The Site of Incorporation of Sialic Acid Residues into Glycoproteins and the Subsequent Fates of These Molecules in Various Rat and Mouse Cell Types as Shown by Radioautography after Injection of [^{3}H]*N*-Acetylmannosamine
II. Observations in Tissues Other than Liver

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ABSTRACT Biochemical evidence from the preceding paper indicated that [^{3}H]*N*-acetylmannosamine may be used as a fairly specific precursor for the sialic acid residues of glycoproteins (and perhaps glycolipids) in radioautographs of rat liver and duodenum. In order to study the site of incorporation of this label in cell types of various tissues, we gave 40-g rats and 15-g Swiss albino mice a single intravenous injection of 8 mCi of [^{3}H]*N*-acetylmannosamine and sacrificed them after 2 and 10 min. To trace the subsequent migration of the labeled glycoproteins, we injected 40-g rats with 4 mCi of [^{3}H]*N*-acetylmannosamine and sacrificed them after 20 and 30 min, 1, 4, and 24 h, and 3 and 9 d. Light microscope radioautographic analysis revealed that in a great variety of cell types the label was initially localized to the Golgi region. Electron microscope radioautographic analysis of duodenal villous columnar and goblet cells, pancreatic acinar cells and Paneth cells, from rats and mice sacrificed 10 min after injection, showed that the silver grains were localized over Golgi saccules (and adjacent secretion granules). In kidney proximal and distal tubule cells reaction was initially localized to the Golgi apparatus in some areas of the kidney cortex whereas in other areas it was more diffuse. In all cells, the proportion of silver grains over the Golgi apparatus decreased with time after injection while an increasing number of grains appeared over secretion products in secretory cells or over the plasma membrane in other cell types. Lysosomes also became increasingly labeled at later time intervals. The above results suggest that in most cell types sialic acid residues are incorporated into glycoproteins (and perhaps glycolipids), primarily in the Golgi apparatus. With time, these newly synthesized molecules migrate to secretion products, to the plasma membrane, or to the lysosomes.

As described in the preceding article (9), light and electron microscope radioautographic studies were carried out on liver tissue of young rats and mice sacrificed at various time intervals after an intravenous injection of [^{3}H]*N*-acetylmannosamine. Concurrent biochemical studies showed this substance to be a fairly specific precursor for sialic acid residues of glycoproteins (and perhaps glycolipids) in radioautographic sections of liver and duodenal tissue. In hepatocytes, at early time intervals after injection, the label was localized to Golgi stacks and adjacent secretory vesicles, but migrated with time to various regions of the plasma membrane and to lysosomes. It was concluded that sialic acid residues were added to the carbohydrate side chains of glycoproteins (and perhaps glycolipids) in the region of the Golgi stacks, and that these molecules then

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had three fates, i.e., migration out of the cell as secretion products, migration to the plasma membrane to become membrane glycoproteins, and migration to lysosomes.

The present article describes the results obtained from light microscope radioautographic studies carried out on other tissues of the above animals, as well as results from electron microscope radioautographic studies on a number of cell types, i.e., duodenal villus columnar and goblet cells, kidney proximal and distal tubule cells, pancreatic acinar cells, and Paneth cells. Some of the results of the present study have been published in preliminary form (1, 2, 5, 7, 8, 13).

MATERIALS AND METHODS

Young (40 g) male Sherman rats and Swiss albino mice (15 g), fed ad lib, were anesthetized with nembutal and given a single injection, via the external jugular vein, of 8 mCi [^{3}H]*N*-acetylmannosamine (New England Nuclear, Boston, Mass., sp act 2.2 Ci/mM) in 0.2 ml saline solution. After 2 or 10 min, the animals were killed by intracardiac perfusion for 15 min at room temperature with a solution of 2.5% glutaraldehyde in 0.05 M Sorensen's buffer, following a 30-s prewash with lactated Ringer's solution. Other young male rats (40 g) were injected as above with 4 mCi of [^{3}H]*N*-acetylmannosamine and sacrificed after 20 and 30 min, 1, 4, and 24 h, and 3 and 9 d.

Sample pieces of all tissues and organs were removed and further fixed by immersion in Bouin's fixative for 24 h before embedding in celloidin and paraffin. In the case of one rat and one mouse sacrificed at 2 min after [^{3}H]*N*-acetylmannosamine injection, the animals were perfused for 30 s with a 0.8% saline solution containing 0.5% nonradioactive *N*-acetylmannosamine but received no perfusion with glutaraldehyde solution; instead sample pieces of tissue were quickly removed and fixed by immersion in Bouin's fixative. Hard tissues from all animals were decalcified in 4.13% disodium EDTA containing 0.44% sodium hydroxide (isotonic at pH 4) at 4°C before embedding. 5-μm thick sections of both soft and hard tissues were stained with hematoxylin and eosin before coating with Kodak NTB2 emulsion for radioautography.

Other sample pieces of liver, duodenum, ascending colon, kidney, pancreas, thyroid, and incisor teeth were removed and immersed in a solution of 2.5% glutaraldehyde in 0.05 M Sorensen's buffer for 2 h at 4°C. The pieces were then washed in 0.15 M Sorensen's buffer, postfixed in 1% OsO_4 in 0.1 M Sorensen's buffer for 1 h at 4°C, dehydrated in graded ethanol solutions, passed through propylene oxide, and embedded in Epon. The teeth were decalcified (as described above) before embedding. Sections of all of the above tissues were then processed for light and electron microscope radioautography as described in the preceding article (9).

RESULTS

Radioautographic Studies

When tissues were examined with the light microscope, a great variety of different cell types exhibited radioautographic reaction after [^{3}H]*N*-acetylmannosamine injection (Table I). At early time intervals in most cell types, the reaction was localized to the region of cytoplasm containing the Golgi apparatus. With time, the distribution of label within the cell changed. In secretory cells the label appeared in secretory material located either in the apical cytoplasm or outside of the cell. In many other cell types the label appeared at the cell surface.

