

DISTRIBUTION OF CELL SURFACE SACCHARIDES ON PANCREATIC CELLS

II. Lectin-Labeling Patterns on Mature Guinea Pig and Rat Pancreatic Cells

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ABSTRACT

The surface saccharide composition of collagenase-dispersed pancreatic cells from adult guinea pig and rat glands was examined by using eight lectins and their ferritin conjugates: Concanavalin A (ConA); Lens culinaris (LCL); Lotus tetragonolobus (LTL); Ricinus communis agglutinins I and II (RCA I, RCA II); Soybean agglutinin (SBA); Ulex europeus lectin (UEL); and wheat germ agglutinin (WGA). Binding studies of iodinated lectins and lectin-ferritin conjugates both revealed one population of saturable, high-affinity receptor sites on the total cell population (~95% acinar cells). Electron microscopy, however, revealed differences in lectin-ferritin binding to the plasmalemma of acinar, centroacinar, and endocrine cells. Whereas acinar cells bound heavily all lectin conjugates, endocrine and centroacinar cells were densely labeled only by ConA, LCL, WGA, and RCA I, and possessed few receptors for LTL, UEL, and SBA. Endocrine and centroacinar cells could be differentiated from each other by using RCA II, which binds to centroacinar cells but not to endocrine cells.

Some RCA II receptors appeared to be glycolipids because they were extracted by ethanol and chloroform-methanol in contrast to WGA receptors which resisted solvent treatment but were partly removed by papain digestion. RCA I receptors were affected by neither treatment. The apparent absence of receptors for SBA on endocrine and centroacinar cells, and for RCA II on endocrine cells, was reversed by neuraminidase digestion, which suggested masking of lectin receptors by sialic acid. The absence of LTL and UEL receptors on endocrine and centroacinar cells was not reversed by neuraminidase.

We suggest that the differential lectin-binding patterns observed on acinar, centroacinar, and endocrine cells from the adult pancreas reflect surface-carbohydrate-developmental programs expressed during morphogenesis and cytodifferentiation of the gland.

KEY WORDS lectin-ferritin conjugates
pancreatic cells—acinar, centroacinar, endocrine
surface saccharides · differential lectin binding

Numerous studies that utilized lectins conjugated to probes visualizable by light or electron microscopy have detected differences in cell surface saccharides among cells from different organs and within a given population of cells as a function of transformation or developmental stage (45; reviewed in reference 34). In many cases the results have been derived from the use of one or a limited number of lectins. To our knowledge, however, no reports have shown differences in cell surface saccharide composition among different cells in the same adult organ. In the mammalian pancreas, this question becomes of interest because the secretory portion of the gland is comprised of three different cell types—acinar, centroacinar and duct cells, and endocrine cells—each involved in different aspects of the gland's exocrine and endocrine function.

To determine whether cell surface saccharide patterns reflect functional specialization of the three classes of cells, we utilized a variety of saccharide-specific lectin-ferritin conjugates, described in the accompanying paper (28), as probes for the plasmalemma of populations of dispersed cells from the adult guinea pig and rat pancreas. The results presented in this paper indicate that the three cell types can be distinguished from each other based on differential lectin binding. So far as we know, this is the first example in which a battery of lectins has been used to characterize different cells from the same organ. This finding has additional implications because the three main cell types of the gland appear to arise from a morphologically homogeneous cell population in the developing pancreatic rudiment (39). Lectin binding at different stages of pancreatic morphogenesis may help define the developmental program for differentiation of the cell surface as studies under way in our laboratory already indicate (27), and may provide clues to potential roles of cell surface saccharides in cell-cell interactions that likely accompany morphogenesis.

MATERIALS AND METHODS

Dissociation Procedure

Cells from adult guinea pig and rat pancreas were obtained by a modification of the tissue dissociation method of Amsterdam and Jamieson (3) which included a decrease by half of collagenase and chymotrypsin

concentrations previously used and omission of hyaluronidase during tissue digestion. For later experiments, chymotrypsin was omitted and the dissociation was carried out only with collagenase devoid of other proteolytic activity (15). A reasonable yield of single cells was obtained with this procedure (5). Dissociated cells from weanling rat pancreas were obtained by collagenase treatment exclusively.

Preparation of Lectins and Lectin-Ferritin Conjugates

Procedures for the purification of lectins and lectin-ferritin conjugates and for radioiodination of lectins with ^{125}I are described in detail in the accompanying paper (28).

Binding Assays

Suspensions of unfixed cells in Krebs-Ringer bicarbonate solution (KRB)¹ which contained 1% bovine serum albumin (BSA) were incubated with either native lectins or the lectin-ferritin conjugates for 30 min at 4°C. Controls for nonspecific binding included 0.2 M of the appropriate hapten sugar. After incubation, the cell suspension was layered over a cushion of KRB which contained 4% BSA and was then centrifuged for 3 min at 150 g at 4°C to sediment cells through the cushion and separate them from unbound reagents. The pellet was subsequently resuspended in KRB-1% BSA and counted in a gamma spectrometer (Beckman Biogamma II, Beckman Instruments Inc., Fullerton, Calif.). Cell number was determined with a hemocytometer.

Extraction of Membrane Lipids with Chloroform Methanol

To remove lipids, and more specifically glycolipids, without destruction of cell morphology and without extensive extraction of membrane proteins, the following procedure was used. Suspensions of dispersed cells from guinea pig pancreas, washed as described above, were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 30 min at room temperature. After repeated washes with KRB-0.1 M glycine, they were enzymatically radioiodinated by the method of Hubbard and Cohn (16). After several washes in KRB-1% BSA the cells were dehydrated at room temperature with two washes of 70% ethanol followed by two additional washes with 95% ethanol (37). They were then extracted for 10 min at room temperature with 20 vol of the lower phase of an 8:4:3 mixture of chloroform, methanol, and an aqueous solution which contained

¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; ConA, concanavalin A; KRB, Krebs-Ringer bicarbonate solution; RCA I and II, Ricinus communis agglutinins I and II; SBA, soy bean agglutinin; WGA, wheat germ agglutinin.

0.4% CaCl_2 and 0.74% KCl (12, 46). After centrifugation for 5 min at 800 g, the cells, which were floating at the surface, were added to 2 vol of 95% ethanol and subsequently pelleted by centrifugation at 800 g for 5 min. They were then washed once more with 95% ethanol and progressively rehydrated by two washes in 70% ethanol followed by two washes in 35% ethanol before transfer to 1% BSA in KRB (37). The supernates of the different ethanol treatments before and after chloroform-methanol extraction were pooled and centrifuged at 10,000 g to remove cell debris. The chloroform-methanol extract, centrifuged at 10,000 g to remove cell debris, was washed with 0.2 vol of a solution which contained 0.04% CaCl_2 , 0.034% MgCl_2 , and 0.74% KCl . The total radioactivity in ethanol, chloroform-methanol, and aqueous fractions represented 38% of the total initial counts. The various extracts were then evaporated to dryness in a rotatory evaporator (Buchler Instruments Div., Searle Diagnostics Inc., Fort Lee, N. J.) before acid hydrolysis in 0.1 N H_2SO_4 at 80°C for 1 h. The hydrolysates were adsorbed on a Dowex AG 1 \times 8 column (Dow Chemical Co., Midland, Mich.) in its formate form, and sialic acid was eluted from the column with 0.3 N formic acid (47). The amount of sialic acid was determined by the Warren reaction (51). The content of sialic acid in control and solvent-extracted cells was determined by a similar procedure.

Papain Digestion

Isolated guinea pig pancreatic cells, previously fixed and radioiodinated as above, were resuspended in 0.05 M Tris-HCl, pH 8.4, which contained 0.01 M cysteine. They were then digested with 0.3% papain (Calbiochem, San Diego, Calif.) for 1 h at 37°C, harvested by centrifugation at 800 g for 5 min, and the supernate was removed. The cells were then washed twice by centrifugation and resuspension in KRB-1% BSA. These two additional washes and the supernate from the first centrifugation were pooled and counted to determine the extent of the digestion.

Neuraminidase Treatment

Dispersed unfixed guinea pig pancreatic cells were incubated for 1 h at 37°C with neuraminidase (Type IV, Sigma Chemical Co., St. Louis, Mo.) from *Clostridium perfringens* in buffer at a final concentration of 0.5 U/ml. At the end of the incubation period, the cell suspension was centrifuged and washed with 2 ml of KRB-1% BSA. The supernates from the two centrifugations were pooled and lyophilized. The amount of sialic acid released by enzymatic treatment was measured by the thiobarbituric reaction (51). The total amount of sialic acid present in intact cells before digestion as well as the amount not removed by neuraminidase digestion was measured by the same reaction after these steps: acid hydrolysis of the cells at 80°C for 1 h with 0.1 N H_2SO_4 , adsorption of the hydrolysate on a Dowex AG 1 \times 8 column in its formate form as previously described,

and sialic acid elution with 0.3 N formic acid (47). The recovery of sialic acid during the different steps of hydrolysis and ion-exchange chromatography was estimated by adding traces of [^3H]N-acetyl neuraminic acid (New England Nuclear, Boston, Mass.) to the cells before acid hydrolysis.

