# Development of Cell Surface Saccharides on Embryonic Pancreatic Cells

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ABSTRACT  Using a battery of seven lectin-ferritin conjugates as probes for cell surface glyco-conjugates, we have studied the pattern of plasmalemmal differentiation of cells in the embryonic rat pancreas from day 15 in utero to the early postpartum stage. Our results indicate that differentiation of plasmalemmal glycoconjugates on acinar, endocrine, and centroacinar cells is temporally correlated with development and is unique for each cell type, as indicated by lectin-ferritin binding. Specifically, (a) expression of adult cell surface saccharide phenotype can be detected on presumptive acinar cells as early as 15 d in utero, as indicated by soybean agglutinin binding, and precedes development of intracellular organelles characteristic of mature acinar cells; (b) maturation of the plasmalemma of acinar cells is reached after intracellular cytodifferentiation is completed, as indicated by appearance of Con A and fucose-lectin binding sites only at day 19 of development; conversely, maturation of the endocrine cell plasmalemma is accompanied by "loss" (masking) of Ricinus communis II agglutinin receptors; and (c) binding sites for fucose lectins and for soybean agglutinin are absent on endocrine and centroacinar cells at all stages examined. We conclude that acinar, centroacinar, and endocrine cells develop from a common progenitor cell(s) whose plasmalemmal carbo-hydrate composition resembles most closely that of the adult centroacinar cell.

Finally, appearance of acinar lumina beginning at ~17 d in utero is accompanied by differentiation of apical and basolateral plasmalemmal domains of epithelial cells, as indicated by enhanced binding of several lectin-ferritin conjugates to the apical plasmalemmal, a pattern that persists from this stage through adult life.

Our previous studies showed that the plasmalemmas of acinar, centroacinar, and endocrine cells of the mammalian pancreas each possess a distinctive glycoconjugate composition as revealed by differential binding of a battery of lectin-ferritin conjugates (15, 16). Whereas acinar cells display receptors for all lectins tested (Con A[1], specific for mannosyl and glucosyl residues; RCA I, galactosyl residues; RCA II and SBA, N-acetyl-galactosaminyl residues; WGA, N-acetyl-glucosaminyl residues; Ulex europeus and Lotus tetragonolobus lectins, fucosyl residues), endocrine and centroacinar cells are devoid of

binding sites for fucose-specific lectins and for SBA, but can be distinguished from each other by the ability of centroacinar cells to bind RCA II. Because these three cell types arise from an endodermal evagination of the embryonic foregut that forms the pancreatic primordial tissue, it was of interest to examine the pattern of differentiation of plasmalemmal glycoconjugates at key stages of pancreatic development in the rat that have been previously defined biochemically and morphologically by others (for a review of these stages, see reference 20).

Our results, obtained by the use of the same set of lectin-ferritin conjugates we employed previously to map cell surface saccharides on cells of the adult rat and guinea pig pancreas (15, 16), indicate that the differential expression of plasmalemmal glycoconjugates is temporally correlated with the development of undifferentiated cells from day-15 embryonic pancreas into three cell types of the mature pancreas (acinar,

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[1] Abbreviations used in this paper: Con A, concanavalin A; RCA I and RCA II, Ricinus communis agglutinins I and II; SBA, soybean agglutinin; WGA, wheat germ agglutinin; BSA, bovine serum albumin; KRB, Krebs-Ringer bicarbonate salt solution supplemented with amino acids and glucose; STI, soybean trypsin inhibitor.

centroacinar, and endocrine B cells) that we have previously examined.

## MATERIALS AND METHODS

### Dissociation of Embryonic Pancreases

Sprague-Dawley male and female rats were mated and the following morning the females were checked for the presence of a vaginal plug and for sperm in the vagina. The females were then isolated; day 0 was defined as the time when sperm-positive vaginas were detected. Females of appropriate gestational age were stunned by a blow to the head and decapitated. Uteri were quickly excised and placed in a petri dish containing Earle's balanced salt solution into which individual embryos were removed. Pancreatic rudiments were dissected in Earle's salt solution with the aid of a binocular microscope and microdissecting knives. 50–60 Embryonic pancreas from each stage examined were dispersed into single cells, using chromatographically purified collagenase (Worthington Biochemical Corp., Freehold, N. J.) and $Ca^{2+}$ chelation, as previously described for the mature gland (1). After tissue dissociation, the cells were washed three times in KRB containing 10 mg/ml BSA and 0.1 mg/ml STI (Worthington Biochemical Corp.) (16) at room temperature before lectin-ferritin labeling. During the various steps of tissue dissociation and of lectin binding, the media were gassed with 95% $O_2$ and 5% $CO_2$.

### Preparation of Lectin-Ferritin Conjugates

Lectin and ferritin conjugates were prepared according to the methods described by us (15).