Cell Types in Which the Label Migrated from the Golgi Region to the Cell Surface

In hepatocytes, light and electron microscope radioautographic studies revealed that most [^{3}H]*N*-acetylmannosamine label was associated with Golgi stacks and adjacent secretory vesicles at early intervals after injection, but migrated with time to all regions of the plasma membrane. The results of these studies have been presented in the preceding paper (9).

In duodenal villous columnar cells from rats and mice sacrificed 2 or 10 min after [^{3}H]*N*-acetylmannosamine injection, light microscope radioautographs revealed a reaction which was strongly localized to the supranuclear Golgi region (Fig. 1). When electron microscope radioautographs of duodenal villous columnar cells from animals sacrificed 10 min after injection were examined, most of the silver grains were seen to be localized over the saccules of Golgi stacks (Figs. 4 and 5). Grain counts revealed that over 74% of the total grains occurred over these stacks (Table II). The lateral plasma membrane and apical microvillous border accounted for 6.0 and 1.8% of the grains respectively, and most of the remaining grains (17.2%) were scattered over the remainder of the cell; many of the latter grains occurred over mitochondria.

Light microscope radioautographs of duodenal villous columnar cells from rats killed 4 h after injection showed a marked change in the distribution of reaction (Fig. 2). Most of the silver grains were now localized over the apical microvillous border, whereas only a few remained over the supranuclear region. Electron microscope radioautographs of columnar cells from the middle portion of duodenal villi at 4 h after injection revealed a heavy reaction over the apical microvilli, with smaller amounts of reaction occurring over the lateral plasma membranes (Figs. 6 and 7). In the cytoplasm, some grains remained over the supranuclear Golgi stacks, others were associated with lysosome-like bodies, and yet others were diffusely scattered over the cytoplasm, often occurring over mitochondria. Grains counts revealed that <7% of the grains now remained over the Golgi stacks, whereas over 46% were found over the apical microvilli, and over 15% occurred over the lateral plasma membrane (Table II). Many lysosome-like bodies were labeled but they accounted for <2% of the total grain count.

In duodenal villous columnar cells of rats killed 24 h after injection (Fig. 3), light microscope radioautographs revealed a distribution of reaction very similar to that seen at the 4-h time interval. Grain counts of electron microscope radioautographs showed that now over 52% of the total grains were localized over the apical microvilli (Table II). Reaction over lysosome-like bodies had also increased and now accounted for 5.5% of the total grain count.

In the surface columnar epithelial cells of the ascending colon, light microscope radioautographs from rats sacrificed 10 min after injection revealed a strongly localized supranuclear Golgi reaction with almost no reaction occurring elsewhere over the cells. By 4 h after injection, reaction over the Golgi region was virtually absent, and a strongly localized reaction occurred over the apical microvillar surface. A similar situation was observed in the ciliated columnar cells of the nasal respiratory epithelium, where a supranuclear Golgi reaction at the 10-min time interval was replaced by a reaction localized over the apical cilia at 4 h.

In kidney proximal tubule cells, the pattern of radioautographic reaction observed at early time intervals varied greatly, depending on which area of the kidney cortex was observed. Near the corticomedullary border, cells of the straight portions of proximal tubules exhibited a fairly heavy reaction in which most of the label was localized to the paranuclear Golgi region (Fig. 8). In the outer cortex, on the other hand, cells of the convoluted portions of proximal tubules exhibited a lighter reaction in which the grains were scattered diffusely over the cytoplasm as well as over the apical microvillous border, and in which little or no localization of grains over the paranuclear Golgi region was evident. Cells of convoluted portions of proximal tubules in middle regions of the cortex often exhibited a reaction of medium intensity in which a cluster of grains in

TABLE I

Comparison of Radioautographic Reactions Observed over the Golgi Region of Various Cell Types of Rats Sacrificed 10 min after Injection of Equal Doses of [^{3}H]Fucose‡ or [^{3}H]N-Acetylmannosamine*

Cells in which label migrated to cell surface	[^{3}H]Fucose (1-mo exposure)	[^{3}H]N-Acetylmannosamine (6-mo exposure)	Cells in which label migrated to secretory material	[^{3}H]Fucose (1-mo exposure)	[^{3}H]N-Acetylmannosamine (6-mo exposure)
Simple epithelia			Mucous cells		
Duodenal villous columnar c.	++++	++++	Duodenal goblet c.	++++	++
Duodenal crypt columnar c.	++	+	Colonic surface goblet c.	++++	+++
Colonic surface columnar c.	++++	++	Colonic crypt goblet c.	+++	++
Colonic crypt columnar c.	+++	+	Conjunctival goblet c.	+++	−
Hepatocyte	++++	++++	Stomach surface c.	+++	++
Kidney proximal tubule c.	+	+/++	Stomach mucous neck c.	++	−
Kidney distal tubule c.	++	++	Submaxillary mucous c.	+++	+
Respiratory ciliated c.	++	++	Sublingual acinar c.	++	++
Vas deferens c.	+	−	Tongue mucous gland c.	+++	+
Choroid plexus c.	++	−	Thyroid follicular c.	+++	+
Ciliary body c.	++	−			
Epididymus c.	++	−	Serous cells		
Submaxillary gland duct c.§	++	−	Pancreatic acinar c.	++	++
Capillary endothelial c.	++	−	Submaxillary serous c.	++	+
Mesothelial c.	++	−	Tongue serous gland c.	+	−
			Lacrimal gland c.	++	−
Stratified epithelia			Stomach chief c.	+	+
Tongue	++	−			
Esophagus	++	−	Cells producing ground substances		
Forestomach	++	−	Ameloblasts	+++	++
Bladder	++	−	Odontoblasts	++	+
Epidermal	++	−	Osteoblasts	++	+
Conjunctival	++	−	Chondroblasts	++	−
			Fibroblasts	++	+
			Fibrocartilage c.	+	++
			Osteoclast c.‖	++	++++

* From paraffin sections.