Preparation of the Cells for Electron Microscopy

Separated cells, after incubation with lectin-ferritin conjugates and subsequent washes through the KRB-BSA cushion as described above under binding assays, were fixed by mixing 1 vol of cell suspension in KRB-1% BSA with 1 vol of 4% glutaraldehyde in 0.2 M Na cacodylate buffer, pH 7.4 (3). After fixing for 1 h at 25°C or overnight at 4°C, the suspensions were centrifuged in polyethylene tubes (Beckman Microfuge 152, Beckman Instruments, Inc.) at 10,000 g for 5 min. Disks of cell pellets cut from the tubes with a razor blade (3) were postfixed for 1-2 h at 4°C with 1% OsO_4 in 0.1 M Na cacodylate, pH 7.4, washed once with 0.15 M NaCl, and stained in block for 1 h at 23°C with 0.5% magnesium uranyl acetate in 0.15 M NaCl. The pellets were then dehydrated in ethanol and propylene oxide and embedded in Epon (26), Epon-Araldite (30), or Spurr's medium (48). Before observation in a Philips 301 electron microscope, thin sections were stained either with bismuth subnitrate (2) or doubly stained with uranyl acetate and lead citrate (21).

RESULTS

Binding of Lectins to Dispersed Guinea Pig Pancreatic Cells

Before binding parameters for lectins and lectin-ferritin conjugates to dispersed pancreatic cells were measured, the time required to reach binding equilibrium was determined and the proportionality between amount of ligand bound and number of cells in the assay was established for each lectin. Data not shown indicate that binding equilibrium was reached at 4°C within 15 min, and that at the highest concentrations of lectins used in the assays, binding was proportional to cell concentration up to 7×10^6 cells/ml. Consequently, equilibrium binding assays contained less than 7×10^6 cells/ml and were carried out for 30 min at 4°C.

Fig. 1 shows a typical binding curve for native lectin and its ferritin conjugate to pancreatic cells, in this example the binding of *Ricinus communis* agglutinin (RCA) II. As can be seen, binding of both RCA II and its conjugate approaches saturation and the level of nonspecific binding in the presence of 0.2 M hapten sugar (N-acetyl galactosamine) does not exceed 7%. Similar binding

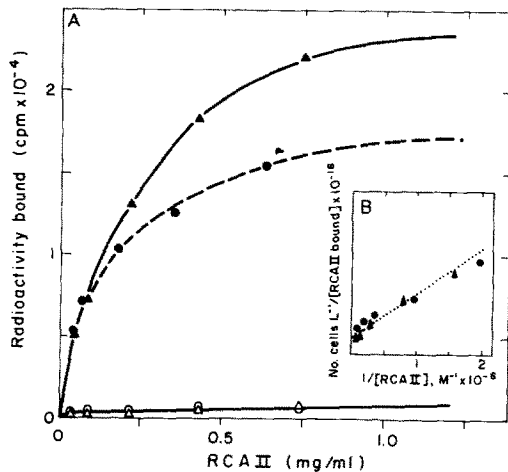


FIGURE 1 Binding of ¹²⁵I RCA II and its ferritin conjugate to dispersed cells from guinea pig pancreas. (A) ▲—▲ ¹²⁵I RCA II; ●—● ¹²⁵I RCA II-ferritin conjugate; △—△ ¹²⁵I RCA II in presence of 0.2 M N-acetyl galactosamine; ○—○ ¹²⁵I RCA II-ferritin conjugate in the presence of 0.2 M N-acetyl galactosamine. Note that the lectin-ferritin conjugate and the native lectin exhibit similar extents of nonspecific binding. (B) The same data as shown in Fig. 1A plotted by the method of Steck and Wallach (49). No. of cells per liter/[bound RCA II] is the concentration of pancreatic cells/liter divided by the molar concentration of RCA II, or RCA II-ferritin conjugate bound to cells calculated from bound radioactivity; 1/free RCA II, or 1/free RCA II-ferritin conjugate is the amount of unbound lectin in the binding assay after subtraction of the amount of lectin or conjugate bound. $K_a = -1/[\text{free RCA II}]$ to 0 provides a value for 1/number of moles of [RCA II] bound per cell. ▲—▲ Specific binding of ¹²⁵I RCA II; ●—● Specific binding of ¹²⁵I RCA II-ferritin conjugate. Note that the data points are fitted by a straight line for the lectin and its conjugate, which indicates, on the one hand, one class of noninteracting binding sites and, on the other hand, very close values for K_a for both the lectin and its conjugate.

properties were obtained with the other lectins used: nonspecific binding never exceeded 5–10% at saturation levels of lectins and their ferritin conjugates except for wheat germ agglutinin (WGA) where background levels of about 30% were found. This is likely due to binding of WGA to sialic acid residues (14, 41) which could not be competed for by N-acetyl glucosamine alone. Specific equilibrium binding (e.g., Fig. 1) was plotted by the method of Steck and Wallach (49) as shown, or by the method of Scatchard (44). Both types of plots were in agreement and indicated that for RCA II (and all other lectins used in this

study), the cell preparation possessed a single population of apparently noninteracting receptor sites because the data points were fitted by a straight line. (These data differed from Dionne and Beaudoin's results [11] which revealed two classes of binding sites for Concanavalin A [ConA].) From these plots we derived both the number of lectin binding sites per cell and their affinity constants as indicated in Table I. It can be seen that while quantitative differences exist among the lectins, both the number of receptor sites per cell and their apparent affinity constants are within approximately an order of magnitude. Table I also shows that the ferritin-lectin conjugates exhibit generally similar binding characteristics compared to those of native lectins, which suggests that our conjugation procedure (28) does not markedly modify the binding properties of lectins. Finally, we should note that the quantitative binding data reflect mainly the lectin binding properties of acinar cells because these comprise about 95% of the cells in the dispersed cell preparation (3). However, as will be mentioned later, the number of receptor sites per acinar cell measured by radioligand binding was likely overestimated because the labeling densities for all lectins are quantitatively similar as determined by counting ferritin particles bound per cell on electron micrographs. The overestimation of lectin binding sites is most likely explained by the trapping and binding of the ligands to fragments of connective tissue, basement membranes, and cell debris which inevitably contaminate the cell pellet observed by electron microscopy. Quantitative binding of lectins to dispersed rat pancreatic cells was not carried out.

Cell Surface Properties of Acinar Cells

After labeling with saturating concentrations of lectin-ferritin conjugates specified in the figure legend, thin sections of the dispersed cell populations from the guinea pig pancreas were observed in the electron microscope. All lectin-ferritin conjugates were homogeneously distributed on the plasmalemma except for ConA- and Lens lectin-ferritin conjugates which appeared to be distributed in patches. No preferential localization of the conjugates to the apical or basal and lateral plasmalemmal regions of the cell surface were detected (Fig. 2 and 3). In general, the density of ferritin labeling appeared to be qualitatively similar for all lectins used. In some instances, however, when basement membrane was not visibly

TABLE I
Binding Properties of Lectins and Lectin-Ferritin Conjugates to Dispersed Guinea Pig Pancreatic Cells

Lectin	Hapten Sugar	No. of molecules* per cell	Affinity constant M ⁻¹
Concanavalin A	glucose, mannose	0.6 × 10 ⁷ (4)‡	6 × 10 ⁵
ConA-ferritin		2 × 10 ⁷ (3)	2 × 10 ⁵
Lens culinaris lectin	glucose, mannose	3 × 10 ⁷ (2)	2 × 10 ⁶
Lens lectin-ferritin		6 × 10 ⁷ (2)	3 × 10 ⁶
Wheat germ agglutinin	N-acetyl glucosamine	8 × 10 ⁷ (4)	8 × 10 ⁵
WGA-ferritin		2 × 10 ⁷ (3)	2 × 10 ⁵
Ricinus communis agglutinin I	galactose	3 × 10 ⁷ (4)	2 × 10 ⁶
RCA I-ferritin		2 × 10 ⁶ (3)	2 × 10 ⁶
Ricinus communis agglutinin II	N-acetyl galactosamine, galactose	5 × 10 ⁷ (2)	3 × 10 ⁵
RCA II-ferritin		3 × 10 ⁷ (2)	8 × 10 ⁵
Soybean agglutinin	N-acetyl galactosamine, galactose	2 × 10 ⁶ (3)	9 × 10 ⁵
§BA-ferritin		ND§	ND
Ulex Europeus lectin	fucose	3 × 10 ⁶ (3)	7 × 10 ⁶
Ulex lectin-ferritin		9 × 10 ⁶ (3)	1 × 10 ⁶
Lotus tetragonolobus lectin	fucose	5 × 10 ⁶ (2)	2 × 10 ⁶
Lotus lectin-ferritin		1 × 10 ⁶ (2)	1 × 10 ⁶

* Data are for specific binding only; nonspecific binding as determined in the presence of 0.2 M appropriate hapten sugar has been subtracted.

† Figures in parentheses indicate the number of separate experiments carried out; data are averages from these experiments.

§ Not determined.

digested by collagenase treatment during cell preparation, dense labeling of the former basal plasmalemmal region of the cell occurred.

To obtain a more precise indication of lectin receptor sites per cell detected by electron microscopy, we counted the number of ferritin particles bound per linear length of plasmalemma (principally basolateral plasmalemma which represents 95% of the acinar cell surface area) (8) and, by assuming a section thickness of 50 nm, we were able to calculate the density of ferritin-lectin binding sites per unit cell surface. Given that the surface area of the guinea pig pancreatic acinar cell is 580 μm² (8), we then estimated the total number of lectin-ferritin binding sites per cell. These results are given in Table II.

Control experiments for all morphologic studies consisted of incubating cells with lectin-ferritin conjugates in the presence of 0.2 M hapten sugar. As shown in Fig. 4A, which is representative of all lectins studied except WGA (Fig. 4B), the cell membrane appears almost totally devoid of ferritin particles, which indicates low nonspecific binding.