### Lectin Labeling

A suspension of unfixed embryonic cells in KRB containing 10 mg/ml BSA and 0.1 mg/ml STI was incubated for 30 min at 4°C with 0.4–0.6 mg/ml lectin-ferritin conjugates. These conditions were previously determined (16) to be optimal for maximum binding of lectins to adult pancreatic cells. After incubation, the cell suspension was layered over a cushion of KRB containing 40 mg/ml BSA and 0.1 mg/ml STI at 4°C and centrifuged for 3 min at 150 g to separate cells from unbound lectin-ferritin conjugates. The pellet of cells was subsequently resuspended in KRB with 10 mg/ml BSA and 0.1 mg/ml STI and washed through the 40 mg/ml BSA-STI cushion once more before preparation for electron microscopy. For each developmental stage examined, at least six pregnant animals were used in each of three separate labeling experiments; in each experiment, dispersed cells were pooled from ~60 embryos. Each lectin study was paired with its hapten sugar control. Competing sugars were present throughout the labeling procedure at concentrations of 0.1 M.

### Neuraminidase Treatment

Dispersed embryonic cells were incubated for 1 h at 37°C with 0.5 U/ml of chromatographically purified neuraminidase (Type VI, Sigma Chemical Co., St. Louis, Mo.) from *Clostridium perfringens* in KRB with 10 mg/ml BSA and 0.1 mg/ml STI as previously described (16) with minor modifications. In our studies on adult pancreatic cells, release of sialic acid by neuraminidase was quantitated (16), although in the present study this was not feasible because of the small amounts of tissue available. The neuraminidase preparation was chromatographically purified and contained <0.003 U caseinase activity/mg protein. Assays for other glycosidases were not performed.

At the end of the incubation period, the cells were washed twice with 1 ml of KRB with 10 mg/ml BSA and 0.1 mg/ml STI. They were then labeled with SBA-ferritin conjugate and processed for electron microscopy as described below.

### Preparation of the Cells for Electron Microscopy

After lectin labeling, the cells were fixed at 4°C with 1% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, containing 0.1 M monosaccharides. When the cells were tagged with Con A-ferritin or WGA-ferritin conjugates, the fixative contained 0.1 M galactose; for the other lectin-ferritin conjugates, the fixatives contained 0.1 M glucose. Inclusion of nonspecific sugars compensated for osmotic activity introduced by the appropriate competing hapten sugars used in control labeling experiments. After fixation for 1 h at room temperature or overnight at 4°C, the cell suspension was centrifuged in polyethylene tubes (Beckman Microfuge 152, Beckman Instruments, Inc., Fullerton, Calif.) at 10,000 g for 5 min. The cell pellets, cut from the bottom of the tube with a razor blade, were postfixed with 1% $OsO_4$ in 0.1 M sodium cacodylate for 1–2 h at 4°C. After two washes in 0.15 M NaCl, the pellets were stained in block overnight at 4°C with 0.5%

magnesium uranyl acetate in 0.15 M NaCl. They were then dehydrated in increasing concentrations of ethanol and propylene oxide before embedment in Epon-Araldite (13). Thin sections were stained with uranyl acetate and lead citrate as before (16) prior to examination in a Philips 301 electron microscope. No fewer than three specimen blocks from cells labeled with each lectin in a given experiment were thin sectioned and at least 50 random micrographs of cells from each separate labeling study at each developmental stage were examined.

Because inclusion of the appropriate competing hapten sugar during labeling and fixation in control experiments completely abolished lectin-ferritin binding, micrographs of each control are not shown (see reference 16 for examples of control preparations).

## RESULTS

### Cell Surface Properties of Embryonic Pancreatic Cells at the Late Protodifferentiated Stage (Day 15)

The protodifferentiated stage, as defined by Pictet et al. (19) and Pictet and Rutter (20), corresponds to an early step in pancreatic development in which cords of epithelial cells, invading the surrounding mesenchyme, appear morphologically undifferentiated. They are characterized by many free ribosomes, a small Golgi complex, and a few elements of the rough endoplasmic reticulum (RER) and are not yet organized into acini with open lumina, as shown in Figure 1 a. The level of digestive enzymes, as well as of insulin (20) and of somatostatin (9), is low but measurable. These cells have been postulated to be progenitor cells of acinar, centroacinar, and duct cells, and of endocrine B and D cells.

When labeled with lectin-ferritin conjugates, these undifferentiated cells exhibited a similar lectin binding pattern in that they all heavily bound ferritin-conjugated RCA I, RCA II (Fig. 2), and WGA; the distribution of lectin-ferritin molecules on the cell surface was homogenous. The plasmalemma of the undifferentiated cells did not contain binding sites for *Ulex* lectin, Con A, or *Lotus* lectin–ferritin conjugates (Fig. 3). Only remains of material that may correspond to basal lamina and that was visible on the basal region of the plasmalemma bound Con A–ferritin conjugates. In contrast to other lectins, SBA revealed two populations of cells. One population, the most abundant, was characterized by a high content of free ribosomes and did not bind the SBA-ferritin conjugate (Fig. 4), whereas the remaining cells, which contained well-defined elements of RER, bound substantial amounts of conjugate (Fig. 5).

Endocrine A cells, which are already present at the protodifferentiated stage (20), exhibited a lectin binding profile generally similar to that of adult endocrine A cells: their plasma membrane was devoid of receptors for *Ulex* lectin (Fig. 6), *Lotus* lectin, and SBA, but heavily bound Con A, WGA, and RCA I–ferritin conjugates. However, in contrast to adult endocrine A cells, embryonic endocrine A cells exhibited a high density of RCA II binding sites on their surface (Fig. 7). We have observed that the adult endocrine A cells possess a similar density of RCA II binding sites but only after neuraminidase removal of terminal sialic acid (16).