‡ Data taken from reference 6.

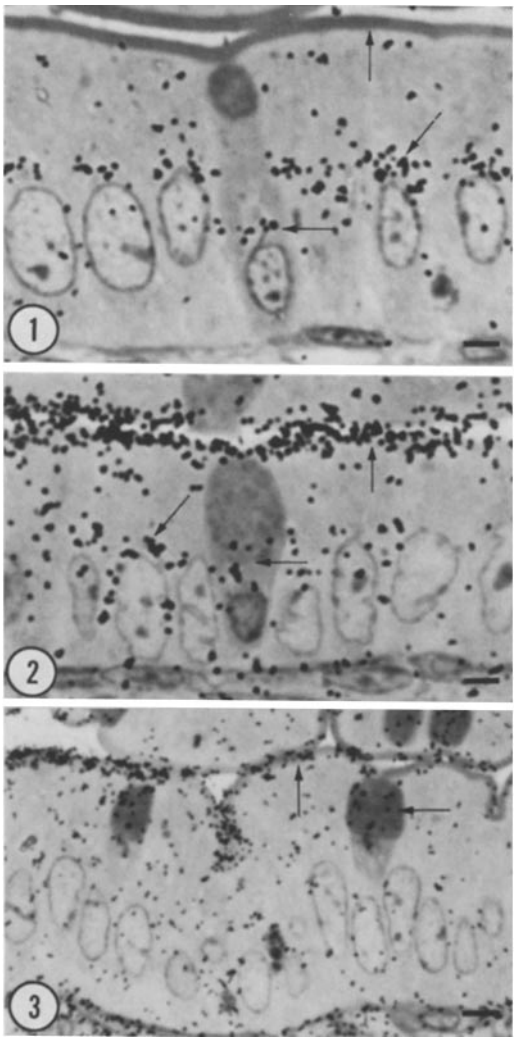
§ Recent electron microscope radioautographic work by A. Hand (1979. *Anat. Rec.* **195**:317–340.) has shown that most [^{3}H]fucose label actually migrates to apical secretion granules.

‖ Reaction was quite diffusely scattered over cytoplasm even at 10 min after injection, and retained this distribution during later time intervals.

the paranuclear Golgi region was accompanied by a lighter diffuse reaction over the remainder of the cytoplasm and the apical microvillous border (Fig. 9). When radioautographs of proximal tubule cells were examined in the electron microscope, the pattern of reaction observed at the 10-min time interval was seen to vary as described above, depending on the region of kidney cortex examined. In tubule cells of some regions, most of the silver grains were found to be associated with Golgi stacks (Fig. 14). In cells of other regions, grain counts revealed that as few as 10% of the silver grains were over Golgi saccules, whereas 10% occurred over lateral and basal membranes, 30% occurred over the apical tubules and microvilli, 10% occurred over lysosomes, 25% occurred over mitochondria, and 15% occurred over the remainder of the cytoplasm.

In light microscope radioautographs of kidney proximal tubule cells from animals sacrificed 1 h after injection, clusters of grains remained over the paranuclear regions, but now the heaviest reaction was localized over the apical microvillous border (Fig. 10). By the 4-h time interval, most of the cytoplasm exhibited little reaction while the microvillous border was heavily labeled, and some grains appeared over the basal surface of the cells (Fig. 11). At 24 h after injection, these cells exhibited a similar light microscope reaction, and when examined in the electron microscope (Fig. 15), revealed many grains over the apical microvilli and over tubules and vacuoles in the apical cytoplasm. In addition, a heavy reaction was seen over the numerous lysosomes. In light microscope radioautographs of animals sacrificed 3 and even 9 d after injection, the proximal tubule cells continued to exhibit a heavy reaction, especially in portions of tubules near the corticomedullary border. At the 9-d time interval, the reaction was sharply localized over the microvillous border, the remainder of the cells being virtually free of label.

In kidney distal tubule cells, the pattern of radioautographic reaction at 10 min after injection likewise varied depending on the area of cortex examined, although not as dramatically as in the case of proximal cells. In the convoluted portions of distal tubules near glomeruli, the cells exhibited a light diffuse reaction, while in straight portions of the tubules located in medullary rays, the cells often exhibited a much heavier reaction, in which most grains were localized to a paranuclear Golgi



FIGURES 1-3 Light microscope radioautographs of Epon sections of duodenal villus cells from rats injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5.0 μm.

FIGURE 1 Cells from the lower half of a villus. 10 min after injection. Exposed 6 mo. The columnar cells exhibit a reaction of medium intensity in which most of the silver grains are localized to the supranuclear (Golgi) region (oblique arrow). No significant reaction occurs over the apical microvillar surface of the cells (vertical arrow). In the goblet cell, a lighter reaction is seen in which all of the grains are localized to the supranuclear (Golgi) region (horizontal arrow).

FIGURE 2 Cells from the lower half of a villus. 4 h after injection. Exposed 3 mo. In the columnar cells, some silver grains remain over the supranuclear region (oblique arrow), but most of the reaction is now localized over the apical microvillar surface (vertical arrow). Some grains occur over the cytoplasm outside of the supranuclear region, and the number of such grains increases as one ascends the villus (i.e., towards the left in this figure). Some of these grains may be over lateral plasma membranes. In goblet cells, the silver grains are localized over the supranuclear region and the base of the mucous theca (horizontal arrow).

FIGURE 3 Cells from the middle portion of a villus. 24 h after injection. Exposed 3 mo. In the columnar cells, most of the reaction is localized over the apical microvillar surface (vertical arrow). In goblet cells, the silver grains now occur over the apical portions of the mucous theca (horizontal arrow). The lamina propria at the base of the figure is now strongly labeled.

region (Fig. 12). When such cells were observed in the electron microscope, the majority of the silver grains were located over Golgi saccules.

