

## **Ultrastructural Visualization of Galactosyl Residues in Various Alimentary Epithelial Cells with the Peanut Lectin-Horseradish Peroxidase Procedure**

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**Summary.** A conjugate of peanut lectin with horseradish peroxidase (PL-HRP) has been employed for ultrastructural localization of glycoprotein with presumed terminal galactose residues in mouse alimentary epithelial cells. The PL-HRP conjugate imparted electron opacity in sites that stain at the light microscopic level, as for example, Golgi cisternae in surface epithelial cells of the stomach and in superficial and deep crypt cells and goblet cells of the large intestine. Ultrastructural staining revealed that Golgi cisternae intermediate between the *trans* and *cis* faces stained selectively in these sites. Secretion stored in secretory granules or Golgi vesicles in the cells lacked affinity for PL-HRP conjugate, however. Selective staining of intermediate Golgi cisternae in cells with unreactive secretory product is interpreted as indicating the site of galactosyl transferase activity and a location where galactose occurs transitorily as the terminal sugar in the glycoprotein side chains. The luminal aspect of the surface epithelial cells in the stomach and columnar cells in the colon also stained, but with some variability. Staining of these surfaces was considered possibly attributable to PL affinity of some of the secretory glycoprotein which, after absorbing to the cell surface, lost terminal sialic acid through action of luminal enzyme. PL-HRP conjugate stained granules in pancreatic zymogen cells near the block surface but not in other cells, presumably because of limited penetration of reagent. Secretion on the surface of pancreatic acinar cells or in the lumen also exhibited affinity for PL-HRP complex as did the luminal surface of gastric chief cells. Staining of secretion in the pancreatic zymogen cells and gastric chief cells for galactose appeared inconsistent with lack of evidence for presence of glycoprotein in these sites which failed to stain with the periodic acid-Schiff or periodic acid-thiocarbohydrazide-silver proteinate methods for demonstrating glycoprotein at the light and electron microscopic levels. This discrepancy points to possible selective binding of PL-HRP conjugate to a moiety other than terminal galactose of glycoprotein in a few histologic sites. These results demonstrate the applicability of the PL-HRP technique at the ultrastructural level and provide information con-

cerning the chemical structure of epithelial cell glycoproteins and their biosynthesis.

## Introduction

Marker-labeled lectins have been employed as light microscopic histochemical reagents for localizing macromolecules with certain sugars on the basis of their specific binding affinity for these saccharide residues. A method employing a conjugate of peanut lectin with horseradish peroxidase (PL-HRP) has recently been found, for example, to demonstrate glycoproteins presumably containing terminal galactose in their oligosaccharide side chains (Stoward et al., in press). The lectin labeling methods have generally proved unsatisfactory for electron microscopy however, probably because of limited penetration into the tissue specimen or into the cells. Preliminary studies of mouse gastric surface epithelium, however, have provided evidence for the applicability of the PL-HRP conjugate at the ultrastructural level (Sato and Spicer 1981). The present study inquires further into the selective ultrastructural staining with this reagent in a variety of cells known to contain different types of glycoproteins.

## Materials and Methods

*Tissue Preparation.* Stomach, jejunum, rectosigmoid colon and pancreas were obtained from adult male black mice (C57 BL/++) under nembutal anesthesia. Specimens were minced to blocks less than 1 mm<sup>3</sup> with a razor blade and fixed 1–2 h at 4°C in either 2.5% glutaraldehyde or freshly prepared 4% paraformaldehyde in 0.1 M sodium cacodylate at pH 7.2. A portion of each fixed tissue was rinsed 1 h with phosphate-buffered saline (PBS) at pH 7.2 prior to sectioning and staining with PL-HRP. A part of some specimens was held overnight in the cacodylate buffer containing 7% sucrose for subsequent staining with other methods of localizing complex carbohydrates.

*Staining with PL-HRP Conjugate.* The procedure employed in this study resembled closely that recently described (Sato and Spicer 1981b). The peanut lectin was isolated by affinity column chromatography on lactosyl-sepharose 4B (Stoward et al. 1980), and conjugated to horseradish peroxidase (Sigma Chem. Co., St. Louis, MO) with glutaraldehyde (Avrameas 1969). Cryostat sections of the fixed specimens were cut at about 10 µm thickness and exposed to a solution of the PL-HRP conjugate (0.06 mg/ml in PBS) for 1 h or overnight at 4°C. The cryostat sections were employed in preference to the necessarily thicker tissue chopper slices because of the lesser distance required for diffusion of staining moieties and the prospect of enhanced diffusion of regents into the tissue after membrane disruption by freezing. Sections were rinsed 1 h with 3 changes of PBS and then incubated 20 min at room temperature in 3,3'-diaminobenzidine-hydrogen peroxide medium for visualizing peroxidase (Graham and Karnovsky 1966). As controls, sections of each tissue block were subjected to the same procedure except for replacing the PL-HRP conjugate with either unconjugated lectin (0.04 mg/ml in PBS) or 0.5 mg% HRP in PBS. After postfixation for 1 h with 1% osmium tetroxide, the sections were dehydrated through ethanol and embedded in Epon. Thin sections were examined without heavy metal counterstaining.

*Staining with Other Methods for Demonstrating Complex Carbohydrate.* The glutaraldehyde-fixed tissues were processed with several comparative methods for localizing and characterizing glycoconjugates at the ultrastructural level. To visualize both sulfated and carboxyl-rich glycoconjugates, 40 µm cryostat sections were stained overnight with the dialyzed iron (DI) solution (Wetzel et al., 1966). The high iron diamine (HID) procedure was employed on cryosections to demonstrate

**Table 1.** Ultrastructural comparison of PL-HRP and other carbohydrate cytochemical procedures in mouse alimentary epithelia

