MIGRATION OF GLYCOPROTEIN FROM THE GOLGI APPARATUS TO THE SURFACE OF VARIOUS CELL TYPES AS SHOWN BY RADIOAUTOGRAPHY AFTER LABELED FUCOSE INJECTION INTO RATS

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ABSTRACT

A single intravenous injection of L-[^{3}H]fucose, a specific glycoprotein precursor, was given to young 35–45 g rats which were sacrificed at times varying between 2 min and 30 h later. Radioautography of over 50 cell types, including renewing and nonrenewing cells, was carried out for light and electron microscope study.

At early time intervals (2–10 min after injection), light microscope radioautography showed a reaction over nearly all cells investigated in the form of a discrete clump of silver grains over the Golgi region. This reaction varied in intensity and duration from cell type to cell type. Electron microscope radioautographs of duodenal villus columnar cells and kidney proximal and distal tubule cells at early time intervals revealed that the silver grains were restricted to Golgi saccules. These observations are interpreted to mean that glycoproteins undergoing synthesis incorporate fucose in the saccules of the Golgi apparatus. Since fucose occurs as a terminal residue in the carbohydrate side chains of glycoproteins, the Golgi saccules would be the site of completion of synthesis of these side chains.

At later time intervals, light and electron microscope radioautography demonstrated a decrease in the reaction intensity of the Golgi region, while reactions appeared over other parts of the cells: lysosomes, secretory material, and plasma membrane. The intensity of the reactions observed over the plasma membrane varied considerably in various cell types; furthermore the reactions were restricted to the apical surface in some types, but extended to the whole surface in others.

Since the plasma membrane is covered by a "cell coat" composed of the carbohydrate-rich portions of membrane glycoproteins, it is concluded that newly formed glycoproteins, after acquiring fucose in the Golgi apparatus, migrate to the cell surface to contribute to the cell coat. This contribution implies turnover of cell coat glycoproteins, at least in nonrenewing cell types, such as those of kidney tubules. In the young cells of renewing populations, e.g. those of gastro-intestinal epithelia, the new glycoproteins seem to contribute to the growth as well as the turnover of the cell coat. The differences in reactivity among different cell types and cell surfaces imply considerable differences in the turnover rates of the cell coats.

INTRODUCTION

The surface of most cells shows no structural peculiarities in the electron microscope and was paid little attention until it was found to stain with histochemical reagents for carbohydrate. First, strong staining with the periodic acid (PA)-Schiff technique was reported on the free surface of epithelial cells in intestine and kidney tubules (36) and on the lateral surfaces of intestinal cells (50). Later, moderate PA-Schiff staining was described on the various surfaces in all cells of the rat (56). Staining with colloidal iron was also described at first on the free surfaces of the epithelia of bladder (13) and intestine (29) and later, on all surfaces of all cells (56). Since the PA-Schiff technique indicates the presence of glycoprotein (39), while colloidal iron detects acidic residues (47), it was concluded that glycoproteins carrying acidic groups were present along the cell surface, forming what was termed the "cell coat" (56). These results were obtained in the light microscope, but were later confirmed in the electron microscope using PA-silver methenamine (55) or the low-pH phosphotungstic acid technique (51), both of which are specific for glycoprotein (52). Similar results were obtained with the cationic dyes, colloidal iron (4) and thorium (55) and with ruthenium red (42), which indicate the presence of acidic groups. The overall conclusion was that a cell coat containing acidic glycoproteins is localized on the outer surface of the plasma membrane of all cells (4, 55).

Direct chemical analysis of surface membranes from animal cells reveals a carbohydrate content of 2–10% (70). A small fraction of this carbohydrate is accounted for by glycolipids (27). Mucopolysaccharides have not been shown to be present at the surface of cells in vivo, although heparin sulfate has been demonstrated at the surface of cultured mammalian cells (35). The absence of uronic acid residues in the isolated microvilli of intestinal cells (which are presumed to contain a high concentration of cell-coat material) clearly rules out the presence of mucopolysaccharides at this site (17). On the other hand, the cell surface has been found to be rich in glycoproteins (27). Separation techniques have shown that different species of glycoproteins are present at the surface of each cell type (1, 20). According to recent evidence (43, 61, 70), the plasma membrane consists of a bimolecular phospholipid layer which is interrupted in places by protein and glycoprotein molecules. Glycoprotein molecules usually have a nonpolar end embedded within the lipid bilayer and a polar end extending outward from the outer surface. This polar end holds the carbohydrate side chains. These in turn are composed of aminosugars, neutral sugars including fucose, and sialic acid residues (63, 70). The acidic groups of these residues account for the staining with colloidal iron, while the glycol groups of the sugars react with PA-Schiff. The carbohydrate-containing portions of the membrane glycoproteins extend outward at the cell surface where they constitute the cell coat.

Several investigations indicate that the cell coat is synthesized by the cell itself. After incubation of cat intestine with a labeled precursor of glycoprotein ([^{3}H]glucose) followed by radioautography, Ito (30) observed in intestinal epithelial cells that the labeled material appeared first in the cytoplasm and later in the microvilli of the apical surface. Neutra and Leblond (48) confirmed thse observations in vivo after injection of labeled galactose into young rats and added that the initial appearance of the label in the cytoplasm was restricted to the Golgi region. The uptake of galactose label in Golgi saccules and the passage of this label to the microvillar surface was further demonstrated in intestinal epithelial cells by Ito (31) and Bennett (5). When the experiment was repeated by Bennett and Leblond (7) using labeled fucose instead of galactose, the initial uptake was again restricted to Golgi saccules and, within 20 min, the label had migrated not only to the microvillar surface, but also to the lateral and basal membranes. Since labeled fucose is an excellent precursor of glycoproteins (2, 24, 32) the results were interpreted as indicating that cell coat glycoproteins are completed in the Golgi apparatus and migrate from there to the whole cell surface.

At about this time, the fate of [^{3}H]fucose was examined in the thyroid gland (23). Again the label was initially observed in the Golgi apparatus, but it later migrated to the luminal colloid. None appeared along lateral and basal membranes, while the label observed along the apical membrane was tentatively assigned to the highly labeled colloid secretion which accumulated there.

Hence, in the two tissues investigated by our

group, the fucose was initially incorporated in the Golgi apparatus, but in the small intestine it migrated to all cell surfaces (7) and in the thyroid, to secretory material (23). Was fucose incorporation at the cell surface a special feature of the intestinal epithelium, or was it a property shared with other cells? Furthermore, since the intestinal epithelium undergoes renewal, its cells would be young and perhaps still in the process of completing their growth; we therefore wondered if the passage of fucose label to the cell surface represented an *addition* of material to an incomplete coat. The alternate explanation would be the *turnover* of an already completed coat.

It was decided to examine the fate of [^{3}H]fucose in a variety of cell types including secretory and nonsecretory as well as renewing and nonrenewing cells. The work was done in two stages. A survey of representatives of these various cell types was carried out in light microscope radioautographs at different times after [^{3}H]fucose injection. Then an electron microscope investigation was carried out on two tissues from the same animals. One was the epithelium of the small intestine, which was reexamined as a model for cells undergoing renewal. The second was the kidney, in which the epithelial cells of proximal and distal tubules served as models for cells which do not undergo renewal.

MATERIALS AND METHODS

Young rats weighing 35–45 g were each given a single intravenous injection of 5.0 mCi of L-[^{3}H]-fucose (New England Nuclear, Boston, Mass.; specific activity 4.3 Ci/mol; radiochemical purity greater than 98%). Under these conditions, the fucose label decreases rapidly in blood and tissues (2), and therefore this procedure approximates pulse labeling. The animals were sacrificed 2–2.5, 5, 10, 20, and 35 min, 1, 4, 22, and 30 h later by glutaraldehyde perfusion through the left ventricle under ether anesthesia. The 2.5% aqueous solution of glutaraldehyde contained 35% of 0.05 M Sörensen's buffer, 0.1% sucrose, and either 0.25 or 1% fucose.

For electron microscope radioautography, short segments of duodenum and small pieces of kidney cortex tissue were removed from the animal and kept for 2 h in the same fixative as used for perfusion. The tissues were then trimmed, washed in 0.15 M Sörensen's buffer, postfixed for 2 h in 1% osmium tetroxide in 0.1 M Sörensen's buffer, dehydrated in acetone, and embedded in Epon. Thin (silver to gold) sections of the lower third of duodenal villi and of kidney cortex tissue were radioautographed with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England) by the method of Kopriwa[1] and, after exposure, poststained with uranyl acetate followed by lead citrate.

For light microscope radioautography, about 30 organs were removed from the animal and placed overnight in Bouin's fixative. They were all routinely embedded in paraffin in one block. Hard tissues were decalcified in 4.13% disodium ethylenediaminetetraacetic acid (EDTA) containing 0.44% sodium hydroxide (isotonic at pH 4) at 4°C until soft (67). These tissues were then similarly embedded in one block. In both cases, sections 5 μm in thickness were stained with hematoxylin and eosin, colloidal iron-Feulgen, or periodic acid-Schiff-hematoxylin. Some sections were treated with saliva for removal of glycogen. Radioautography was carried out by the coating technique (33) using liquid Kodak NTB 2 emulsion (Eastman Kodak Co., Rochester, N. Y.).

For quantitation of the intensity of reaction over the various cell types at different time intervals (Tables I and II), sections from all of the experimental animals used in this study were radioautographed at one time using the same batch of Kodak NTB 2 emulsion and were exposed for the same period of time.

Half-micron thick, unstained sections of the Epon-embedded duodenal and kidney tissue were also processed for light microscope radioautography in the same manner as paraffin sections. After radioautography, the sections were poststained through the emulsion with toluidine blue.

RESULTS

When the tissues were examined with the light microscope, nearly all cell types exhibited some radioautographic reaction after [^{3}H]fucose injection. At the earliest time intervals, a reaction of variable intensity occurred in the form of a discrete, paranuclear clump of silver grains. The location of this clump corresponded to that of the Golgi apparatus whenever its location was known in the cell under study. No significant reaction was detected over the nucleus and the rest of the cytoplasm. With time, the distribution of label in the cells changed. In most cells, the label appeared at the cell surfaces (Table I). In others, it was in the apical secretory material (Table II). A few scattered silver grains were over the rest of the cytoplasm, but not over the nucleus.

[1] B. Kopriwa. 1973. A reliable, standardized method for electron microscope radioautography. *Histochemie.* In press.

Cell Types in Which the [³H]Fucose Label Migrated from the Golgi Region to the Cell Surface (Table I)

This pattern of radioautographic reaction was best seen in the columnar epithelial cells of *intestinal epithelium*. In the *duodenum*, reaction was heaviest over the *cells covering the lower third of the villi* and decreased gradually toward the apex (Fig. 1). At 2–10 min after [³H]fucose injection, there was a dense accumulation of silver grains in the supranuclear Golgi region (Fig. 2). At 1 h after injection, silver grains appeared in fair number over the apical (microvillar) and lateral cell surfaces but were rare over the basal surface (Fig. 3). By 4 h after injection, the number of silver grains markedly decreased over the Golgi region but reached a peak over the cell surfaces (Fig. 4). The apical and lateral surfaces still exhibited a definite but smaller number of grains 30 h after injection.

In the *large intestine*, the columnar cells making up *surface and crypt epithelium* exhibited at the 2-min interval a strong Golgi reaction increasing somewhat from the base of the crypts to the surface (Fig. 5). At later time intervals, this Golgi reaction decreased while a heavy reaction appeared over the apical cell surface (Fig. 6). In contrast to duodenal cells, little reaction was seen over lateral cell surfaces.

In the *columnar cells lining the vas deferens*, a localized reaction in the supranuclear Golgi region at 2 min (Fig. 29) was later replaced by one at the apical cell surface (Table I). In the epithelial cells lining the *choroid plexus* the label was detected first in the paranuclear Golgi region (Fig. 10) and later along the apical and, to a lesser extent, lateral cell surfaces (Fig. 11). In *hepatocytes* at the earliest time intervals, localized grain clusters were located near bile canaliculi (Fig. 7). By 20 min after injection, a moderate number of grains (Fig. 8) and by 4 h, a large number (Fig. 9) occurred over the cell surfaces facing the sinusoids. Similar patterns of reaction occurred over *adrenal cortex cells* and over the epithelial cells covering the *ciliary body of the eye* or lining the *trachea*, *epididymis*, *oviducts*, and *ducts in the submaxillary and other glands of the body* (Table I).

