence on the microvillar surface of the epithelial cells lining the villi and the crypts. In contrast to the results obtained with the *R. communis* lectin, only a very few cells in the lamina propria region reacted with the *L. tetragonolobus* lectin (Fig. 11). No fluorescence was seen in the control sections treated in the presence of L-fucose. Sections of the intestine at the 2-cm level resembled the sections adjacent to the pylorus; however, at the 5-cm level, the brush borders of the epithelial cells at the tips of the villi

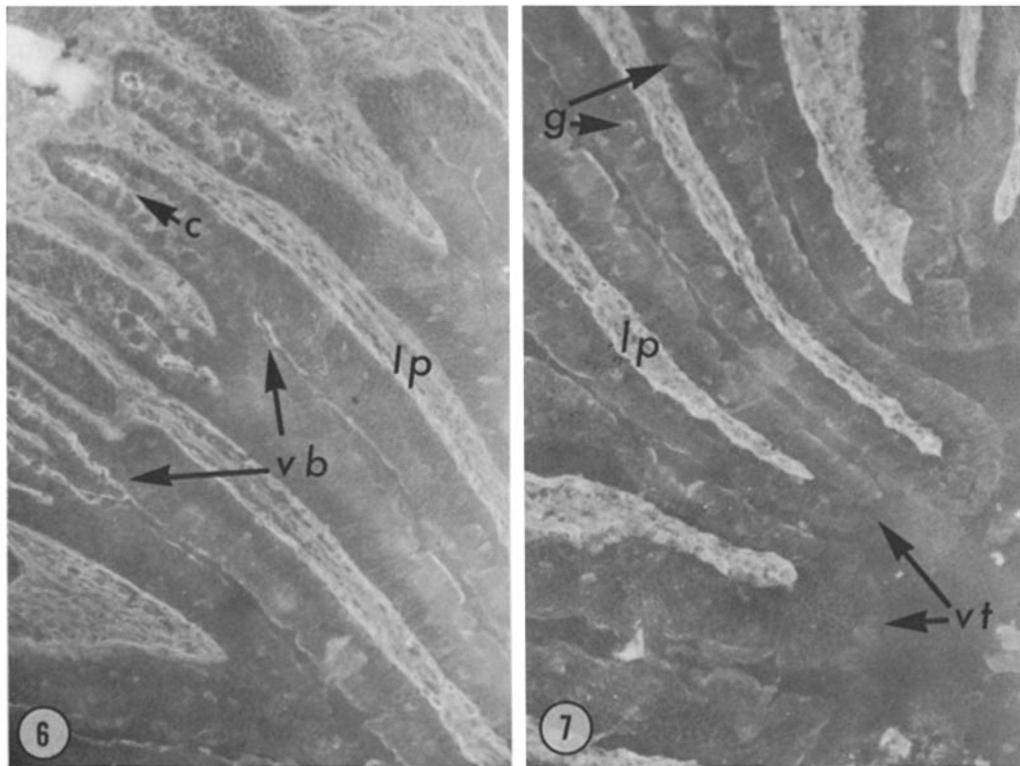


FIGURE 6 Section of intestine 10 cm from pylorus treated with FITC-RCA$_I$ lectin. Fluorescence is in brush borders of cells at bases of villi (*vb*) and in crypts (*c*). Fluorescence is also in lamina propria (*lp*). × 180.

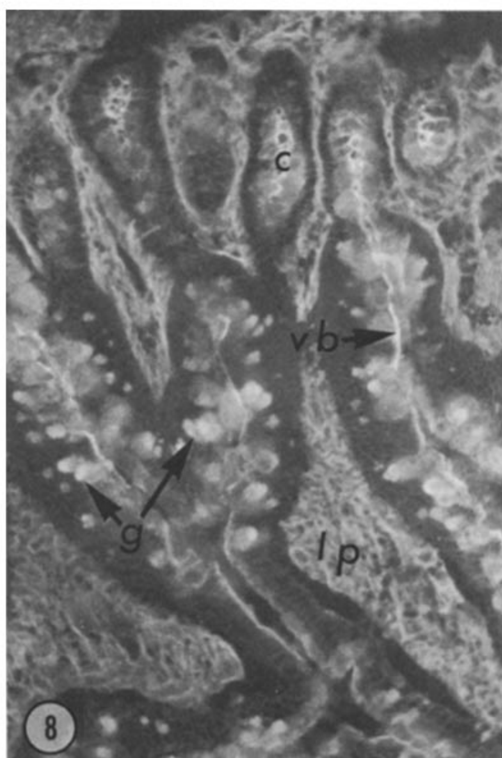
FIGURE 7 Tops of same villi shown in Fig. 6. Fluorescence is found mainly in lamina propria (*lp*) of villi which extend diagonally toward lower right. Very little fluorescence is in brush border of epithelial cells lining the villi or at villi tips (*vt*). Note very weak fluorescence in some goblet cells (*g*). × 180.

showed weak fluorescence in contrast to the strong fluorescence obtained in the brush borders of the other epithelial cells on the villi. At the 10-cm level, the fluorescence was weak in the epithelial cells of the top half of the villi (Fig. 12), and by the 15-cm level the fluorescence was confined to the luminal surface of the epithelial cells at the bases of the villi and in the crypts (Fig. 13). This pattern is maintained throughout the distal region of the intestine although in the ileum the fluorescence is much weaker than noted in more proximal sections.