Dispersed acinar cells from adult rat pancreas were also labeled with the same lectin-ferritin conjugates as applied to guinea pig pancreatic cells with the same results (Fig. 5). Again, as for

guinea pig cells, the distribution of ferritin particles did not reveal any marked differences in the density of lectin binding sites between the apical and basolateral surfaces of cells. When, however, acinar cells from newborn and weanling rat pancreas were examined after exposure to ferritin conjugates of RCA I, WGA, and Lotus lectin, a much higher density of labeling was found on the apical plasmalemmal region compared to that of the basolateral plasmalemma (Fig. 6A and B).

Nature of the Lectin Receptor on Pancreatic Acinar Cells

Because it has been shown that lectins can potentially bind to membrane glycoproteins as well as to membrane glycolipids (7, 10, 40, 41, 42, 50), we attempted to determine the nature of lectin receptors on the plasmalemma of acinar cells by treatment of the cells with papain or after chloroform-methanol extraction of membrane lipids. Our results indicated that papain digestion released ~80% of the radioactivity bound to the surface of pancreatic cells which had previously been fixed and radioiodinated. The papain-treated cells were then labeled with WGA, RCA I and II, and their ferritin conjugates because the hapten sugars for these lectins are common to glycopro-

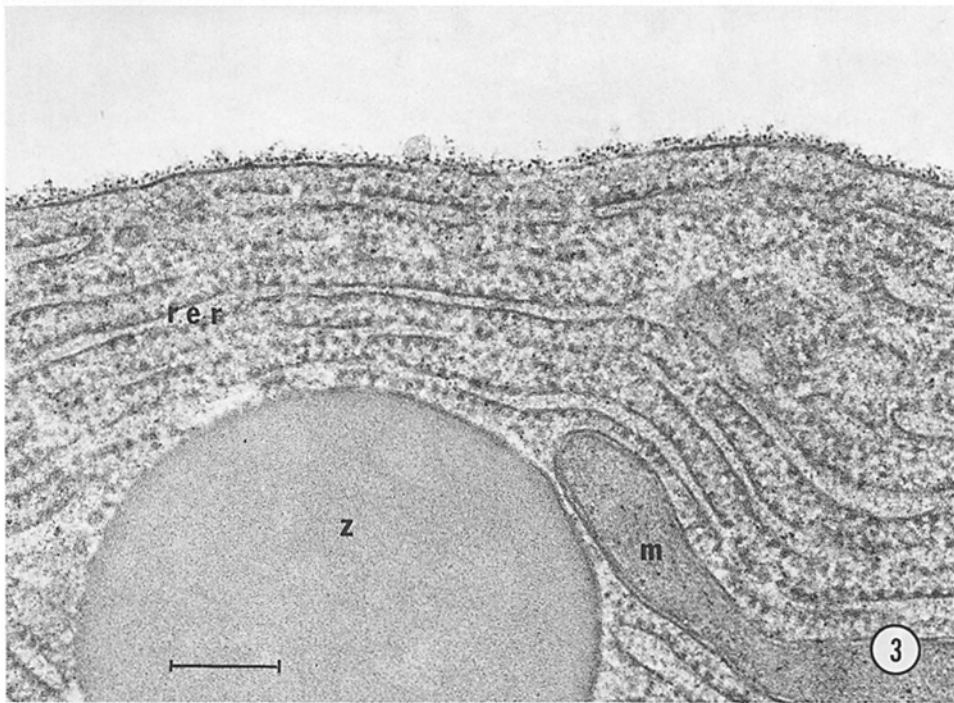
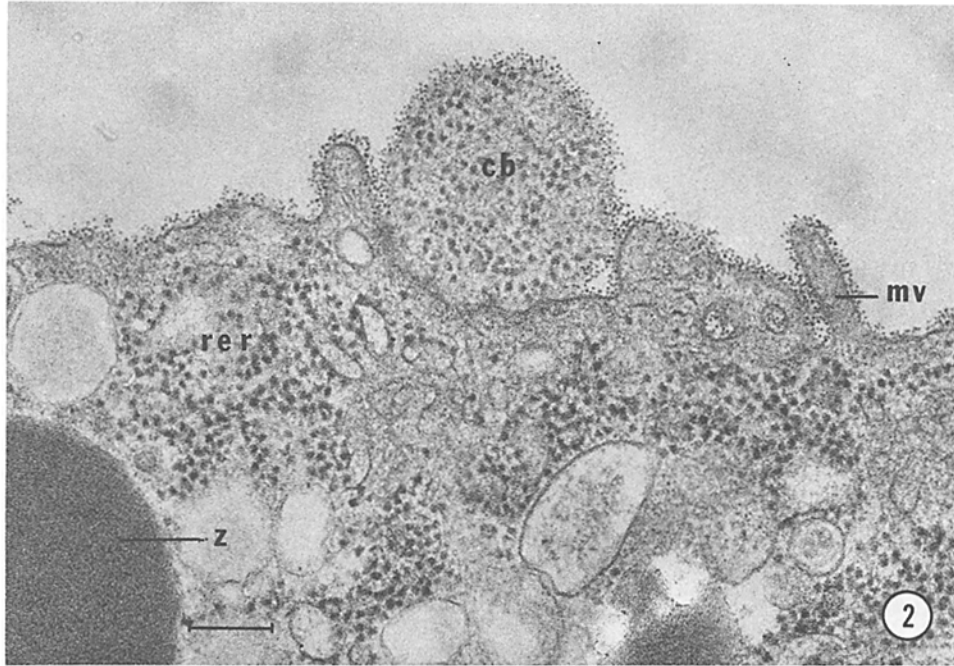


FIGURE 2 Apical portion of an acinar cell labeled with WGA-ferritin conjugate. The cell was incubated at 4°C for 30 min with a saturating concentration of conjugate (0.49 mg/ml). Note the high density of ferritin particles on the plasma membrane. The apical region is easily recognized by the presence of microvilli (*mv*) and zymogen granules (*z*). Rough endoplasmic reticulum (*rer*); cytoplasmic blebs (*cb*) derived from neighboring cells. Section stained with Bismuth subnitrate. $\times 53,000$. Bar, 0.2 μm .

FIGURE 3 Electron micrograph which shows an acinar cell after incubation with a saturating concentration of RCA I-ferritin conjugate (0.67 mg/ml). Note the heavy binding of the conjugate to the cell surface. Zymogen granules (*z*); rough endoplasmic reticulum (*rer*); mitochondria (*m*). Section stained with uranyl acetate and lead citrate. $\times 65,000$. Bar, 0.2 μm .

TABLE II
Density of Ferritin-Lectin Conjugates on the Surface of Pancreatic Cells

Cell type	Lectin conjugate				
	WGA*	RCA I*	RCA II	Lotus	SBA
	<i>ferritin particles/μm²</i>				
Acinar	1871 ± 380 (n = 5) (1.1 × 10 ⁶)‡	1841 ± 135 (n = 5) (1.1 × 10 ⁶)‡	1483 ± 196 (n = 5) (0.9 × 10 ⁶)‡	1616 ± 301 (n = 5) (0.9 × 10 ⁶)‡	1683 ± 178 (n = 5) (1.0 × 10 ⁶)‡
Endocrine	1671 ± 209 (n = 5)	1367 ± 99 (n = 5)	459 ± 189§ (n = 5)	111 ± 70 (n = 5)	164 ± 85 (n = 5)
Centroacinar	2139 ± 133 (n = 4)	1767 ± 164 (n = 5)	1832 ± 386 (n = 5)	80 ± 12 (n = 4)	124 ± 44 (n = 5)

Data are given ± SD; n, number of cells counted.

* Although some differences in label density were seen between the cell types, these differences were not as significant as those indicated above.

‡ Total number of lectin-ferritin particles per cell given the surface area of an acinar cell ≈ 580 μm² (8).

§ Labeling of endocrine cells was significantly less than that of both acinar and centroacinar cells. *P* < 0.0001. Student's *t* test.

|| Both endocrine and centroacinar cells were labeled significantly less than acinar cells. *P* < 0.0001. Student's *t* test.

tein and glycolipid carbohydrate chains (24, 31, 36). Equilibrium binding assays were carried out with the iodinated lectins on cells similarly treated with papain but not iodinated. The data plotted by the method of Steck and Wallach (49) indicated that 24% of WGA receptors and 20% of RCA II binding sites had been removed by papain digestion. In contrast, the number of RCA I receptor sites had decreased by <2% after proteolysis.

To estimate the extent of binding of the lectins to glycolipids, we extracted fixed, radioiodinated pancreatic cells with ethanol and chloroform-methanol as described under Methods. This treatment led to the extraction of 38% of the total radioactivity with 29% appearing in the ethanol extract, 6% in the chloroform-methanol extract, and 3% in the aqueous solution. The remainder was presumably associated primarily with plasmalemmal proteins. The radioactivity in the aqueous solution was assumed to be distributed among gangliosides, glycoproteins, and free iodine. The extent of protein extraction by the solvent treatments was not determined.

The extent of glycolipid extraction was approximated by measuring the amount of sialic acid in the organic-solvent and aqueous-extract phases, given the assumption that extractable sialic acid is primarily associated with gangliosides. 35% of the total sialic acid content in the cell preparation was found in the extracts with 81% of that associated

with the ethanol extract. When labeled with lectin-ferritin conjugates, the chloroform-methanol-treated cells showed a loss of RCA II receptors on the cell surface (Fig. 7A). However, the number of WGA and RCA I (Fig. 7B) receptors was unaltered. This result suggests that RCA II binds preferentially to glycolipids on the surface of pancreatic acinar cells.