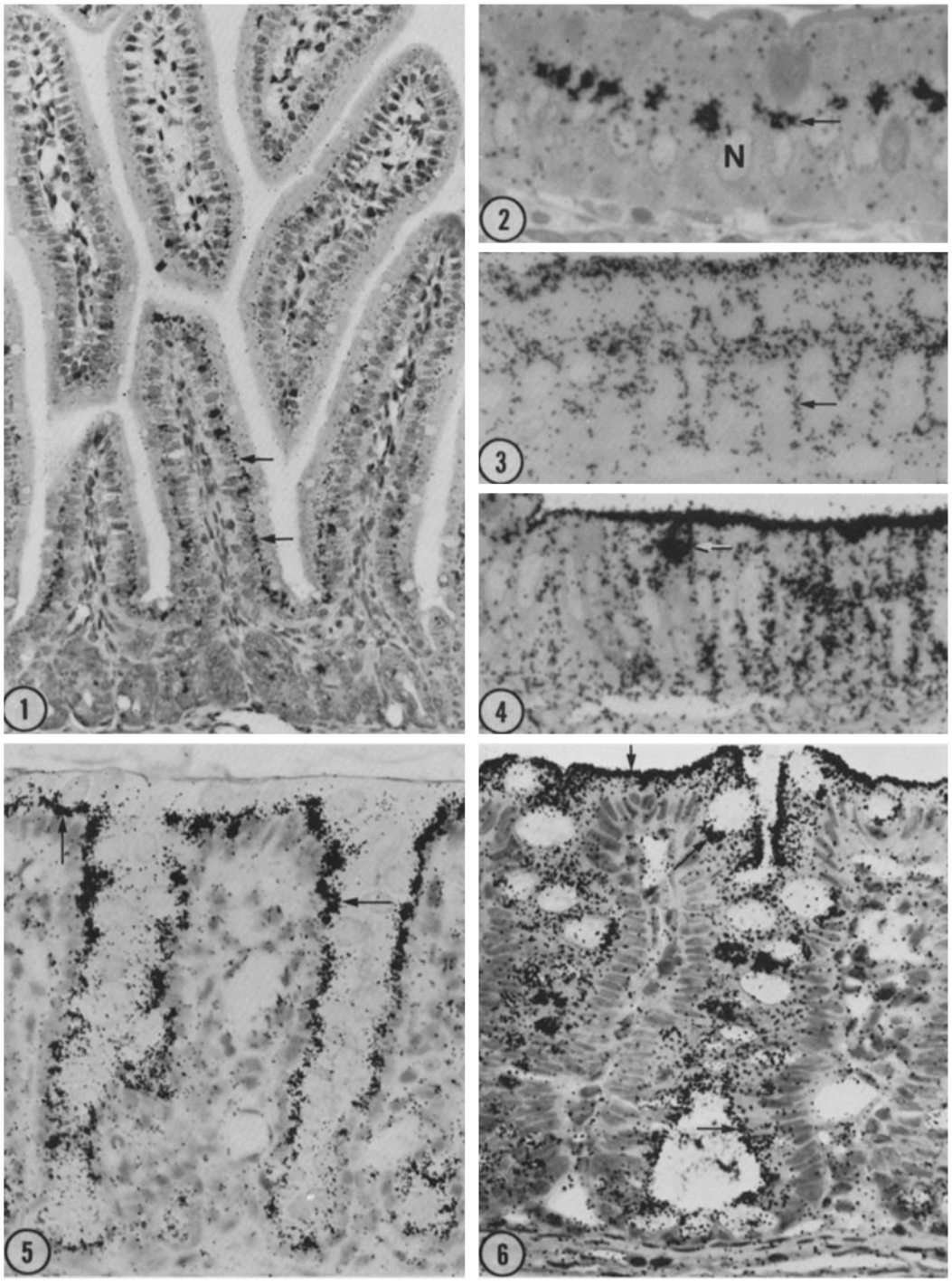
In the *kidney*, at early time intervals a heavy Golgi localized reaction occurred over *distal tubule cells* (Fig. 14); a similar, but usually lighter reaction appeared over *proximal tubule cells* (Fig. 15). In both cell types the reaction later became localized over the cell surfaces, especially the apical one (Figs. 16 and 17). However, the intensity of all these reactions greatly varied from tubule to tubule.

Even *simple sqaumous epithelial cells* such as *capillary endothelial cells* exhibited definite paranuclear localizations of silver grains at 10 min after injection (Fig. 21); by 4 h the silver grains covered the entire luminal surface of the cells (Fig. 22).

In *stratified epithelial membranes*, small paranuclear grain clusters were observed at early time intervals in cells of the surface layer in *bladder transitional epithelium* (Fig. 18), the spinosum layer of the *epithelium of the forestomach* (Fig. 23) and the *inner and outer root sheaths of hair follicles* (Fig. 24). At later time intervals the grains became almost entirely localized to the periphery of the cells, as shown in the stratified epithelium of the tongue (Fig. 27), the oral cavity (Fig. 25), and the inner root sheath (Fig. 26) and bulb of hair follicles (Fig. 28).

The partly differentiated *seminiferous epithelium* of the young animals used showed variation in degree and pattern of labeling. In some seminiferous tubules the reactions occurred over the supranuclear cytoplasm of Sertoli cells (Fig. 30). In other, more advanced tubules the reactions occurred over primary spermatocytes (Fig. 31). At later times the reaction was localized over cell surfaces (Fig. 32).

Certain nonepithelial cell types also exhibited radioautographic reactions in which the label was localized at first to the Golgi region and later to the cell surface. This pattern showed up in the *nerve cell bodies* of *peripheral ganglia* in which a weak to moderate reaction was at first localized in paranuclear grain clusters (Fig. 19) and later became localized to the cell surface (Fig. 20). A similar pattern was not observed in the neurons of the *central nervous system* except in rare instances, possibly because fucose does not cross the blood-brain barrier readily. In *muscles*, a paranuclear reaction was occasionally observed in cardiac muscle as well as in smooth muscle. With time strong reactions appeared outside the fibers, but it could not be decided whether they were due to reactivity of the fiber surface or of the interstitial connective tissue. As for such connective tissue reactions, they were prominent at the later time intervals. They seemed to be due to the ground substance secreted by the fibroblasts. The reac-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tions of these cells will be considered with those to be examined presently.

Cell Types in Which the [^{3}H]Fucose Label Migrated from the Golgi Region to Secretory Material (Table II)

The heaviest radioautographic reactions of this type occurred over *mucus secreting cells*. In duodenal (Fig. 2) and colonic (Fig. 5) goblet cells, the label which was sharply localized at early time intervals to the supranuclear Golgi region, later migrated to the base of the theca where it is depicted at the 1-h interval in the colon (Fig. 6) and later still to the apical portion of the theca as shown at 4 h in the duodenum (Fig. 4). Heavy reactions also occurred *over mucous acinar cells of salivary glands*. The label which was initially in the Golgi region (Fig. 12) later migrated to the mucous secretion.

In *serous cells*, lesser reactions were usually seen after [^{3}H]fucose injection than in mucous cells, as shown by comparing early reactions over serous acinar cells with those over mucous acinar cells in Fig. 12. A somewhat heavier supranuclear reaction could be discerned, however, at early time intervals over *pancreatic acinar cells* (Fig. 13). With time, this label migrated apically toward the acinar lumen.

In the *reticular epithelial cells of thymus medulla*, paranuclear clusters of grains were seen at early times, while at later time intervals, the reaction became heavier, more diffuse, and often associated with PA-Schiff positive cytoplasmic material.

In *connective tissue cell types*, Golgi localized reaction was observed at early time intervals, followed by migration of the label to extracellular secretory products at later time intervals (see Table II). In *fibroblasts*, such as those occurring in the lamina propria beneath epithelial membranes (Fig. 18), definite paranuclear localization of silver grains could be seen at early time intervals after injection. At later times, the connective tissue exhibited an intense diffuse reaction, as illustrated beneath the epithelium in Fig. 25 and between the seminiferous tubules in Fig. 32. As mentioned above, when such reactions occurred over connective tissue which permeated between other cell types

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FIGURES 1–6 Radioautographs of gastro-intestinal epithelia at various times after [^{3}H]fucose injection.

FIGURE 1 Duodenal mucosa, 2 min after injection. Paraffin section stained with hematoxylin and eosin. 6-wk exposure. A supranuclear (Golgi) reaction is observed in the columnar cells located toward the base of the villi (arrows). Towards the tips of the villi, the reaction decreases in intensity and becomes negligible. × 125.

FIGURE 2 Duodenal epithelium at the base of a villus, 2 min after injection. Epon section stained with toluidine blue. 8-wk exposure. Above the row of nuclei (*N*), clusters of silver grains overlie the Golgi region of the columnar cells. Reaction is also seen over the Golgi region of a goblet cell (arrow). × 1,000.

FIGURE 3 Duodenal epithelium at the base of a villus, 1 h after injection. Epon section stained with toluidine blue. 3-wk exposure. The columnar cells show a moderate Golgi reaction at this time, but a reactive band appears along the top of each cell, corresponding to the microvilli of the striated border, while perpendicular bands (arrow) correspond to the lateral cell membranes. × 1,000.

FIGURE 4 Duodenal epithelium at the base of a villus, 4 h after injection. Epon section stained with toluidine blue. 11-wk exposure. The Golgi reaction is negligible, while the reactions over apical and lateral surfaces have intensified. A heavy reaction also occurs over the apical half of the theca of a goblet cell (arrow) whose Golgi region is now unlabeled. × 1,100.

FIGURE 5 Colonic mucosa, 10 min after injection. Paraffin section stained with colloidal iron and Feulgen. 16-wk exposure. A heavy supranuclear reaction appears over the Golgi region of surface and upper crypt columnar cells (horizontal arrow); the reaction is less intense over cells near the base of the crypts. Heavy supranuclear reactions also occur over goblet cells (vertical arrow). × 500.

FIGURE 6 Colonic mucosa, 1 h after injection. Paraffin section stained with hematoxylin and eosin. 2-wk exposure. Reaction is now localized at the apex of columnar cells in surface (vertical arrow) or crypt (horizontal arrow). In goblet cells the radioactivity remains at the base of the mucous theca (oblique arrow). × 500.

TABLE I

Cell Types in Which [^3H]Fucose Label Migrates from Golgi Region to Cell Surface

	Radioautographic location at various times after an injection of [^{3}H]fucose					
	2–10 min		20–35 min		4 h	
	Golgi region	Cell surface	Golgi region	Cell surface**	Golgi region	Cell surface**
I *Cells of simple epithelial membranes*						
Duodenal villus columnar c.	+++	−	+++	+++A, ++L	++	+++A, ++L
" crypt columnar c.	++	−	++	+++A, +L	−	++A
Jejunal villus columnar c.	++	−	++	+++A, ++L	+	+++A, +L
" crypt columnar c.	++	−	++	++A	−	+++A
Colonic surface columnar c.	+++	−	+++	+++A	−	+++A
" crypt columnar c.	++	−	++	++A	−	++A
Vas deferens c.	++	−	++	+A	+	+A, +B
Choroid plexus c.	+	−	++	+A	+	+A, +L, ++B
Hepatocytes	+++	−	++	++A	−	++A, +L
Adrenal cortical c.	+	−	+	++A	+	++A, +L
Adrenal medulla c.	+	−	+	++A	abs	abs
Islets of Langerhans c.	+	−	+	−	−	++A, +L
Ciliary body c.	+	−	++	++A, +L	−	++A, +L
Tracheal epithelial c.	+	−	+	++A	−	++A
Rete Testis c.	+	−	abs	abs	+	++A
Epididymal epithelial c.	+	−	++	++A	+	++A
Oviduct epithelial c.*	++	−	++	+++A	abs	abs
Submaxillary gland duct c.	++	−	++	++A, +B	−	+++A, +L, +B
Kidney proximal tubule c.	+	−	+	+A	−	+++A, +B
Kidney distal tubule c. (loop of Henle)	++	−	+++	+++A	−	+++A, +B
Capillary endothelial c.‡	+	−	+	+A	−	++A
Lung alveolar c.§	+	−	+	+	−	++
Schwann c.	+	−	+++	+‖	−	++
Mesothelial c.	+	−	+	+	−	+
II *Cells of stratified epithelial membranes*						
Tongue surface epithelial c.	+	−	+	+	−	++
Esophagus epithelial c.	+	−	+	+	−	+
Forestomach epithelial c.	++	−	++	+	−	+
Bladder epithelial c.	+	−	++	++A	−	++
Urethral epithelial c.	+	−	+	++	−	++
Corneal epithelial c.	−	−	+	−	−	++
Conjunctival epithelial c.	+	−	++	+	−	++
Epidermal c.	++	−	++	−	−	++
Inner root sheath c.	+	−	+	+	−	++
Outer root sheath c.	+	−	+	+	−	+
Spermatocytes in Seminiferous Epithelium	+	−	++	−	−	++
III *Other cell types*						
Ganglionic c. (Auerbach plexus)	+	−	+	−	+	++
CNS Neurons¶	−	−	+	−	+	+
Lymphocytes	+	−	+	+(?)	−	+(?)