By 4 h after injection, the paranuclear reaction previously seen over distal tubule cells had disappeared and been replaced by a heavy reaction over the apical cytoplasm and microvillar surface, as well as a lighter reaction over the basal surface (Fig. 13). Electron microscope radioautographs of these cells at 24 h after injection showed the presence of many grains over the apical microvillar surface. A somewhat lighter reaction appeared over the lateral and basal plasma membrane, and lysosomes were heavily labeled.

In light microscope radioautographs of animals sacrificed 3 d after injection, the distal tubule cells continued to exhibit a fairly heavy reaction over their apical surface. By the 9-d time interval, however, this reaction had diminished considerably.

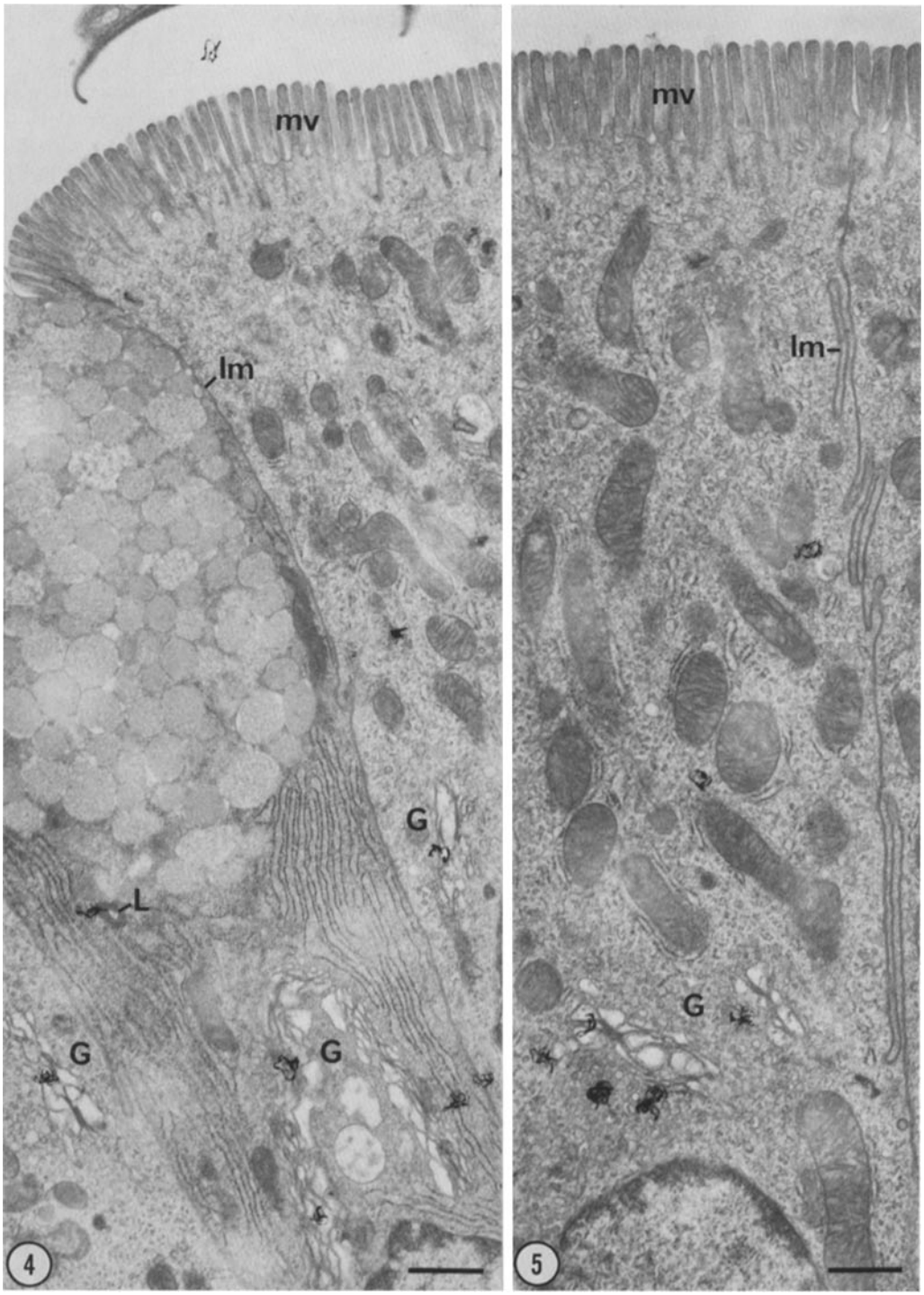
Cell Types in Which the Label Migrated from the Golgi Region to Secretion Products

The heaviest radioautographic reactions of this type occurred over mucus-secreting cells (Table I). In intestinal goblet cells (Fig. 1) and the mucous acinar cells of salivary glands (Fig. 21), the [^{3}H]*N*-acetylmannosamine label was localized at early time intervals to the supranuclear Golgi region. 4 h after injection, the reaction of goblet cells was still supranuclear but now appeared to be over the base of the mucous theca (Fig. 2). By 24 h, the label had migrated to apical portions of the mucous theca (Fig. 3). In electron microscope radioautographs of goblet cells, from rats sacrificed 10 min after injection, most of the silver grains were localized over saccules of the supranuclear Golgi stacks (Fig. 4), although some occurred over adjacent newly formed mucous granules (see Fig. 2 *a* of Bennett [1]). 4 h after injection, these stacks were unlabeled and grains now overlay secretion granules located in the base of the mucus theca (Fig. 6). In addition, a number of grains occurred over the lateral plasma membranes separating the goblet cells from neighboring columnar cells.