### Cell Surface Properties of Embryonic Pancreatic Cells at the Secondary Transition Stage (Day 17)

Beginning at day 16 and progressing through day 19, the epithelial cells of the rudiment begin to undergo cytodifferentiation and morphogenesis into acini with recognizable lumina. The content of endoplasmic reticulum increases markedly in
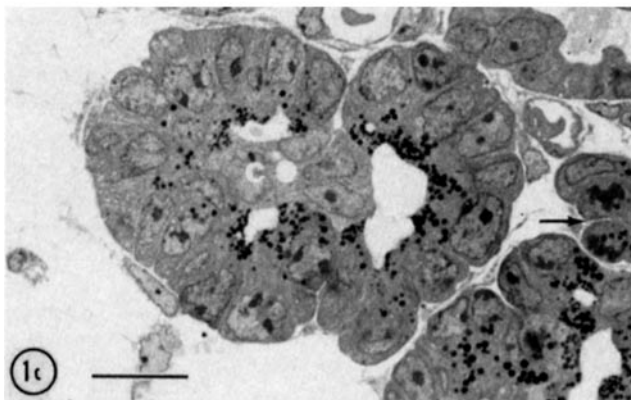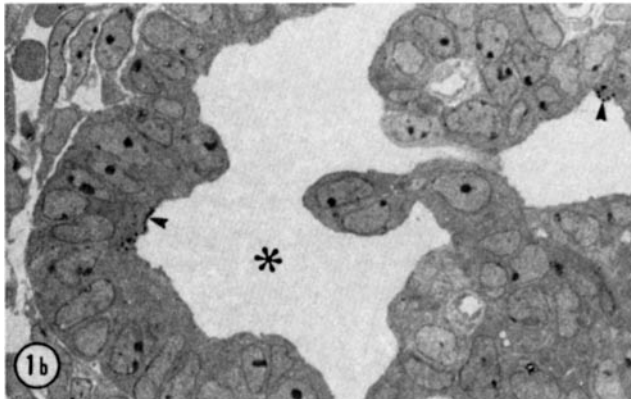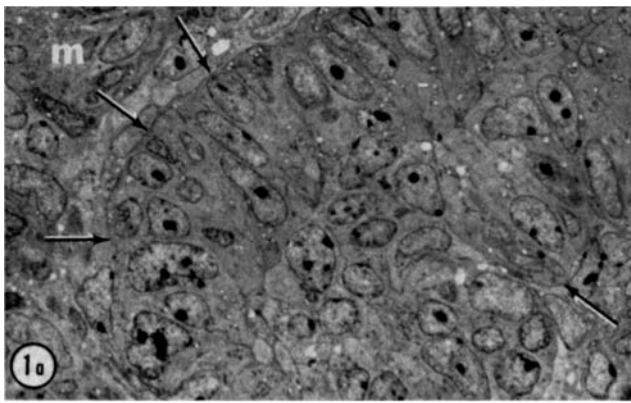
FIGURE 1 Light micrograph of thick plastic sections through rat pancreatic rudiments at day 15 (*a*), day 17 (*b*), and day 19 (*c*) of gestation. Note in Fig. 1 *a* the undifferentiated columnar epithelial cells (arrows) invading the surrounding mesenchymal tissue (*m*). A lumen is not visible in the epithelial cell mass. Fig. 1 *b* shows a widely dilated acinar lumen (asterisk) surrounded mainly by undifferentiated epithelial cells; small zymogen granules are present in several forming acinar cells (arrowheads). In Fig. 1 *c*, acinar cells are clearly recognizable by their content of zymogen granules; centroacinar (ductlike) cells (*c*) are also recognizable. The arrow indicates two cells in mitosis that contain zymogen granules. Sections stained with toluidine blue. Bar, 20 μm. × 620.

differentiating acinar cells (Fig. 8), and a few small zymogen granules are visible in the apical regions of some of the cells lining the acinar lumen at day 17 (Figs. 1 *b*, 8 *b*). the levels of digestive enzymes and proenzymes and of insulin increase substantially (20). Endocrine B cells are also visible at this developmental stage.

When labeled with lectin-ferritin conjugates, the differentiating acinar cells, now clearly recognizable by their abundant

complement of rough-surfaced endoplasmic reticulum (Fig. 8 *a*), well-developed Golgi complex, and occasional zymogen granules (fig. 8 *b*), displayed binding sites for RCA I, RCA II, and WGA-ferritin conjugates. Although most of the acinar cells were labeled with SBA-ferritin conjugate (Fig. 8 *a*–*c*), some still lacked binding sites for this lectin. This may be a result of the asynchronous differentiation of cells within the acini (20). However, all cells comprising an acinus, including presumptive acinar and centroacinar cells, were still devoid of receptors for Con A, *Lotus*, and *Ulex* lectin-ferritin conjugates.

