

**MIGRATION OF GLYCOPROTEIN FROM GOLGI
APPARATUS TO CELL COAT IN THE COLUMNAR
CELLS OF THE DUODENAL EPITHELIUM**

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A layer of carbohydrate material appears at the surface of a variety of mammalian cells (1) and is particularly abundant at free surfaces such as the striated border of the columnar cells of the intestine (2). In electron micrographs of intestinal cells, this material appears as a discrete coating covering the tips and sides of the microvilli (3). From the various histochemical tests applied (3-10), it has been concluded that this "cell coat" consists of glycoprotein rich in acidic residues (1, 9).

After incubation of cat intestine with a labeled precursor of complex carbohydrates (glucose-³H) followed by EM radioautography, Ito (11) observed radioautographic reaction first over the cytoplasm of columnar cells and, after 1 hr, over the cell coat of the free surfaces. In this laboratory, by means of light microscopic radioautography, after injection of galactose-³H into young rats, label was localized, at early time intervals, to the Golgi region of duodenal columnar cells, and later traced

to the striated border (12, 13). Jersild (14) confirmed this early localization of galactose-³H to the Golgi apparatus of intestinal cells with the electron microscope. In recent in vitro EM radioautographic studies, Ito (15) reported incorporation of tritium-labeled mannose and galactose into the Golgi apparatus of cat intestinal cells at early time intervals and into the apical surface coat later.

The present in vivo study, on young rats, is an attempt to locate more exactly the source of apical cell coat material in rat duodenal columnar cells, and to learn about its mode of transport to the cell surface.

MATERIALS AND METHODS

Single injections of galactose-³H (3.33–5.0 mCi/animal; specific activity 1.17–2.79 Ci/mmmole) were given to young male albino rats, between 2 and 3 wk of age (weighing 25–40 g), and whose diet consisted of mother's milk and/or Purina laboratory chow. The injections were administered either intraperitoneally or intravenously via the jugular vein. After time intervals varying from 2½ to 60 min following injection, the animals were anesthetized with ether and perfused through the left ventricle with either 2.5% phosphate-buffered (pH 7.0) glutaraldehyde (0.1% sucrose, 1% galactose) or 4% phosphate-buffered (pH 7.0) paraformaldehyde. After 15 min perfusion, short segments of duodenum were removed from the animal and fixed by immersion for a further 2 hr in the same fixative that was used for the perfusion. The tissues were then further trimmed, washed in buffer wash (0.15 M Sorensen's buffer), and then postfixed for 2 hr in phosphate-buffered 1% osmium tetroxide, dehydrated in acetone, and embedded in Epon.

Thin sections were prepared of the lower one-third of the duodenal villus; they were radioautographed by the coating technique, using Ilford L4 emulsion, and poststained with uranyl acetate followed by lead citrate.

RESULTS

When radioautographs of duodenum from animals sacrificed 2½ min after galactose-³H injection were examined with the light microscope, all villus columnar cells were seen to incorporate label in the supranuclear Golgi region of their cytoplasm. The intensity of reaction decreased gradually from the base to the apex of a villus. Goblet cells throughout crypt and villus as well as columnar crypt cells also showed supranuclear radioautographic reactions but of a much lighter intensity than those over villus columnar cells. Comparison

of radioautographs of animals injected intravenously with those of animals injected intraperitoneally revealed no differences in over-all intensity or distribution of label.

Electron microscope radioautographs of duodenal columnar cells from animals sacrificed 2½ min after galactose-³H showed that over 70% of the silver grains were concentrated over the Golgi apparatus (Fig. 1, *G*). In these cells, the Golgi apparatus consists of parallel arrays of three to five flattened saccules whose ends are often dilated (Fig. 2). Along one side of the arrays of saccules, there are Golgi vacuoles (*GV*) measuring about 1400 Å in diameter as well as small coated vesicles (*CV*). Within the Golgi apparatus, silver grains were mainly localized over saccules and vacuoles (Fig. 2). The labeling of endoplasmic reticulum was relatively slight (about 15% of grains), as was also the case at later time intervals.

By 10 min after galactose-³H injection, some radioautographic reaction was still seen over the Golgi apparatus, but a considerable number of grains were localized over the apical region of the cytoplasm (Fig. 3). Beneath the striated border in these cells is an area of cytoplasm which is relatively free of mitochondria and endoplasmic reticulum, but contains smooth-surfaced vesicles (Figs. 3–4, *SV*) which average about 1400 Å in diameter, like the vacuolar elements of the Golgi apparatus. At 10 min after galactose-³H injection, many silver grains appeared to be located over these vesicles.