In serous cells, such as pancreatic acinar cells and Paneth cells, light microscope radioautography localized the label to the supranuclear region at 10 min after injection (Figs. 18 and 20), while electron microscope radioautography in both of these cell types showed that most of the silver grains were localized over Golgi stacks and nearby immature secretion granules (Fig. 22). 4 h after injection, in pancreatic acinar cells, the label had migrated to secretion granues in the apical cytoplasm. Much reaction remained at this site at 24 h after injection (Fig. 19), but by 3 d after injection all of the label had been secreted.

In thyroid follicular cells, only a light radioautographic reaction was seen at 10 min after injection, but the grains were localized to a paranuclear Golgi region (Fig.16). By 4 h, the cells themselves had lost their label, and the reaction now formed a ring over the peripheral portion of the luminal colloid (Fig. 17).

In pancreatic islets, heavy, well-localized, paranuclear reactions appeared at 10 min after injection (Fig. 23) over cells which were mostly located toward the central portion of the islets, and therefore tentatively identified as β-cells (20). 24 h after injection, these central cells exhibited a heavy diffuse reaction over their cytoplasm, whereas cells at the periphery of islets were much less labeled (Fig. 24). The cytoplasmic reaction



FIGURES 4 and 5 Electron microscope radioautographs of duodenal villous epithelial cells from a rat sacrificed 10 min after injection of [^{3}H]*N*-acetylmannosamine. Stained with uranyl acetate and lead citrate. Exposed 10 mo. × 10,800. Bar, 1 μm.

FIGURE 4 A goblet cell in the middle of the figure is flanked on both sides by portions of columnar cells. In all three cells, the silver grains are localized over Golgi stacks (*G*). In the goblet cell one grain lies over a lysosome (*L*), and one lies over the lateral membrane (*lm*); the mucous theca is unlabeled. In the columnar cells, no grains are seen outside of the Golgi region, and the apical microvillous border (*mv*) is unlabeled.

FIGURE 5 Most of the silver grains in the photograph are associated with the Golgi stacks (*G*) of a columnar cell. The lateral plasma membrane (*lm*) and the apical microvillous border (*mv*) are unlabeled.

TABLE II

Distribution of Silver Grains over Structures in Duodenal Villous Columnar Cells of Rats Sacrificed at Various Time Intervals after [^{3}H]N-Acetylmannosamine

Time after injection	Total grains counted	Percentage distribution of grains over organelles				
		Golgi stacks	Lateral plasma membrane	Microvillous border	Lysosomes	Remainder of cell
10 min	574	74.3	6.0	1.8	0.7	17.2
4 h	2,019	6.9	15.4	46.2	1.9	29.6
24 h	1,102	6.6	13.7	52.6	5.5	21.6

over the central cells persisted with time and remained heavy even at 9 d after injection.

In connective tissue cell types, Golgi localized reactions were observed at early time intervals followed by migration of the label to extracellular secretory products at later time intervals. Thus, in osteoblasts, an early paranuclear reaction was followed by the appearance of a band of reaction over the edge of forming bone matrix. In ameloblasts from the region of inner enamel secretion of incisor teeth, reaction was initially localized over the supranuclear Golgi region (Fig. 25), but by 4 h after injection, had become concentrated over the distal Tomes' processes, with some grains appearing over the adjacent enamel (Fig. 26). In odontoblasts, a light supranuclear reaction at early time intervals was followed by appearance of a band of reaction over the dentin just above its junction with the predentin; a lighter diffuse reaction occurred over the predentin.

In the fibroblasts in loose connective tissue a light paranuclear reaction was frequently observed at early time intervals after injection. At later times, a very heavy diffuse reaction occurred over the loose connective tissue in a variety of locations, i.e., the stroma of glands and other organs, between muscle cells, and beneath epithelial membranes (Fig. 3). This labeling persisted for long intervals after injection, with the result that, at 3 and 9 d after injection, a heavy reaction remained over the connective tissue surrounding the acini of glands or over the lamina propria of intestinal villi, after the epithelial cells themselves had either lost their label or been replaced by new unlabeled cells.

Finally, the multinucleated osteoclast cells of bone exhibited a heavy reaction after [^{3}H]*N*-acetylmannosamine. As seen in light microscope radioautographs, the reaction was quite diffusely scattered over the cytoplasm even in animals sacrificed at short time intervals after injection, and this distribution was retained during later time intervals. The significance of this reaction has not yet been determined.