It should be noted that the distribution of lectin-ferritin conjugates was not homogeneous on the plasmalemma of cells



FIGURE 2 Portions of two undifferentiated pancreatic cells from day-15 embryo labeled with RCA II–ferritin conjugate (0.6 mg/ml). Note the high density of ferritin particles on the plasma membrane. These cells are characterized by a large number of free ribosomes and paucity of RER. Section stained with uranyl acetate and lead citrate. Bar, 0.2 μm. × 42,000.
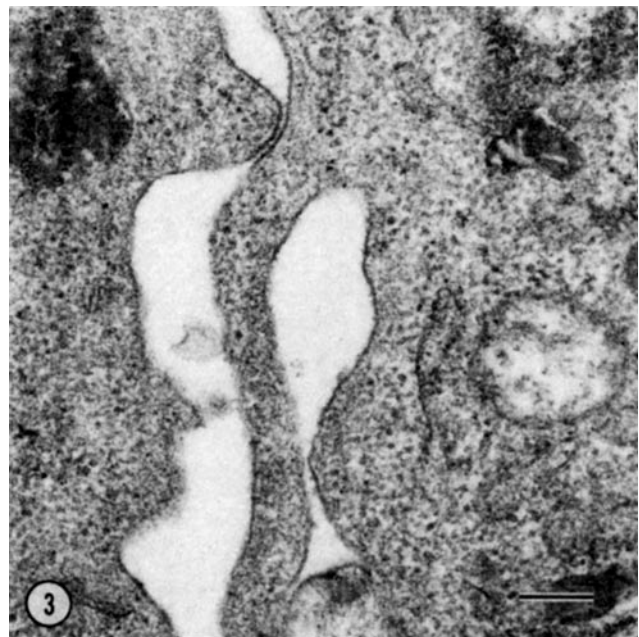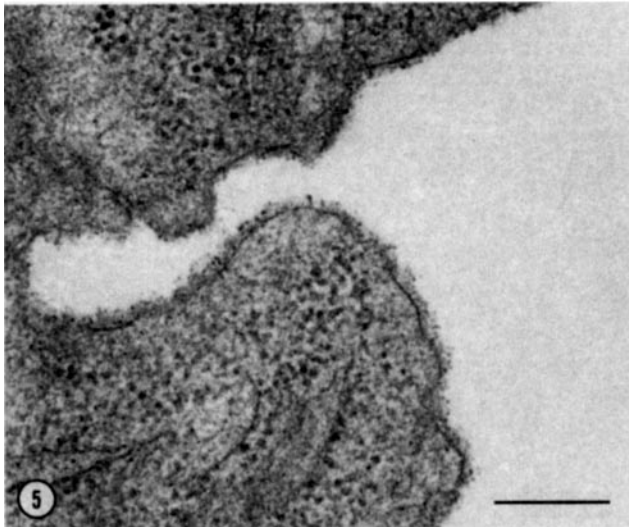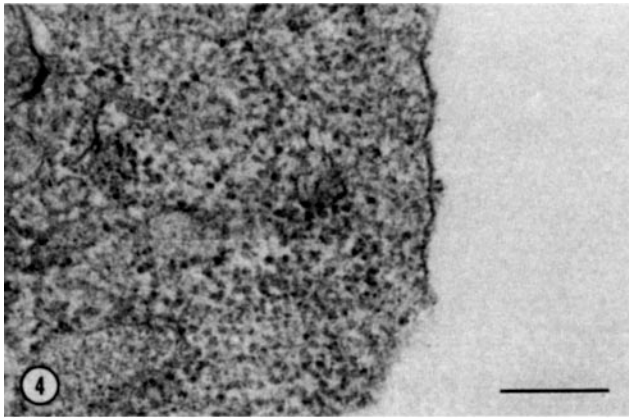


FIGURE 3 Undifferentiated pancreatic cells (day-15 embryo) incubated with *Lotus* lectin-ferritin conjugate. Note that the plasma membranes of the cells do not bind the lectin-ferritin conjugate. Section stained with uranyl acetate and lead citrate. Bar, 0.2 μm. × 50,000.

characteristic of mature acinar cells, including a large number of zymogen granules in the apical cytoplasm. Centroacinar cells are recognizable by their location in the acini, absence of zymogen granules, and paler staining cytoplasm at the light microscope level (Fig. 1 c). At this stage, the plasma membrane of all acinar cells has acquired a density of Con A binding sites similar to that on adult cells, but two populations of acinar cells are still qualitatively distinguished by their ability to bind Lotus and Ulex lectins. Some cells heavily bound these lectin-ferritin conjugates, whereas adjacent cells, joined to the former



FIGURES 4 and 5   Differential distribution of SBA-ferritin conjugate on the surface of undifferentiated cells from day-15 embryo. Note that the cell (Fig. 4) characterized by many free ribosomes and little RER does not bind the lectin, whereas the cell with some RER elements (Fig. 5) exhibits many ferritin particles on its surface. Section stained with uranyl acetate and lead citrate. Bars, 0.2 μm. Fig. 4, X 73,000; Fig. 5, X 79,000.