Cell Surface Properties of Endocrine and Centroacinar Cells

In contrast to the uniform labeling of acinar cells by the lectin-ferritin conjugates used in this study, distinct differences in lectin binding were observed on endocrine and centroacinar cells² which account for the remaining 2-5% of cells in our dispersed cell preparation.³ Thus, while endocrine A and B cells bind WGA- and RCA I-ferritin conjugates extensively, and to a lesser extent those of ConA, very few ferritin particles

² In our study, centroacinar cells are by definition cells which, associated with acinar cells by junctional complexes, contain abundant mitochondria and possess numerous apical microvilli which become distorted and arborized after cell dispersion.

³ During our cell dispersion procedure, blood vessels and intact ducts are filtered out. Very few recognizable ducts and endothelial cells contaminate the cell preparation. Labeling of these cells by the lectins is not considered in the paper.

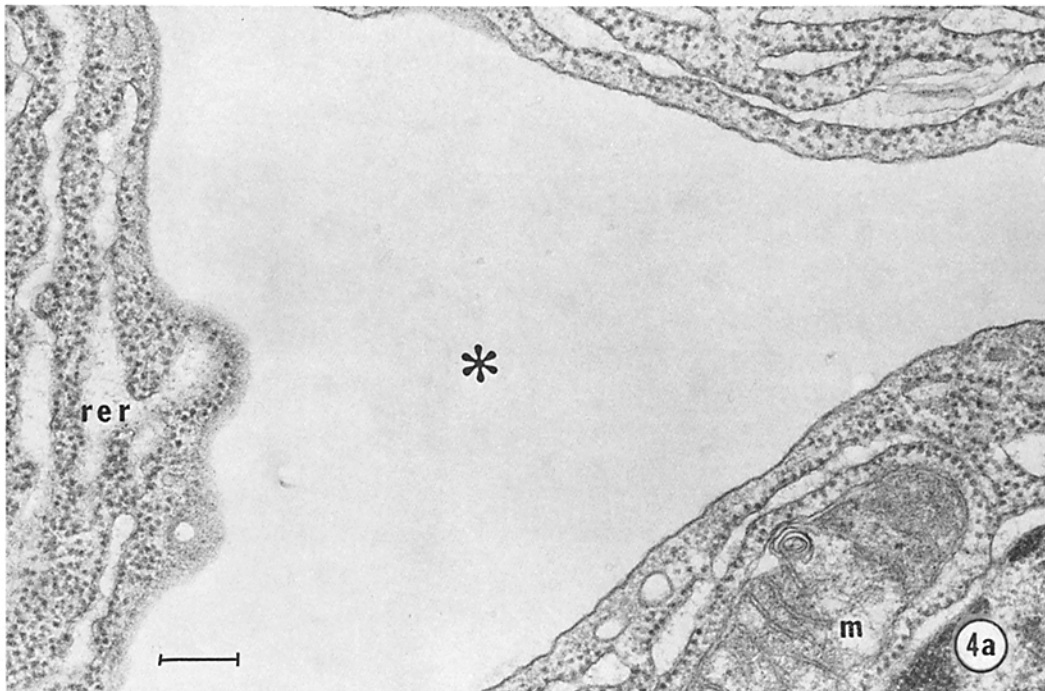


FIGURE 4 Control experiments in which pancreatic cells were incubated with lectin-ferritin conjugates in the presence of hapten sugar. Note the absence of ferritin particles on the surface of the acinar cells incubated with RCA I-ferritin conjugate in presence of 0.2 M galactose (Fig. 4a). However, Fig. 4b shows some ferritin particles on the acinar cell surface after labeling with WGA-ferritin conjugate in the presence of 0.2 M N-acetyl glucosamine. Rough endoplasmic reticulum (*rer*); mitochondria (*m*); intercellular space (*). Bismuth subnitrate section staining. Fig. 4a \times 42,000. Fig. 4b \times 67,500. Bar, 0.2 μ m.

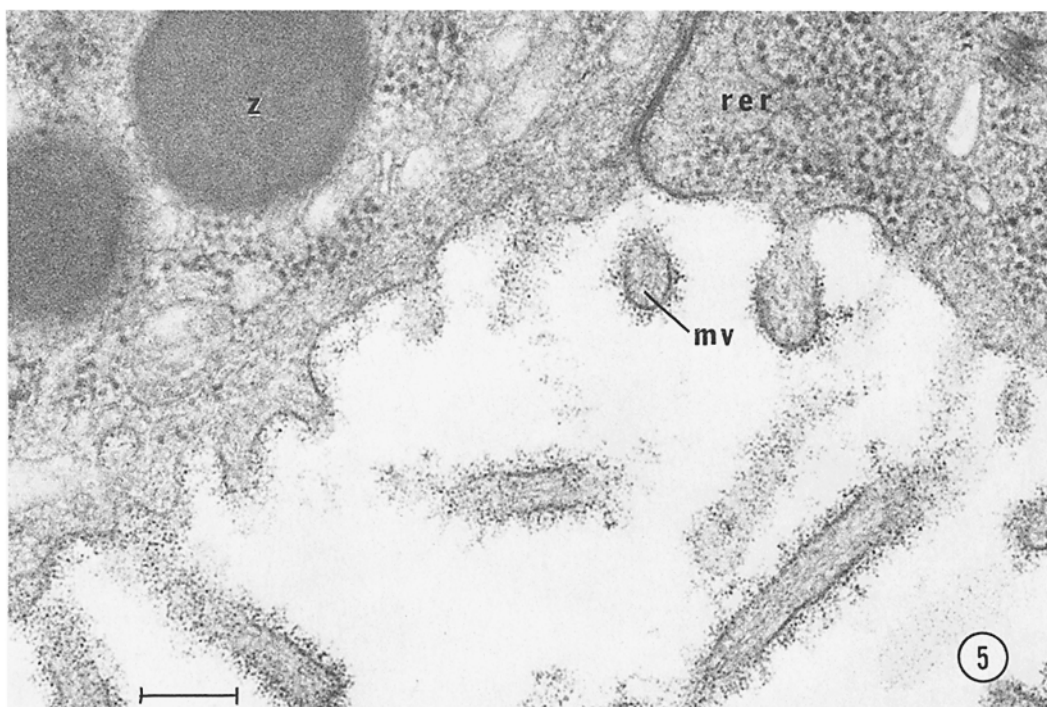


FIGURE 5 Electron micrograph which shows the apical region of two acinar cells from rat pancreas labeled with WGA-ferritin conjugate (0.46 mg/ml). Note that these cells bind the conjugate heavily as already observed for guinea pig acinar cells (Fig. 2). Zymogen granule (*z*); rough endoplasmic reticulum (*rer*); microvilli (*mv*). Section stained with uranyl acetate and lead citrate. $\times 60,000$. Bar, $0.2 \mu\text{m}$.

were associated with the plasmalemma of endocrine cells exposed to RCA II (Fig. 8), soy bean agglutinin (SBA), and Ulex- and Lotus-lectin-ferritin conjugates (Fig. 9). Quantitative values for the labeling densities of lectin-ferritin conjugates on endocrine cells are given in Table II and are to be compared to those for acinar cells.

The lectin labeling pattern of centroacinar cells was generally similar to that of endocrine cells as these cells labeled heavily with WGA-ferritin conjugates (Fig. 10) but were almost devoid of binding sites for SBA-, Ulex-, and Lotus- (Fig. 11) lectin-ferritin conjugates. In contrast to endocrine cells, however, centroacinar cells were heavily labeled with RCA II-ferritin (Fig. 12). Except for this lectin, the density of binding sites on centroacinar cells for SBA, and for Lotus and Ulex lectins, resembles that of endocrine cells (Table II).

Effect of Neuraminidase on Lectin Binding

The apparent absence of binding sites for Lotus and Ulex lectins and for SBA on centroacinar cells, and for these lectins plus RCA II on endo-

crine cells, could in principle be caused by masking of lectin sites by sialic acid-containing surface saccharides as has been noted by others for RCA I and II (1, 17, 33, 43). To examine this hypothesis, unfixed pancreatic cells were incubated with neuraminidase (which contained low or negligible levels of protease) from *Cl. perfringens*, as described under Methods, which liberates 56% of the total cell sialic acid in the dispersed cell population. After digestion, cells were labeled with SBA, RCA II, and Ulex lectin-ferritin conjugates as before. In contrast to the unhydrolyzed controls (e.g., Fig. 8), the number of receptor sites for SBA and RCA II on endocrine cells increased dramatically (Fig. 13A and B). A comparable increase in SBA receptor sites was apparent on centroacinar cells (Fig. 14) while the density of RCA II sites, already substantial in controls, increased significantly. The density of receptor sites for SBA and RCA II also increased on acinar cells after neuraminidase treatment. These results suggest that sialic acid masks receptor sites for SBA- and RCA II-ferritin conjugates, in whole or in part, on endocrine and centroacinar