DISCUSSION

Addition of Sialic Acid Residues to Glycoproteins in the Golgi Region

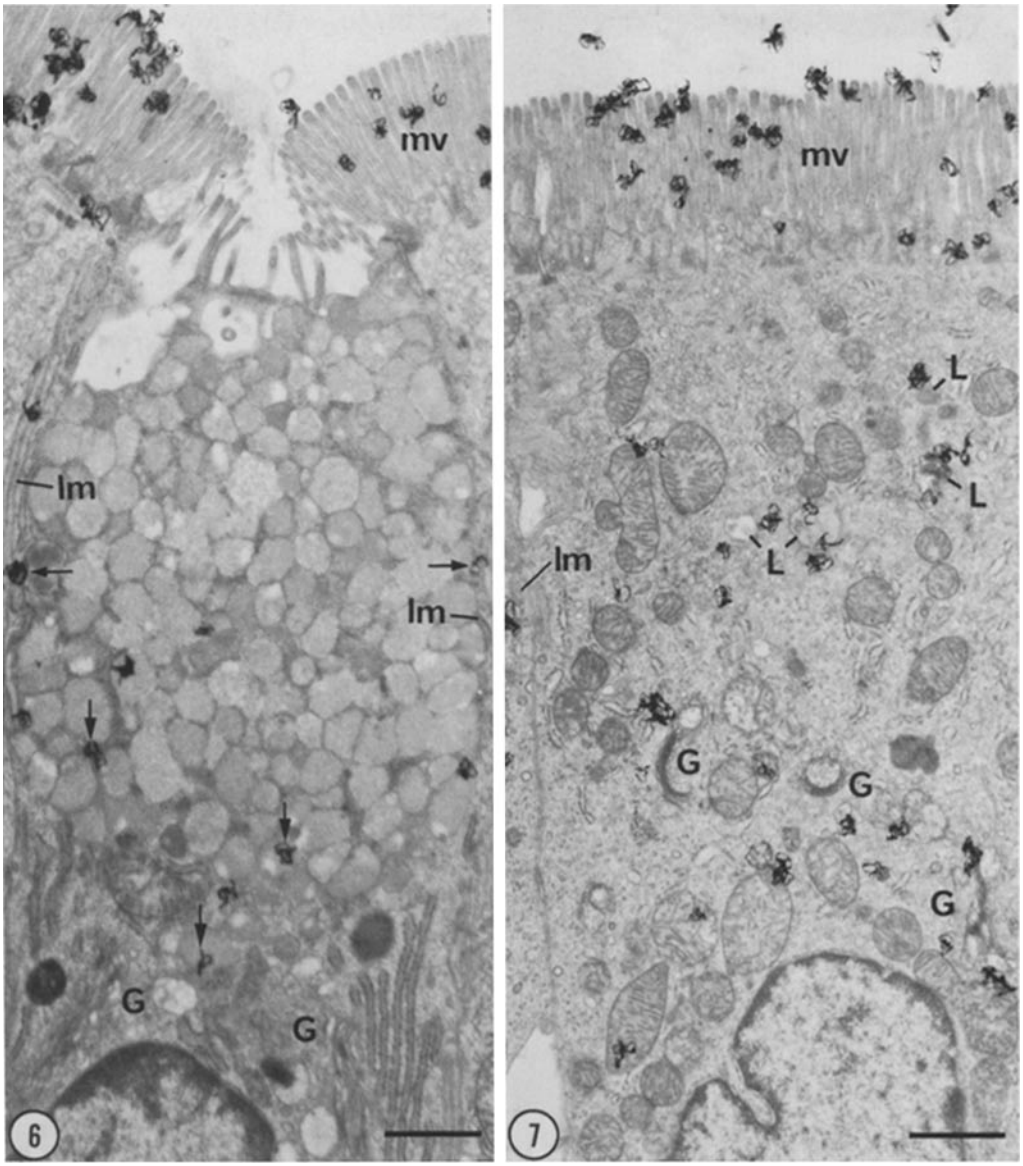
In most of the cell types examined in the present study, the light microscope radioautographic reaction, at short time intervals after [^{3}H]*N*-acetylmannosamine injection, was clearly localized to the Golgi region (Table I). When cells were examined in the electron microscope the grain distribution within this region varied somewhat depending on the cell type examined. In duodenal villous columnar cells (Figs. 4 and 5) and some kidney proximal tubule cells (Fig. 14) and distal tubule cells, the initial label tended to be homogeneously distributed throughout the Golgi stacks, a situation similar to that previously observed after [^{3}H]fucose injection (3, 4, 11). In pancreatic acinar and Paneth cells (Fig. 22), on the other hand, there appeared to be a preferential localization of the initial label to the trans face of the Golgi stack and adjacent immature secretion granules, a situation similar to that described in hepatocytes at 10 min after [^{3}H]*N*-acetylmannosamine in the preceding article (9). In rat pancreatic cells, Novikoff and Novikoff (18) and Hand and Oliver (12) have shown the presence of a GERL element along the trans face of Golgi stacks. This GERL element was in continuity with immature secretion granules and appeared to play a role in their formation. The initial localization of label to the trans face of the Golgi stacks in the present study allows the possibility that the GERL may represent a site of addition of sialic acid residues to glycoproteins in these cell types.

Early Labeling of Structures Outside of the Golgi Region

In most cell types examined, only a small proportion of the label at 2 or 10 min after [^{3}H]*N*-acetylmannosamine injection appeared outside of the Golgi region. Thus, in electron microscope radioautographs of duodenal villous columnar cells at 10 min after injection, 7.8% of the grains were associated with the lateral and apical plasma membranes, while ~17% occurred over the remainder of the cytoplasm, many of the latter being associated with mitochondria (Table II). The possible roles of these sites in the addition of sialic acid residues to glycoproteins has been discussed in the preceding article (9).

In certain cell types, however, notably in proximal tubule cells in certain regions of the kidney cortex, light microscope radioautographs showed that the reaction was diffusely scattered over the cytoplasm, even at 2 and 10 min after [^{3}H]*N*-acetylmannosamine injection, with little or no localization to the Golgi region. The reaction observed at 2 min after injection of [^{3}H]*N*-acetylmannosamine was too weak to permit electron microscope analysis, but grain counts in electron microscope radioautographs from animals sacrificed 10 min after injection revealed that as few as 10% of the silver grains were over Golgi saccules, while lateral and basal plasma membranes accounted for 10%, apical tubules and microvilli for 30%, lysosomes for 10%, mitochondria for 25%, and the remainder of the cytoplasm for 15%. In a similar electron microscope radioautographic study of rat kidney after [^{3}H]*N*-acetylmannosamine injection, Nayyar and Koenig reported in abstract form (15) that the label was initially taken up in the Golgi apparatus and lysosomes, with nearly 100% of the lysosomes being labeled by 5 min. The explanation for this early diffuse reaction in certain cell types is not as yet clear. Nonspecific binding of the [^{3}H]*N*-acetylmannosamine label by glutaraldehyde fixative is unlikely since the amine group of the molecule is acetylated. Furthermore, a similar diffuse reaction was observed in kidney tissue taken from animals not subjected to glutaraldehyde perfusion, but fixed by immersion in Bouin's fixative. The diffuse reaction is thus assumed to reflect true macromolecular incorporation of label.

Because kidney tissue is rich in glycolipids, it is possible that a substantial portion of the [^{3}H]*N*-acetylmannosamine label



FIGURES 6 and 7 Electron microscope radioautographs of duodenal villous epithelial cells from a rat sacrificed 4 h after injection of [^{3}H]*N*-acetylmannosamine. Stained with uranyl acetate and lead citrate. Exposed 8 mo. × 12,500. Bar, 1 μm.