FIGURE 6   Electron micrograph of endocrine A cell from day-15 embryonic pancreas. Note that, like the adult cells, the embryonic endocrine A cell does not exhibit binding sites for Ulex lectin. Section stained with uranyl acetate and lead citrate. Bar, 0.2 μm. X 55,000.

in acini from day-17 embryos in that the density of binding sites for WGA, RCA I, RCA II, and SBA, when present, was higher on the apical plasmalemma than on the lateral and basal surfaces (Fig. 8 a–c). This asymmetric distribution of lectin binding sites is maintained at least up to 3 wk after birth (see Fig. 6 in reference 16) and recent studies (Muresan and Jamieson, unpublished observations) indicate that the apical plasmalemma of acinar cells is highly enriched in sialoglyco-proteins throughout adult life.

Endocrine B cells present at this stage exhibited the same complement of lectin binding sites as did adult endocrine B cells, i.e., they bound RCA I, WGA, and Con A, but their surface was lacking receptors for Ulex lectin, Lotus lectin, and SBA. As was found for the embryonic endocrine A cells, they also exhibited a high density of RCA II binding sites on their plasmalemma, whereas adult endocrine B and A cells do not bind this lectin (16).

## Cell Surface Properties of Embryonic Pancreatic Cells at the Prenatal Stage (Day 19)

Before birth (19–21 d of gestation), acinar cells of the pancreatic rudiment contain the full complement of organelles
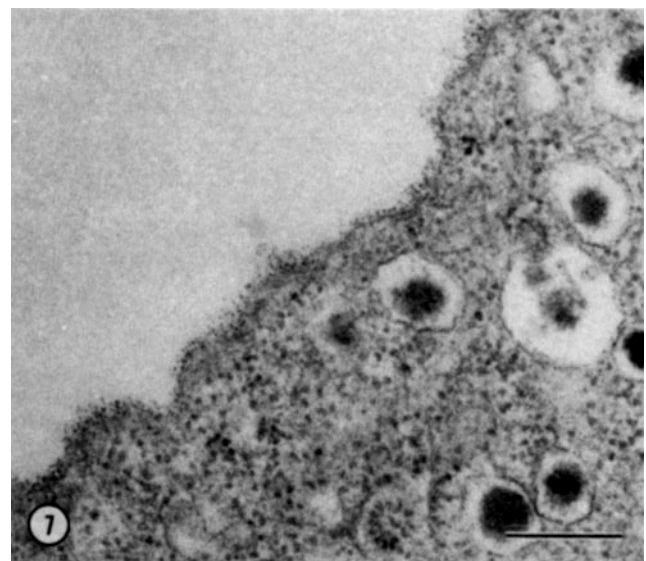


