HISTOCHEMICAL LOCALIZATION OF GLUCOSE-6-PHOSPHATE RESIDUES IN HUMAN PLACENTA

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The important role played by glycoconjugates in activity of the living cell is well known. They are responsible for structural organization of the tissues and for processes of immune recognition and intercellular interaction. Many methods of analyzing these substances at the morphological level are available. Labeled lectins [7], labeled enzymes [3], and various histochemical techniques have been used [8]. A technique involving the use of labeled enzymes, with sugars as their substrates [2], is simple and provides a specific approach. Only glycoconjugates that are not washed out in the course of histochemical processing [3], i.e., the sugar components of protein molecules, are detected. The aim of this investigation was to study, by means of a reagent developed by the writers previously (glucose-6-phosphate dehydrogenase labeled with colloidal gold [2]), the distribution of sugar residues in the human placenta at the ultrastructural level.

EXPERIMENTAL METHOD

Colloidal gold was obtained by Frens' method [5]. The specimens obtained contained monodispersed gold particles measuring 10 nm. A conjugate of colloidal gold with glucose-6-phosphate dehydrogenase (G6PDH) was obtained by the following method [2].

The flocculation point for G6PDH ("Merck," 700U/ml) was determined. The flocculation point for bovine serum albumin was next determined and a mixture of this protein with G6PDH was added to the colloidal gold solution in a concentration 100 times greater than the flocculation points determined The resulting solution was centrifuged at 10,000*g* for 1 h to remove unstabilized particles, and the residue thus obtained was collected and kept at 4°C for 1-7 days.

Samples of placenta were taken immediately after full-term natural childbirth from five healthy women, from the central part of the placenta. The samples were fixed in 4% paraformaldehyde ("Serva," Germany) and embedded in the water-soluble resin LR White ("Serva," Germany) [9], by a somewhat modified version of our own method for subsequent ultrastructural investigation.

Ultrathin sections were cut from the material embedded in LR White, mounted on nickel grids, and preincubated for 20 min at 37°C in a 0.01% solution of bovine serum albumin in a humid chamber, after which they were transferred to a drop of dye diluted 5 times with distilled water. Incubation was carried out for 5-7 min, after which the grids were carefully washed and counterstained with uranyl acetate.

Cytochemical controls were set up by the following methods: 1) the grids were incubated in a concentrated solution of albumin, labeled with colloidal gold; 2) the grids were first incubated in solution of unlabeled G6PDH (30 min, 37°C), and then with the dye; 3) G6PDH labeled with colloidal gold was mixed with glucose-6-phosphate, and the sections were incubated after 20 min in this combination.

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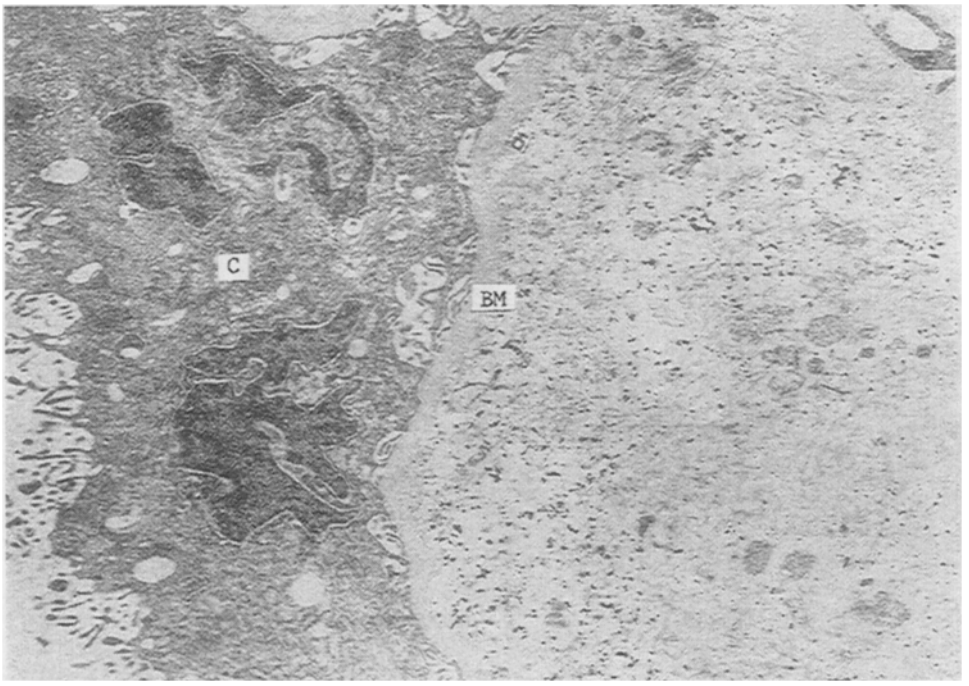

Fig. 1. Distribution of glucose-6-phosphate residues in human placenta. Collagen molecules in stroma of villi actively labeled. Basement membrane (BM) and syncytial cell (C) not labeled. 4000×. Section treated with G6PDH, labeled with colloidal gold.