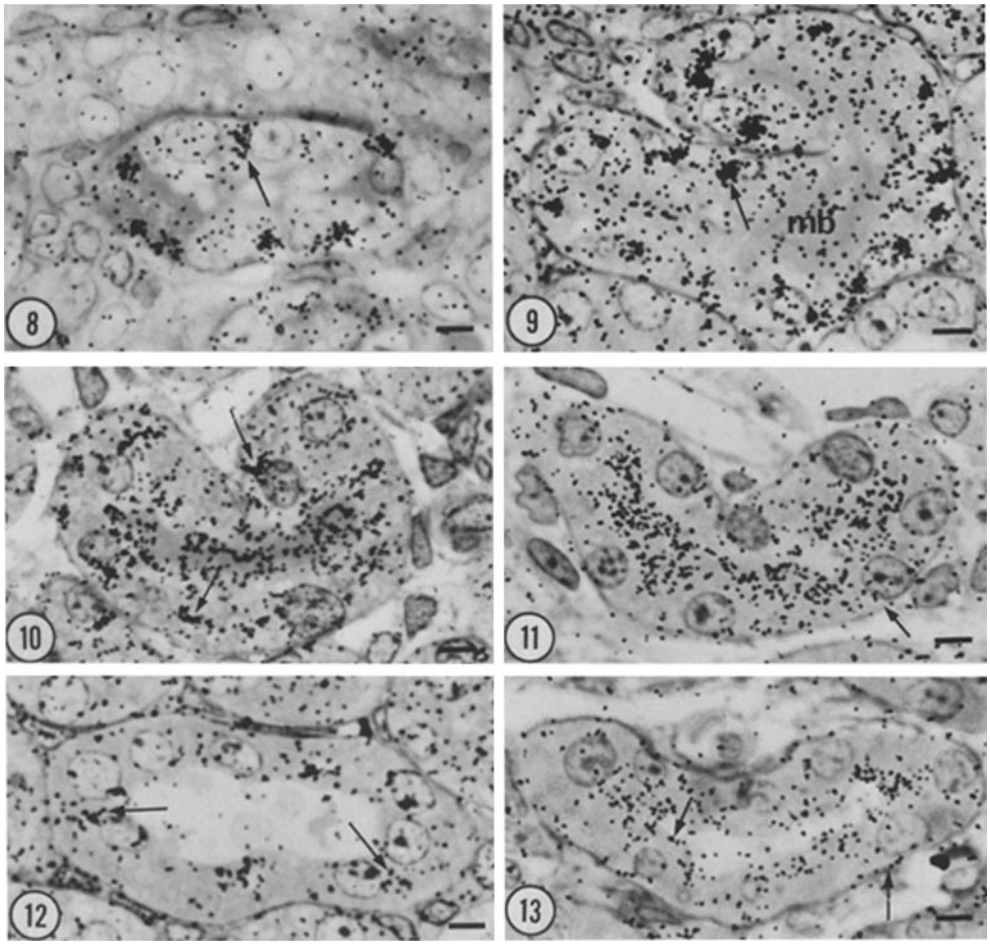
FIGURE 6 A goblet cell in the center of the figure is flanked on both sides by portions of columnar cells. The Golgi stacks (*G*) of the goblet cell are unlabeled, while a number of silver grains lie over mucous granules in the basal half of the mucous theca (vertical arrows). Other grains (horizontal arrows) lie over the lateral plasma membranes (*lm*) separating the goblet cell from the adjacent columnar cells. In the columnar cells, a substantial reaction occurs over the apical microvillous border (*mv*).

FIGURE 7 In this columnar cell, some silver grains are associated with Golgi stacks (*G*), others occur over lysosomes or lysosome-like vacuoles (*L*), and yet others are scattered over the remainder of the cytoplasm. In addition, a substantial reaction is seen over the apical microvillous border (*mv*). One grain lies adjacent to a lateral plasma membrane (*lm*).

had been incorporated into glycolipids, and these latter molecules may be handled differently than glycoproteins. Whatever the explanation, the above radioautographic results indicate that in some cell types, such as certain kidney proximal tubule cells, substantial addition of sialic acid residues to glycoproteins (and possibly glycolipids) may occur outside of the Golgi region.

Migration of Labeled Glycoproteins

MIGRATION TO SECRETION PRODUCTS: In many cell types, label appeared in secretion products at later time intervals after [^{3}H]*N*-acetylmannosamine injection. The secretion products of different cell types are very diverse in nature but a great many have been shown to contain glycoproteins (19). The speed at which such secretion products migrated out of the cells varied greatly among different cell types, being fairly rapid in cells such as osteoblasts, ameloblasts (Fig. 25), odontoblasts and thyroid follicular cells (Fig. 17), but much slower in cells such as pancreatic acinar cells (Fig. 19), salivary mucous acinar cells, and goblet cells (Figs. 2, 3, and 6). In goblet cells, the rate of intracellular migration of labeled glycoproteins was slower than that previously observed by Neutra and Leblond (16). The difference may possibly be caused by the fact that our rats



FIGURES 8–13 Light microscope radioautographs of Epon sections of kidney cortex tissue from rats injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5 μm.

FIGURE 8 Proximal tubule, 10 min after injection. Exposed 2 mo. In the cells of this portion of proximal tubule, most of the silver grains are localized to the paranuclear (Golgi) region (arrow); no significant reaction is seen over the apical microvillous border.

FIGURE 9 Proximal tubule, 10 min after injection. Exposed 6 mo. In the cells of this different portion of proximal tubule, a paranuclear cluster of silver grains is present (arrow), but in addition a lighter diffuse reaction covers the remainder of the cytoplasm and the apical microvillous border (*mb*).