Cytologic site	PL-HRP	PA-TCH-SP	DI	HID	Glycoconjugate demonstrated
<i>Surface Epithelial Cell</i>					
Luminal surface	(-)(+) <sup>a</sup>	(+)	(-)	(-)	Neutral glycoprotein with terminal galactose in the most superficial cells <sup>a</sup>
Stored mucous droplets	(-)	(+)	(-)	(-)	Neutral glycoprotein
Secreted mucus	(-)(+) <sup>b</sup>	(+)	(-)	(-)	Neutral glycoprotein
Golgi cisternae	(+)	(+)	(-)	(-)	Neutral glycoprotein with galactose transiently terminal during biosynthesis
<i>Stomach</i>					
<i>Isthmus Cell</i>					
Luminal surface	(-)	(+)	(+)	(+)	Sulfated glycoprotein
Secretory granule	(-)	(+)	(+)	(+)	Sulfated glycoprotein devoid of galactose
Golgi cisternae	(-)	(+)	(+)	(+)	Sulfated glycoprotein devoid of galactose
<i>Parietal Cell</i>					
Apical plasmalemma	(-)	(+)	(-)	(-)	Neutral glycoprotein
Basolateral plasmalemma	(-)	(+)	(+)	(-)	Carboxylated glycoprotein
<i>Chief Cell</i>					
Luminal surface	(+)	(+)	(+)	(-)	Carboxylated glycoprotein with terminal galactose <sup>c</sup>
Zymogen granule	(-)	(-)	(-)	(-)	None
<i>Small Intestine</i>					
<i>Columnar Epithelial Cell</i>					
Luminal surface	(-)(+)	(+)	(+)	(-)(+) <sup>d</sup>	Carboxylated glycoprotein (with sparse sulfated glycoprotein at the tips of microvilli)
Golgi cisternae	(-)(+)	(+)	(-)	(-)	Neutral glycoprotein with galactose transiently terminal during biosynthesis
<i>Goblet Cell</i>					
Mucous goblet	(-)(+) <sup>e</sup>	(+)	(+)	(+)	Sulfated glycoprotein
Golgi cisternae	(+)	(+)	(+)	(+)	Sulfated glycoprotein with galactose transiently terminal during biosynthesis
<i>Colon</i>					
<i>Luminal Epithelial Cell</i>					
Luminal surface	(-)(+)	(+)	(+)	(-)	Carboxylated glycoprotein
Small vesicles in the apical cytoplasm	(-)	(+)	(+)	(-)	Carboxylated glycoprotein
Golgi cisternae	(+)	(+)	(+)	(-)	Carboxylated glycoprotein with galactose transiently terminal during biosynthesis

**Table 1.** (continued)

Cytologic site	PL-HRP	PA-TCH-SP	DI	HID	Glycoconjugate demonstrated
<i>Deep Crypt Cell</i>					
Luminal surface	(-)(+)	(+)	(+)	(-)	Carboxylated glycoprotein
Mucous droplet	(-)	(-)	(+)	(-)	Carboxylated periodate-negative glycoconjugate
Golgi cisternae	(+)	(+)	(+)	(-)	Carboxylated glycoprotein with galactose transiently terminal during biosynthesis
<i>Goblet Cell</i>					
Mucous goblet	(-)(+) <sup>f</sup>	(+)	(+)	(+)	Sulfated glycoprotein
Golgi cisternae	(+)	(+)	(+)	(+)	Sulfated glycoprotein with galactose transiently terminal during biosynthesis
<i>Pancreas</i>					
<i>Acinar Cell</i>					
Apical plasmalemma	(-)	(+)	(-)	(-)	Neutral glycoprotein
Zymogen granule	(-)(+) <sup>g</sup>	(-)	(-)	(-)	Component binding PL-HRP unidentified
Secretion in the intercellular canaliculari and on the luminal surface	(+) <sup>h</sup>	(-)	(-)	(-)	Component binding PL-HRP unidentified

PL-HRP=Peanut lectin-horseradish peroxidase; PA-TCH-SP=Periodic acid-thiocarbohydrazide-silver proteinate; DI=Dialyzed iron; HID=High iron diamine

(-)(+) indicates range of reactivity among individual structures (see text)

- <sup>a</sup> Luminal surface of the foveolar surface epithelial cells revealed little or no densification with PL-HRP, whereas that of the most superficial surface epithelial cells disclosed moderate to intense reactivity
- <sup>b</sup> Secreted mucus in the gastric pit showed little or no staining, whereas that in the gastric lumen revealed moderate to intense staining
- <sup>c</sup> Presence of terminal galactose and sialic acid in the chief cell surface, although an apparent contradiction, could be attributed to branching of oligosaccharide side chains or heterogeneity of side chains in a glycoprotein or of glycoproteins in the glycocalyx
- <sup>d</sup> Only the tips of the microvilli making up the apical surface of some columnar cells appeared stained with HID
- <sup>e</sup> Theca stained in all regions of small intestine except proximal duodenum
- <sup>f</sup> Caps of mucus extruded into the intestinal lumen were densified with peanut lectin, whereas ordinary mucous goblets were not stained
- <sup>g</sup> Stained zymogen granules varied in degree of reactivity and appeared randomly distributed among unstained granules
- <sup>h</sup> Luminal surface did not stain in profiles of acinar lumen filled with densified secretion but was coated with aggregates of reaction product when the lumen looked empty

sulfated mucosubstances specifically (Spicer et al. 1978a). For controls, a 0.1 M MgCl<sub>2</sub> or 1.2% FeCl<sub>3</sub> solution was applied in place of the DI or HID solution respectively. To localize glycoproteins with hexoses rich in *vicinal* hydroxyls, Thiéry's periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) method (Thiéry 1967) was employed on ultrathin sections of an aliquot of each glutaraldehyde-fixed specimen that was not postfixed with osmium tetroxide. Control sections were exposed to the TCH-SP sequence without prior periodate oxidation. Thin sections of cytochemical preparations were examined in an Hitachi HS-8 electron microscope without heavy metal counterstaining.

## Results

The peanut lectin-horseradish peroxidase (PL-HRP) method consistently densified a number of epithelial cell sites in mouse alimentary organs at the ultrastructural level (Table 1). The staining intensity varied somewhat in different preparations however. Paraformaldehyde-fixed tissues generally afforded stronger staining than the glutaraldehyde-fixed specimens, although providing poorer ultrastructural preservation. In tissues exposed 1 h to PL-HRP conjugate, observations were restricted to the outer cell layer at the edge of the cryostat section, and little or no staining was observed within certain cell types. Overnight immersion of the cryostat sections generally provided intra- and extracellular localization of lectin positive sites.