FIGURE 7   Endocrine A cell from day-15 embryonic pancreas labeled with RCA II-ferritin. Note the heavy binding of the lectin to the surface of the cell. Section stained with uranyl acetate and lead citrate. Bar, 0.2 μm X 77,000.
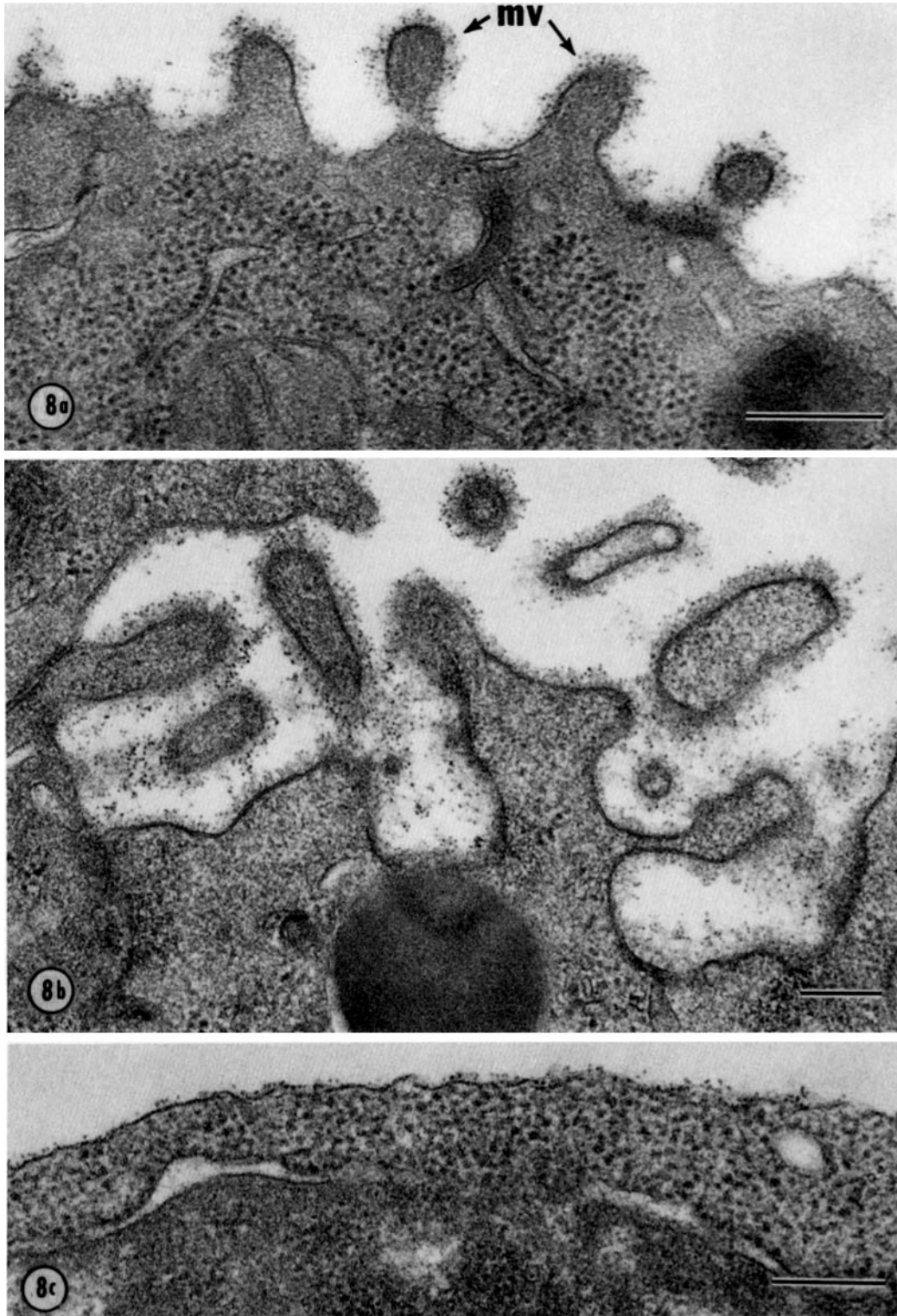
FIGURE 8   (a) Apical region of an undifferentiated acinar cell (day-17 embryo) labeled with SBA-ferritin conjugate. Note the high density of ferritin particles on the plasma membrane. The apical region is easily recognized by the presence of microvilli (mv) coated with a fuzzy glycocalyx. (b) Apical region of an acinar cell whose cytodifferentiation is more advanced than that of the cell shown in Fig. 8 a, as indicated by an apically located zymogen granule. Note the thick fuzzy coat, especially surrounding microvilli that are labeled with SBA-ferritin cojugate. (c) Basal region of an acinar cell labeled with SBA-ferritin conjugate. In contrast to the heavy labeling of the apical plasmalemma, the basal plasmalemmal labeling consists of a monolayer of ferritin particles separated from the plasma membrane by a 10-nm zone. Sections stained with uranyl acetate and lead citrate in Fig. 8 a, b, and c. Bars, 0.2 μm. Fig. 8 a, × 105,000; Fig. 8 b, × 58,500; Fig. 8 c, × 83,000.
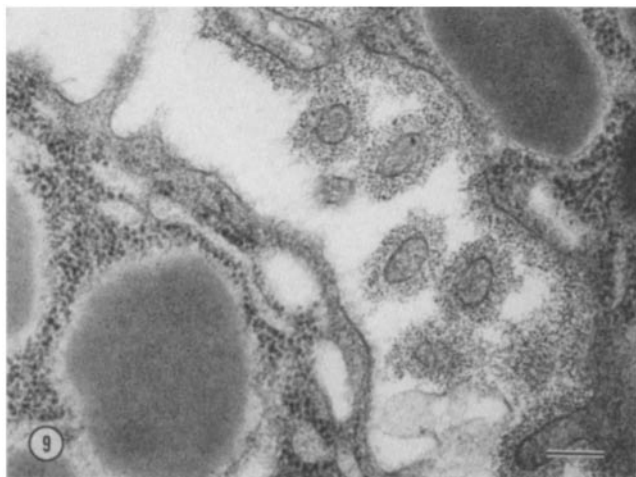
through junctional complexes, were devoid of binding sites for these lectins, as shown in Fig. 9. All acinar cells acquired binding sites for these two fucose-specific lectins only after 2–3 wk of postpartum development.

Between days 17 and 19 of gestation, no changes in lectin labeling patterns of endocrine cells were observed, although the numbers of secretory granules in each cell appeared to increase.

## Effect of Neuraminidase on SBA Binding to Embryonic Cells

The absence of binding sites for SBA on the surfaces of cells in the protodifferentiated stage could be the result of the masking of these sites by sialic acid residues as we have previously noted for SBA binding on adult centroacinar and endocrine cells. To examine this hypothesis, pancreatic cells from 15-d embryos were enzymatically dispersed and incubated after dispersion with neuraminidase. After digestion, the cells were labeled with SBA-ferritin conjugates. In contrast to the unhydrolyzed control (Fig. 4), all neuraminidase-treated cells from 15-d embryos displayed a high density of SBA binding sites (Fig. 10), including endocrine A cells, which are present at this stage. The density of SBA binding sites was also increased on acinar cells from day 17 and 19 embryos after neuraminidase treatment. These results suggest that sialic acid masks binding sites for SBA on undifferentiated cells. However, the presence of this carbohydrate residue apparently does not prevent the binding of RCA I and RCA II to the surface of undifferentiated cells.

We are aware that the neuraminidase preparation used here contains low but detectable levels of proteases that in principle could unmask SBA binding sites by cleavage of glycopeptides other than those containing sialic acid. Although we cannot rule out this possibility, we believe that the effect of proteolysis is not significant, for the following reasons: (*a*) neuraminidase treatment was carried out in the presence of BSA and STI, which should competitively reduce proteolytic activity, and (*b*) nonspecific proteolysis might be expected to reduce binding of SBA, which was not observed.