Fig. 4 illustrates a radioautograph of an animal sacrificed 1 hr after galactose-³H injection. By this time interval, most silver grains were seen over the microvilli, and some were seen over the hazy surface material covering the sides and tips of microvilli (*SC*). A few grains were still seen over the apical cytoplasm as well as the Golgi apparatus; some were also seen over the lateral and basal cell surfaces.

DISCUSSION

The reaction of the apical cell coat material of the duodenum with the PA-Schiff stain in the light microscope (2) and PA-silver stain in the electron microscope (9) is indicative of its glycoprotein nature. Galactose and mannose residues have been found in ameba surface coat (16), as in many other glycoproteins (17). It was, therefore, felt that galactose would provide a good label for the study of cell coat glycoprotein synthesis. Indeed,

in various in vitro experiments, labeled galactose has been shown to be incorporated into glycoproteins (18-22).

In the present study, most of the galactose-³H label at 2½ min after injection was localized over the Golgi apparatus of duodenal columnar cells (Fig. 1). It has been shown that the incorporation of sugars into glycoproteins proceeds by the stepwise addition of simple sugars, each mediated by a specific glycosyl transferase (18, 21). Recent studies on bovine liver and epididymal cells (23) have shown that galactosyl transferase activity is concentrated in the Golgi apparatus. Thus, the evidence from the present study suggests that in duodenal columnar cells the Golgi apparatus is the site of incorporation of galactose moieties into synthesizing glycoproteins.

By 10 min after galactose-³H injection, many silver grains were seen over the ectoplasmic region of the cytoplasm just below the microvillar border, while occasional grains were seen over the microvilli themselves (Fig. 3). The light reaction over the cytoplasm between the Golgi apparatus and the apical region suggests a fairly rapid migration of the label between these two regions. Ito (15), in his in vitro studies, had already suggested a rapid transit of label from the Golgi apparatus to the microvillar border itself. The present evidence further suggests that the rapidly migrating label slowed down in the apical ectoplasmic region prior to reaching the microvillar border.

The role of the smooth-surfaced vesicles seen in Figs. 1-3 is not yet clear. The possibility exists that these vesicles are derived from pinocytosis of the cell surface. However, their frequent association with silver grains suggests that they may