### *PL-HRP Reactivity*

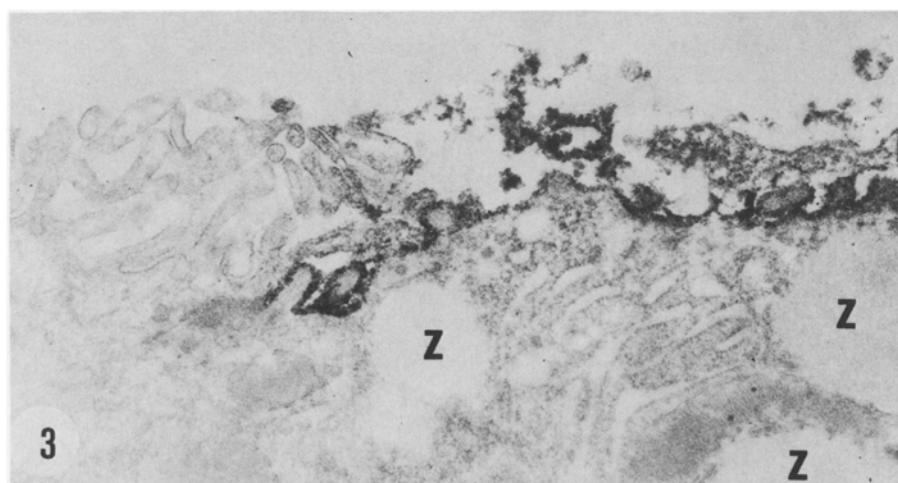
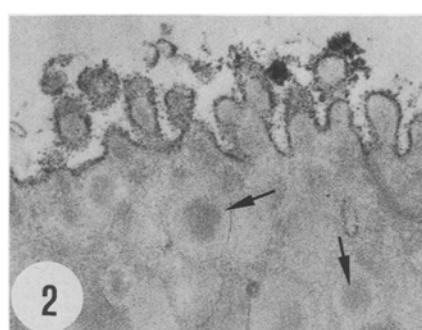
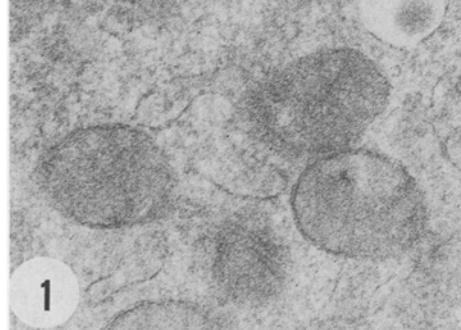
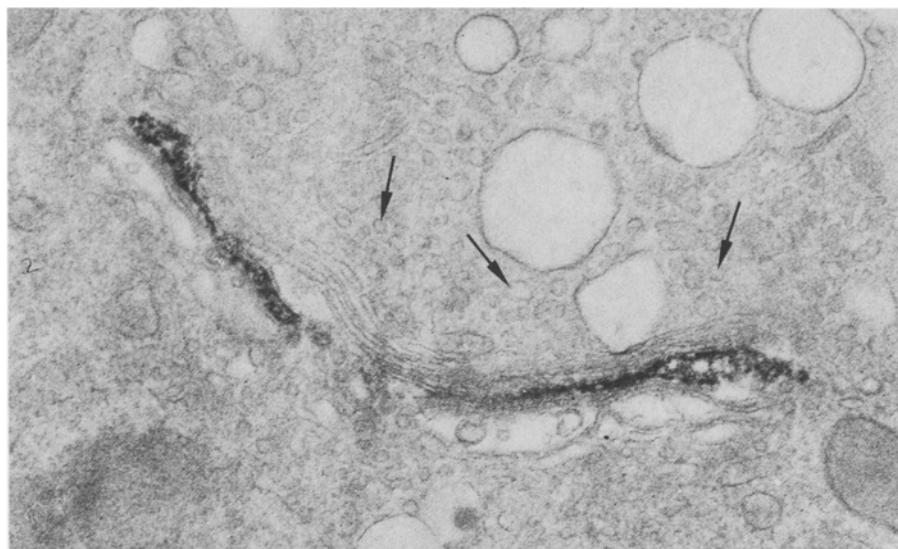
In the stomach, the superficial and foveolar surface epithelium displayed affinity for PL-HRP conjugate selectively in intermediate Golgi cisternae (Fig. 1). Dense reaction product was generally restricted to one or two cisternae sandwiched by unstained saccules at the maturing and forming faces. Stored mucous droplets in superficial and foveolar surface epithelium were consistently devoid of PL-HRP affinity. However, the luminal surface of the superficial surface epithelium and adherent mucus in the main lumen stained (Fig. 2), although the surface of foveolar epithelium and secreted mucus in the gastric pits did not.

Isthmus cells which have been clearly distinguished from mucous neck cells by the cytochemical and fine structural features of the secretory granules (Spicer et al. 1978; Sato 1981a), were uniformly nonreactive throughout. Parietal cells also failed to stain, however, the luminal face of the chief cell's apical plasmalemma stained strongly and contrasted with the nonreactive chief cell secretory granules (Fig. 3).

In the jejunum, Golgi cisternae of a few columnar absorptive cells revealed PL-HRP reactivity, but other cisternae in the same cell and all cisternae in other cells failed to stain. The luminal surface of the microvilli of the columnar cells frequently disclosed light affinity for PL-HRP conjugate (Fig. 4) showing finely particulate reaction product. Density of luminal content was also increased by the cytochemical procedure.

Goblet cells of the proximal duodenum and the rectosigmoid colon consistently evidenced reactivity in the Golgi zone. This staining was restricted to two or three saccules at the forming face of the Golgi stack or between the forming and the maturing face that rimmed the closely packed mucous droplets in the supranuclear area (Figs. 5 and 6). The droplets throughout the mucous goblet invariably lacked staining with PL-HRP conjugate except in the occasionally observed mucous caps that were partially secreted and appeared extruded into the intestinal lumen (Fig. 7).

In the colon, nongoblet columnar epithelial cells constituting the principal cell type at the luminal surface and the nongoblet, mucous-filled epithelial cells predominantly deep within the colonic crypts resembled each other in PL reactivity. These cells, referred to hereafter as the luminal epithelial cell and the deep crypt cell respectively, revealed staining occasionally on the microvillous surface and consistently in the Golgi cisternae, but not in the secretory



droplets of the deep crypt cell nor in small vesicles in the apical cytoplasm of the luminal epithelial cell (Table 1).

Pancreatic acini revealed selective sites of PL binding in agreement with light microscopic findings. Ultrastructural observations were confined to the outer cell layer at the edge of the cryosection, however, because of the limited penetration of the PL-HRP conjugate. Profiles of zymogen granules in the acinar cells varied in degree of reactivity and strongly positive granules intermingled with negative granules in the luminal cytoplasm (Fig. 8). Generally, the heavily stained granules disclosed a coarser meshwork of reaction product than the less reactive ones. Rather strangely, the staining of secretion in most intercellular canaliculi appeared to alternate with that on the luminal surface of the acinar cells bordering the lumen. Thus, when the acinar lumen was filled with densified secretory material, the luminal surface failed to stain with PL-HRP and an electron-lucent space intervened between the luminal secretion and the plasmalemma (Fig. 9). However, when the lumen of the intercellular canaliculus contained little or no densified secretion, the luminal aspect of the plasmalemma was stained in a stratum comparable to the lucent space of the filled canaliculi (Fig. 10, cf. Fig. 9).

No densification was detected in control sections exposed initially to either unconjugated PL or HRP in place of the PL-HRP conjugate.

#### *Comparative Complex Carbohydrate Cytochemistry*

Reactivity for PL-HRP in mouse alimentary epithelium was compared with that for other methods that permit classifying complex carbohydrates into glycosaminoglycans and glycoproteins of neutral, carboxylated and sulfated types (Table 1).