FIGURE 8 Section of intestine 20 cm from pylorus treated with FITC-RCA$_I$ lectin. Note bright fluorescence on luminal surfaces of cells in crypts (*c*) and along bases of villi (*vb*). Fluorescence is also in goblet cells (*g*) and lamina propria (*lp*). × 180.

FIGURE 9 Section of intestine 20 cm from pylorus treated with FITC-RCA$_I$ lectin. Note absence of fluorescence in brush borders of epithelial cells lining the villi in contrast to bases of villi from this same level shown in Fig. 8. Fluorescence is in goblet cells (*g*) and lamina propria (*lp*). × 180.

FIGURE 10 Section of intestine 5 cm from caecum treated with FITC-RCA$_I$ lectin. Fluorescence is in lamina propria (*lp*) and weak fluorescence is in goblet cells (*g*). No fluorescence is seen in brush borders of cells lining villi. In this view bases of villi (*vb*) are at right. × 180.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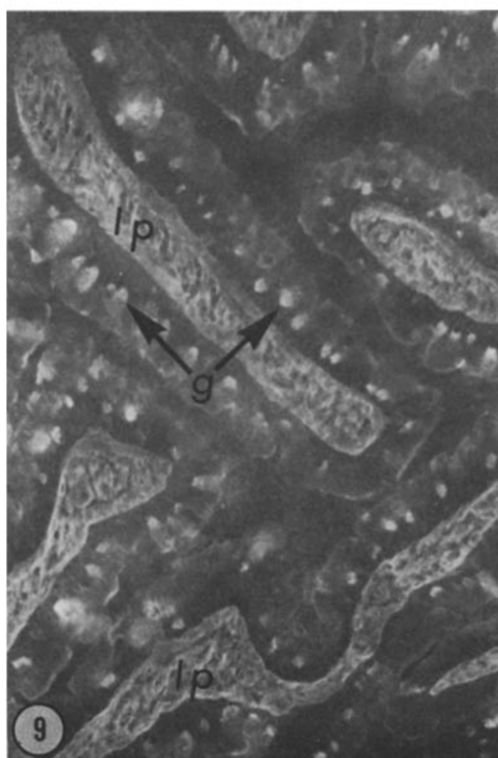
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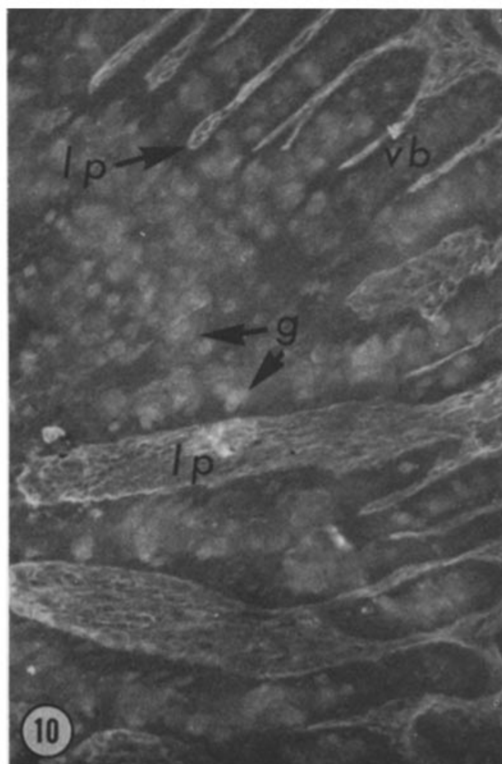
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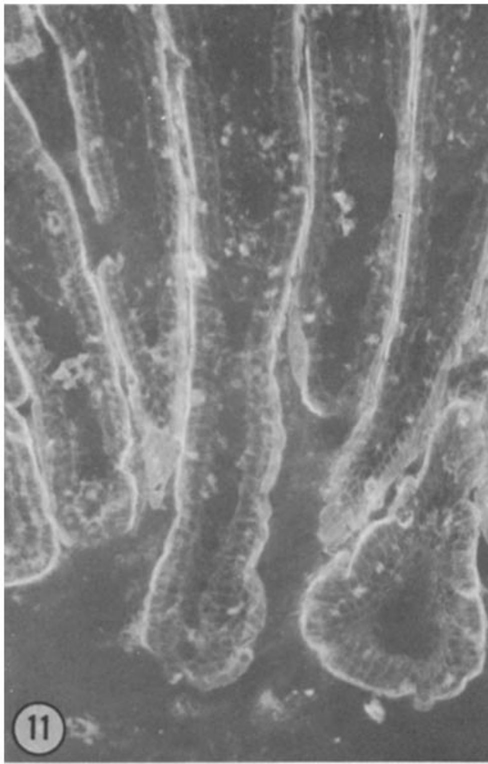
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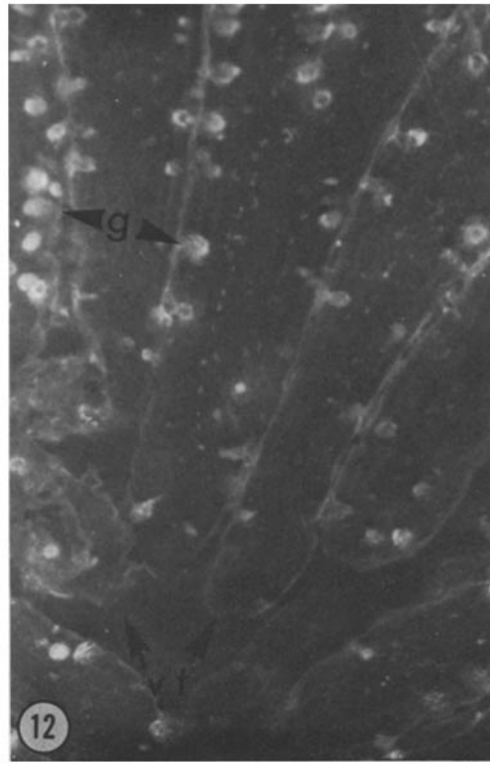
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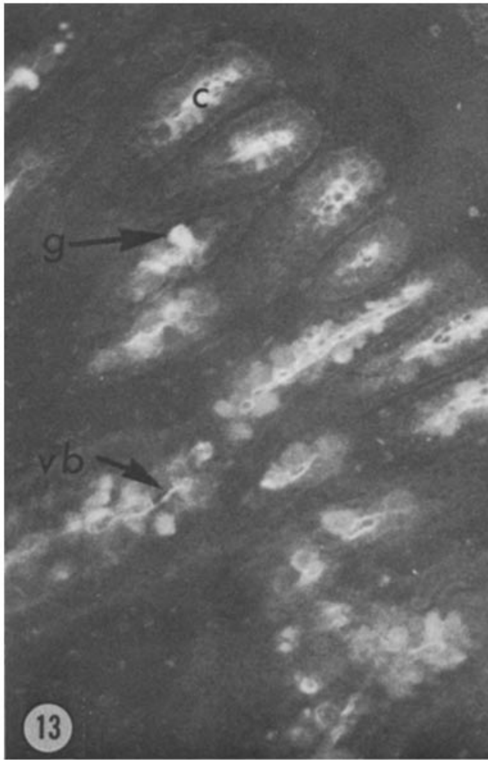
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The difference in reactivity of *L. tetragonolobus* lectin with epithelial cells found at various levels of the intestine resembles the results with the *R. communis* lectin; however, comparisons