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USE OF A LECTIN-HISTOCHEMICAL METHOD TO CHARACTERIZE GLYCOSYLATION OF BIOPOLYMERS IN DUODENAL GLAND CELLS

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The use of lectins as a new class of histochemical reagents has led to considerable progress in recent years in our understanding of the structure and function of complex carbohydrates and carbohydrate-containing biopolymers of cells and tissues [4, 6, 8, 11]. With the aid of lectins not only can specific glycoconjugates and the cells accumulating them be identified, but they can also act as quite sensitive molecular probes, which can be used to study the redistribution of carbohydrate-containing biopolymers under physiological and pathological conditions [4]. In the present investigation an attempt was made to utilize lectins for the morphologic analysis of certain stages of glycosylation of carbohydrate-containing biopolymers in duodenal gland cells.

EXPERIMENTAL METHOD

Samples of duodenum from sheep and cows were studied. The choice of animals was determined by previous data showing that the duodenal glands of herbivorous mammals have the broadest spectrum of lectin receptors [14]. Pieces of duodenum from healthy mature animals were excised from the proximal third of the duodenum, not more than 30 min after death. Since the functional state of the glands, connected with digestive processes, affects the results of histochemical detection of carbohydrates in glandulocytes [1], material for investigation was taken only from animals with no chyme in their stomach. Histologic specimens were fixed in 10% neutral formalin and embedded in paraffin wax.

Serial sections 7 μ thick were treated with the following lectins: concanavalin A (con A), lentil (LCA) – both specific for αD-mannose, peanut (PNA), caster oil (RCA) – both specific for βD-galactose, soy (SBA) – specific for N-acetyl-D-galactosamine, wheat germ agglutinin (WGA) – specific for N-acetyl-D-glucosamine and N-acetylneuraminic acid, Laburnum anagyroides (LAA) – specific for αL-fucose [4]. The corresponding glycopolymers were detected by means of lectins labeled with horseradish peroxidase, followed by visualization in a system of diaminobenzidine – H_2O_2 [3]. Conjugates of lectins with peroxidase, and also purified peroxidase, for indirect detection of con A receptors were obtained at the L'vov branch of the A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR. The cytotopography of the lectin receptors was analyzed separately in gland cells located at the periphery of the terminal portions and in the central part of the glandular lobules separately. Microscopy and recording of the results were carried out by two workers independently of each other.

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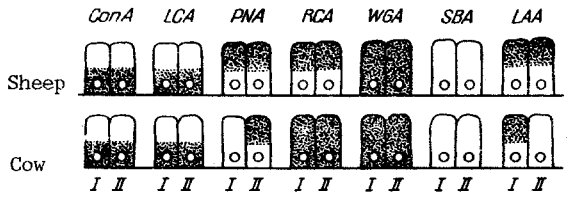

Fig. 1. Localization of receptors of lectins in cells of peripheral (I) and central (II) terminal regions of duodenal glands.

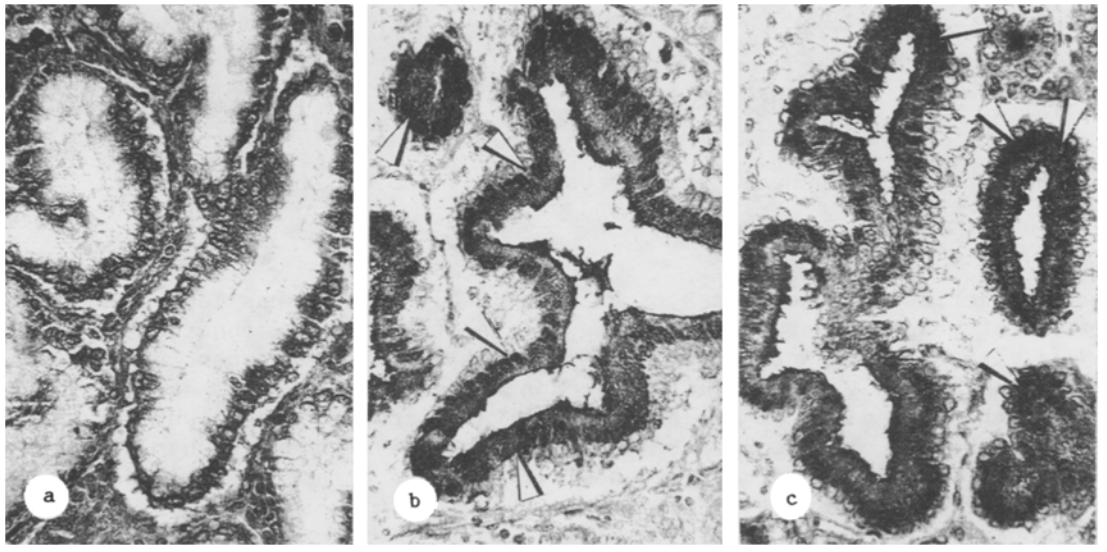

Fig. 2. Duodenal glands of sheep: a) localization of LCA receptors in basal part of cells; b) localization of PNA receptors in apical pole of cells with intensification of reaction in supranuclear zone of cytoplasm (arrows); c) localization of LAA receptors in apical part of cells, supranuclear zone of cytoplasm is stained more intensively (arrows). Magnification: 240 ×.