Gastric surface epithelium in both superficial and foveolar regions disclosed neutral glycoprotein with intense periodate reactivity but no basophilia in the apical plasmalemma, adherent secreted mucus, stored mucous droplets and Golgi cisternae. As previously observed (Sato and Spicer 1981 b), Golgi cisternae dis-

**Fig. 1.** Golgi region of a mouse gastric surface epithelial cell. Electron-dense reaction product fills selectively the intermediate Golgi cisternae which are sandwiched by the unstained lamellae at the maturing and forming faces of the stack. Monozonal (upper right) and bizonal (center) mucous droplets and small vesicles (arrowheads) bordering the mature face of the Golgi stack lack affinity for PL-HRP as do the mitochondria at lower left. Glutaraldehyde fixation. Cryostat section immersed overnight in PL-HRP conjugate. Unstained thin section.  $\times 36,000$

**Fig. 2.** Apical portion of a superficial surface epithelial cell of mouse stomach processed as in Fig. 1. Dense reaction product for PL-HRP is located on the luminal surface of the plasmalemma along with adherent mucus. Stored mucous droplets in the apical cytoplasm are difficult to recognize in this uncounterstained section but are barely identifiable by their cores with faint inherent density (arrows).  $\times 28,000$

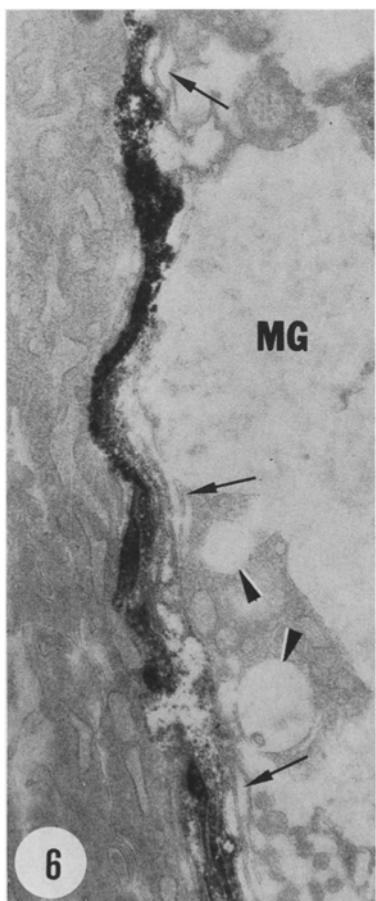
**Fig. 3.** Gastric gland processed as in Fig. 1 except specimen was fixed with paraformaldehyde. Intense staining on the luminal surface and in adherent mucus of the chief cell at right contrasts with the lack of surface staining in the neighboring parietal cell at left. Chief cell zymogen granules ( $Z$ ) show no reactivity.  $\times 28,000$



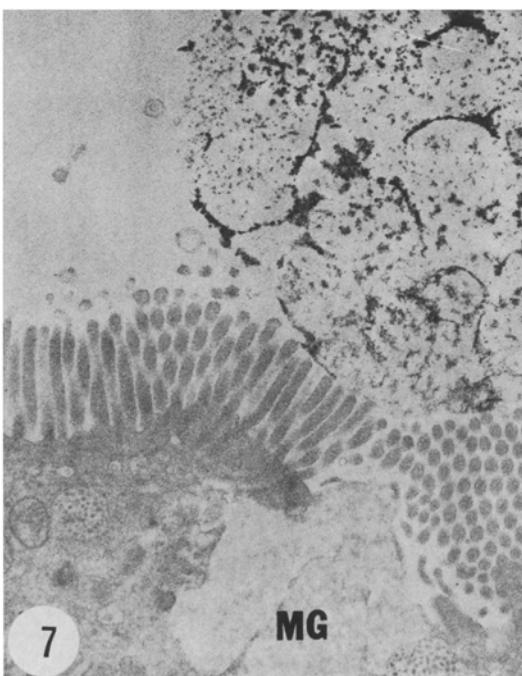
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5



6



7

played progressively more reactivity toward the maturing face in the surface epithelial cells.

Isthmus cells, recently characterized in rat and guinea pig stomachs (Spicer et al. 1978b; Sato and Spicer 1981a), occur in mouse gastric epithelium also. Isthmus cells exhibited sparse apical secretory granules and a thick glycocalyx both of which stained for heavily sulfated periodate reactive glycoprotein (Fig. 11).

Gastric parietal cells revealed a periodate reactive neutral glycoprotein on the luminal surface of the numerous microvilli protruding into the main lumen and intracellular canaliculi. In contrast, the glycocalyx of the basolateral plasma-lemma consisted of nonsulfated, acidic (i.e. carboxylated) complex carbohydrate with periodate reactivity. The acidic glycoprotein on this basolateral plasma membrane reversed the situation prevailing in most epithelia where basophilia of the luminal plasmalemma greatly exceeds that of the basolateral plasmalemma.

The luminal surface of the apical plasma membrane of gastric chief cells evidenced basophilia and periodate reactivity indicative of carboxylated glycoprotein (Fig. 12). Chief cell zymogen granules failed to stain with any of the methods and apparently lacked complex carbohydrate.

Absorptive enterocytes of the jejunum displayed acidic, periodate-reactive complex carbohydrate on the luminal surface of the microvilli (Fig. 13). Paradoxically Golgi cisternae in these cells lacked a capacity to bind the cationic reagents but possessed PA-TCH-SP reactivity.