of adjacent sections at each level of the intestine treated with these two lectins show that the *L. tetragonolobus* lectin reacts with epithelial cells higher on the villi than does the *R. communis* lectin at the 5- and 10-cm levels (Table II). By the 15-cm level, both lectins react with only those epithelial cells in the crypts and at the bases of the villi. These results indicate that in the rat duodenum there is a progressive change in reactivity of villi epithelial cells with *R. communis* and *L. tetragonolobus* lectins within the first 15 cm of intestine. The change in reactivity with the *L. tetragonolobus* lectin occurs more gradually than does the difference with *R. communis* lectin.

As in the case of the *R. communis* lectin, the goblet cells of the middle levels of the intestine reacted with *L. tetragonolobus* lectin and the reactivity of the goblet cells with this lectin was also seen as high as the 10-cm level (Fig. 12). Occasionally, goblet cells at the 5-cm level reacted with the lectin.

Immunofluorescence with FITC-Wheat Germ Lectin

The FITC-labeled wheat germ lectin reacted with the brush borders of the epithelial cells all along the villi at each level of the intestine studied (Figs. 14-17), with the exception of the 75-cm (Fig. 18) and ileal sections where the fluorescence was very weak and was confined to the epithelial cells at the bases of the villi. In each case, the fluorescence extended into the crypts and was specifically inhibited by *N*-acetyl-D-glucosamine. There was also some fluorescence in the lamina propria region.