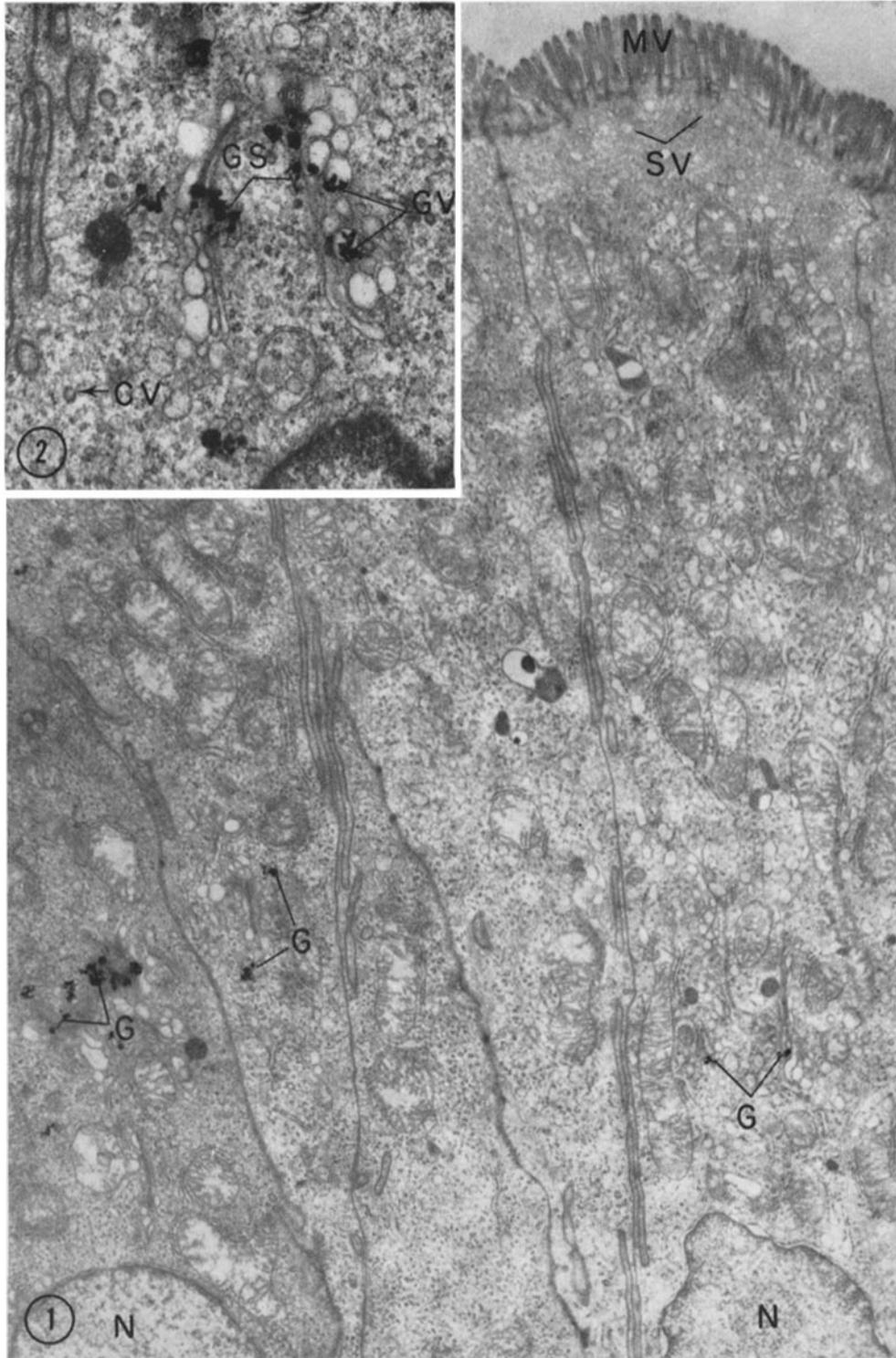
transport labeled material from the Golgi apparatus to the cell surface. While such vesicles have not been described in radioautographs of the intestinal cells of adult animals (11, 15), recent morphological studies have given indications that vesicles such as these may be important in transferring material to the cell surface in other intestinal absorptive cells. Thus, an electron microscope study of mouse colonic columnar cells by Wetzel et al. (6) showed smooth-surfaced vesicles in the apical cytoplasm beneath the striated border (their Fig. 3) which closely resemble the vesicles observed in the present study. When their tissue was stained with iron stains for acidic carbohydrate material, reaction occurred on the surface coat and in these apically located vesicles, as well as on similar vesicles adjacent to Golgi saccules, and on the Golgi saccules themselves. Those authors suggested that the iron-stained material is synthesized in the Golgi apparatus and transported to the cell surface by means of these vesicles. Similar results were obtained with colloidal thorium in the mouse colon by Weinstock (24), in the rat by Berlin (8), and in *Xenopus* by Bonneville and Weinstock (25).

Thus, in summary, it is suggested that in duodenal columnar cells of young rats the carbohydrate portion of glycoproteins destined for the surface coat is synthesized in the Golgi apparatus where it combines to the protein portion. This material then migrates through the cytoplasm to the apical surface, and is added to the apical cell coat. The presence of some silver grains over the lateral surfaces raised the possibility that the lateral and basal cell coats also received some of this material.

In preliminary light microscope radioauto-

FIGURE 1 Electron microscope radioautograph of duodenal columnar cells 2½ min after galactose-³H injection (exposure 35 days). Parts of several columnar cells are seen in longitudinal section. The nuclei of two of the cells can be seen at the bottom of the figure (*N*), while at the top the microvillous border of one of the cells is seen (*MV*). At this time interval, most of the silver grains are localized over the Golgi apparatuses (*G*). At the apex of the cells is an ectoplasmic zone of cytoplasm which is devoid of endoplasmic reticulum and mitochondria but in which are seen smooth-surfaced vesicles (*SV*). × 12,000.

FIGURE 2 Golgi region of a columnar cell from the same preparation. The Golgi apparatus in these cells is composed of three types of elements: parallel arrays of flattened saccules (*GS*), whose ends may be somewhat dilated, smooth-surfaced vacuoles (*GV*), and small coated vesicles (*CV*) scattered around the other elements. The silver grains at this time interval (2½ min after injection) appear to be localized mainly over Golgi saccules and vacuoles. × 24,000.