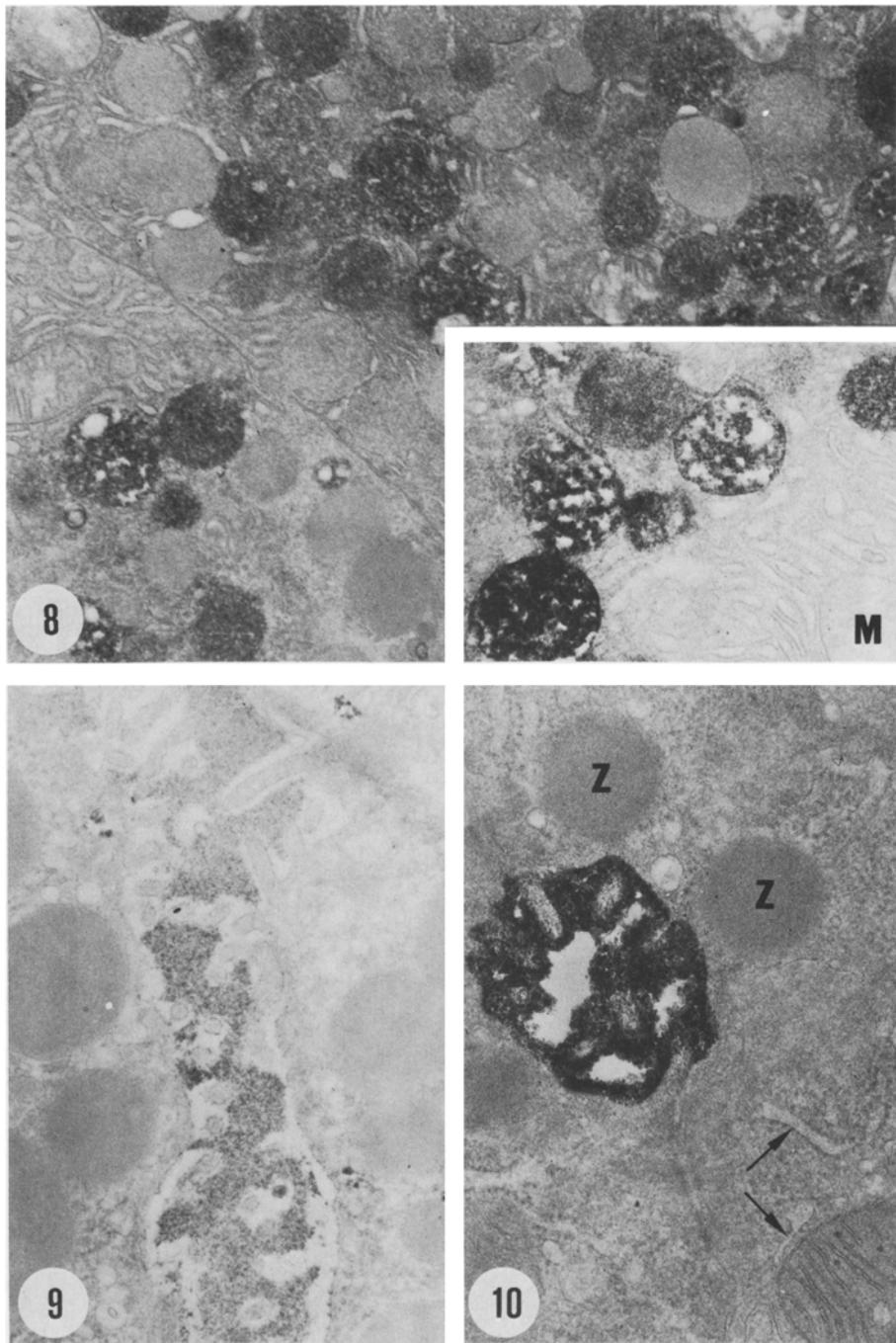
Goblet cells in the jejunum and in the rectosigmoid colon stained similarly. The mucous droplets comprising a goblet in these cells and the forming granules bordering the Golgi zone disclosed moderate to intense DI and HID affinity

**Fig. 4.** Luminal aspect of a jejunal absorptive cell revealing finely particulate reaction product at the surface of the microvilli. Luminal content at lower right also demonstrates PL-HRP affinity. Paraformaldehyde fixation. Cryostat section treated 1 h with PL-HRP. Unstained thin section.  $\times 28,500$

**Fig. 5.** Supranuclear area of a mouse proximal duodenal goblet cell with abundant mucous droplets rimmed in a U by several stacks of Golgi saccules. Electron-dense reaction product is located selectively in the intermediate Golgi cisternae situated between the unreactive saccules of the forming face (convex side) and those of the maturing face of the stack. The mucous droplets surrounded by the Golgi stack lack PL-HRP affinity as does a mitochondrion (*M*). Glutaraldehyde fixation. Cryosection exposed overnight to PL-HRP conjugate. Thin section not stained.  $\times 18,000$

**Fig. 6.** Portion of a colonic goblet cell processed as in Fig. 5 except tissue was fixed with paraformaldehyde. A Golgi complex with heavily stained cisternae at the forming face (on the left) and unstained cisternae at the maturing face (arrows). The mucous goblet (*MG*) and relatively small granules (arrowheads), presumably forming granules, bordering the inside of the Golgi stack also fail to stain (cf. Figs. 15 and 17).  $\times 28,000$

**Fig. 7.** An area of mouse jejunum processed as in Fig. 5 showing a goblet cell between the columnar enterocytes. The mass of mucous droplets extruded into the intestinal lumen (upper right) contains granular reaction product for PL-HRP, contrasting with the lack of reactivity in the stored mucous goblet (*MG*).  $\times 14,000$



and also strong PA-TCH-SP reactivity and, thus, contained sulfated glycoprotein (Figs. 14–17). Golgi saccules rimming the mucous goblets exhibited similar sulfated glycoconjugate in increasing amount in cisternae toward the maturing face.

The luminal epithelial cell and the nongoblet deep crypt cell of the rectosigmoid colon possessed affinity for DI, as previously reported (Wetzel et al. 1966), revealing intense staining in the apical minute vesicles of the luminal epithelial cell, in mucous droplets of the deep crypt cell and in the Golgi complex and luminal surface in both cell types (Table 1). The Golgi elements and apical plasmalemma of these cells failed to stain with HID but consistently disclosed PA-TCH-SP reactivity (Fig. 14) and, accordingly, contained carboxylated glycoprotein. The small apical vesicles of luminal epithelial cells stained similarly for carboxylated glycoprotein but the mucous droplets of the deep crypt cell differed in lacking the expected PA-TCH-SP staining. From its sialidase lability at the light microscope level the mucous droplets here apparently consist of periodate unreactive sialoprotein (Stoward et al. 1980).

The apical plasmalemma of pancreatic acinar cells lacked affinity for the cationic reagents but showed PA-TCH-SP reactivity in a fine layer unlike the heavily PL-HRP-positive coat seen on some cells (Fig. 18, cf. Fig. 10). The other PL-HRP-positive cytologic sites, including zymogen granules and secretion in the intercellular canaliculi, failed to stain with any of the methods employed here. The PL-HRP-negative stratum between apical plasmalemma and luminal secretion appeared unreactive and empty also with the PA-TCH-SP method (Fig. 18, cf. Fig. 9).