Although no difference among levels of the first 50 cm of intestine was noted in the ability of wheat germ to react with the epithelial cells, there was a difference found among these levels in the reactivity of goblet cells. In sections close to the pylorus, the wheat germ lectin reacted with approximately 20% of the goblet cells (Fig. 14) whereas at the lower levels the lectin reacted with most of the goblet cells (Figs. 16-18).

DISCUSSION

The data presented above suggest that: (*a*) throughout most of the adult rat small intestine, changes occur in the carbohydrate portion of the microvillar surface of the epithelial cells as these cells differentiate and move up the villi; (*b*) these changes in cell surface are related to the region of the intestine in which the villi are located; and (*c*) the carbohydrate nature of the secretory material of the goblet cells varies in different regions of the small intestine. These conclusions are based upon the differential reactivities of the cell surfaces and secretory components of the rat intestinal epithelium with four different lectins (plant agglutinins). These lectins are the *D. biflorus* lectin with a specificity for terminal nonreducing α-*N*-acetyl-D-galactosamine residues (5, 7), the wheat germ agglutinin with a reported specificity for terminal nonreducing β-*N*-acetyl-D-glucosamine (2) and *N*-acetyl-neuraminic acid residues (10), the *L. tetragonolobus* lectin with a specificity for terminal nonreducing α-L-fucose (25, 33), and the *R. communis* lectin. The *R. communis* lectin (RCA_I) used in this study has a specificity predominantly for terminal nonreducing β-D-galactosyl residues although it also reacts with the α-anomer (27, 28). Preliminary experiments with FITC-labeled antibodies to blood group B substance indicate that these antibodies, which recognize terminal nonre-

FIGURE 11 Section of intestine adjacent to pylorus treated with FITC-*Lotus tetragonolobus* lectin. Fluorescence is in brush border (microvilli) of epithelial cells lining the villi. × 180.

FIGURE 12 Section of intestine 10 cm from pylorus treated with FITC-*Lotus tetragonolobus* lectin. Note weak fluorescence in brush borders of epithelial cells lining the upper portion of the villi. Villus tips are designated (*vt*). Fluorescence is stronger in cells toward villus base. Fluorescence is also in goblet cells (*g*). × 180.

FIGURE 13 Section of intestine 15 cm from pylorus treated with FITC-*Lotus tetragonolobus* lectin. This micrograph shows the crypts and bases of the villi. Note fluorescence on luminal surfaces of cells in crypts (*c*) and at villus base (*vb*). Fluorescence is also in goblet cells (*g*). × 180.

TABLE II
Summary of Fluorescence Microscopy Data

Distance from pylorus	Lectins reacting with microvillar surface of epithelial cells				Lectins Reacting with Goblet Cells
	Crypts	Base of Villus	Sides of Villus	Top of Villus	
cm					
0	WGA	WGA	WGA	WGA	WGA*
	Lotus	Lotus	Lotus	Lotus	—
	RCA_I	RCA_I	RCA_I	RCA_I*	—
	—	—	—	—	Dolichos
2	WGA	WGA	WGA	WGA	WGA*
	Lotus	Lotus	Lotus	Lotus	—
	RCA_I	RCA_I	RCA_I	RCA_I*	—
	—	—	—	—	Dolichos
5	WGA	WGA	WGA	WGA	WGA
	Lotus	Lotus	Lotus	Lotus*	Lotus
	RCA_I	RCA_I	RCA_I‡	—	RCA_I*
	—	—	—	—	Dolichos
10	WGA	WGA	WGA	WGA	WGA
	Lotus	Lotus	Lotus‡	—	Lotus
	RCA_I	RCA_I	—	—	RCA_I*
	—	—	—	—	Dolichos
15	WGA	WGA	WGA	WGA	WGA
	Lotus	Lotus	—	—	Lotus
	RCA_I	RCA_I	—	—	RCA_I
	—	—	—	—	Dolichos
20 and 25	WGA	WGA	WGA	WGA	WGA
	Lotus	Lotus	—	—	Lotus
	RCA_I	RCA_I	—	—	RCA_I
	—	—	—	—	Dolichos
50§	WGA	WGA	WGA	WGA	WGA
	Lotus	Lotus	—	—	Lotus
	RCA_I	—	—	—	RCA_I*
75§	WGA	WGA	WGA	—	WGA
	Lotus	Lotus*	—	—	Lotus*
	RCA_I	—	—	—	RCA_I*
100	WGA	WGA*	—	—	WGA
	Lotus	—	—	—	Lotus*
	RCA_I*	—	—	—	RCA_I*
	—	—	—	—	Dolichos

WGA refers to wheat germ agglutinin.
* Weak fluorescence.
‡ Weak fluorescence in cells at top half of villus.
§ Sections at this level were not treated with the *Dolichos biflorus* lectin.

ducing α-D-galactose residues (15), show very little reaction with the intestinal tissue, thus indicating that the reactivity of the *R. communis* lectin may be primarily due to terminal β-D-galactosyl-like residues on the cells.