* In region of isthmus.

‡ Reaction is most prominent in nervous tissue but occurs in nearly all tissues.

§ It is possible that some of these reactions are over capillary endothelial cells.

‖ A diffuse reaction extends over all of the peripheral nerve tissue.

¶ Reaction was seen only in some neurons.

** A, reaction along apical cell surfaces; L, lateral cell surface; B, basal cell surface; abs, tissue absent from the experiment.

e.g. striated muscle cells (Fig. 25), the possible presence of surface reactions was masked. In the *chondroblasts* of epiphyseal cartilage, the radioautographic reaction at early time intervals took the form of discrete paranuclear grain clusters (Fig. 33). By 4 h after [^{3}H]fucose injection most of the reaction was at the surface of the chondroblasts or over the adjacent cartilage matrix (Fig. 34), and by 30 h (Fig. 35), it was distributed uniformly throughout the cartilage matrix. An early paranuclear reaction in *osteoblasts* (Figs. 33, 36), was later followed by the appearance of reaction bands over bone matrix (Figs. 34, 35, 37).

Electron Microscope Radioautographic Results

The results obtained in *duodenal villus columnar cells* confirmed those previously reported (7) and provided additional quantitation (Fig. 38). Thus at 2 min after injection it was found that almost 80% of the silver grains over the cells were localized over the saccules of the Golgi apparatus itself, while only a negligible number of grains was counted at the cell surfaces. With time, however, the distribution of label in the cell changed rapidly; by 20 min after inection the percentage of grains localized to the Golgi apparatus had dropped to 30%, while 32% of the grains now occurred over the lateral and apical cell surfaces. At this time, several silver grains appeared over the cytoplasm between the Golgi apparatus and cell surfaces (Fig. 38: "remainder of cytoplasm"); frequently these grains occurred over smooth surfaced vesicles measuring about 140 nm in diameter. Label also appeared at this and later time intervals in dense and multivesicular bodies scattered throughout the cell. A high percentage of these bodies ultimately became labeled (8) but they never accounted for more than 5% of the total grain count over the cell.

By 4 h after injection, reaction over the Golgi apparatus had further decreased (Fig. 39, *G*) and now only 8% of the silver grains occurred over this organelle (Fig. 38). The lateral and apical cell surfaces, on the other hand, were now intensely labeled (Fig. 39, *lmb* and *mv*) and together accounted for approximately 70% of the total label. In addition, some silver grains occurred over dense bodies (*D*) and vesicles (vertical arrows) in the cytoplasm. The intensity of labeling of the apical microvillus plasma membrane was found to vary considerably from cell to cell; thus over some cells the reaction was intense, while over others almost no reaction was seen (Fig. 40). Close inspection showed that the sites at which the radioautographic reaction changed in intensity coincided exactly with the lateral limits of individual cells. After staining the apical microvilli of mouse jejunal cells with colloidal iron, great differences in staining were similarly observed between individual cells (57). The individual differences may reflect different functional states in adjacent cells.

In the *distal convoluted tubule cells of kidney*, the label at 2.5 min after [^{3}H]fucose injection was confined to the saccules of the Golgi apparatus (Fig. 41). As in the case of duodenal villus columnar cells, the distribution of label in the cells changed with time and, at the later time intervals after injection, the great majority of the silver grains occurred over the apical and lateral membranes (Figs. 42 and 43) as well as over the infolded basal plasma membrane (Fig. 43).

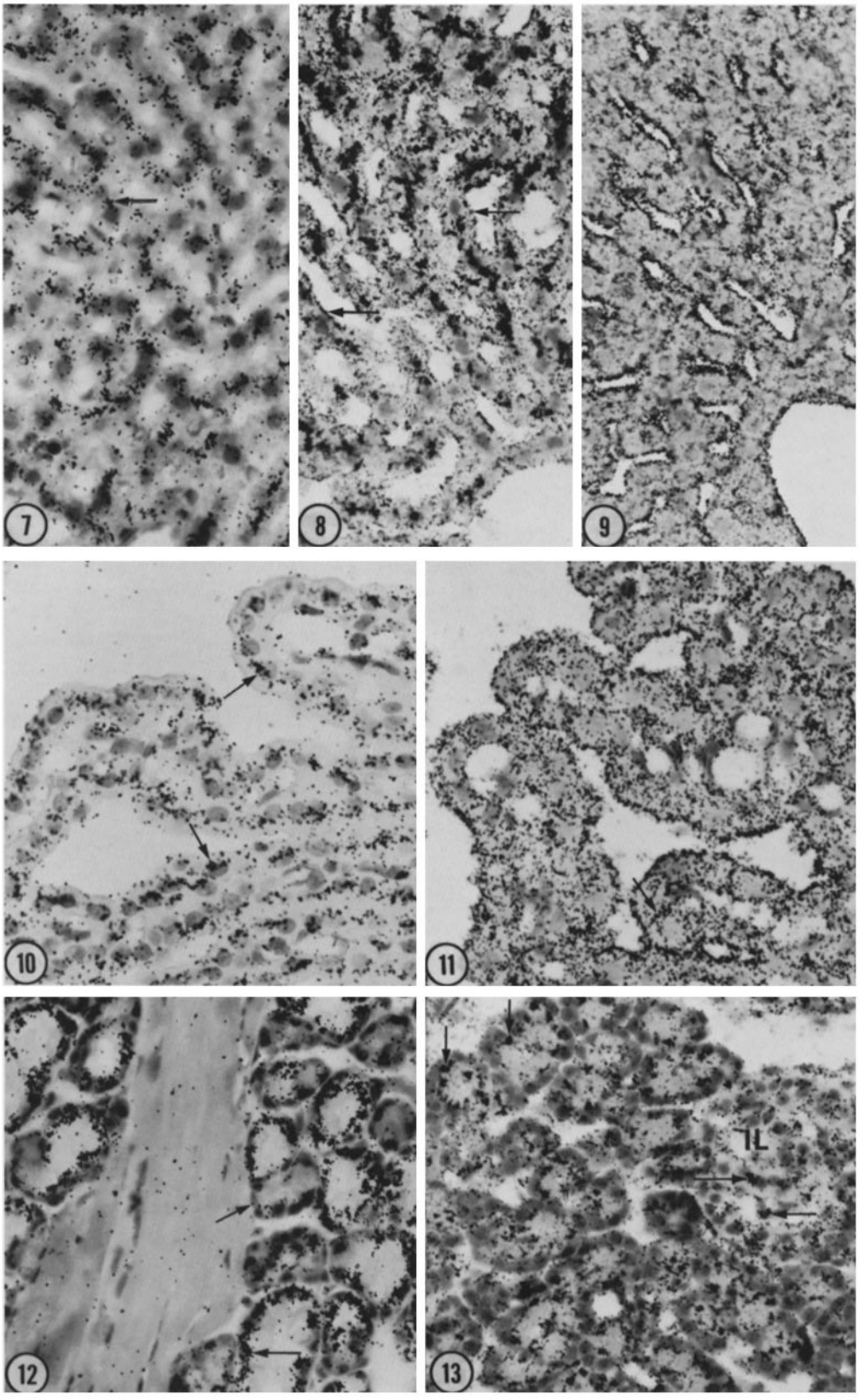
Finally, in *proximal convoluted tubule cells* the label was again localized to the Golgi saccules at early time intervals (Fig. 44). At later time intervals (Fig. 45) the label became associated with the apical microvillar membrane (*mv*) as well as with light (horizontal arrow) and dense (vertical arrow) invaginations of the cell surface, and with many of the numerous lysosomes present in these cells.

DISCUSSION

Significance of the Uptake of Fucose Label

Although free fucose has not been detected in the blood so far (26), it has been observed in milk (25) and urine (3). Fucose probably becomes available to the circulation as a result of the digestion of those food glycoproteins and glycolipids which contain fucose residues. In this regard, it is of interest that exogenously administered fucose can be utilized by animal tissues (2, 9, 14).

After administration of labeled fucose to rats, there is no appreciable breakdown to metabolites nor conversion to other sugars which could in turn be broken down; rather the sole fate of fucose is to be incorporated as fucose residues into newly synthesized glycoproteins (2, 24, 32) and to a lesser extent into glycolipids (11, 12). Thus, after incubation of thyroid lobes with labeled fucose for 30 min, all of the label was recovered as fucose residues whereas, after similar incubation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with other labeled hexoses or hexosamines, from 15–50% appeared in different sugars or in amino acids (Herscovics, private communication). For these reasons, labeled fucose is a more reliable precursor of glycoproteins than other labeled hexoses or hexosamines.

In the side chains of glycoproteins in which fucose occurs, the amino sugars *N*-acetylglucosamine and *N*-acetylgalactosamine are usually combined with mannose to form the inner residues; galactose residues usually occur next to the free ends, whereas fucose occupies a terminal position (63, 70).

The uptake of each sugar into the glycoprotein requires its phosphorylation and subsequent conversion to a nucleotide sugar, which in the presence of the appropriate specific transferase, donates the sugar to the forming side chain. In the course of glycoprotein synthesis, the polypeptide chain is made first and the sugar residues of the side chains are then added one by one while the polypeptide migrates through endoplasmic reticulum cisternae and Golgi saccules (24, 63, 64, 69). Since fucose occupies a terminal position in the side chains in which it occurs, it would be the last sugar added.

In the present experiment, a modest amount of carrier (0.19 mg) was present in the injected dose of tritiated fucose. It is not known whether this amount is of the order of what is usually provided in the food, but it is known that fucose administered in similar doses is readily taken up into glycoproteins (2, 9).

Since cells may contain not only free fucose, but also GDP-fucose in addition to glycoproteins and glycolipids, all these substances may carry label. Free and GDP-fucose as well as glycolipids are soluble in some of the reagents used for histological processing and, therefore, likely to be extracted from the investigated tissues, whereas glycoproteins should be retained throughout the procedure. The observed radioautographic reactions would thus be due to the fucose label incorporated into these glycoproteins.

The cellular site of fucose uptake into glycoproteins was revealed in radioautographs from animals sacrificed early (2–10 min) after [^{3}H]-fucose injection. In the light microscope, most cells exhibited a discrete, paranuclear reaction, the location of which corresponded to that of the Golgi apparatus whenever this was known in the

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FIGURES 7–13 Radioautographs of paraffin sections after [^{3}H]fucose injection.

FIGURE 7 Liver, 2 min after injection. Hematoxylin and eosin stain. 10-wk exposure. Along the liver cords, localized reactions occur adjacent to hepatocyte nuclei (horizontal arrow). These reactions are distributed in the same manner as after specific staining of the Golgi apparatus. × 455.

FIGURE 8 Liver, 20 min after injection. Hematoxylin and eosin stain. 10 wk exposure. Localized paranuclear reactions are heavy; in addition, some reaction can now be seen along the cell surfaces facing the sinusoids (horizontal arrows). × 425.