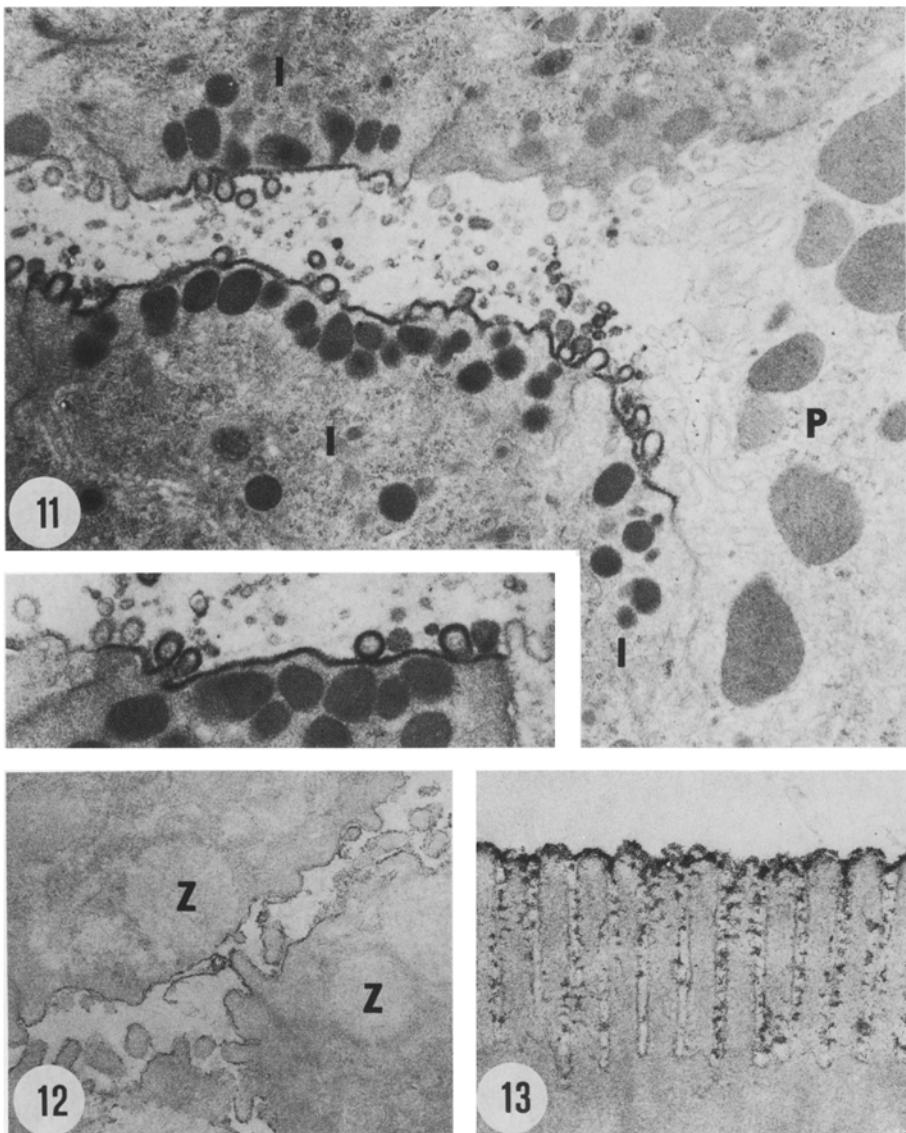
## Discussion

Peanut lectin conjugated with horseradish peroxidase (PL-HRP) has recently been utilized at the light microscopic level to visualize glycoproteins presumably

**Fig. 8.** Portions of the two pancreatic acinar cells processed as for Fig. 5 display many profiles of zymogen granules with a variable degree of PL-HRP affinity. The stained zymogen granules appear randomly distributed among the unreactive ones in the apical cytoplasm.  $\times 13,000$  Inset shows in more detail variable PL-HRP staining of zymogen granules. The intensely densified granules reveal a coarser meshwork of reaction product compared with the less densified ones. The mitochondrion (*M*) and rough ER are unstained.  $\times 17,000$

**Fig. 9.** An intercellular canaliculus between pancreatic acinar cells is filled with densified secretory material of fine granular texture. An electron-lucent space (arrowhead) intervenes between the stained luminal secretion and the unstained, microvillous and intermicrovillous plasmalemma. Paraformaldehyde fixation. Cryostat section immersed 1 h in PL-HRP conjugate. Thin section not stained.  $\times 26,250$

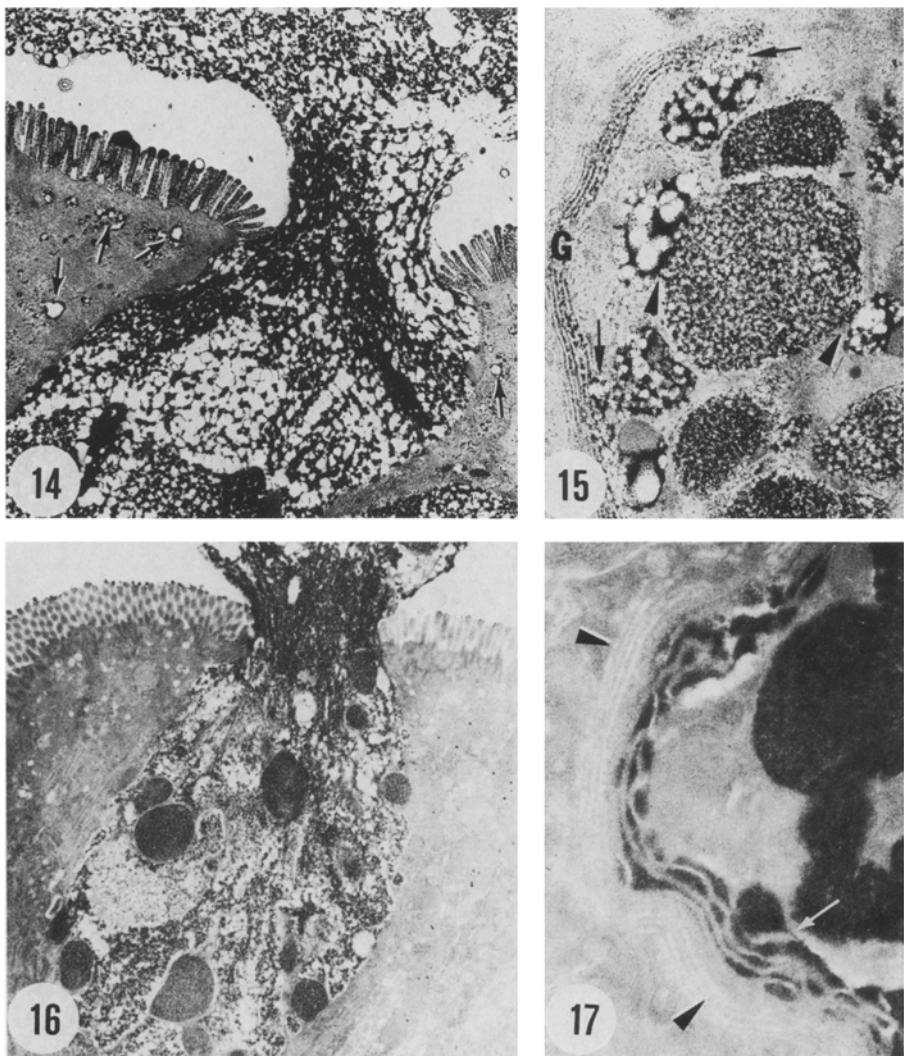
**Fig. 10.** Cross section of an intercellular canaliculus surrounded by two pancreatic acinar cells. This canaliculus, stained in a procedure similar to that for Fig. 9, differs in lacking reaction material free in the lumen and showing a heavily stained layer (arrowhead) on the surface of the microvilli. This reactive stratum corresponds in position with the unstained space denoted by an arrowhead in Fig. 9. Zymogen granules (*Z*) and the mitochondrion at the lower right fail to stain as does the rough surfaced ER (arrows). Glutaraldehyde fixation. Cryostat section stained overnight with PL-HRP. Unstained thin section.  $\times 20,800$