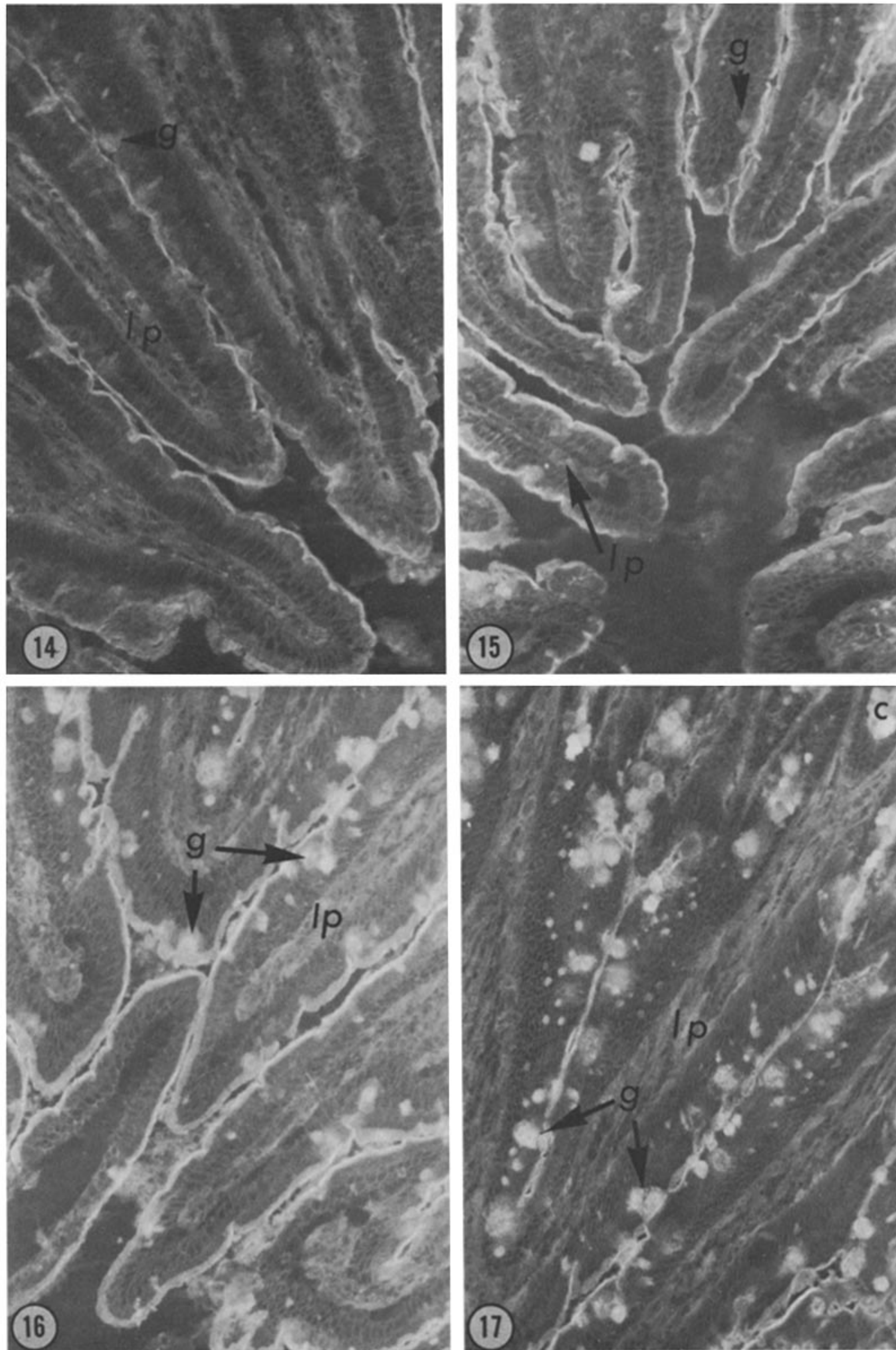
The microvilli of the intestinal epithelial cells are covered with a carbohydrate-rich material that appears to be synthesized by the cells as an integral part of the membrane (13). At each level of the intestine studied (with the possible exception of the most distal levels), the luminal surfaces of the cells in the crypts reacted with the *R. communis*, *L. tetragonolobus*, and wheat germ lectins, thereby suggesting that the microvillar surfaces of these relatively undifferentiated cells are rich in terminal nonreducing β-D-galactosyl, α-L-fucosyl, and β-*N*-acetyl-D-glucosaminyl, and/or sialic acid residues. This interpretation is supported by previous work on isolated epithelial cells from various levels of the villi and crypts of rat intestine in which galactosyl, fucosyl, and *N*-acetylglucosaminyl transferases were found to be associated predominantly with the cells from the crypts (39).