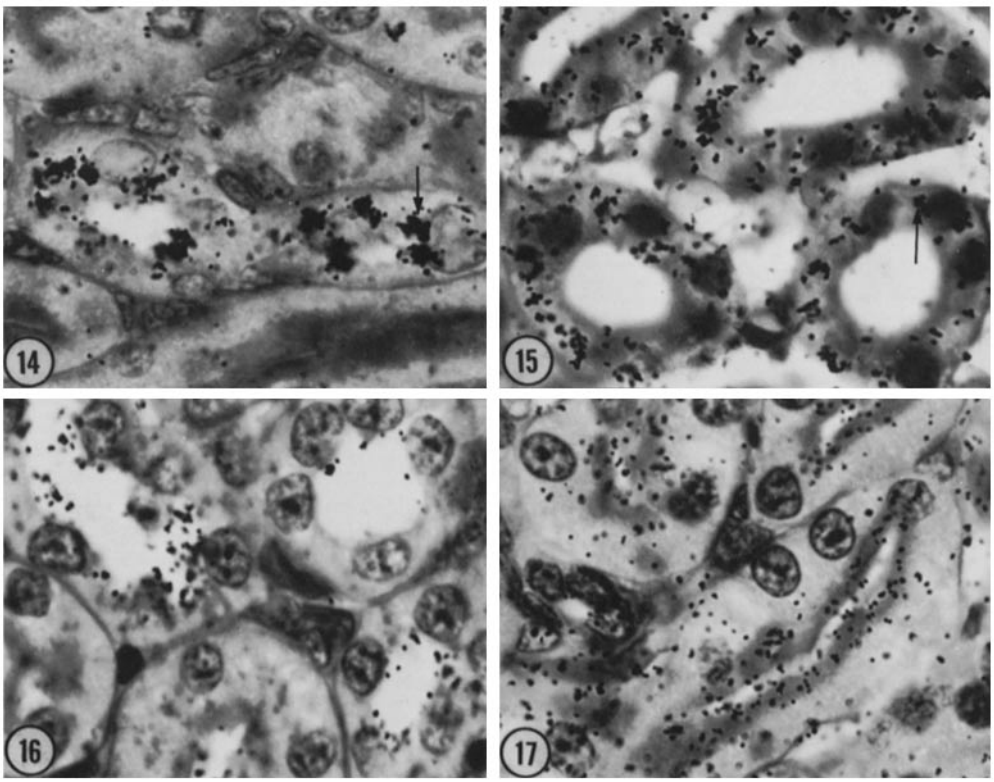
FIGURE 9 Liver, 4 h after injection. Hematoxylin and eosin stain. 5-wk exposure. The Golgi localized reactions are no longer seen, while the reaction along the sinusoidal surfaces of the cells is intense. × 425.

FIGURE 10 Choroid plexus, 10 min after injection. Hematoxylin and eosin stain. 5-wk exposure. The epithelial cells show a supranuclear reaction (arrows). × 550.

FIGURE 11 Choroid plexus, 4 h after injection. Colloidal iron and Feulgen stain. 6-wk exposure. A reaction is seen along the free cell surfaces. Some reactivity of lateral membranes is observed in places (arrow). × 525.

FIGURE 12 Glands of tongue, 2 min after injection. Hematoxylin and eosin stain. 23-wk exposure. A heavy supranuclear reaction is seen over the cells of mucous acini (horizontal arrow). Much less reaction is seen over serous acinar cells (oblique arrow) although some supranuclear grains are present. × 650.

FIGURE 13 Pancreas, 20 min after injection. Hematoxylin and eosin stain. 10-wk exposure. A supranuclear reaction is observed in the cells of the pancreatic acini (vertical arrows). Paranuclear reactions are also seen (horizontal arrows) over islet of Langerhans cells (*IL*). × 425.



FIGURES 14–17 Radioautographs of kidney cortex obtained at various times after [^{3}H]fucose injection. Paraffin sections stained with periodic acid-Schiff and hematoxylin. × ~800.

FIGURE 14 Distal tubules, 5 min after injection. Well localized reactions occur over the supranuclear Golgi region of distal tubule cells (vertical arrow).

FIGURE 15 Proximal tubules, 10 min after injection. Paranuclear localizations of silver grains are seen over proximal tubule cells (vertical arrow).

FIGURE 16 Distal tubules, 4 h after injection. Reaction is now localized to the apical surface of distal tubule cells.

FIGURE 17 Proximal tubules, 30 h after injection. Reaction is now localized over the apical brush border of proximal tubule cells.

cell under study. The intensity of the paranuclear reactions was very high in some cells (e.g., colonic epithelium) and weak in others (e.g., lymphocytes) with all intermediates between these extremes. In the electron microscope, the silver grains were initially localized over Golgi saccules in distal and proximal tubule cells of kidney (Figs. 41, 44) as well as in the epithelial cells of small intestine (Fig. 38). A similar Golgi localization of early reactions was reported in thyroid follicular cells (23), hepatocytes (8) and odontoblasts (68). Briefly, in all cell types in which the initial reaction was strong enough to be clearly localized, the uptake of fucose label could be assigned to the Golgi apparatus. These observations are interpreted as indicating that fucose is incorporated into glycoproteins in the Golgi apparatus. The presence of glycoproteins in this structure had already been demonstrated by histochemical staining (54).

That fucose incorporation takes place in the Golgi apparatus receives support from the finding in HeLa cells of fucosyl transferase within the smooth microsome fraction (11), which presumably includes Golgi membranes.

Two minor exceptions to the rule that fucose

label is taken up in the Golgi apparatus may be noted. In thyroid cells, a very small proportion of the label at early time intervals after [^{3}H]fucose injection appeared in the rough endoplasmic reticulum near the Golgi apparatus (23). It is possible that fucosyl transferase is synthesized in the rough endoplasmic reticulum and becomes concentrated in the Golgi apparatus. Low levels of this enzyme in the rough endoplasmic reticulum, especially near the Golgi apparatus, might enable small amounts of fucose to be incorporated into forming glycoproteins before their actual arrival into Golgi saccules. The second exception is that fucose incorporation into glycoproteins has recently been reported in the rough endoplasmic reticulum of thyrotrophs in thyroidectomized rats

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FIGURES 18–22 Radioautographs of paraffin sections at various times after [^{3}H]fucose injection.

FIGURE 18 Bladder epithelium, 10 min after [^{3}H]fucose injection. Hematoxylin and eosin stain. 6-wk exposure. Paranuclear reactions occur in the cells of the surface layer (horizontal arrow). Small reactions can also be seen adjacent to the nuclei of fibrocytes in the underlying connective tissue (oblique arrow). × 250.

FIGURE 19 Ganglion cells of Auerbach's plexus of colon, 20 min after injection. Periodic acid-Schiff and hematoxylin stain. 5-wk exposure. Paranuclear reactions are seen over most cells (arrow). × 750.

FIGURE 20 Ganglion cells in connective tissue of salivary glands, 30 h after injection. Periodic acid-Schiff and hematoxylin stain. 6-wk exposure. Reaction is localized to the periphery of the ganglion cells. × 800.

FIGURE 21 Capillaries in cerebral nervous tissue, 10 min after injection. Colloidal iron and Feulgen stain. 16-wk exposure. Paranuclear reactions occur over the endothelial cells of large and small capillaries (arrows). × 500.

FIGURE 22 Capillaries in cerebral nervous tissue, 4 h after injection. Hematoxylin and eosin stain. 10-wk exposure. Surface reaction occurs over the endothelial cells (arrow). × 525.

FIGURES 23–28 Radioautographs of paraffin sections of stratified epithelia obtained at various times after [^{3}H]fucose injection.

FIGURE 23 Forestomach epithelium, 10 min after injection. Hematoxylin and eosin stain. 6-wk exposure. Paranuclear dot-like reactions (dark horizontal arrow) are seen in the stratum spinosum. They should not be confused with the keratohyalin of the granular layer (white arrow). Small reactions also often occur adjacent to nuclei of fibrocytes in the underlying connective tissue (vertical arrow). × 475.

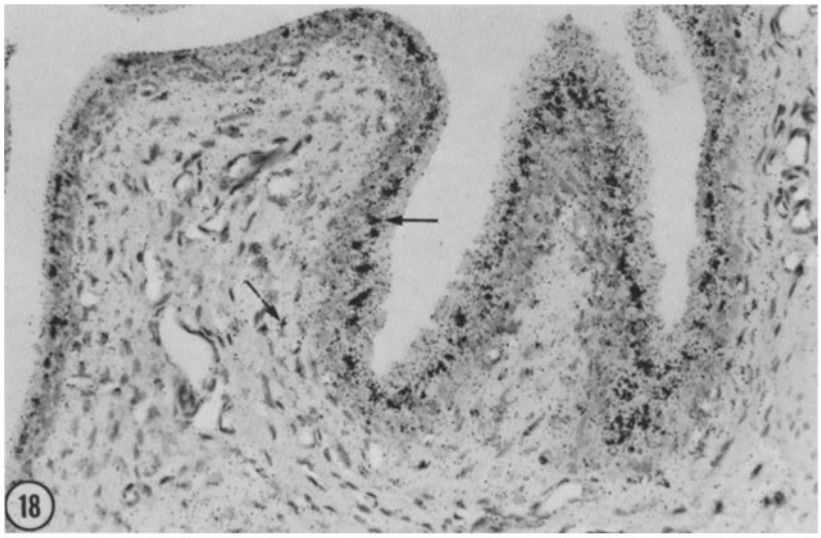
FIGURE 24 Root of hair follicle, 10 min after injection. Colloidal iron and Feulgen stain. 16-wk exposure. Dot-like paranuclear reactions are seen in a region corresponding to the stratum spinosum in the inner (arrow) and outer root sheaths (arrowhead). Only weak reactions occur over the cells about to transform into elements of the hair cortex (*C*). × 475.

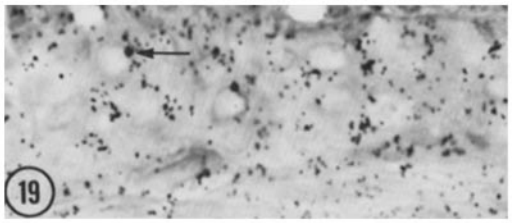
FIGURE 25 Stratified epithelium of oral cavity, 30 h after injection. Hematoxylin and eosin stain. 7-wk exposure. In the stratum spinosum, the reaction outlines the cells precisely. A strong diffuse reaction is also seen at this time over the underlying lamina propria (*LP*). This reaction outlines the fibers of striated muscle cut in cross section (*M*). × 400.

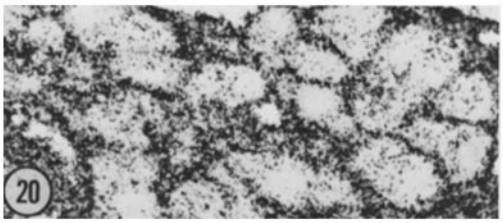
FIGURE 26 Inner root sheath of hair follicle, 4 h after injection. Periodic acid-Schiff and hematoxylin stain. 6-wk exposure. Reaction appears at the periphery of the cells in the inner root sheath seen in the center of the figure. × 325.

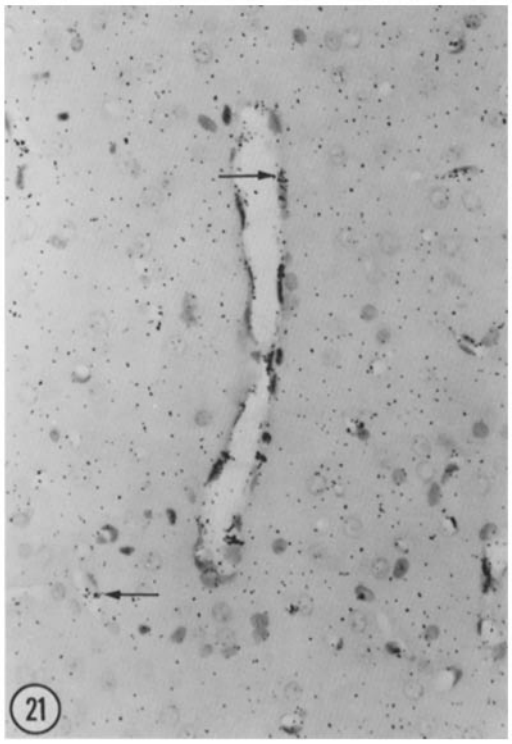
FIGURE 27 Tongue epithelium, 4 h after injection. Hematoxylin and eosin stain. 6-wk exposure. A reaction is again seen at the periphery of the cells in the stratum spinosum. × 400.