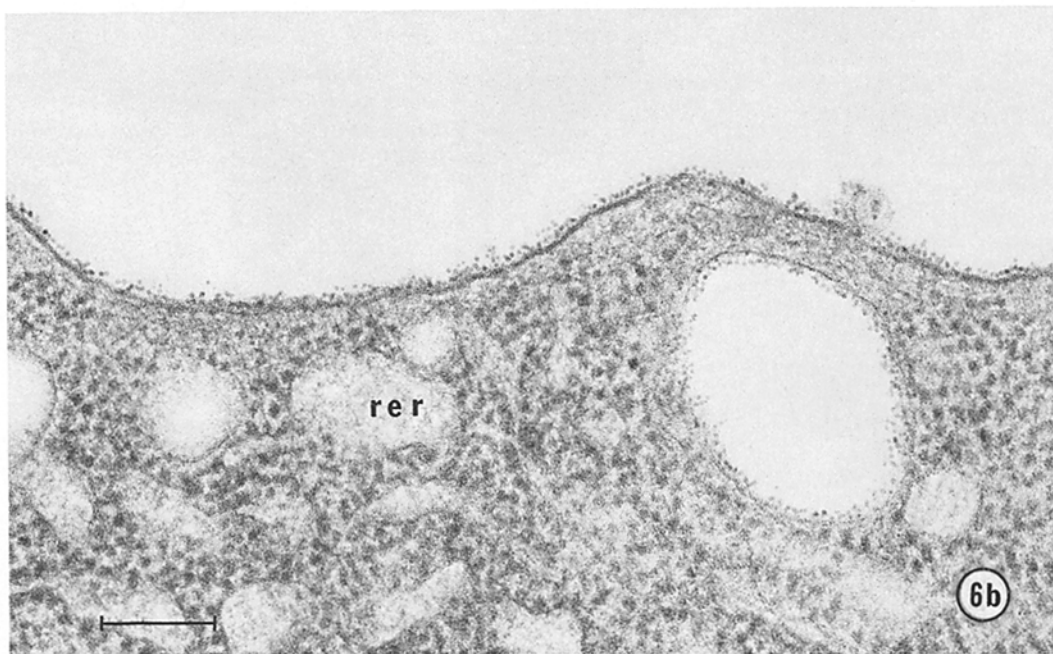
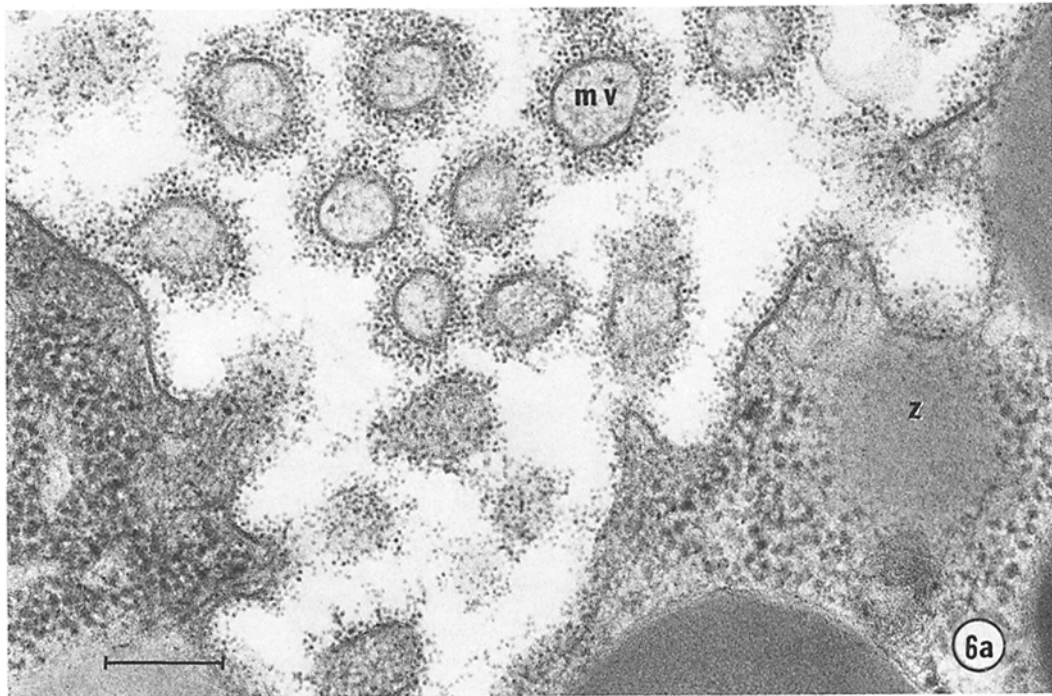


FIGURE 6 Differential distribution of WGA-ferritin conjugate on the surface of acinar cells from weanling rat pancreas. Fig. 6a shows that the apices of the cells are covered with a thick layer of ferritin particles, in contrast to the more discrete binding of the conjugate to the basolateral region (Fig. 6b). Zymogen granule (*z*); microvilli (*mv*); rough endoplasmic reticulum (*rer*). Section stained with uranyl acetate and lead citrate. Figure 6a and b $\times 75,000$. Bar, $0.2 \mu\text{m}$.

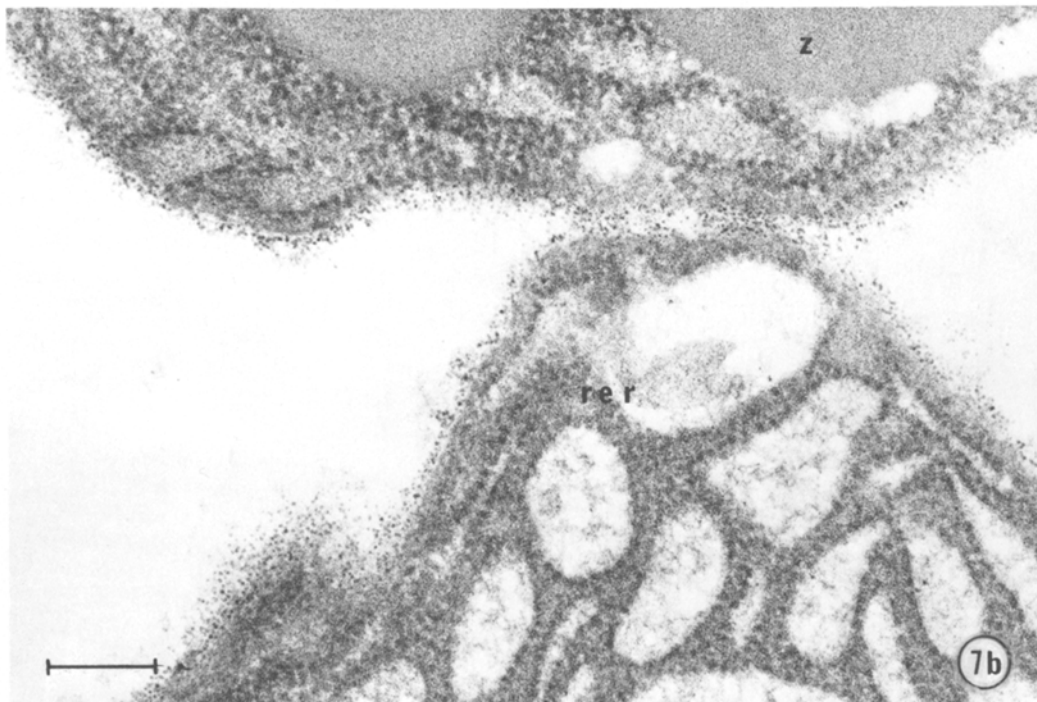
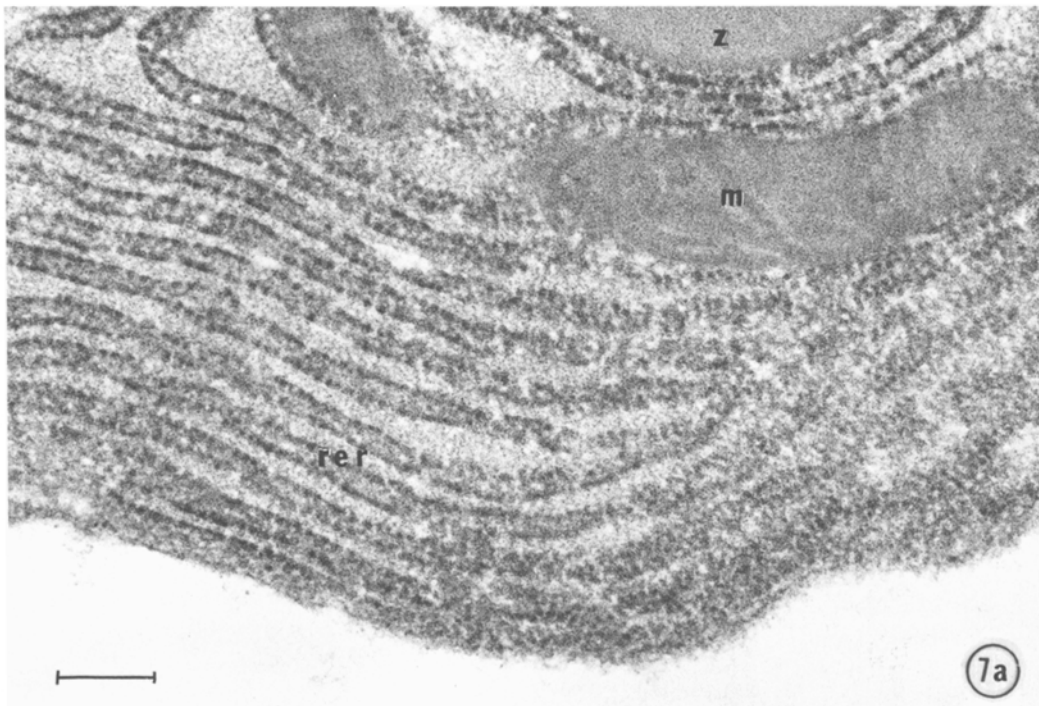


FIGURE 7 Pancreatic acinar cells labeled with RCA II (Fig. 7a) and RCA I (Fig. 7b) ferritin conjugate after chloroform methanol extraction. Note that RCA II receptors have disappeared from the cell surface after solvent treatment. In contrast, the extent of RCA I binding is not altered. Note also that the trilaminar appearance of all cell membranes is lost after solvent extraction. Rough endoplasmic reticulum (*rer*); mitochondria (*m*); zymogen granule (*z*). Fig. 7a $\times 60,000$. Fig. 7b $\times 67,500$. Bar, 0.2 μm .

