FORMATION OF CELL COAT MATERIAL FOR THE WHOLE SURFACE OF COLUMNAR CELLS IN THE RAT SMALL INTESTINE, AS VISUALIZED BY RADIOAUTOGRAPHY WITH L-FUCOSE-3H

G. BENNETT and C. P. LEBLOND. From the Department of Anatomy, McGill University, Montreal, Canada

INTRODUCTION
Some 30 years ago, Chambers proposed that the cell surface is covered by a thin amorphous "cell coat" (1). The evidence in support of this view was mostly indirect until the cell coat was demonstrated in numerous rat cells by light microscopy with the periodic acid-Schiff or colloidal iron procedure (2) and by electron microscopy with periodic acid-silver methenamine (3) or phosphotungstic acid at low pH (4). The staining of the cell coat by these techniques was attributed to the presence of glycoprotein material (3) tightly attached to the outer leaflet of the plasma membrane (4).

The cell coat was found to be most prominent at the apical surface of cells which are in contact with a lumen (3, 5). In the small intestine of cat and some other species, a thick coating of periodic acid-Schiff-positive fuzzy material was described on the tips and sides of the microvilli making up the apical surface of columnar cells (5). To investigate the formation of this material, Ito (6) incubated pieces of cat intestine with glucose-3H and, using electron microscope radioautography, observed a reaction first over the cytoplasm and, after 1 hr, along the apical surface. Light microscope radioautographic studies carried out in our department showed that, minutes after injection of galactose-3H into young rats, the label was localized in the Golgi region of duodenal columnar cells and, soon after, was present at the apical border (7, 8). The early localization of the galactose label to the Golgi apparatus of intestinal cells was later confirmed in the electron microscope (9-11). These investigations led to the belief that the apical surface of intestinal columnar cells is characterized by a rapidly forming carbohydrate layer.

In the course of a recent study of this problem with galactose-3H (11), there were indications that the label appeared not only at the apical surface, but also at the other cell surfaces. However, this phenomenon was not so clear as one might wish because of scattered labeling in the cytoplasm. This scatter may be explained by unpublished observations of A. Weinstock showing rapid passage of some galactose label into glucose, which would then be utilized for glycogen synthesis or, after transformation into amino acids, for protein synthesis. On the other hand, recent experience with fucose-3H revealed that there was little transformation of this sugar into glycogen (12) or amino acids (Herscovics, unpublished) and that it was, in fact, rather stable (13). Accordingly, it was hoped that this sugar would provide radioautographic pictures with a minimum of scattered labeling in the cytoplasm. Furthermore, since in many glycoproteins fucose is located at the end of the carbohydrate side chains, whereas galactose is next to the end (14), it was thought that the uptake and migration of fucose label might resemble that of galactose label. This was, indeed, found to be the case.

MATERIALS AND METHODS

Young rats weighing 30-40 g and aged 2-3 wk were given a single intravenous injection of l-fucose-3H (5.0 mCi/animal; specific activity 4.3 mCi/m mole).
A single intravascular injection of the labeled sugar was used since under these conditions fucose label is known to decrease rapidly after injection (13), and, therefore, the injection would approximate a pulse labeling. The animals were sacrificed 2.5, 5, 20, and 35 min, 1, 4, and 24 hr following injection. The animals were anesthetized with ether and perfused for 15 min through the left ventricle with 2.5% aqueous solution of glutaraldehyde containing 37% of 0.05 M Sorensen buffer, 0.1% sucrose, and either 0.25 or 1% fucose. Short segments of duodenum were removed from the animals and kept for 2 hr in the same fixative as used for perfusion. The tissues were then trimmed, washed in 0.15 M Sorensen’s buffer, postfixed for 2 hr in 1% osmium tetroxide in 0.1 M Sorensen’s buffer, dehydrated in acetone, and embedded in Epon. Semithin (0.5 μ) sections were radioautographed for light microscopy with use of Kodak NTB2 emulsion. Thin (silver-to-gold) sections were prepared of the lower third of duodenal villi, radioautographed with use of Ilford L4 emulsion, and, after exposure, poststained with uranyl acetate followed by lead citrate.

In preliminary quantitation, silver grains were recorded as being over either the Golgi apparatus, or the lateral and basal surfaces, or the apical surface (defined as comprising the microvilli and, for reasons made clear below, an equal thickness of adjacent cytoplasm). Silver grains which were not over these structures, i.e. were farther away than a distance equal to their own diameter, were listed as being over the remainder of the cell (Table I).