## DISCUSSION

We describe here stage-specific changes of cell surface glycoconjugates during the development of rat embryonic pancreas in vivo using seven defined lectin-ferritin conjugates. Our observations indicate that at the protodifferentiated stage, undifferentiated epithelial cells of the pancreatic rudiment all exhibit identical lectin binding profiles. The results suggest the existence of a common progenitor cell(s) for acinar, endocrine B, and ductlike cells from which these cell types develop and in the process acquire their own specific cell surface saccharide profiles (16). The existence of a single stem cell for exocrine and endocrine pancreas has already been suggested. For instance, Rutter and Pictet (22) showed that if pancreatic primordia, deprived of mesenchyme, are cultured in a simple medium, ~65% of the cells produced by the embryonic epithelia are endocrine cells, with the remaining population divided among acinar and ductlike cells. On the other hand, the majority (~80%) of cells produced from similar epithelia cultured in a medium containing mesenchymal factor (22) are acinar cells along with smaller numbers of endocrine (~7%) and ductlike cells, (~13%) indicating that the rudiment contains a pleuripotential stem cell or cells. Along the same line, Walther et al. (24), and Githens et al. (6) showed that pancreatic rudiments from day-15 embryos cultured for several days in the presence of bromodeoxyuridine, an inhibitor of differentiation, contain mostly ductlike cells, suggesting that these cells may be progenitors for both acinar and duct cells. Our data are also consistent with the latter finding, as shown by a comparison of the lectin labeling patterns of undifferentiated cells with those of adult pancreatic cells (Table I). Among the adult pancreatic cells, the surface carbohydrate composition (as defined here by lectin binding) of centoacinar (i.e., ductlike cells) resembles most closely that of the undifferentiated cells
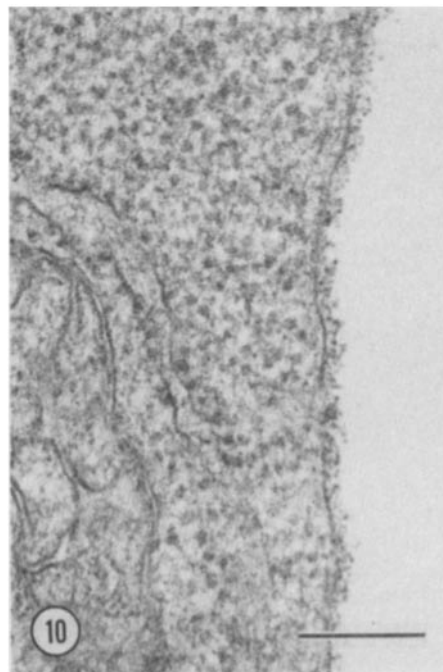
| Developmental age | Lectin-ferritin conjugates and their saccharide specificities | | | | | | |
|---|---|---|---|---|---|---|---|
| | Con A (Mann) | Lotus (Fuc) | Ulex (Fuc) | RCA I (Gal) | RCA II (NAcGal) | SBA (NAcGal) | WGA (NAcGlu) |
| **15 d** | | | | | | | |
| Undiff | ~ | – | – | +++ | +++ | +/– | +++ |
| Endo | + | – | – | +++ | +++ | – | +++ |
| **17 d** | | | | | | | |
| Undiff | ~ | – | – | +++ | +++ | ++/– | +++ |
| Endo | ++ | – | – | +++ | +++ | – | +++ |
| Ac | – | – | – | +++ | +++ | ++ | +++ |
| **19 d** | | | | | | | |
| Endo | ++ | – | – | +++ | +++ | – | +++ |
| CAc | ++ | – | – | +++ | ++ | – | +++ |
| Ac | ++ | +++/– | +++/– | +++ | +++ | ++ | +++ |
| **Adult** | | | | | | | |
| Endo | ++ | – | – | +++ | – | – | +++ |
| CAc | ++ | – | – | +++ | ++ | – | +++ |
| Ac | ++ | +++ | +++ | +++ | +++ | ++ | +++ |

Abbreviations used: Undiff, undifferentiated cells characterized by abundant free polysomes and sparsely developed Golgi complex and rough endoplasmic reticulum; Endo, endocrine A and B cells; Ac, acinar cells; CAc, centroacinar cells characterized by numerous mitochondria. Symbols used: + to +++, lectin-ferritin labeling density varying from a single, often interrupted layer to a heavy, multilayered pattern; –, absence of detectable labeling; ++/–, etc., labeling patterns in which part of the cell population is labeled while the rest is unlabeled.

with the exception of Con A binding sites, which appear to be absent from undifferentiated cells but present on adult centroacinar cells. Absence of Con A binding to undifferentiated pancreatic cells was unexpected, as in many differentiating systems (for example, see reference 7) Con A receptor density decreases during development.