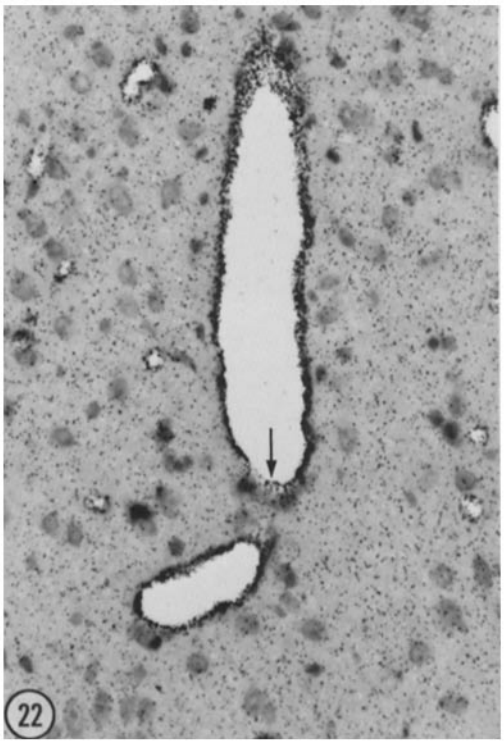
FIGURE 28 Bulb of hair follicle, 4 h after injection. Periodic acid-Schiff and hematoxylin stain. 6-wk exposure. Reaction occurs at the periphery of cells in the stratum spinosum of the hair bulb and above it in the inner root sheath. × 325.



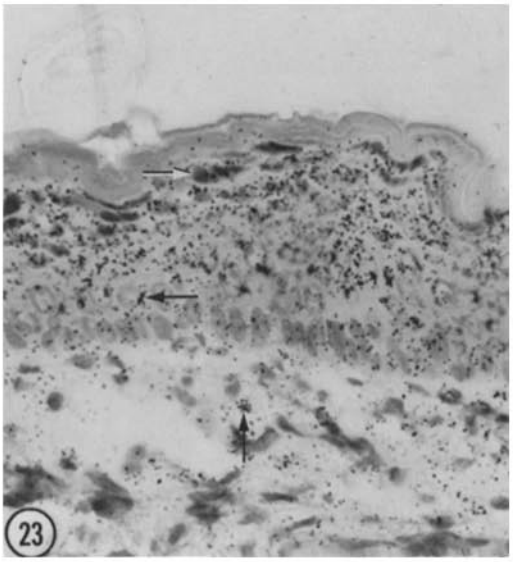








Legends to Figs. 18–28 appear on page 269.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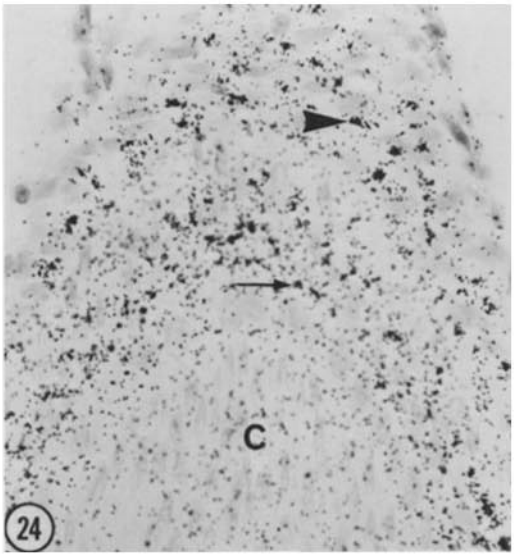
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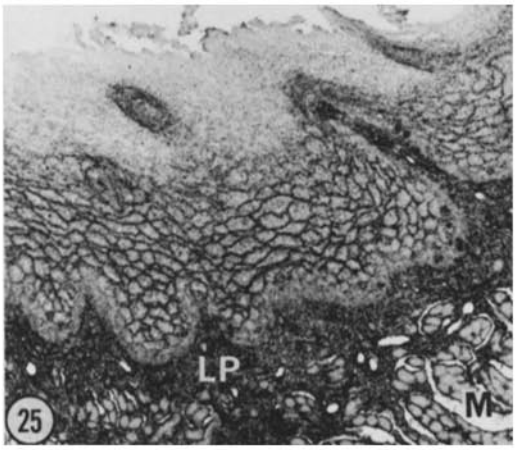
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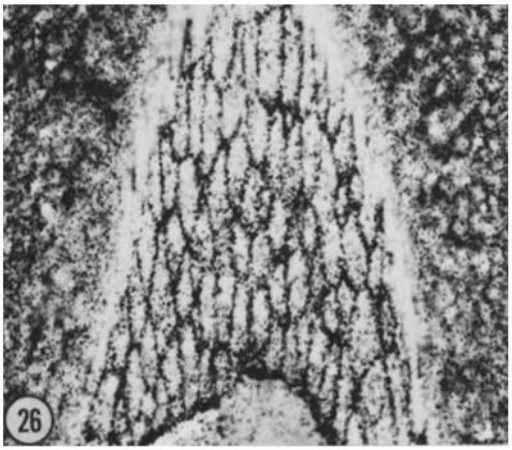
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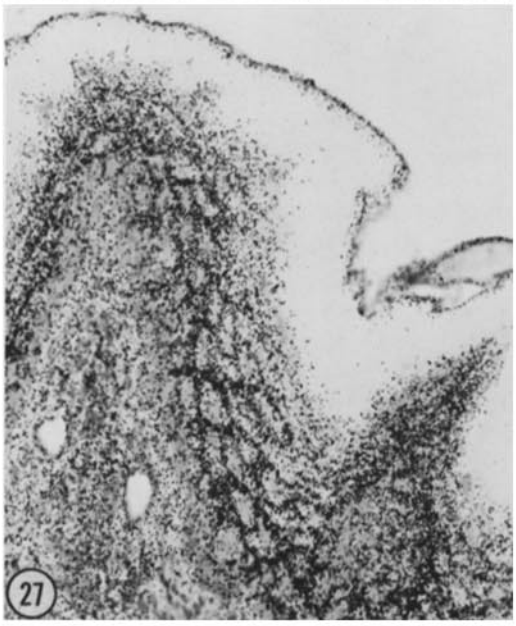
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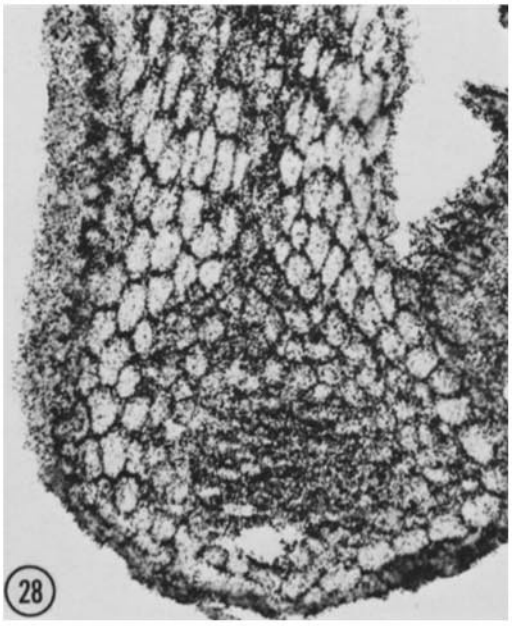
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(49). However, these cells were under the artificially strong stimulation provided by thyroidectomy, which may have altered the distribution of fucosyl transferase.

Hughes (27) has pointed out that, since Golgi membranes may fuse with the plasma membranes, glycosyl transferases could be carried from the Golgi apparatus to the cell surface. Some transferases, although not fucosyl transferase, have been detected biochemically at this site (10, 58, 59); however, they are only present in small amounts and their function may not be to participate in glycoprotein synthesis but perhaps to intervene in cell adhesion (10, 58, 59). In our study, no significant radioautographic reaction was observed over cell surfaces at very early time intervals (2–5 min after [^{3}H]fucose injection) indicating a lack of significant incorporation of fucose into glycoproteins at the cell surface. In conclusion, *the incorporation of fucose into glycoproteins almost invariably occurs in the Golgi apparatus.* Moreover, since fucose is the last sugar added to the side chains in which it occurs, *the Golgi apparatus would be the site of completion of the carbohydrate side chains ending in fucose.*

The prominent role of the Golgi apparatus in the building of carbohydrate side chains is also shown by the high concentration in Golgi fractions of transferases catalyzing the addition of other terminal or near terminal sugars to carbohydrate side chains; these include galactosyl transferase (16, 40, 46, 60) and sialyl transferase (60). It is, therefore, likely that most side chains ending in sialic acid, like those ending in fucose, are completed in the Golgi apparatus. If so, this organelle would be the site of completion of the whole glycoprotein.

Migration of Fucose Labeled Glycoproteins out of the Golgi Apparatus

As time elapsed after [^{3}H]fucose injection, all cells examined showed a decrease in the amount of Golgi label. Meanwhile, label appeared in other parts of the cells.

In some cells, the label was relocated in *secretory products* (Table II), such as mucus which is known to be rich in glycoprotein (63), thyroid colloid which contains the glycoprotein thyroglobulin (62), bone matrix, cartilage matrix and connective tissue ground substance, all three of which contain glycoproteins (19). In many cell types fucose label also appeared in *lysosomes*, as observed here in proximal convoluted tubule cells and as already described in liver and duodenal epithelial cells (8). The label would then be incorporated into lysosomal hydrolases, many of which have been shown to be glycoproteins (22).

In numerous cells, the relocation of the label was observed along one or more *cell surfaces* (Table I). Thus, in the cells of both proximal and distal convoluted tubules of kidney, the label which had first been observed in Golgi saccules later appeared at the apical surfaces and later still at the lateral and basal surfaces (Figs. 42, 43, and 45). The interpretation of these results is that some of the glycoproteins completed in the Golgi apparatus migrated to the cell surfaces to be added to the cell coat. It had already been shown by histochemical techniques that glycoproteins are present at the various surfaces of kidney cells (55). Furthermore, biochemical techniques used after centrifugal separation had revealed that the brush border which constitutes the apical surface of proximal tubule cells contains at least ten glycoproteins, one of which is the enzyme γ-glutamyltransferase (21).

In the duodenum, the relocation of label was measured by grain counts over the components of epithelial cells. While the Golgi count decreased from about 80% of the total cell count 2 min after injection to about 8% at 4 h, the combined counts over the cell surfaces increased from less than 2% at 2 min to about 70% at 4 h (Fig. 38). These data again illustrate the transport of glycoprotein from Golgi apparatus to cell coat. There is some evidence that this transport may be effected by smooth-surfaced vesicles with a diameter of about 140 nm. The vesicles were frequently seen near or under silver grains located between the Golgi region and the apical cell surface. Grains in this location were most abundant at a time intermediate between the decrease in Golgi counts and the rise to a maximum of the counts at cell surfaces (Fig. 38) and may represent labeled glycoproteins in transit from the Golgi apparatus to the cell surface. The associated vesicles would be their carriers. Histochemical techniques had already demonstrated the presence of glycoproteins at the surface of the epithelial cells of the small intestine (55, 56). In fact, when sections of intestinal epithelium from [^{3}H]fucose injected animals were treated with low pH-phosphotungstic

acid (one of the techniques for glycoprotein detection) and then radioautographed for electron microscopy, nearly all of the silver grains observed were localized over the stained cell surfaces or over other stained structures (Golgi saccules, lysosomes; 53). Biochemical studies (1, 18) had also shown the presence of several different glycoproteins in the microvillar apical border of these cells, including the digestive enzymes maltase, sucrase, β-naphthylamidase and alkaline phosphatase (18). These enzymes could be removed from the still intact membranes of the centrifuged microvilli by a short treatment with papain, a result indicating that they are located at the surface (18) where they presumably constitute some of the cell coat glycoproteins.

On examination of Table I, it is apparent that the various cell types listed show great differences in the intensity of surface labeling. The rate at which the label appeared also differed from cell type to cell type. Thus, the apical labeling of kidney cells usually reached a peak at 30 min in distal tubules and at 4 h in proximal tubules. Furthermore, while in some cell types the labeling was of equal intensity along all cell surfaces, as for instance in the cells of oral stratified epithelium (Fig. 25), hair follicle (Fig. 28), and seminiferous epithelium (Fig. 32) as well as in neurons (Fig. 20), in other cell types, the different surfaces were labeled to different extents. For instance, in the colonic epithelium (Fig. 6), only the apical surface was significantly labeled; in the epithelial cells of duodenum (Fig. 4) and kidney tubules (Fig. 16), the labeling was maximal at the apical surface, but was also significant on the others. Recently, [^{3}H]fucose radioautographs of nerve endings in chicken ciliary ganglia showed a heavier labeling of the presynaptic portion than of the remainder of the plasma membrane (6). In conclusion, glycoproteins may be added at different rates on different surfaces of a given cell.