**Fig. 11.** Isthmic region of mouse fundic gland exhibits strong staining for sulfated glycoconjugate on the luminal surface of isthmus cells (*I*), contrasting with the nonreactivity of the surface of a neighboring parietal cell (*P*). Sparse cytoplasmic granules in the isthmus cells are mainly just below the apical plasmalemma and intensely stained. High iron diamine (HID) method.  $\times 12,250$ . A higher magnification inset shows the characteristically thick glycocalyx and sparse apical secretory granules of an isthmus cell both of which contain sulfated glycoprotein with HID affinity.  $\times 16,800$

**Fig. 12.** Gastric chief cells surrounding the lumen in a black mouse stomach reveal staining demonstrative of the presence of acid mucosubstance on the luminal surface of the apical plasmalemma. Zymogen granules (*Z*) lack reactivity. Dialyzed iron (DI) stain.  $\times 22,200$

**Fig. 13.** Luminal portion of a mouse jejunal columnar cell discloses strong staining indicative of acid glycoconjugate in discrete deposits on the tips and sides of the microvilli. DI stain.  $\times 44,300$

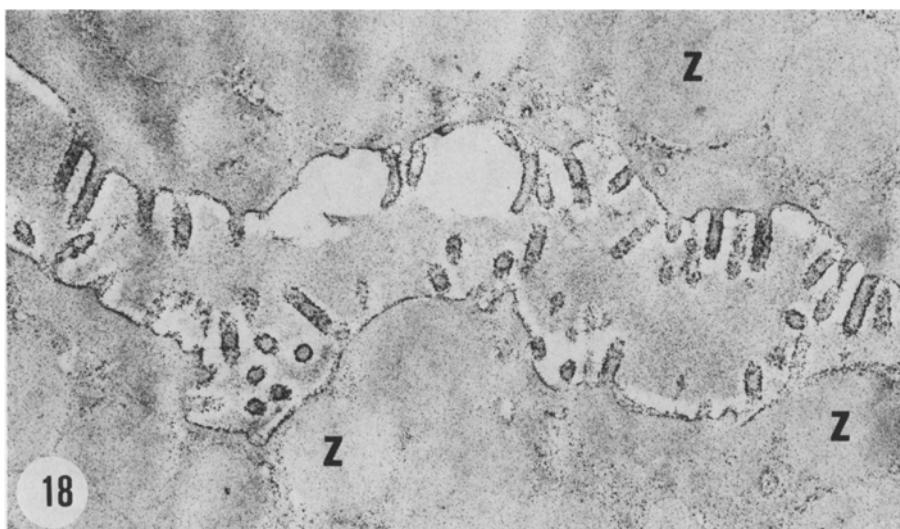


**Fig. 14.** An area of rectosigmoid colon contains a goblet cell filled with stored mucus consisting of a reticulum of intensely reactive glycoprotein. Secreted mucus above has the same staining and texture as that in the mucous goblet. Apical small vesicles (arrows) in the luminal nongoblet epithelial cells (at both sides of the goblet cell) reveal periodate-reactive glycosubstance mainly at their periphery. Surface of the microvilli of the cells also is moderately stained. Periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) sequence.  $\times 7,000$

**Fig. 15.** Golgi cisternae (G) display progressively more reactivity toward the maturing face in a jejunal goblet cell. Presumed forming granules bordering the mature face of the Golgi apparatus enclose stained and unstained foci, and evidence continuity with Golgi cisternae (arrows). These nascent granules appear to fuse also with mucous droplets (arrowheads) that show a tighter reticular pattern of staining. PA-TCH-SP stain.  $\times 15,000$

**Fig. 16.** An area of mouse jejunal mucosa showing a goblet cell sandwiched by columnar enterocytes. Mucous droplets in the goblet cell vary in degree of cytochemical densification. The extruded mucus above shows intense staining. Note the positive material on or attached to the tips of the microvilli of the columnar cells. HID stain.  $\times 5,600$

**Fig. 17.** Portion of a goblet cell of mouse rectosigmoid colon discloses intense staining restricted to the 3-4 cisternae at the maturing face of the Golgi stack. Saccules at the forming face lack reactivity (arrowheads). The innermost cisterna appears continuous with a strongly positive structure (arrow), presumably a nascent granule forming from the cisterna. The edge of the mucous goblet in cytoplasm at the right also stains heavily. HID stain.  $\times 25,300$



**Fig. 18.** Longitudinal section of an intercellular canaliculus of mouse pancreas. The apical plasmalemma of the acinar cells shows a densified surface coat demonstrative of periodate-reactive glycoprotein. Zymogen granules (Z) and secretion in the lumen lack reactivity, raising a question as to the basis for PL-HRP affinity in this site (cf Figs. 8–10). Note the periodate unreactive lucent stratum covering the apical plasmalemma (see also Fig. 9). PA-TCH-SP sequence.  $\times 23,750$

containing oligosaccharide side chains with terminal galactosyl residues (Lotan et al. 1975; Novogrodsky et al. 1975; Stoward et al. 1980). The present study has shown the applicability of the lectin method to demonstration of selective reactivity of cellular organelles at the electron microscopic level. Staining appeared comparable for light and electron microscopy.