The programming of stem cells toward a specific developmental pathway probably occurs early in the protodifferentiated stage and is reflected by early onset of plasmalemmal glycoconjugate differentiation (Table I). For example, in addition to lectin binding sites present on progenitor cells and common to all differentiated cells in the adult pancreas (i.e., binding sites for RCA I and WGA), the plasma membranes of presumptive acinar cells acquire unique cell-type-specific lectin binding sites as the first expression of their cytodifferentiation program. This event precedes formation of intracellular organelles characteristic of adult acinar cells. Thus, SBA receptors appear as early as day 15 in utero, and their density increases significantly during the secondary transition stage (about day 17), when some cells of the population can be clearly identified as acinar cells by the presence of zymogen granules. However, other lectin binding sites (such as those for Con A, Lotus lectin, and Ulex lectin) appear on acinar cells later in differentiation and are expressed beginning only at day 19 in utero when the cells have already undergone cytodifferentiation. In this connection, secretagogue responsiveness of acinar cells in the developing rat pancreas is also acquired in the late prenatal period (i.e., at day 21 in utero or 1 d before birth) although the intracellular apparatus for effecting exocytosis is functional 2 d earlier (5). The relationship between development of secretagogue responsiveness and maturation of plasmalemmal glycoconjugates remains unknown.

The appearance of SBA binding sites on presumptive acinar cells could, in principle, result from the activity of an endogenous neuraminidase acting on the cell surface, because we have shown that exogenously added neuraminidase from Cl. perfringens reveals cryptic SBA binding sites on undifferentiated embryonic cells, these sites presumably being "masked" by sialic acid–containing glycoconjugates. The expression of SBA binding sites could also result from a reduction of sialyltransferase activity or reflect changes in the charge, conformation, etc., of the same or adjacent cell surface glycoconjugates, allowing access to the SBA-ferritin probe. It is more likely, however, that the appearance of SBA binding sites results from the insertion of new membrane constituents containing N-acetyl galactosaminyl groups. This notion is supported by the fact that both RCA II and SBA recognize N-acetyl galactosaminyl residues, yet the former lectin binds to acinar cells at all stages of their development, suggesting that two different classes of N-acetyl galactosamine–containing glycoconjugates are finally present on mature acinar cells.

It is of interest to note that at the protodifferentiated stage of development, all domains of the surface of the undifferentiated cells appear equivalent in that they label homogeneously with lectin-ferritin conjugates. Early in the secondary transition of the gland rudiment (i.e., between days 16 and 17 in utero), however, the plasmalemma of presumptive acinar cells becomes polarized into discrete apical and basolateral domains coinciding with onset of formation of acinar lumina. This is reflected morphologically by acquisition of a "fuzzy" coating on the apical plasmalemma (19) and by relatively higher binding of WGA, RCA I, RCA II, and SBA to this domain. Differentiation of the apical plasmalemma is not completed until near birth when fucosyl residues become expressed, as detected by binding of Ulex and Lotus lectin-ferritin conjugates. In the adult gland, maintenance of polarized distribution of membrane constituents between apical and basolateral plasmalemmal domains depends on integrity of tight junctions between adjacent acinar cells (11, 21). Whether regional specialization of the plasmalemma in the developing pancreatic acinus precedes or follows establishment of tight junction belts encircling the apical poles of pancreatic acinar cells has yet to be determined, though studies by Cereijido et al. (3) indicate that establishment of epithelial cell polarity (both structurally and functionally) depends on formation of tight junctional complexes.

In the case of endocrine cells, the cell surface glycoconjugate pattern is also developmentally regulated. Thus, although endocrine A and B cells appear never to display binding sites for SBA (however, see below) or for *Ulex* and *Lotus* lectins (fucosyl residues), they display binding sites for RCA II at a time when the sites can be first identified by their content of characteristic secretory granules. These binding sites appear to be "lost" late in development (i.e., about the time of birth) and after cytodifferentiation has been completed. Loss of RCA II binding sites is, however, only apparent because RCA II binding on post-natal endocrine cells can be restored after neuraminidase treatment (16). It is conceivable that the apparent disappearance of RCA II binding sites is the result of insertion of additional membrane constituents that contain sialic acid leading to "masking" of RCA II binding sites and that terminal differentiation is related to this event. Because the absence of SBA binding to endocrine cells at all stages of development, including the adult, is also only apparent and can be reversed by neuraminidase treatment, the mature endocrine cell probably possesses two different classes of sialic acid–containing molecules. Further biochemical studies are required to substantiate this and to determine whether or not these molecules are sialoglycoproteins or sialoglycolipids. Finally, in contrast to acinar cells, in which accumulation of secretory product precedes both onset of secretagogue responsiveness (5) and terminal maturation of plasmalemmal glycoconjugates, endocrine B cells of the developing pancreas synthesize insulin and respond to secretagogues as early as day 16 of gestation (4), indicating that secretory competency and final differentiation of plasmalemmal glycoconjugates are not temporally coordinated.

From our studies it is impossible to determine if lectin binding sites that are present at all stages of development, including those on cells of the adult gland, are located on the same molecules or if they represent different species that are synthesized and inserted into the cell surface at different times of development. Along this line, Mintz and Glaser (12) have shown that even though Con A receptors are present throughout differentiation of neural retinal cells, the biochemical composition of these receptors changes during development of the retina.

Finally, changes of cell surface carbohydrate composition during development have also been reported in other systems such as chick embryo neural retina and liver (8, 17), neural crest cells (23), early embryos (2, 10, 18, 25), and cerebellum (7). The relationship of these changes to normal tissue histogenesis remains to be elucidated.

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