Secretory cells often appeared to lack a distinct surface membrane reaction. In many instances, reaction appeared along one or more surfaces but it was not possible to decide whether the labeling was of the cell coat or of secretory material or of a nearby structure. Thus, a reaction seen over the lateral membrane of goblet cells may be due to label in the adjacent membrane of a neighboring columnar epithelial cell. Reaction over the basal membranes of many acinar cells may be due to the heavily labeled connective tissue nearby. Finally, in the case of the luminal surface of secretory cells, the reaction was mostly due to recently released labeled secretory products but might hide a surface reaction.

Renewal of Cell Coat

Surface reactions were found to occur over nearly all cell types with the exception, perhaps, of secretory cells. Why do such reactions occur? In new cells, the reactions could represent addition of glycoprotein to a forming cell coat. In other cells, however, it may be assumed that the cell coat has been completed; in this case the addition of new glycoproteins must be balanced by a corresponding loss, i.e., cell coat components turn over. For the examination of this problem, cell populations may be classified as static, expanding, and renewing (37, 38).

Static cell populations undergo no cell division and are, therefore, formed of "old" cells. The [^{3}H]-fucose label was observed to pass to the surface of some of these cells, as illustrated by the neurons of Auerbach's plexus (Fig. 20). Such reaction is attributed to turnover of cell coat glycoproteins. Recently, reactions observed along the surface membrane of nerve terminals were found to decrease between 6 and 23 days after [^{3}H]fucose administration, thus demonstrating that turnover had taken place (6).

Expanding cell populations are those in which the frequency of cell divisions decreases with age, resulting in a gradual increase in the proportion of "old" cells. Passage of label to the surface of the cells of this group was also observed, as exemplified by liver (Fig. 9) and kidney (Figs. 42, 45). In the young 35–45 g rats used for the present work, cell division is still relatively frequent; it is estimated from previous data (15) that about one cell in twelve is added each day to their liver and kidney. Because the new cells must be growing, it is possible that some of the surface reactions represent addition of glycoprotein for completion of the cell coat. In the liver, however, reactions occurred along the surface of cell cords in a regular manner (Fig. 9), suggesting that new and old cells produce cell coat at the same rate. Furthermore, in kidney radioautographs the abundance of silver grains over the apical surface of distal tubule cells was definitely less at 30 than 4 h after [^{3}H]fucose injection, indicating that surface glycoproteins had turned over.

Renewing cell populations are those in which cell

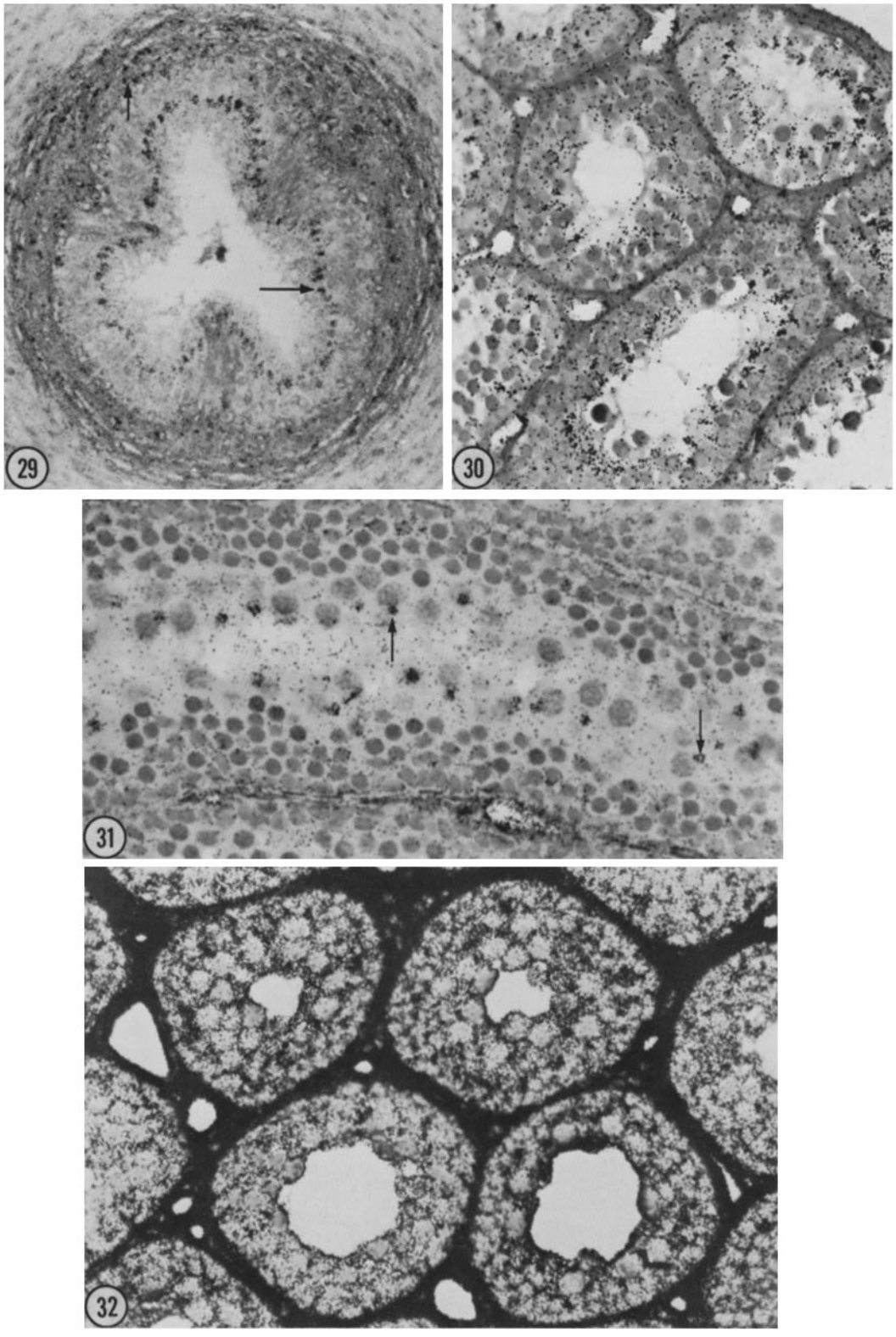
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TABLE II

Cell Types in Which [^3H]Fucose Label Migrates from Golgi Region to Secretory Material

	Radioautographic location at various times after an injection of [^{3}H]fucose					
	2–10 min		20–35 min		≥4 h	
	Golgi region	Secretion	Golgi region	Secretion	Golgi region	Secretion
I *Cell producing mucous secretion*						
Duodenal goblet c.	+++	−	+++	+++	−	+++
Jejunal goblet c.	++	−	++	++	−	++
Colonic goblet c.	+++	−	+++	+++	−	+++
Stomach surface c.	+++	−	+++	++	−	++
Stomach mucous neck c.	+	−	+	+	−	+
Submaxillary acinar c.	+++	−	+++	+++	−	+++
Sublingual acinar c.	++	−	++	++	−	++
Tongue mucous gland c.	+	−	+++	+++	−	+
Conjunctival globlet c.	++	−	+++	+++	−	+++
II *Cells producing serous secretion*						
Pancreatic acinar c.	+	−	++	+	−	+
Submaxillary secr. tubule c.	+	−	++	+	+	+
Tongue serous gland c.	+	−	+	−	+	+
III *Cells producing other secretions*						
Thyroid follicular c.*	++	−	+	+	−	++
Thymus medulla reticular c.	+	−	+	+	+(?)	++
IV *Cells producing ground substance*						
Fibroblasts	+	−	+	+	−	+
Osteoblasts	+	−	+	+	−	++
Odontoblasts‡	++	−	+	+	−	++
Ameloblasts§	+++	−	++	++	−	+++
Chondroblasts	+	−	+	−	+	+

* Based on observations by Haddad et al. (23).

‡ Based on observations by Weinstock et al. (68).

§ Based on unpublished observations of A. Weinstock.

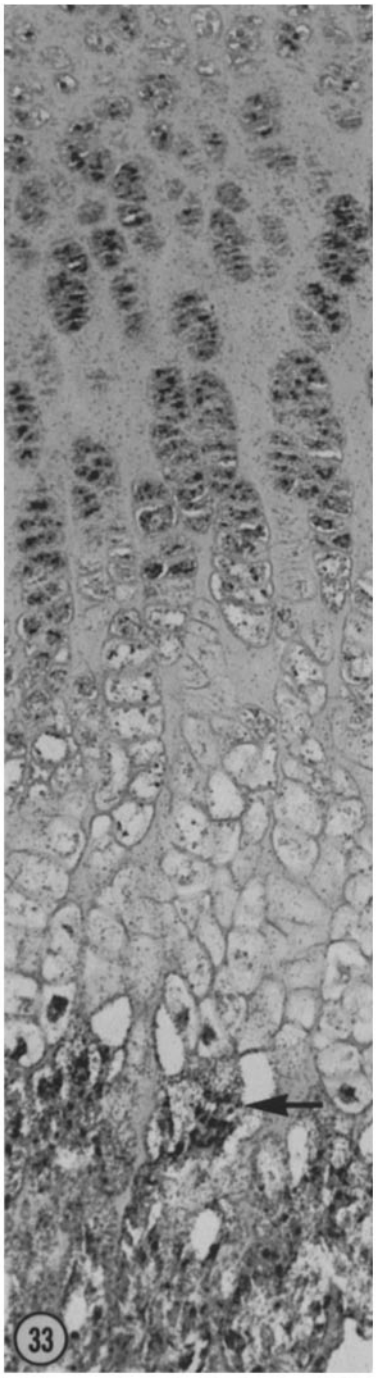
FIGURES 29–32 Radioautographs of paraffin sections of male structures at various times after [^{3}H]-fucose injection.

FIGURE 29 Vas deferens, 35 min after injection. Periodic acid-Schiff and hematoxylin stain. 6-wk exposure. Paranuclear reactions occur over the epithelial cells (horizontal arrow); in addition small dot-like paranuclear reactions occur in the surrounding connective tissue sheath (vertical arrow). × 200.

FIGURE 30 Seminiferous tubules cut in cross section, 20 min after injection. Periodic acid-Schiff and hematoxylin stain. 16-wk exposure. In these young tubules, Sertoli cells and spermatogonia occupy the peripheral part of the tubules; the central region is mostly vacant but contains a few young spermatocytes. Substantial reactions occur in the supranuclear cytoplasm of Sertoli cells. × 400.

FIGURE 31 Seminiferous tubule somewhat older than that in Fig. 30 and cut in longitudinal section. 1 h after injection. Hematoxylin and eosin stain. 6-wk exposure. Sizable paranuclear reactions are seen over the centrally located pachytene spermatocytes (vertical arrows). × 475.