In addition to differences in transferase activities, epithelial cells isolated from the crypts and from various locations on the villi have shown a difference in sugar incorporation (38) and in ability to agglutinate with concanavalin A (30, 37); however, whether these differences are due to the microvillar surface of the cells remains to be established. In the present study, using sections through intact intestinal epithelium, throughout most of the intestine differences in reactivities with the various lectins were found between the microvilli of the crypt cells and the cells on the villi. These differences suggest that as the crypt cells differentiate and move up the villi, their terminal carbohydrate residues are altered. These residues may be degraded by various glycosidases of the brush border (8), modified by such processes as acetylation or sulfation, or they may serve as receptors for other transferases such as the sialyltransferase which has been shown to increase in activity as the cells move up the villi (39).

The nature of the surface of the epithelial cells lining the villi was found to vary with the distance of the villi from the pylorus. A sharp difference in cell surface was found within the first 10–15 cm of the adult intestine; for the rats used in this investigation, this segment represents only the first 10% of the small intestine. In successive sections from the proximal to the distal end of this intestinal segment, the reactivity of the *R. communis* lectin with the epithelial cells lining the villi diminished beginning with the cells at the tips of the villi and progressing to the cells at the bases of the villi. A similar but more gradual diminution in reactivity with the *L. tetragonolobus* lectin was found (Table II), so that by the 15-cm level of the intestine these two lectins reacted only with the surfaces of the cells in the crypts and at the bases of the villi. These differences in cell surface are concomitant with other changes that occur along a proximal-distal axis within this segment of the intestine, including a change in alkaline phosphatase activity (24, 35) and a sharp decrease in villus length (1). Estimates of the villus transit time of epithelial cells from different regions of the intestine have shown either no great difference between various levels of intestine (19) or a very gradual decrease in transit time along the length of intestine (1, 3). Because of the sharp decrease in villus height within the first 10% of the intestine, it is possible that the rate of cell migration varies between the proximal and distal portions of this segment; the changes in cell surface properties may thus possibly be related to the rate of cell migration.

The reaction of the wheat germ lectin with the luminal surfaces of the epithelial cells on the villi throughout the proximal half of the intestine is in accordance with the finding that isolated epithelial cells from the villi of rat small intestine are agglutinated with this lectin (37). The present study shows a difference between the epithelial cells on the villi at proximal and distal regions of the intestine in their ability to react with the wheat germ lectin. The reaction of the wheat germ lectin with the epithelial cells may be due to the presence of terminal nonreducing β-*N*-acetyl-D-glucosamine and/or sialic acid residues on these cells. Competition experiments raise the possibility that both of these sugars may bind to identical sites on the lectin (10); thus, whether the nature of the wheat germ receptor(s) changes or remains the same at various levels of the intestine or as the cells migrate from the crypts to the tops of the villi cannot be determined by the present data.

Differences in goblet cell secretory material were also noted at various regions of the intestine. The *L. tetragonolobus*, *R. communis*, and wheat germ lectins did not react or reacted weakly with



FIGURES 14–17 Sections of intestine treated with FITC-wheat germ lectin. Sections were taken adjacent to pylorus (Fig. 14), and 10 cm (Fig. 15), and 20 cm (Figs. 16 and 17) from the pylorus. Fig. 17 shows the bases of the same villi shown in Fig. 16. At each level fluorescence is in brush border (microvilli) of epithelial cells lining the villi and crypts (*c*). Note weak fluorescence in goblet cells (*g*) at 0- and 10-cm levels (Figs. 14 and 15) and strong fluorescence in goblet cells (*g*) at the 20-cm level (Figs. 16 and 17). Weak fluorescence is in lamina propria (*lp*). × 180.

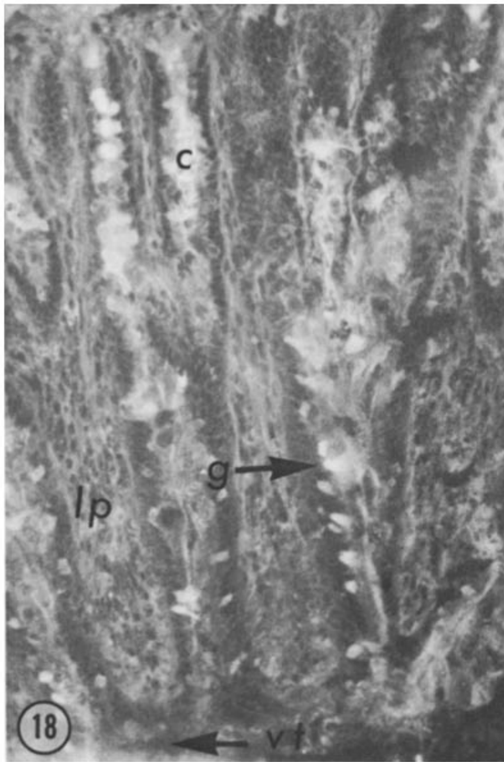


FIGURE 18 Section of intestine 75 cm from pylorus treated with FITC-wheat germ lectin. Fluorescence is in goblet cells (*g*) and in cells in crypts (*c*). Note absence of fluorescence in brush borders of cells lining villi. Villi tips (*vt*) are at bottom of micrograph. Weak fluorescence is in lamina propria (*lp*). × 180.