RESULTS

At 2.5 and 5 min after fucose-3H injection, the light microscope showed strong reaction over the supranuclear Golgi region in columnar cells of duodenal villi. The intensity of reaction was maximal in the cells of the lower third of the villi and gradually decreased toward the apex, as was previously observed with galactose-3H (11). Crypt columnar cells exhibited only light reaction. In electron microscope radioautographs of columnar cells from the lower third of villi, silver grains were concentrated over the stacks of flattened saccules to confuse dense bodies of various sizes (D) with grains, occasional grains may be seen over nucleus (N) and cytoplasm. Below the microvilli (mv), there is one silver grain (horizontal arrow at upper left). Over the lateral cell membranes (lmb), only one grain is seen (horizontal arrow at upper right). No grains are seen over the basal cell membrane (bmb). X 8,000.
and the vesicles making up the Golgi apparatus (Fig. 1). In contrast, few silver grains were seen over the remainder of the cell, or over the apical, lateral, and basal surface membranes (Table I).

At later time intervals, the distribution of silver grains was greatly altered. Although there was some variation from animal to animal, a definite pattern was recognized. By 20 min after fucose-4H injection, light microscopic radioautographs showed a reaction not only over the Golgi region but, in addition, over all of the cell surfaces. This was confirmed in the electron microscope, for now in addition to silver grains over the Golgi apparatus (Fig. 3), several could be seen over the microvillar border (Fig. 2), over the lateral surface membranes (as seen in cells cut longitudinally—Fig. 3—or in cross-section—Fig. 4), and finally, over the basal surface membrane (Figs. 5 and 6). Silver grains were few over the cytoplasm outside of the Golgi apparatus except for a region immediately below the microvillar border (Fig. 2). Here the grains often seemed to be associated with smooth-surfaced, light vesicles, similar to those seen in the Golgi region (Fig. 3). Since labeling of this apical cytoplasm occurred at about the same time as that of the microvillar border, it was classified, for purposes of quantitation, as part of the latter.

At 35 min and 1 hr after fucose-4H injection, the distribution of silver grains remained basically similar to that observed at the 20-min time interval. By 4 hr after injection, however, the number of silver grains over the Golgi apparatus had greatly decreased, while over the apical and other cell surfaces they remained prominent (Fig. 7, Table I).

At 24 hr, reactions were examined in the light microscope in the upper region of villi on the assumption that the cells had migrated there from the lower third. A weak reaction definitely occurred over the apical surface membrane and possibly over lateral and basal surface membranes.

**DISCUSSION**

Fucose is known to be a component of many glycoproteins (14–17), in which it occurs as a sugar residue at the end of some of the carbohydrate side chains (14). Biochemical studies (12–13) have demonstrated that the rat small intestine incorporates labeled fucose into glycoprotein. Since sugar residues are taken up stepwise during the biosynthesis of a glycoprotein (14, 18), fucose is likely to be one of the last ones added. Hence, in the present study, the uptake of fucose label in the Golgi apparatus of columnar cells within 2.5 min after injection indicated that glycoprotein—or at least some of the side chains of glycoprotein—was being completed in this organelle.

By 20 min, a substantial number of grains appeared over surface membranes (Figs. 2 and 3). It was realized that this might be caused by either the labeling of the plasma membrane itself or the labeling of material associated with its outer surface (cell coat) or inner surface (cytoplasm). It was unlikely that the cytoplasm was responsible since the remainder of the cell cytoplasm (outside of the Golgi and apical regions) was not significantly labeled. The presence of glycoprotein on the outer surface as the "cell coat," on the other hand, made it likely that this was the site of deposition of the label.

The grains were not only over the apical surface as already observed with labeled galactose (7, 8, 10, 11), but also were over all other surfaces. The relatively heavier reaction over the apical surface (Fig. 2) reflected the greater amount of surface membrane on the microvillar border than on the rest of the cell. It may be seen in Table I that the percentage of silver grains over cell surfaces increased with time, while that over the Golgi apparatus decreased, suggesting that the glycoprotein material appearing at the cell surface had migrated from the Golgi region. An alternate possibility was a direct uptake of fucose for completion of a glycoprotein at the cell surface. If this were the case, however, it would be difficult to explain the fact that at 2.5 min after injection, silver grains were rare over the surface membranes and that when present they were usually over