FIGURE 32 Seminiferous tubules in cross section, 4 h after injection. Periodic acid-Schiff and hematoxylin stain. 16-wk exposure. In the tubules, spermatocytes show a heavy surface reaction. An intense reaction is diffused over the intertubular spaces. × 400.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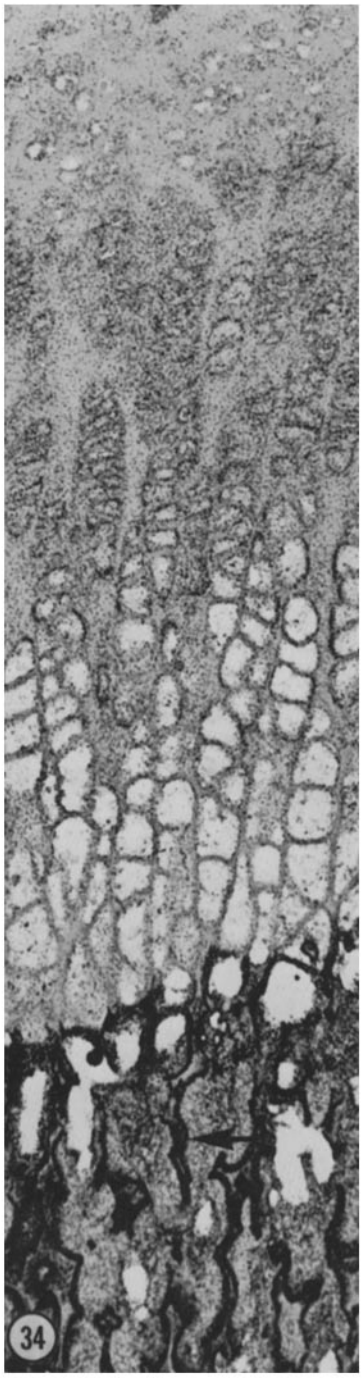
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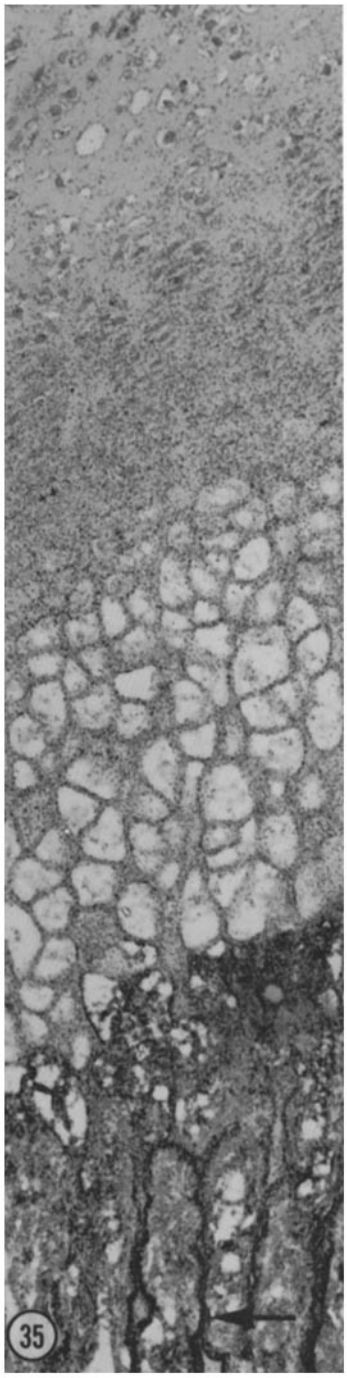
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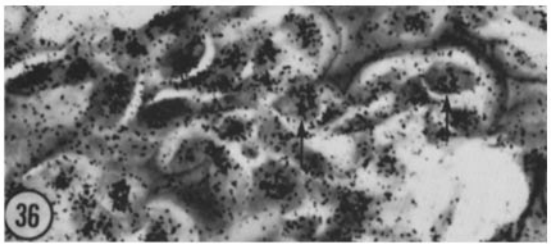
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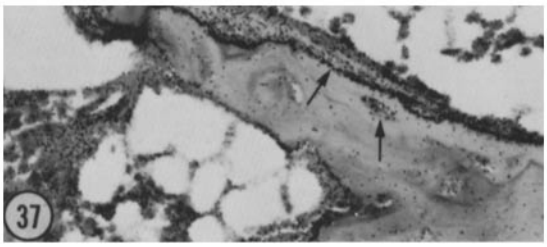
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FIGURES 33–37 Radioautographs of paraffin sections of bone and cartilage tissues obtained at various times after [^{3}H]fucose injection.

FIGURE 33 Subepiphyseal region, 35 min after injection. Hematoxylin and eosin stain. 6-wk exposure. A paranuclear reaction is seen in the cells of hyaline cartilage in the upper half of the figure. A similar reaction in osteoblasts is shown at the arrow. × 200.

FIGURE 34 Subepiphyseal region, 4 h after injection. Hematoxylin and eosin stain. 6-wk exposure. An indication of a cell surface reaction is seen in cartilage cells in the lower part of the proliferation zone, the cell hypertrophy zone, and the upper part of the degenerating zone. This surface reaction, however, cannot be readily distinguished from reaction over the matrix adjacent to the cells. Dark reactive bands are seen in bone spicules (arrow). × 200.

FIGURE 35 Subepiphyseal region, 22 h after injection. Hematoxylin and eosin stain. 6-wk exposure. Diffuse reactivity is now seen over the matrix of the lower half of the cartilage including the partitions of matrix between degenerating cells. Reactive bands remain over the older bone spicules (arrow). × 200.

FIGURE 36 Subepiphyseal bone tissue, 2 min after injection. Periodic acid-Schiff and hematoxylin stain. 50-wk exposure. Paranuclear reactions occur over osteoblasts (arrows). × 350.

FIGURE 37 Subepiphyseal bone tissue, 30 h after injection. Periodic acid-Schiff and hematoxylin stain. 2-wk exposure. A band of reaction (oblique arrow) now appears over the matrix of the bone spicules at some distance from the osteoblasts at the periphery of the spicule. Reaction also appears over an osteocyte and the matrix around it (vertical arrow). × 350.

divisions continually provide "young" cells. Again passage of label to the cell surface was observed in these populations, as shown in small intestine (Fig. 39) and in stratified epithelia (Fig. 25). In the small intestine, the reaction was found to be heavier over the younger cells at the base of intestinal villi (Fig. 1) than over the older cells nearer the tip. Therefore, synthesis is most active in these younger cells and perhaps most of the reaction represents addition of glycoprotein to a newly forming cell coat. However, this interpretation does not fully account for what happens. Since the cells migrate from the base to the upper region of villi in about 30 h, the apical reaction observed over the cells of the base 4 h after injection could be compared with that in the upper region in animals sacrificed 30 h after injection. The comparison demonstrated a decrease in reaction intensity with time, indicating that a certain amount of turnover of the cell coat does occur in these cells. The same conclusion was reached by Ito after in vitro experiments using [^{3}H]galactose (31). Biochemical studies have also shown that the glycoproteins of apical microvilli turn over more rapidly than can be accounted for by the turnover of the cells (1). In conclusion,

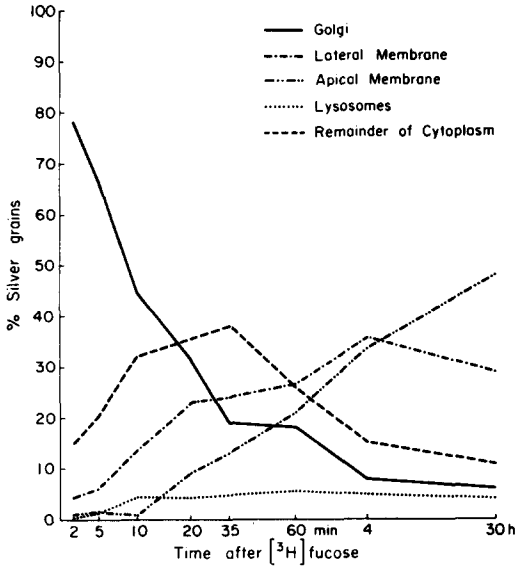


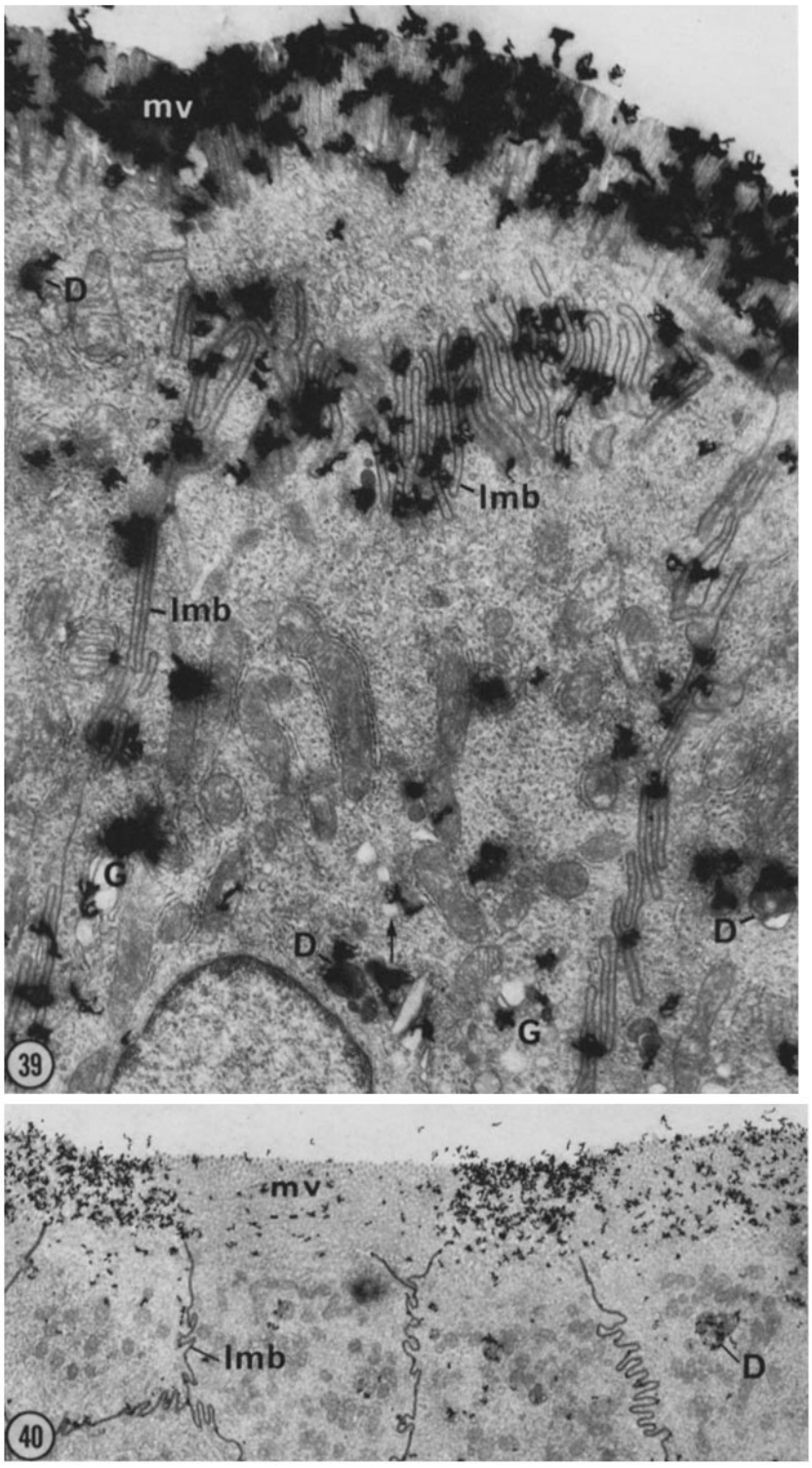
FIGURE 38 Distribution of silver grains over columnar cells of duodenal villi at various time intervals after an injection of [^{3}H]fucose. At 2 min, most of the silver grains are over the Golgi apparatus. The remainder of the cytoplasm reaches a peak at 10–20 min, while the lateral and apical membranes do so at 4 h or later. A small proportion of silver grains is over lysosomes.

surface reactions indicate turnover, even in the young cells of renewing populations.

These various observations are in line with reports indicating that the membrane components of cultured cells are synthesized at the same rate whether the cells are actively dividing or have stopped doing so (28, 66). This remarkable observation implies that, when cells are growing, membrane components are added mainly for growth, but when cells are in a stationary phase, membrane components turn over.

The turnover of cell coat glycoproteins may be associated with a purposeful secretion of these substances. Observations on ascites cells in culture have shown that they release glycoproteins to the medium continually (27, 34, 45). It is possible that in the body too, cells release glycoproteins to the extracellular space or to a lumen when present. Thus, in liver cells, the radioautographic reaction at the surface facing the sinuses seemed to be less intense at 30 than 4 h, perhaps because surface glycoproteins are released to the circulation, where they may constitute some of the serum glycoproteins. In the case of distal and proximal tubule cells of rat kidney, the luminal urine may sweep along surface material and thus acquire the carbohydrates observed in rat urine (41, 44). In the small intestine, the turnover of the glycoprotein hydrolases present at the surface of microvilli (1) may result from their removal by the passage of chyme. In fact, one of these hydrolases, alkaline phosphatase, has been identified in duodenal juice (65).