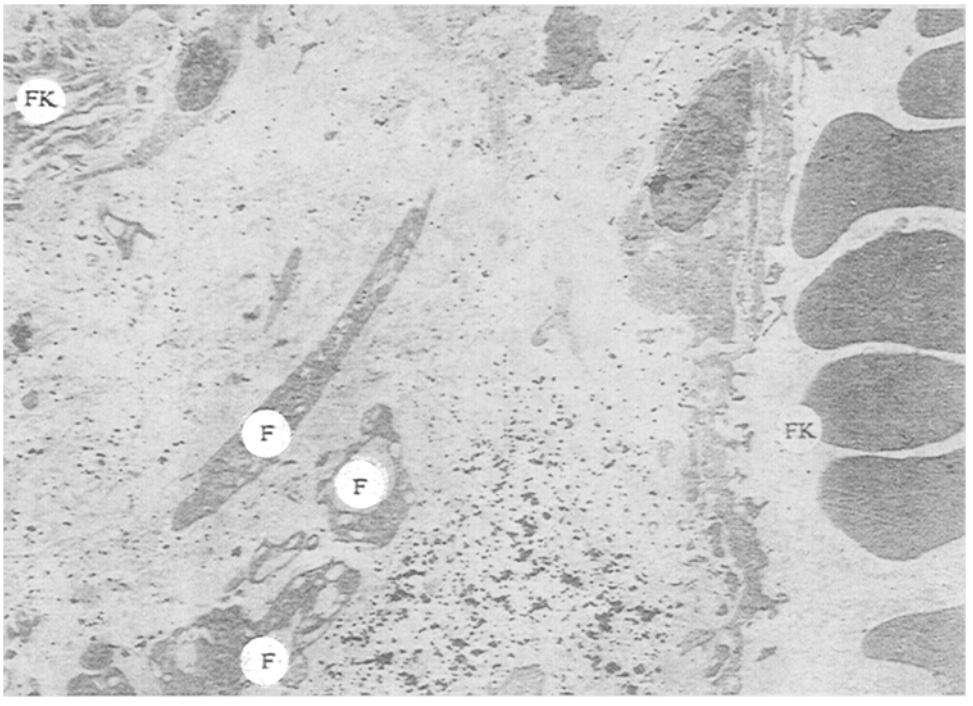

Fig. 2. Unevenness of specific staining of stroma of villi – area of active labeling borders of area in which collagen molecules contain very small amounts of glucose-6-phosphate residues. Cytoplasm and cisterns of fibroblasts are not labeled. 3000×. Section treated with glucose-6-phosphate, labeled with colloidal gold.