1 The authors of both works implied that this glycoprotein may be a mucin (12, 13). However, in the present investigation, the mucin-secreting goblet cells of duodenum, although reactive, took up less label than columnar cells did. Furthermore, goblet cells comprised only about one-twentieth of the epithelial cell population (unpublished). Thus, the glycoprotein analyzed by Coffey et al. (12) at 1 hr after injection of labeled fucose, that is, at a time when our experiments showed much of the label at the cell surfaces, must have consisted mainly of cell coat material produced by columnar cells.
FIGURE 4 EM radioautograph of cross-section through the nuclear region of columnar cells 20 min after an injection of fucose-3H. Most of the silver grains are associated with the lateral cell membranes (lmb). Rare grains are over nucleus (N) or cytoplasm. × 12,400.

surfaces close to the Golgi apparatus. This finding suggested that these nearest surfaces were rapidly reached by migrating label.

Since the cell cytoplasm outside of the Golgi apparatus was only lightly labeled 20 min after injection, the migration of labeled glycoprotein to the surface must occur quickly (10). The occasional association of cytoplasmic silver grains with vesicles (Fig. 3) indicated that the vesicles might be carriers of the material. In the apical cytoplasm beneath the microvillar border, both vesicles and silver grains were numerous (Fig. 2), suggesting perhaps a slowing down before passage into the surface coat (11). Additional support for the role of these vesicles was given by the finding that, in these cells, the periodic acid–silver methenamine technique—a technique believed to be specific for glycoprotein—stained some Golgi sacculles and vesicles, similar vesicles scattered in the cytoplasm, and the cell surface itself (19).

In the present study, the number of silver grains at the apical surface increased up to 4 hr after injection of fucose-3H, but by 24 hr appeared to have decreased. This observation suggested that the cell coat material on this surface turns over, perhaps owing to shedding into the lumen. This conclusion is supported by in vitro studies on cat ileal columnar cells, in which the galactose label

Figures 2 and 3 EM radioautographs of columnar cells 20 min after injection of fucose-3H.

Figure 2 Apical surface of columnar cells. Numerous silver grains are now over the microvilli (mv) as well as over a region about 1 μ thick immediately below. Many of the silver grains over this region are associated with smooth-walled vesicles (oblique arrows). A few grains seem to be associated with the lateral cell membranes (lmb). At lower right, a multivesicular body (B) is unlabeled. × 11,000.

Figure 3 Longitudinal section of columnar cells. At this time, silver grains are still numerous over the Golgi apparatus. Most of the other grains in this figure are over the lateral cell membranes, which are convoluted in many places (lmb), or the basal cell membrane (bmb). The few grains seen over the cytoplasm are often associated with smooth vesicles (oblique arrows). × 10,200.

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FIGURES 5 and 6  EM radioautographs of the basal region of columnar cells 20 min after an injection of fucose-3H. Besides silver grains over the lateral cell membranes (lmb), some are found over the basal cell membrane (bmb) in contact with the basement membrane (BM, Fig. 6) or over folds of the basal cell membrane distinct from the basement membrane (Fig. 5, arrows). \( \times 18,000. \)

disappeared from the surface coat by 4–10 hr after cessation of incubation with labeled precursor (20).

In addition to the epithelial cells of the duodenum, many other cell types in the fucose-3H-injected animals were shown by light microscope radioautography to incorporate label at early times in the Golgi region. Some of these cells (liver, epididymis, stratified epithelia, and others) later exhibited labeling at their surfaces. The pattern described in this report may, therefore, be of widespread occurrence and may be the general mechanism by which cells produce their cell coat. This pattern would be analogous to that seen in plant cells in which the Golgi apparatus has been found to be the source of carbohydrate cell wall material (e.g. 21).

In summary, it is proposed that, in rat duodenal columnar cells and perhaps in some other cells, glycoprotein material whose synthesis is completed in the Golgi apparatus is transported to all cell surfaces to become part of the cell coat.

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FIGURE 7 EM radioautograph of duodenal columnar cells 4 hr after injection of L-fucose-3H. By this time interval, reaction over the Golgi apparatus (G) has greatly decreased. Three Golgi stacks are seen over which only two grains are present (each beneath a letter G). About 10 silver grains are seen over lateral membranes (lmh), and many also occur over the microvillar border (mv). In contrast to the situation in Fig. 2, little reaction is present over the cytoplasm immediately beneath the microvillar border. (Gob: Goblet cell). × 5,700.

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