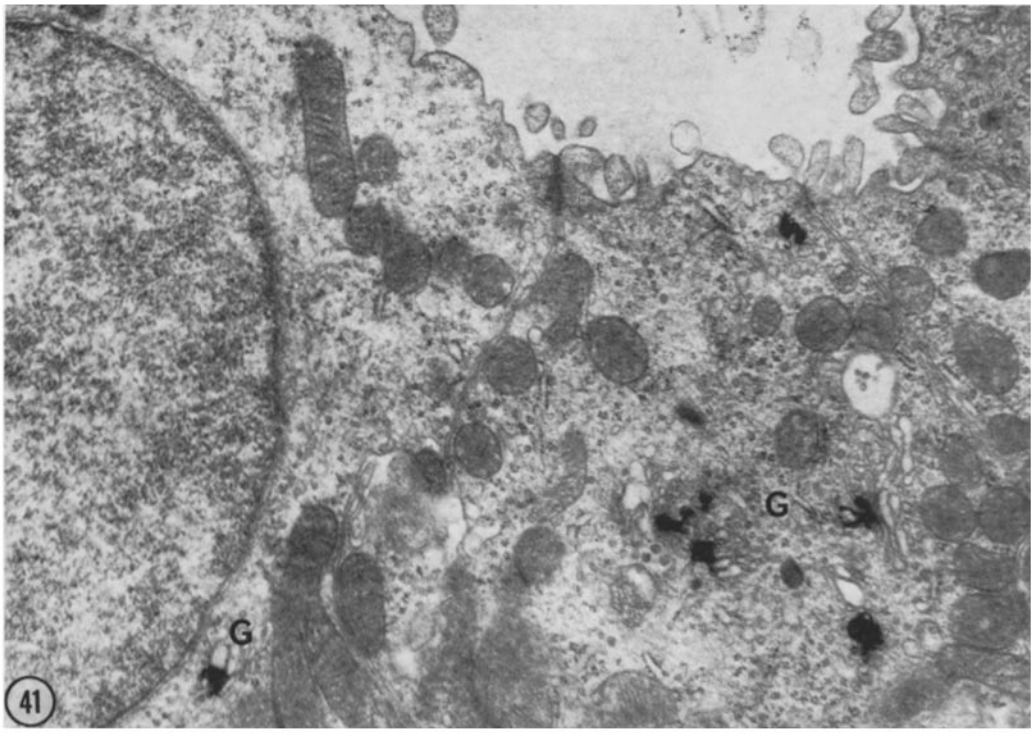
The turnover of cell coat glycoproteins may also be due to a loss of these substances resulting from their participation in surface activities, such as intercellular adhesion, uptake of antigens, interaction with viruses and other noxious agents, active transport across the membrane, pinocytosis, and phagocytosis (27, 70). For instance, adhesion to other cells (or conversely the breakdown of the bonds required for the mobility of motile cells) may consume glycoproteins. Pinocytosis and phagocytosis require the formation of vesicles which are derived from the plasma membrane and carry off cell coat glycoproteins. The glycoproteins thus lost have to be replaced. The procedure evolved by nature is to ensure a constant supply of new glycoproteins and, in this way, anticipate their wear or damage. It was recently shown that the rate of formation of surface glycoproteins in ascites cells remains constant,

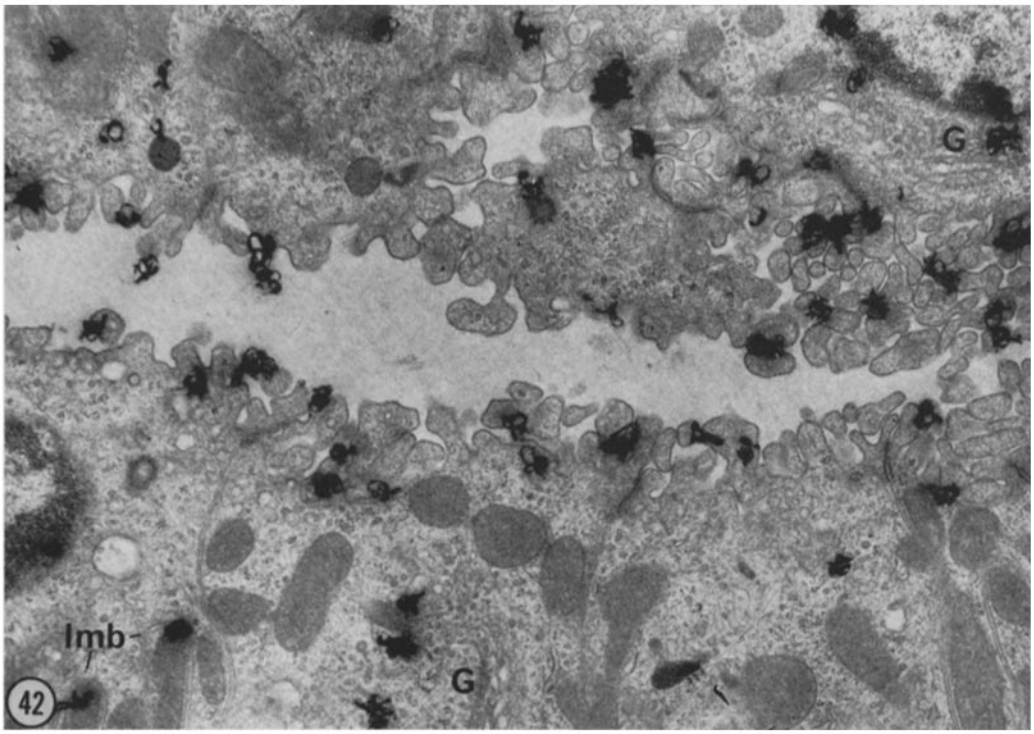


FIGURES 39–40 Electron microscopic radioautographs of duodenal villus columnar cells, 4 h after [^{3}H]-fucose injection. Epon sections stained with uranyl acetate and lead. 14-wk exposure.

FIGURE 39 Apical region of cells near base of villus. A heavy reaction is localized over the microvilli (*mv*) at the free surface of the cell and over lateral membranes (*lmb*). Dense bodies (*D*) and some vesicles (vertical arrows) are also labeled. Some radioactivity remains over the Golgi apparatus (*G*). × 12,000.

FIGURE 40 Apical region at low magnification. The lateral membranes are outlined in ink (*lmb*). The intensity of reaction over the microvilli (*mv*) is uniform within any given cell, but differs dramatically in different cells (*D*, dense body). × 5,000.





FIGURES 41–42 Electron microscopic radioautographs of kidney distal convoluted tubule cells after [^{3}H]fucose injection. Epon sections stained with uranyl acetate and lead.

FIGURE 41 10 min after injection. All but one of the seven silver grains are over, or very close to, Golgi stacks (*G*). The free surface is unlabeled. × 15,000.

FIGURE 42 30 min after [^{3}H]fucose injection. Most of the silver grains are associated with the small microvilli of the free surface. Some others remain in the Golgi regions (*G*) and two grains are seen over or near a lateral cell membrane (*lmb*). × 15,000.

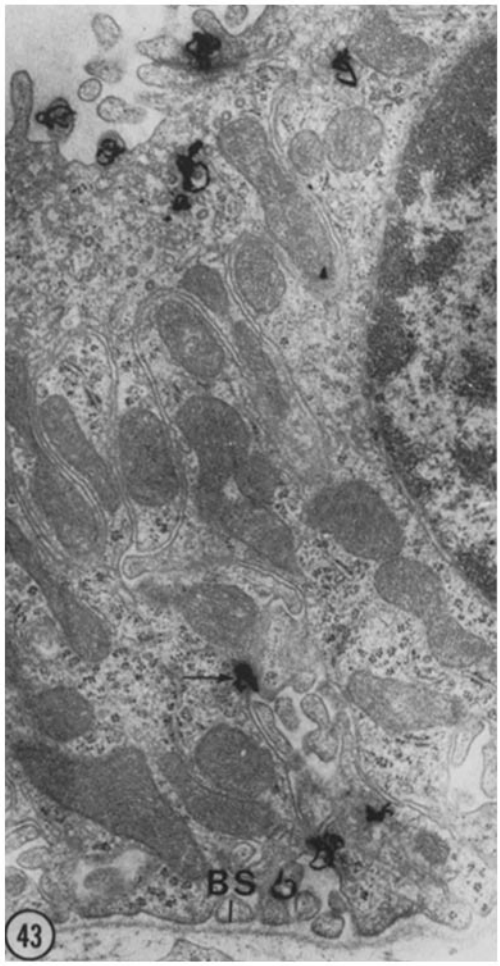


FIGURE 43 Electron microscopic radioautograph of kidney distal convoluted tubule cells, 30 h after [^{3}H]-fucose injection. Epon section stained with uranyl acetate and lead. At this time most silver grains are found on the free surface or on the folds of the membrane of the basal surface (*BS*). One grain is on the lateral surface (arrow). × 18,000.

whether the surface sialic acid is intact or has been removed by neuraminidase (28). Thus, regardless of whether or not surface glycoproteins have been damaged or lost, their renewal occurs continually.

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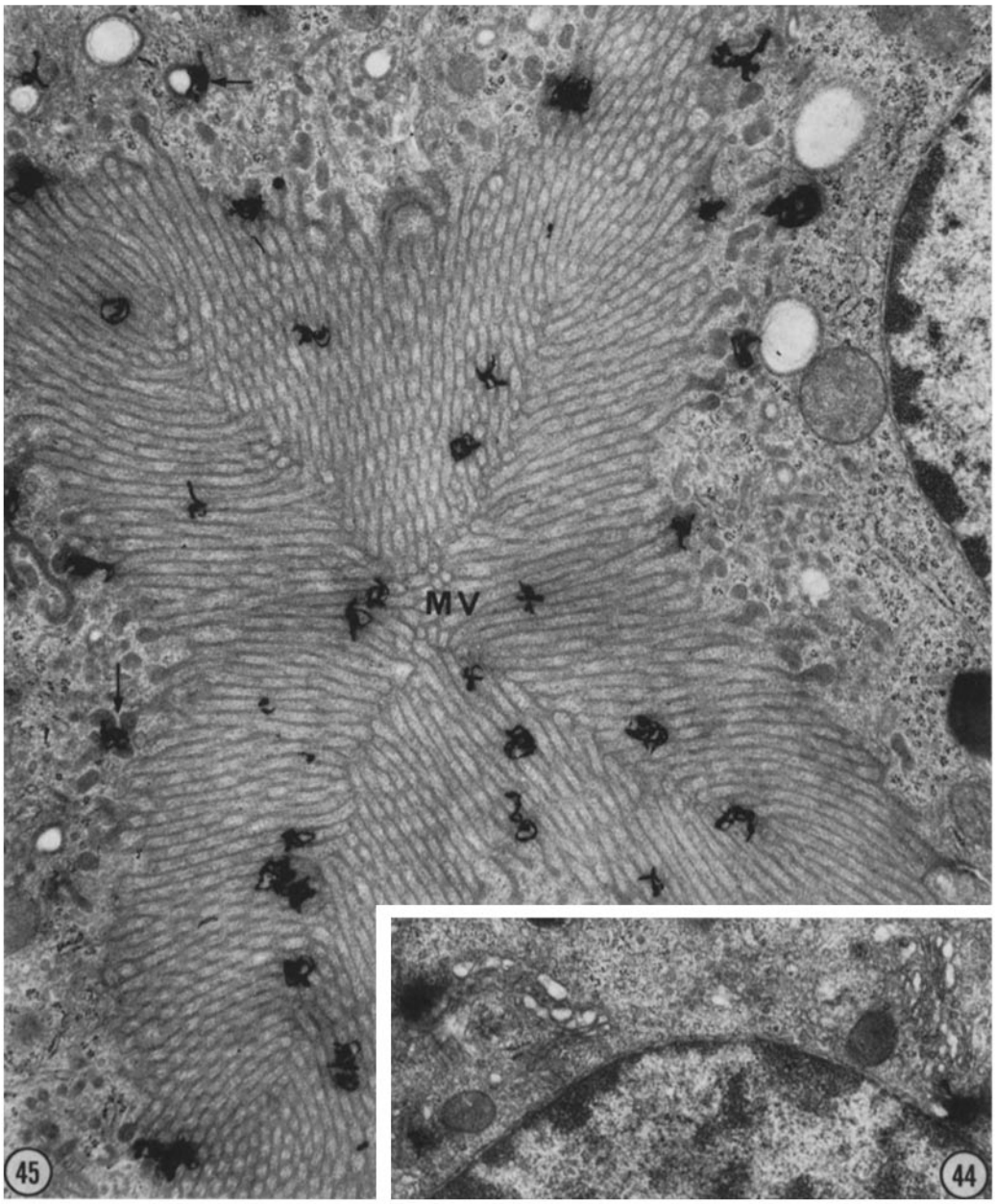

FIGURES 44–45 Electron microscope radioautographs of kidney proximal convoluted tubule cells, at various times after [^{3}H]fucose injection. Epon sections stained with uranyl acetate and lead.

FIGURE 44 10 min after injection, three silver grains are localized over Golgi saccules. × 22,000.

FIGURE 45 30 h after injection. Most of the silver grains are localized over the apical microvillar border (*MV*). Some grains occur over light (horizontal arrow) or dense (vertical arrow) invaginations of the cell surface. × 15,000.

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