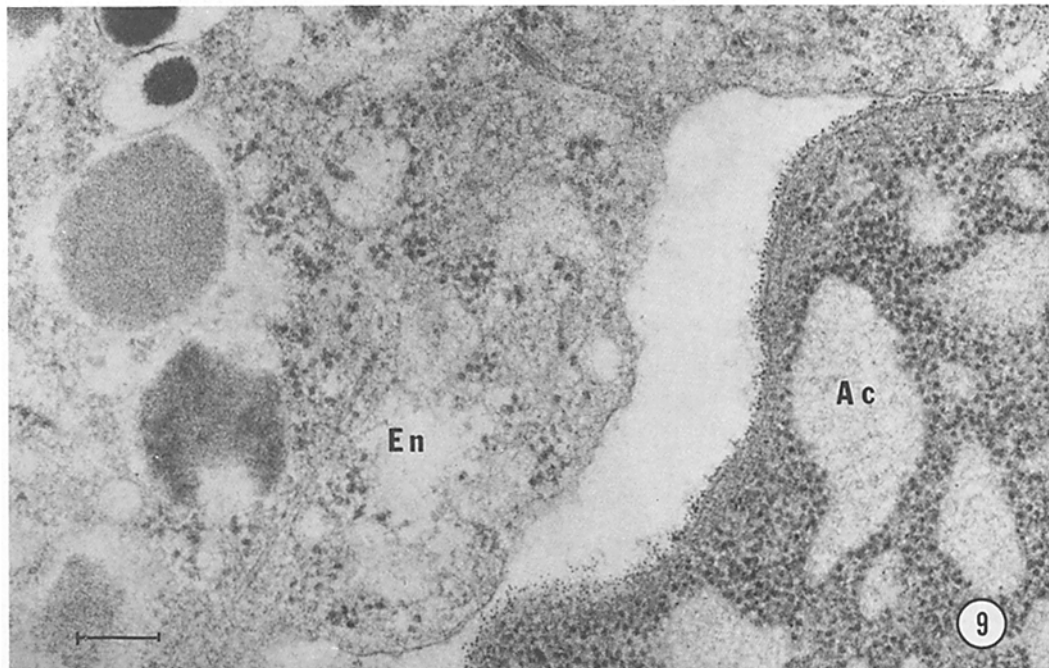
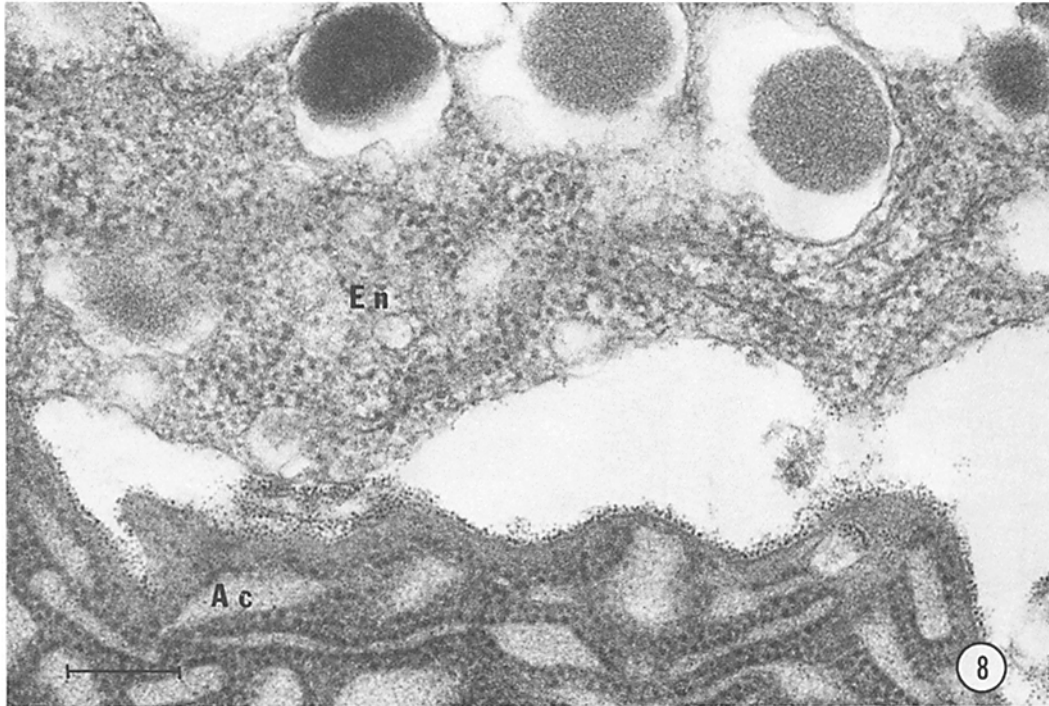


FIGURE 8 Pancreatic acinar cell (*Ac*) and endocrine B cell (*En*) labeled with RCA II-ferritin conjugates. Note the high density of RCA II receptor sites on the surface of the acinar cell. In contrast, virtually no ferritin particles can be seen on the surface of the endocrine B cell. Section stained with uranyl acetate and lead citrate. $\times 67,500$. Bar, $0.2 \mu\text{m}$.

FIGURE 9 Acinar cell (*Ac*) and endocrine B cell (*En*) labeled with Lotus lectin-ferritin conjugate. The endocrine cell plasmalemma appears devoid of Lotus lectin receptors, whereas a large number of fucosyl residues can be visualized on the acinar cell surface. Section stained with uranyl acetate and lead citrate. $\times 50,000$. Bar, $0.2 \mu\text{m}$.

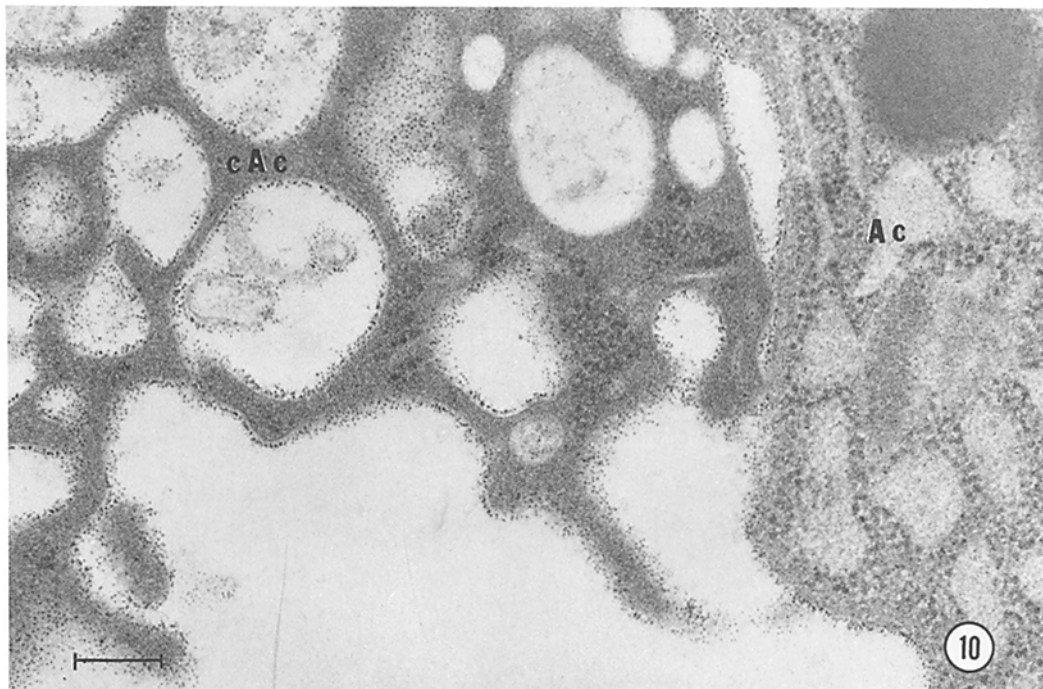


FIGURE 10 Electron micrograph which shows part of an acinar cell (Ac) and a centroacinar cell (cAc) labeled with WGA-ferritin conjugate. Note the heavy binding of the lectin to the surface of both cell types. Section stained with uranyl acetate and lead citrate. $\times 53,000$. Bar, $0.2 \mu\text{m}$.

cells and to a lesser extent on acinar cells. On the other hand, neuraminidase treatment did not reveal cryptic L-fucose receptor sites on endocrine and centroacinar cells as shown by the absence of binding of Lotus and Ulex lectin-ferritin conjugates (Fig. 15), which suggests true absence or low levels of these sites rather than masking of sialic acid. Some increase of receptors for Ulex lectin were, however, noted on acinar cells (Fig. 15).

DISCUSSION

The data reported in this study indicate that both qualitative and quantitative differences exist in the cell surface saccharide composition of pancreatic acinar, centroacinar, and endocrine cells as determined by the binding of a variety of lectin-ferritin conjugates (summarized in Table III). The surface of acinar cells appears to possess a high density of receptors for lectins that recognize the majority of sugars usually present in membrane glycoproteins (23) and glycolipids (24), whereas the surfaces of centroacinar and endocrine cells possess a markedly lower number of binding sites for SBA, and Ulex and Lotus lectins. In addition, endocrine and

centroacinar cells can be differentiated from each other with RCA II, which binds heavily to centroacinar cells but to a much lesser extent to endocrine cells.