only a few of the goblet cells at the proximal levels of the intestine, whereas these lectins reacted with most of the goblet cells at lower levels of the intestine (Table II). These regional differences in goblet cell reactivity indicate a difference in goblet cell secretions at various regions of the intestine. Such differences have also been noted by other investigators using various stains and autoradiography (14, 21, 23, 32).

The *D. biflorus* lectin reacted with approximately 20–25% of the goblet cells at each level of duodenum and jejunum studied. The reactive goblet cells appeared to be randomly distributed among the goblet cells on the villi at each level of intestine. Autoradiographic studies of goblet cells from rats injected with [^{3}H]glucose indicate that the goblet cells continue to synthesize and release mucus throughout their life on the villus but that the secretory cycles of goblet cells at any one portion of the villus are not in synchrony with one another (26). The ability of the various goblet cells to react with only one or more than one lectin may be due to the particular phase of mucus synthesis they are in, although it is also possible that different cells produce different secretory products.

The variations shown in this study among the cell surface and secretory components of epithelial cells at various levels of the intestine and at different portions of the villi reflect only differences in terminal nonreducing carbohydrate residues. It should be pointed out that these terminal residues may be common to a number of different carbohydrate components, and there may thus be a number of cell surface and secretory components represented by the reactivity with each lectin. More information on these components should come from work now in progress on the isolation and characterization of the cell surface and secretory components of rat intestinal mucosa by the use of affinity columns made with the various lectins in this study (6).

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REFERENCES

1. ALTMAN, G. G., and M. ENESCO. 1967. Cell number as a measure of distribution and renewal of epithelial cells in the small intestine of growing and adult rats. *Am. J. Anat.* **121**:319.
2. BURGER, M. M., and A. R. GOLDBERG. 1967. Identification of a tumor-specific determinant on neoplastic cell surfaces. *Proc. Natl. Acad. Sci. U. S. A.* **57**:359.
3. CLARKE, R. M. 1970. Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat. *J. Anat.* **107**:519.
4. DAHLQVIST, A., and C. NORDSTROM. 1966. The distribution of disaccharidase activities in the villi and crypts of the small intestinal mucosa. *Biochim. Biophys. Acta.* **113**:624.
5. ETZLER, M. 1972. Horse gram (*Dolichos biflorus*) lectin. *In* Methods in Enzymology. Vol. XXVIII (Pt. B). V. Ginsberg, editor. Academic Press, Inc., New York. 340.
6. ETZLER, M. E. 1974. Use of plant agglutinins in characterization of glycoproteins and glycolipids

from mammalian cells. *Ann. N.Y. Acad. Sci.* **234:** 260.