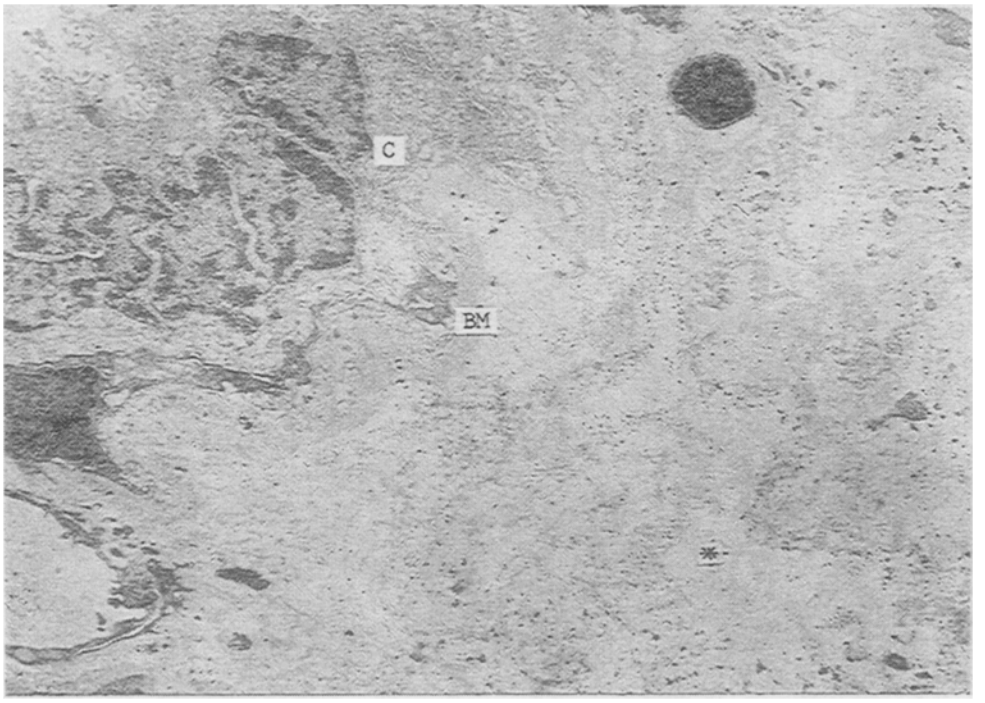

Fig. 3. Glycoprotein residues form a channel in stroma of villus not containing glucose-6-phosphate residues (*). C) Syncytial cell, BM) basement membrane. 2500×. Sections treated with glucose-6-phosphate, labeled with colloidal gold.

In the control sections only discrete particles of gold, haphazardly scattered over the section, could be found.

EXPERIMENTAL RESULTS

Ultrastructural analysis showed that glucose-6-phosphate residues were localized along collagen fibers (Fig. 1) of the connective-tissue stroma of the villi. The basement membrane of the syncytium and of the endothelial cells had some degree of labeling, but only in the lowest layer, bordering the connective tissue of the stroma of the villi (lamina reticularis). Some degree of nonuniformity of staining could also be observed: areas with many glucose-6-phosphate residues adjoined palely stained areas (Fig. 2). As a rule, areas containing many glucose-6-phosphate residues corresponded to regions of accumulation of collagen molecules. However, the individual concentrations of collagen molecules were largely glycosylated, whereas in neighboring regions the degree of glycosylation of the molecules was slight. Channels free from colloidal gold particles also were observed (Fig. 3). Areas of the syncytial membranes had very considerable and uniform labeling of their stroma. It is remarkable that the cells of the villi – syncytial cells, cytotrophoblastic cells, endothelial cells, macrophages, and fibroblasts – were virtually not positively labeled for glucose-6-phosphate residues. Meanwhile the blood nuclear cells and also platelets, occasionally found in the lumen of the fetal capillaries, stained very strongly for specific sugar residues in both nucleus and cytoplasm. Erythrocytes were palely stained.

This investigation showed that positive staining for sugar residues of glycoproteins, represented by glucose-6-phosphate, in the human placenta is concentrated almost exclusively in the extracellular space of the stromal connective tissue. Since the specific stain was not found in fibroblasts, the morphological picture which we saw evidently corresponds to that of extracellular glycolysis of collagen. The nonenzymic reaction through which glucose combines with the free amino groups of collagen has been well studied [6], and more recently the attention of research workers has been concentrated on extracellular glycosylation of proteins in diabetes [6, 11]. Correlation has been shown to exist between the blood glucose concentration in hyperglycemic animals and the degree of gly-

cosylation of the collagen molecules [6], and the disturbance in this case of the physicochemical properties of collagen is linked with processes of aging and diabetes [13].

Self-assembly of collagen is an extracellular process, by contrast with the addition of glucose to glucosaminoglycans [7]. With the dye that we used we could not detect any glucose-6-phosphate residues on the surface of the syncytial cells or endothelium and in fibroblasts, typical sites of localization of glucosaminoglycans, detectable with the aid of histochemical stains (ruthenium red, and so on) [7, 10]. Proteoglycans in the placenta may perhaps contain very small numbers of glucose-6-phosphate residues.

Collagen and proteoglycans are not the only compounds which can incorporate glucose residues into their composition. Hemoglobin, apolipoproteins of circulating plasma proteins, membrane proteins, structural proteins, intracellular tubulins, and nucleic acids are all capable of undergoing glycosylation [12, 13]. Embryonic cells can also incorporate glucose into the composition of their proteins [14]. The absence of a histochemical marker stain in cells of extraembryonic organs is thus a specific phenomenon, possibly characteristic only of extraembryonic tissues. Evidence in support of this view is given by the intensive staining of the cytoplasm and nuclei of blood cells in fetal capillaries, and also the presence of large numbers of glucose-6-phosphate residues demonstrated previously in nuclei and cytoplasm of hepatocytes [1]. The physiological role of the absence of glycosylation of cells in extraembryonic organs requires elucidation. The present investigation showed (Fig. 3) that during glycosylation some collagen molecules in the placenta remain weakly glycosylated, where they form intrastromal channels, along which fluid and substances dissolved in it may perhaps circulate.

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