#### *Staining of Golgi Cisternae in Relation to Glycosylation Sequence in Glycoprotein Synthesis*

At the ultrastructural level, the PL-HRP staining in cells secreting mucusubstance was localized to central Golgi cisternae which were sandwiched by unreactive cisternae at the forming and maturing faces of the Golgi stack. Since PL-HRP conjugate is considered to bind to terminal galactosyl residues in glycoproteins and the Golgi lamellae are regarded as the main site of stepwise attachment of sugar residues to growing oligosaccharide side chains of the glycoprotein molecules (Neutra and Leblond 1966; Roseman 1970; Letts et al. 1974; Hill et al. 1977; Kramer and Geuze 1977; Schachter et al. 1977), the PL-reactive lamellae in the middle of the Golgi stack might be the sites where galactosyl residues are added to the nascent side chains. PL-HRP negative and PA-TCH-SP-positive and cisternae at the *cis* face of the Golgi complex presumably contain nascent glycoprotein to which galactose has not been added. Consistent lack of staining in the cisternae at the maturing face of Golgi stacks, and also in the secretory product, can be explained as resulting from addition of a terminal residue to subterminal galactose so that the stained cisternae

are those where the side chains transitorily possess terminal galactose. Fucose, mannose, N-acetylhexosamines or sialic acid, which generally comprise the units of the carbohydrate side chains of epithelial secretory glycoprotein (Spiro 1969; Snary and Allen 1971; Letts et al. 1974; Schrager and Oates 1974; Schachter et al. 1977), might be added to galactose at the end of incomplete side chains in the maturing lamellae to mask the PL-HRP reactivity in the complete glycoprotein.

Carbohydrate cytochemical properties of the maturing Golgi cisternae and final secretory product afford information concerning potential candidates for the terminal residues in the oligosaccharide side chains of secretory glycoprotein. In the mouse gastric surface epithelial cells, the presence of PA-TCH-SP reactivity and lack of DI or HID affinity has prompted the suggestion that fucose occupies the terminal position (Sato and Spicer 1981).

In the present study, luminal epithelial cells and nongoblet mucous cells deep in the crypts of the rectosigmoid colon disclosed PA-TCH-SP reactivity and affinity for DI but not for HID in the maturing Golgi cisternae. These observations strongly point to sialic acid as a candidate for the terminal residue in the final secretory product in these two cell types. This interpretation fits with the loss of basophilia in the mucous cells at the light microscopic level following sialidase digestion (Spicer 1965).

In proximal duodenal and colonic goblet cells, the Golgi cisternae reacted positively with the PA-TCH-SP method and evidenced affinity for the basic DI and HID reagents. In this case a sulfated residue might cap galactose in the terminal position but terminal sulfated sugars are not well documented. A terminal sialic acid in sulfated glycoprotein is not detectable by basic dye methods (Poddar and Jacob 1979) but a terminal sialic acid and subterminal galactose has been demonstrated in the sulfated glycosubstance of colonic goblets by the induction of PL-HRP reactivity following sialidase digestion (Stoward et al. 1980). The PL-HRP staining of Golgi cisternae in colonic goblet cells, thus, apparently reveals galactose residues transitorily terminal prior to addition of sialic acid.

#### *Mucous Secreting Cells Lacking PL-HRP Positive Golgi Cisternae*

All types of mucus secreting cells surveyed here, except the gastric isthmus cell, have shown PL-HRP affinity in Golgi saccules by both light and electron microscopy. Absence of staining of any Golgi cisterna in isthmus cells of the mouse and rat can be regarded as evidence that the secretory glycoprotein (Schulte and Spicer unpublished observations) produced in this cell differs from that in the other mucous cells in being devoid of galactose. If PL-HRP staining of Golgi cisternae demonstrates sites of galactosyl transferase activity as suggested, the absence of reactive Golgi cisternae in a secretory cell indicates production of galactose-free secretory glycoprotein. Isthmus cells in rat and guinea pig differ markedly from other gastric secretory cells in containing highly sulfated, apparently nonsialylated glycoprotein, in their secretory granules and on the apical plasmalemma (Spicer et al. 1978b; Sato and Spicer 1981a). The

apparent absence of galactose and of sialic acid from isthmus cell glycoproteins are compatible observations because neuaminic acid consistently occupies a terminal position with attachment to penultimate galactose in histochemically demonstrable sialomucins (Stoward et al. 1980).

The lack of PL affinity in the intracellular secretion contrasts with occasional reactivity at the intestinal luminal surface and densification of extruded caps of goblet mucus, suggesting that degradation of oligosaccharide side chains of glycoprotein occurs in the lumen. As previously noted (Sato and Spicer 1981b), the PL-HRP reactivity of the luminal surface and adherent secretion could be attributed to removal of a terminal residue masking galactose in the complete glycoprotein through cleavage by enteric bacterial glycosidases in the lumen. The intestinal tract supports complex bacterial populations (Savage et al. 1968), and many species of enteric bacteria are known to produce glycosidases (Hawksworth et al. 1971; Prizont et al. 1976).

#### *PL-HRP Staining of Zymogen Cells in the Pancreas*

In pancreatic acini the PL-HRP staining indicative of galactose residues in zymogen granules and secreted material in the intercellular canaliculi contradicted the lack of reactivity for *vicinal* glycol-containing hexoses with the PA-TCH-SP method. Galactose possesses a hydroxyl radical on C-2 and C-3, and this *vicinal* glycol should be oxidized to dialdehyde with periodic acid (van Lis and Kalsbeek 1975) and show PA-TCH-SP reactivity. Since PL binding is thought to require a hydroxyl at C-2 of galactose (Lotan et al. 1975; Stoward et al. 1980), the PA-TCH-SP-negativity and PL-HRP-positivity could be explained on the basis of terminal galactose with a substituent or linkage to the neighboring sugar residue at C-3. Difference in sensitivity of the PL-HRP and PA-TCH-SP methods seems unlikely to explain the discrepancy.

Pancreatic acinar cells have long been considered the classic example of a purely serous cell because of the lack of demonstrable carbohydrate in zymogen granules (Munger 1964). However, biochemical analysis of bovine and porcine pancreatic juice has revealed the presence of glycoproteins low in carbohydrate in zymogen granules, and has identified the glycoprotein as an isoenzyme of ribonuclease containing fucose, mannose, galactose, and some aminosugars (Plummer and Hirs 1963; Plummer 1968; Reinhold et al. 1968). Furthermore, radioautography has shown uptake of  $H^3$ -galactose in the Golgi region of rat acinar cells (Neutra and Leblond 1966). Such observations support the PL-HRP evidence for glycoprotein in pancreatic secretion. Zymogen granules in some acinar cells resembled luminal secretion in reactivity with the PL-HRP method as could be expected since the luminal material derives from the granules (Marshall 1954; Munger 1973). The staining of a stratum on the luminal surface of microvilli in pancreatic intercellular canaliculi alternated with staining of content free in the lumen. No explanation for this reciprocal relationship can be offered other than to suggest that this variability may relate to stages of surface adherence of zymogen granule content after secretion or to a difference in histologic location. Granules of some cells failed to stain but this very likely reflected nonpenetration by the conjugate as stained cells lay near the block

edge and the granules stained fairly uniformly in light microscopic paraffin sections after Carnoy fixation (Stoward et al. 1980). Electron microscopic radioautography with labeled galactose tracing isotope into granules would provide direct evidence for glycoprotein in pancreatic zymogen secretion.

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