7. ETZLER, M. E., and E. A. KABAT. 1970. Purification and characterization of a lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus*. *Biochemistry*. **9**:899.
8. FORSTNER, G. G., S. M. SABESIN, and K. J. ISSELBACHER. 1968. Rat intestinal microvillus membranes. Purification and biochemical characterization. *Biochem. J.* **106**:381.
9. GOLDSTEIN, I., J. D. HOLLERMAN, and E. C. SMITH. 1965. Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. *Biochemistry*. **4**:876.
10. GREENAWAY, P. J., and D. LEVINE. 1973. Binding of *N*-acetyl-neuraminic acid by wheat germ agglutinin. *Nat. New Biol.* **241**:191.
11. IMONDI, A. R., M. E. BALIS, and M. LIPKIN. 1969. Changes in enzyme levels accompanying differentiation of intestinal epithelial cells. *Exp. Cell Res.* **58**:323.
12. IMONDI, A. R., M. LIPKIN, and M. E. BALIS. 1970. Enzyme and template stability as regulatory mechanisms in differentiating intestinal epithelial cells. *J. Biol. Chem.* **245**:2194.
13. ITO, S. 1969. Structure and function of the glycocalyx. *Fed. Proc.* **28**:12.
14. JENNINGS, M. A., and H. W. FLOREY. 1956. Autoradiographic observations on the mucous cells of the stomach and intestine. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **41**:131.
15. KABAT, E. A., and S. LESKOWITZ. 1955. Immunochemical studies on blood groups. XVII. Structural units involved in blood group A and B specificity. *J. Am. Chem. Soc.* **77**:5159.
16. KAPLAN, M., and E. A. KABAT. 1966. Studies on human antibodies. IV. Purification and properties of anti-A and anti-B obtained by absorption and elution from insoluble blood group substances. *J. Exp. Med.* **123**:1061.
17. LEBLOND, C. P., and C. E. STEVENS. 1948. The constant renewal of the intestinal epithelium in the albino rat. *Anat. Rec.* **100**:357.
18. LEVINE, D., M. J. KAPLAN, and P. J. GREENAWAY. 1972. The purification and characterization of wheat germ agglutinin. *Biochem. J.* **129**:847.
19. LORAN, M. R., and T. L. ALTHAUSEN. 1960. Cellular proliferation of intestinal epithelia in the rat two months after partial resection of the ileum. *J. Biophys. Biochem. Cytol.* **7**:667.
20. MARCHESI, V. T. 1972. Wheat germ (*Triticum vulgare*) agglutinin. *In* Methods in Enzymology. V. Ginsberg, editor. Vol. XXVIII (Part B) Academic Press, Inc., New York. 354.
21. MARTIN, B. F. 1961. The goblet cell pattern in the large intestine. *Anat. Rec.* **140**:1.
22. MESSIER, B., and C. P. LEBLOND. 1960. Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. *Am. J. Anat.* **106**:247.
23. MOE, H. 1968. The goblet cells, Paneth cells, and basal granular cells of the epithelium of the intestine. *Int. Rev. Gen. Exp. Zool.* **3**:241.
24. MOOG, F., and K. YEH. 1973. Intestinal alkaline phosphatase of the rat: development and distribution of activity with phenylphosphate and β-glycerophosphate. *Comp. Biochem. Physiol.* **44**:657.
25. MORGAN, W. T. J., and W. M. WATKINS. 1953. The inhibition of the haemagglutinins in plant seeds by human blood group substances and simple sugars. *Brit. J. Exp. Pathol.* **34**:94.
26. NEUTRA, M., and C. P. LEBLOND. 1966. Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope radioautography of goblet cells from rats injected with glucose-^{3}H. *J. Cell Biol.* **30**:119.
27. NICOLSON, G. L., and J. BLAUSTEIN. 1972. The interaction of *Ricinus communis* agglutinin with normal and tumor cell surfaces. *Biochim. Biophys. Acta.* **266**:543.
28. NICOLSON, G. L., J. BLAUSTEIN, and M. E. ETZLER. 1974. Characterization of two plant lectins from *Ricinus communis* and their interaction with a murine lymphoma. *Biochemistry*. **13**:196.
29. PALAY, S. L., and L. J. KARLIN. 1959. An electron microscopic study of the intestinal villus. I. The fasting animal. *J. Biophys. Biochem. Cytol.* **5**:363.
30. PODOLSKY, D. K., and M. M. WEISER. 1973. Specific selection of mitotically active intestinal cells by concanavalin A-derivatized fibers. *J. Cell Biol.* **58**:497.
31. RINDERKNECHT, H. 1962. Ultra-rapid fluorescent labeling of proteins. *Nature* (*Lond.*). **193**:167.
32. SPICER, S. S. 1963. Histochemical differentiation of mammalian mucopolysaccharides. *Ann. N.Y. Acad. Sci.* **106**:379.
33. SPRINGER, G. F., and P. WILLIAMSON. 1962. Immunochemical significance of L- and D-fucose derivatives. *Biochem. J.* **85**:282.
34. TOMITA, M., T. KUROKAWA, K. ONOZAKI, N. ICHIKI, T. OSAWA, and T. UKITA. 1972. Purification of galactose binding phytoagglutinins and phytotoxin by affinity column chromatography using Sepharose. *Experientia*. **28**:84.
35. TRIANTAPHYLLOPOULOS, E., and J. TUBA. 1959. Studies on the distribution and kinetics of the alkaline phosphatase of rat small intestine. *Can. J. Biochem. Physiol.* **37**:699.
36. WEBSTER, H. L., and D. D. HARRISON. 1969. Enzymic activities during the transformation of crypt to columnar intestinal cells. *Exp. Cell Res.* **56**:245.

37. WEISER, M. M. 1972. Concanavalin A agglutination of intestinal cells from the human fetus. *Science* (*Wash. D.C.*). **177**:525.

38. WEISER, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* **248**:2536.

39. WEISER, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. II. Glycosyltransferases and endogenous acceptors of the undifferentiated cell surface membrane. *J. Biol. Chem.* **248**:2542.

40. YARIV, J., A. J. KALB, and S. BLUMBERG. 1972. *Lotus tetragonolobus* L-fucose-binding proteins. *In* Methods in Enzymology, V. Ginsberg, editor. Vol. XXVIII (Part B) Academic Press, Inc., New York. 356.