EXPERIMENTAL RESULTS

The results are summarized in the scheme in Fig. 1 and illustrated in Figs. 2 and 3. The regular cytotopography of receptors of individual lectins in particular parts of the cells will be noted. For instance, receptors for con A and LCA were located mainly in the basal and perinuclear zones of the cytoplasm (Figs. 1-3). Receptors of PNA, RCA, and LAA as a rule accumulated in the apical pole of the cells (Fig. 1), and most intensively in the supranuclear part of the cytoplasm. A characteristic condensation of receptors was observed there, in the form of a "cap," which is evidently part of the Golgi complex (Fig. 2b, c). According to electron microscopic data [13], it is in this part of the cytoplasm that the lamellar complex of duodenal gland cells is located. It is a striking fact that staining of this kind, in the form of a "cap" above the nucleus, also was found during immunochemical detection of certain enzymes involved in biosynthesis of glycoconjugates and, in particular, of galactosyl transferase [5]. Incidentally, duodenal gland cells of the cow stained only weakly, and diffusely, with RCA. In this case, however, the reaction also was stronger in the supranuclear part of the cells. A marked diffuse distribution in the cytoplasm also was characteristic of WGA receptors (Fig. 3c).

The results indicate a definite sequence of processing of glycopolymers in the direction of the basal to apical axis of the cell. Thus the preferential localization of con A and LCA receptors in the basal part of the cytoplasm indicates that D-mannose residues can be added in elements of the rough endoplasmic reticulum, for it is in that region of the duodenal gland cells that this organoid is located [13]. N-acetyl-D-glucosamine residues also are probably added to the oligosaccharide chains of glycoconjugates in elements of the endoplasmic reticulum, as follows from the character of distribution of WGA receptors in the gland cells.

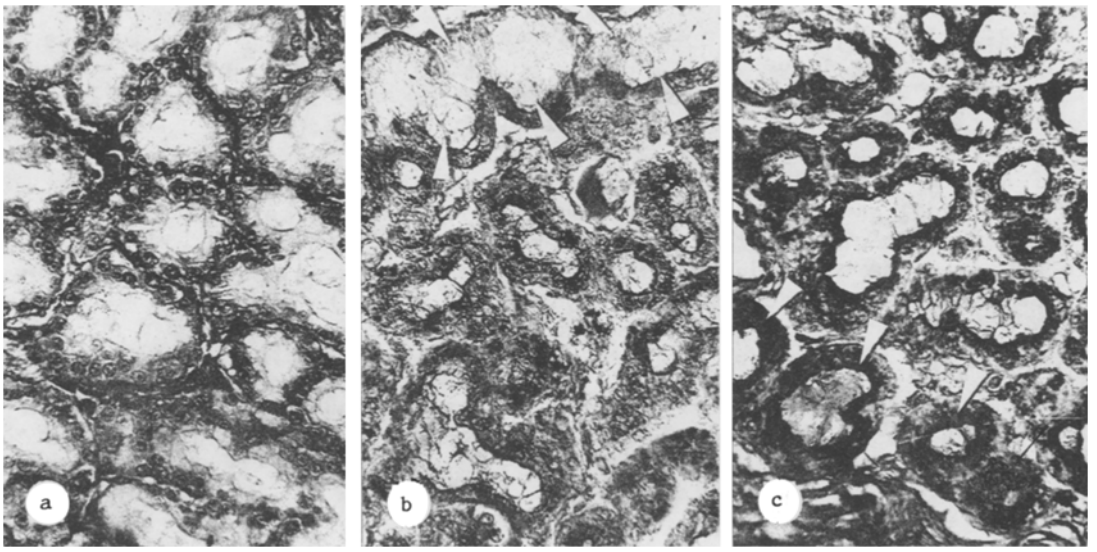

Fig. 3. Duodenal gland of the cow. a) Localization of LCA receptors in basal part of cells; b) absence of receptors in peripheral terminal regions (arrows), intensive reaction in acinar cells located in central part of glandular lobules; c) diffuse localization of WGA receptors with some increase in intensity of reaction in peripheral terminal regions (arrows). Magnification: 240 ×.