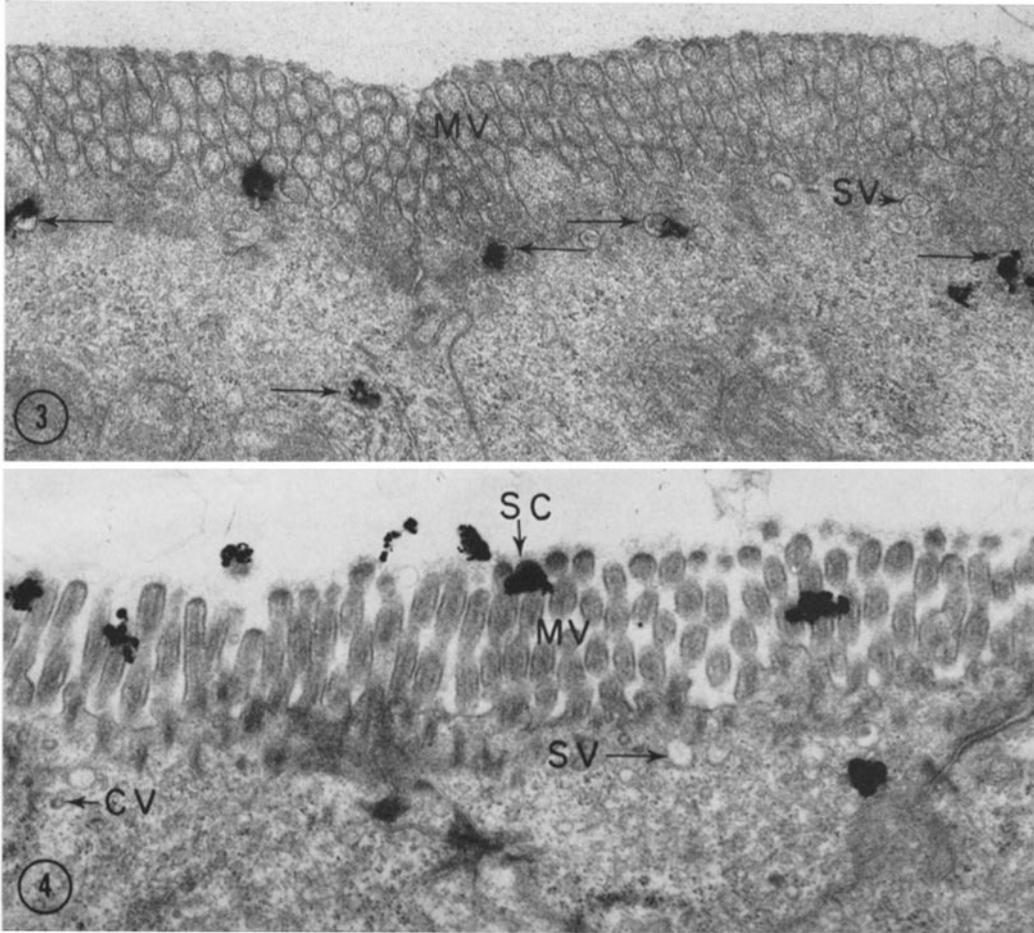


FIGURE 3 Electron microscope radioautograph of the apical portion of a duodenal columnar cell 10 min after galactose-³H injection (exposure 66 days). At the top of the picture, the microvillous border (*MV*) is seen cut in a somewhat oblique section. Beneath this border is a zone which is free of mitochondria and endoplasmic reticulum but in which smooth-surfaced vesicles (*SV*) can be seen. At this time interval, grains can be seen over the apex of the cytoplasm, but few are seen over microvilli and none over their free edge. Many grains lie over vesicles (arrows). $\times 23,000$.

FIGURE 4 Apical portions of duodenal columnar cells 60 min after galactose-³H injection (exposure 75 days). In this section, the apical surface coat (*SC*) is shown clearly above the tips of the microvilli (*MV*) and extending down between them. In the region below the microvillar border, smooth-surfaced vesicles (*SV*) similar to those seen in Fig. 2 are present. Some small coated vesicles (*CV*) can also be seen. By this time interval, many grains are seen over the microvilli and some have reached the hazy material of the surface coat at the tips and sides of microvilli (*SC*). $\times 24,000$.

graphic studies with galactose-³H, several other rat cell types showed migration of label from the Golgi region to the cell surface; thus, in surface columnar cells of the large intestine, kidney proximal and distal tubule cells, duct cells of various glands and epididymal cells, label was

localized at early time intervals to the Golgi region but later migrated to the apical surface membrane. The pattern of Golgi synthesis and migration to free surfaces may, therefore, be of widespread occurrence. Furthermore, the speed at which the passage of material takes place from

the Golgi apparatus to the cell coat in intestinal columnar cells and perhaps other cells suggests that the rate of renewal of the cell coat may be quite rapid.

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