In intact tissue, the plasmalemma of the acinar cells appears to be divided into two spatially and functionally separate regions comprised of the apical zone, where exocytosis normally occurs (20), and the basolateral zone, where it is assumed that the initial events in stimulus-secretion coupling take place. It was therefore somewhat surprising that the former apical- and basolateral-plasmalemmal zones of dispersed adult rat and guinea pig acinar cells were uniformly labeled with the lectin-ferritin conjugates employed despite the observation that both the regional differentiation of the plasmalemma and of intracellular organelles appears to be retained in dispersed guinea pig acinar cells (3, 4). The apparent homogeneity of lectin receptor sites could, however, be artefactual and secondary to redistribution of membrane constituents in the plane of the membrane which results from the conditions of cell dissociation (38). The inability of lectins to visualize cell polarity cannot be caused by the use of saturating

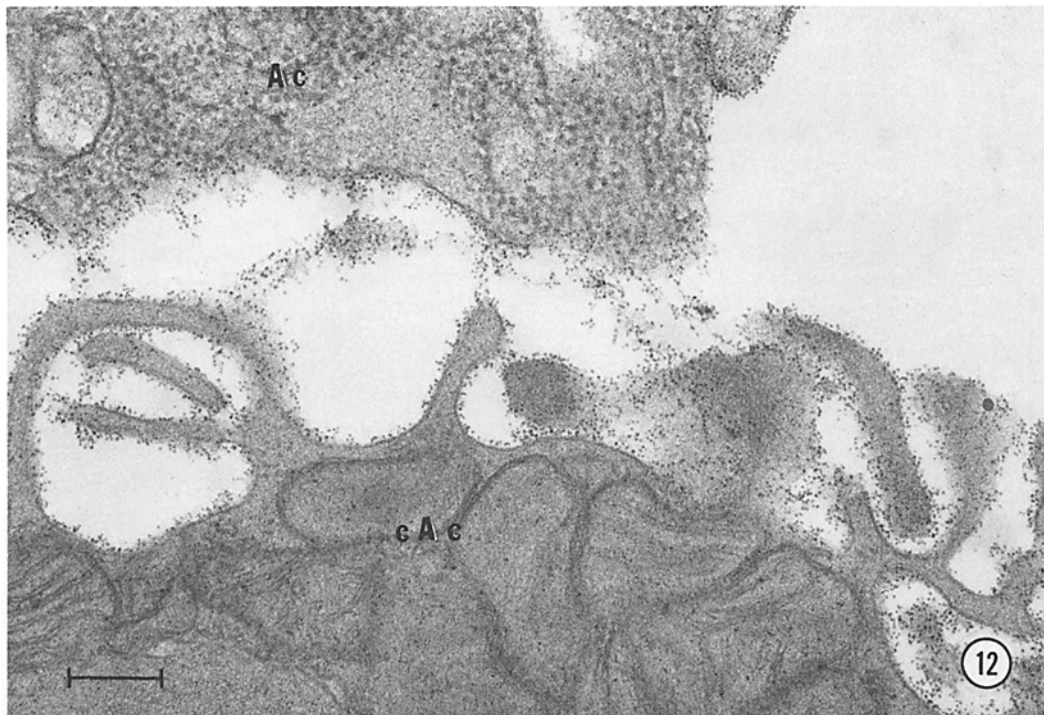


FIGURE 11 Electron micrograph which shows part of an acinar cell (*Ac*) and of a centroacinar cell (*cAc*) labeled with Lotus lectin-ferritin conjugate. Note the marked differences in surface properties, i.e., density of exposed fucosyl residues, between these two pancreatic cell types. Section stained with Bismuth subnitrate. $\times 61,000$. Bar, $0.2 \mu\text{m}$.

FIGURE 12 Centroacinar (*cAc*) and acinar (*Ac*) cells labeled with RCA II-ferritin conjugate. Note that both cell types bind heavily the lectin-ferritin conjugate. Section stained with uranyl acetate and lead citrate. $\times 58,000$. Bar, $0.2 \mu\text{m}$.

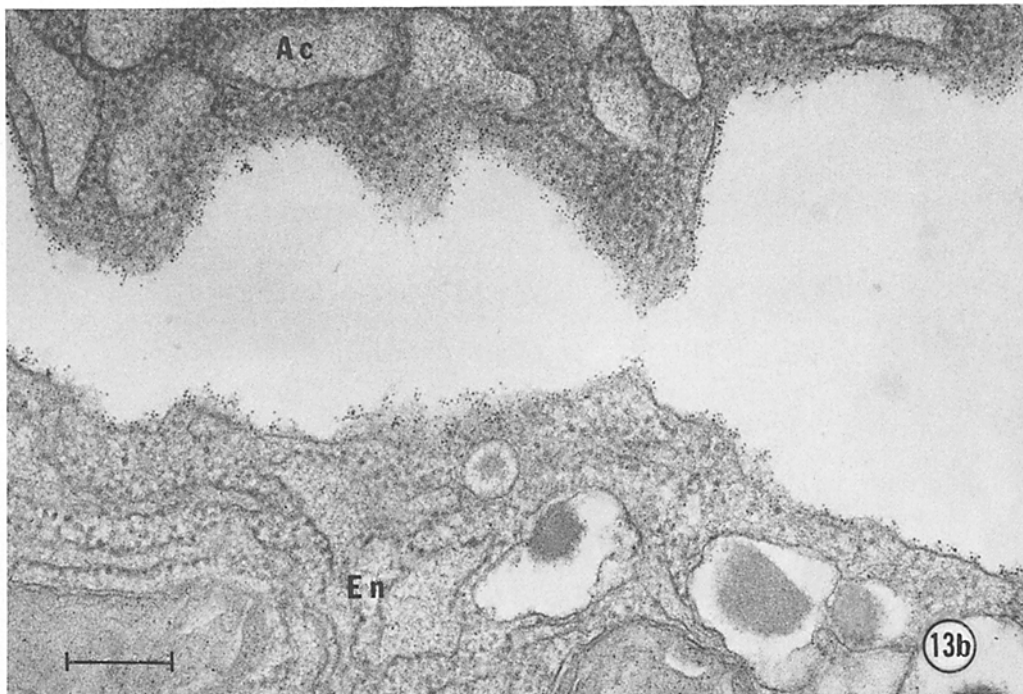
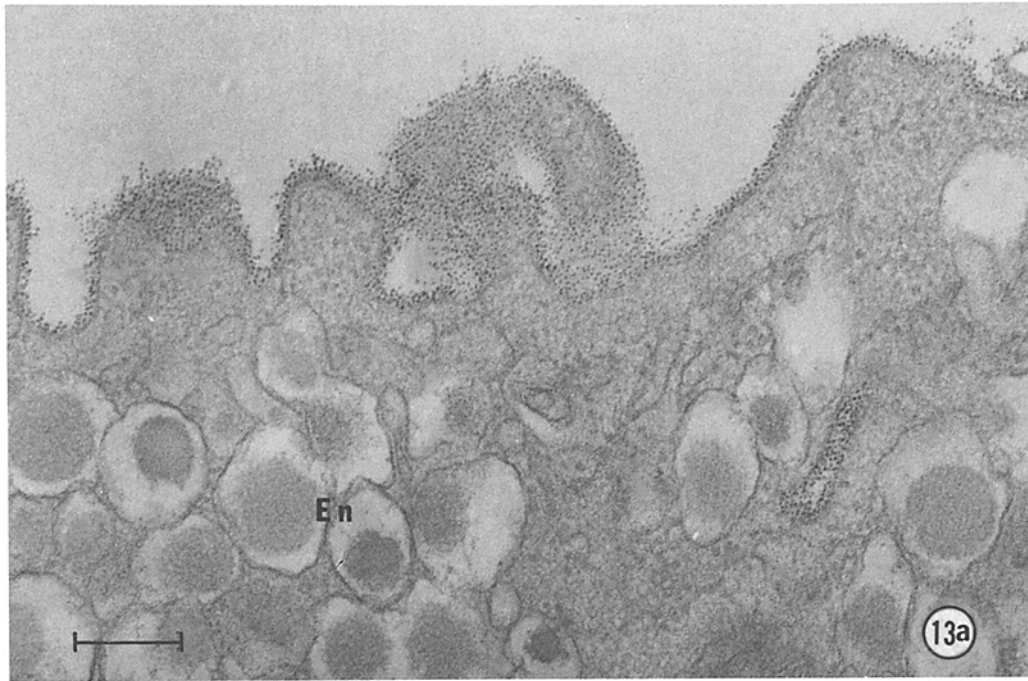


FIGURE 13 Effect of neuraminidase treatment on the distribution of RCA II (Fig. 13a) and SBA-ferritin conjugates (Fig. 13b) on the endocrine cell (*En*) plasmalemma. Note the thick coat of ferritin particles on the cell surface compared to a control shown in Fig. 8. Section stained with uranyl acetate and lead citrate $\times 68,000$. Bar, $0.2 \mu\text{m}$.