On the whole, this conclusion is confirmed by biochemical data on the course of the initial stages of glycosylation in elements of the rough endoplasmic reticulum, during which N-acetyl-D-glucosamine and D-mannose residues are added to the asparagine of the polypeptide chain with the aid of a glycoside bond [5].

Since the apical part of the cytoplasm of the cells remains areactive on staining with conA and LCA, it can be tentatively suggested that in these regions of the gland cells, in the course of the subsequent stages of glycosylation, either the D-mannosides are removed from the oligosaccharide chains or they are masked by residues of other monosaccharides. This conclusion is in good agreement with the results of biochemical investigations [10]. It is possible that the D-mannoside residues in the duodenal glandular cells of these particular species of animals are masked by residues of D-galactose, L-fucose, or N-acetyl-D-glucosamine, for it is mainly in the apical part of the cytoplasm that receptors for PMA, RCA, and LAA, and also some of the WGA receptors, are localized. It will be evident that the addition of these monosaccharide residues, which are receptors for the lectins mentioned, to oligosaccharide chains takes place actually within the elements of the Golgi complex, in agreement with the results of biochemical detection of the corresponding glycosyl transferases in the membranes of this organoid [5, 9], and also with data indicating the more intensive staining of the supranuclear zone of the cytoplasm of the gland cells by PNA and LAA.

Most of the glycoproteins synthesized in mammalian epithelial glandular cells are characterized by the presence of terminal sialic acid residues in the carbohydrate-containing prosthetic groups [12]. The process of sialation of glycoconjugates takes place in the final stages of glycosylation both in elements of the Golgi complex and also in the composition of secretory granules [5]. The distribution of WGA receptors binding not only N-acetyl-D-glucosamine residues but also N-acetyl-neuraminic (sialic) acid residues, diffusely over the whole apical cytoplasm, which we discovered, also points to such a possibility.

By the use of the lectin-histochemical method some species differences were discovered in the character of glycosylation of biopolymers in the animals studied. Investigation of the cytotopography of lectin receptors revealed differences in the course of this process in gland cells of the terminal regions, located in different parts of the glandular lobules. In the duodenal glands of cows, for instance, cells of the secretory divisions, located in the center of the lobules, have no receptors for LAA unlike cells of the peripheral terminal regions. This indicates absence of terminal L-fucose residues here, having been either removed in the course of glycosylation or masked by other monosaccharides. In the same species of animals gland cells from all parts of the glands bind WGA, evidence that N-acetyl-D-glucosamine is incorporated into the comosition of the biopolymers synthesized. This lectin also

has affinity for N-acetylneuraminic acid residues. The latter, if occupying the terminal position in the oligosaccharide chain (before the D-galactose residue), will block binding of PNA but will have no effect on binding of RCA [11]. Hence it can be concluded that sialation of terminal D-galactosyl residues of oligosaccharide chains takes place in cells of the peripheral terminal regions of the glands in cows, bonding WGA and RCA but a reactive relative to PNA (Figs. 1 and 3b). It is remarkable that when standard histochemical methods, used for analysis of the composition of carbohydrate-containing biopolymers, were adopted and, in particular, the PAS reaction and staining with alcian blue, no sialomucins could be detected in the cow's duodenal glands [7]. Similar anlaysis with respect to the sheep's glands indicates absence of the sialation stage in the processing of glycoconjugates in glandular cells of animals of this species.

We observed absence of binding by SBA-cells of the terminal divisions of the duodenal glands, evidence of absence of N-acetyl-D-galactosamine residues in the comosition of the terminal regions of glycoconjugates. As a rule these residues are found in undifferentiated cells, and only rarely in secretory glycoproteins [3, 5]. A positive reaction with this lectin was therefore found only in cells of the initial regions of the efferent ducts, which, according to data in [2], are cambial. The results definitely indicate a change in glycosylation processes during cell differentiation, manifested in particular as removal of N-acetyl-D-galactosamine residues or their masking by other monosaccharide residues (D-galactose, L-fucose, N-acetyl-D-glucosamine, sialic acid), taking place in more highly differentiated gland cells.

The results thus demonstrate that methods of lectin histochemistry can be used to study glycoconjugate processing in actively functioning, and also in differentiating glandular cells. The results are evidence that the method is sufficiently sensitive and that its use is indicated for a more objective analysis of the comosition and topography of carbohydrate-containing biopolymers of cells and tissues.

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