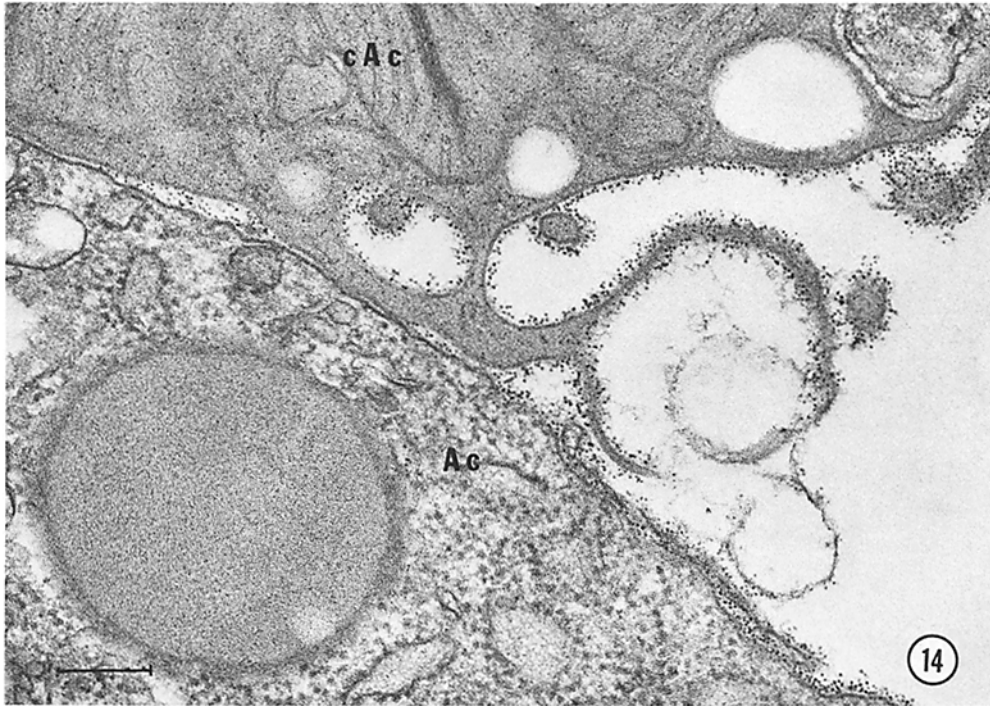


FIGURE 14 Acinar (*Ac*) and centroacinar (*cAc*) cells labeled with SBA-ferritin conjugate after neuraminidase incubation. Note that the enzyme has revealed many receptors for the lectin on the centroacinar cell surface. Section stained with uranyl acetate and lead citrate. $\times 60,000$. Bar, $0.2 \mu\text{m}$.

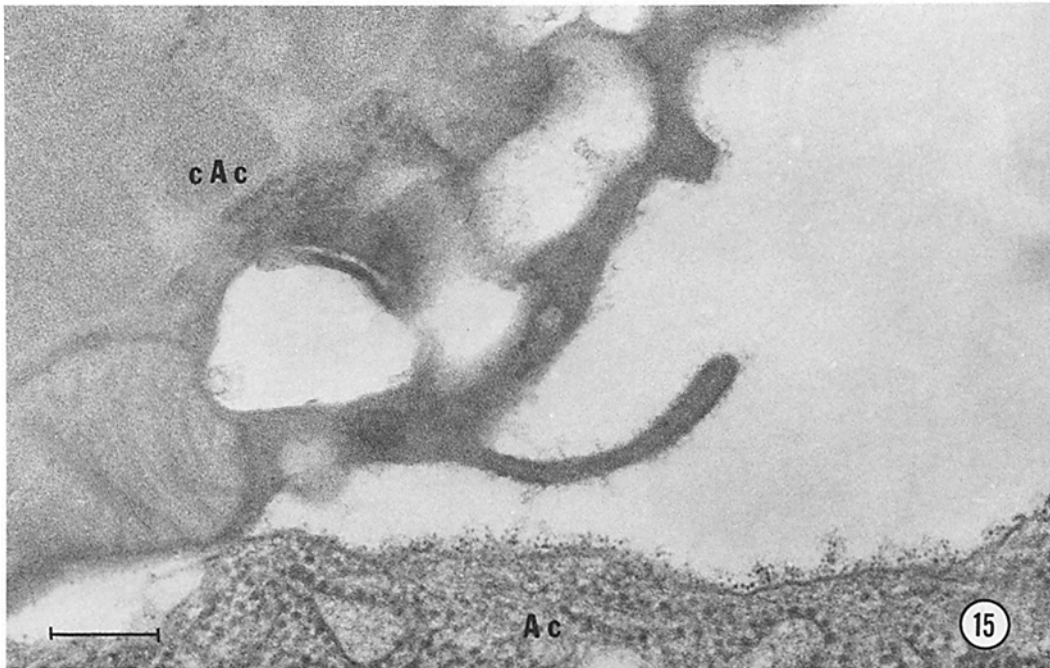


FIGURE 15 Electron micrograph which shows an acinar (*Ac*) and a centroacinar (*cAc*) cell labeled with Ulex lectin-ferritin conjugate after neuraminidase incubation. The enzyme treatment has no effect on the labeling pattern of Ulex lectin on the centroacinar cell, but the density of ferritin particles has increased on the acinar cell plasmalemma. Section stained with uranyl acetate and lead citrate. $\times 70,000$. Bar, $0.2 \mu\text{m}$.

TABLE III
Lectin Receptor Content of Acinar, Centroacinar,
and Endocrine Cells of the Rodent Pancreas

Lectin	Acinar	Endocrine	Centroacinar
ConA	++	++	++
Lens lectin	++	++	++
Lotus lectin	+++	-	-
Ulex lectin	+++	-	-
RCA I	+++	+++	+++
RCA II	+++	-	+++
SBA	+++	-	-
WGA	+++	+++	+++

-, no binding.

++, moderate binding.

+++ , high receptor density.

concentrations of lectin-ferritin conjugates because identical binding patterns of all conjugates were observed on cell preparations labeled with concentrations of lectin-ferritin conjugates 20-times below saturation. In addition, studies from our laboratory (3, 4) and those of Galli et al. (13), indicate that removal of Ca^{+2} required for tissue dispersion of the pancreas into individual cells is attended by disruption and migration of tight junctions from an apical to basolateral position, and by equilibration of the normal asymmetric distribution of intramembranous particles between apical- and basolateral-plasmalemmal zones. An exception to the homogeneous distribution of lectin binding sites on adult acinar cells was noted, however, on acinar cells from newborn and weanling (5-wk-old) rats. At these ages, the apical plasmalemma was more heavily labeled with WGA, RCA I, and Lotus lectin-ferritin conjugates than was the remainder of the plasmalemma, even though the same tissue dispersion procedure was involved. In these cases, the conjugates appeared to label apical plasmalemmal constituents which extended 60 nm from the cell surface compared to the adult where the label was within 35 nm of the surface. The elements labeled in young animals resemble the glycocalyx of the intestinal brush border (18, 19). Whether these extended apical binding sites represent a less mature form of glycoproteins, as might be the case in rapidly turning-over epithelia such as the intestinal epithelium (6, 25), which are hydrolyzed or replaced by a less extensive carbohydrate chain during maturation, remains to be examined.

In the course of our studies we have performed a limited series of experiments to determine the nature of the receptor sites for the lectins used.

First, we have found that after treatment of unfixed cell suspensions by neuraminidase, the apparent absence of binding sites for SBA and RCA II lectin-ferritin conjugates on endocrine cells, and for SBA on centroacinar cells, is reversed. Unmasking of receptor sites for SBA- and RCA II-ferritin conjugates by neuraminidase may be caused by exposure of subterminal galactosyl residues on oligosaccharide chains (1, 17, 33, 43). Alternatively, unmasking by neuraminidase could be accounted for by removal of sialic acid residues on the same or adjacent oligosaccharides which normally prevent conjugate binding by charge repulsion or steric restriction. Although the neuraminidase used in our study is not likely to be free of contaminating proteases (9), we feel that the effect of this enzyme preparation cannot be accounted for by proteolysis as discussed below. We should also note here that while SBA and RCA II both recognize N-acetyl galactosamine which contains glycoconjugates, preferential binding of RCA II to centroacinar cells and not endocrine cells could be explained by different extents of masking by sialic acid of SBA and RCA II receptors on the two cell types. The apparent absence of unmasking of binding sites for Ulex and Lotus lectins on endocrine and centroacinar cells by neuraminidase suggests that fucosyl residues are quantitatively lower in the plasmalemma of these two cell types. Additional studies with a variety of exoglycosidases (22) are, however, required to demonstrate this point conclusively.

Second, attempts were made to differentiate between surface glycoproteins and glycolipids as receptors for several lectins used in this study. For this purpose, fixed cells were treated with papain or extracted with ethanol and chloroform-methanol to remove lipids, including glycolipids (46), before labeling with WGA-, RCA I-, and RCA II-ferritin conjugates.

The results showed that papain removed about 20-24% of WGA and RCA II sites from acinar cells and at the same time hydrolyzed 80% of radioiodinated peptides from the cell surface, which indicates that some WGA and RCA II receptors are glycoproteins. In contrast, RCA I binding was unaffected by papain treatment. The resistance of RCA I receptors to proteolysis has already been observed for hepatoma cells (32) and fibroblasts (33, 43). Solvent extraction of fixed pancreatic cells did not decrease the number of WGA and RCA I binding sites but diminished RCA II binding to the cells, thus suggesting that

RCA II binds primarily but not exclusively to glycolipids. For the future it will be important to determine precisely the extractability of glycoproteins and glycolipids by the procedure because in principle it may enable one to classify lectin receptors as to their chemical nature. Finally, in this connection it should be noted that the initial cell dispersion technique used in this study employed commercial chromatographically purified collagenase (which contains variable amounts of the protease clostripain [15]) plus added chymotrypsin. Although it could be argued that the labeling patterns we observed with the lectins are the result of differential proteolysis which occurred during tissue dispersion, later experiments used highly purified collagenase (15) with very low or undetectable nonspecific levels. The lectin labeling patterns remained the same under both conditions, which suggests that the tissue dissociation procedure does not seriously affect our results.

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