FIGURE 10 Proximal tubule, 1 h after injection. Exposed 3 mo. Localized grains clusters remain over the paranuclear region of the tubule cells (arrows), but now the heaviest reaction occurs over the apical microvillous border.

FIGURE 11 Proximal tubule, 4 h after injection. Exposed 1 mo. A heavy reaction is seen over the apical microvillous border of the tubule cells, while no grain clusters are seen over the paranuclear region of the cytoplasm. A number of silver grains appear to overlie the basal cell surface (arrow).

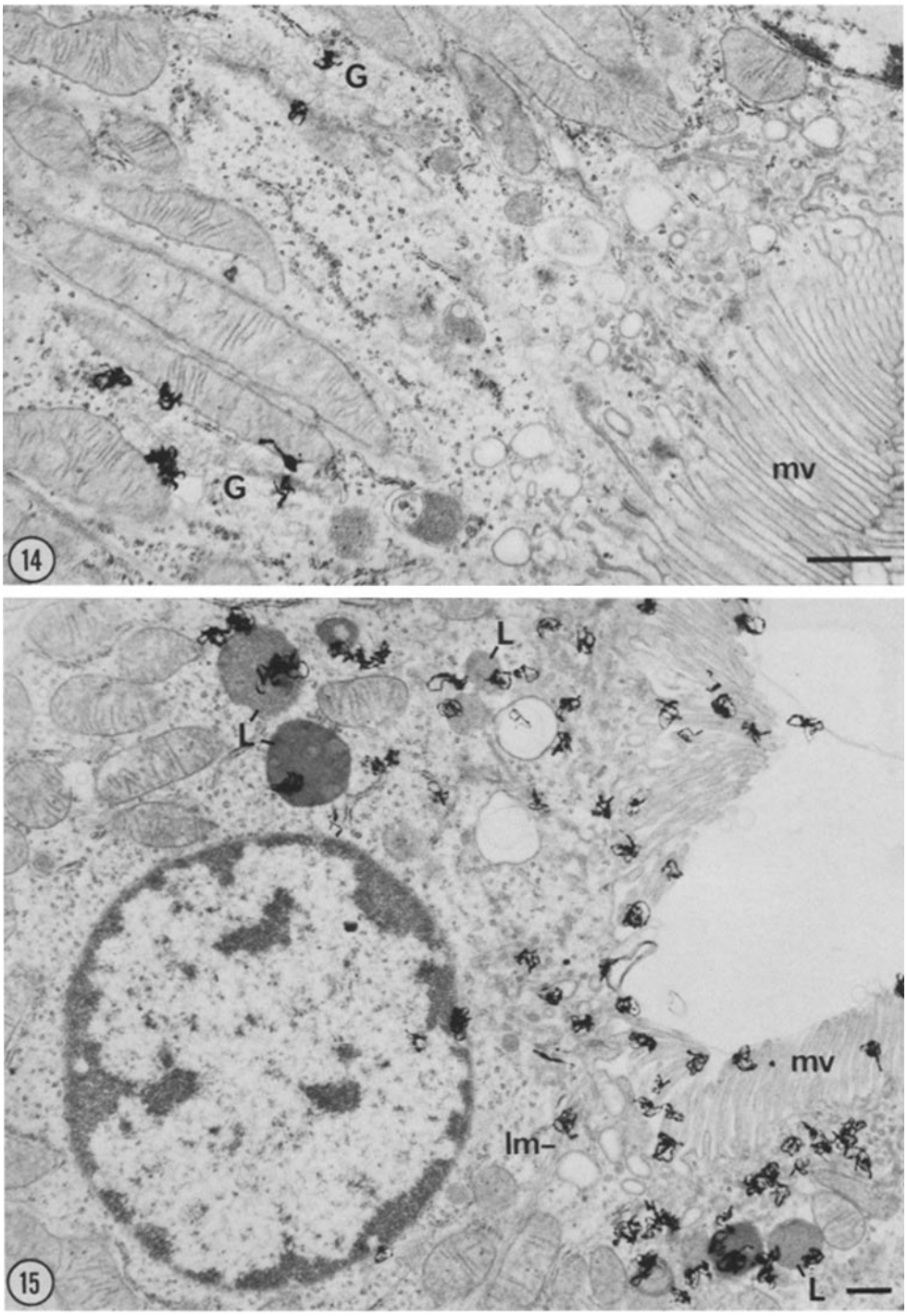
FIGURE 12 Distal tubule, 10 min after injection. Exposed 8 mo. Most of the silver grains over the tubule cells are localized into paranuclear grain clusters (arrows). No significant reaction occurs over either the apical or the basal cell surfaces.

FIGURE 13 Distal tubule, 4 h after injection. Exposed 1 mo. No paranuclear grain clusters are present over the tubule cells, and reaction is localized over the apical (oblique arrow) and basal (vertical arrow) cell surfaces.

were larger (40 g) than those used by Neutra and Leblond (10 g). Recently, Neutra et al. (17) have provided light microscope radioautographic evidence for the uptake of label into goblet cells of human rectal epithelium after in vitro exposure to [^{3}H]*N*-acetylmannosamine. In the cells of this system the intracellular migration of labeled glycoproteins was found to be considerably closer than that previously seen in the rat (16), requiring ~24 h for labeled mucous granules to reach the apical surface, as opposed to 4–8 h in the rat.

In fibroblasts of loose connective tissue, only a light Golgi localized reaction was observed after long radioautographic exposure times (Table I). Yet, at later times, a very heavy diffuse reaction occurred over the loose connective tissue in a variety of locations (Fig. 3). A similar phenomenon had previously been observed after [^{3}H]fucose injection (6). In several instances, the amount of label present appeared too great to be accounted for by local synthesis and may possibly have been in part due to labeled plasma proteins manufactured by liver hepatocytes and transported to these locations by the blood.

MIGRATION TO THE PLASMA MEMBRANE: In many cell types, the localized reaction initially observed over the Golgi region was replaced by reaction over the cell surface at later time

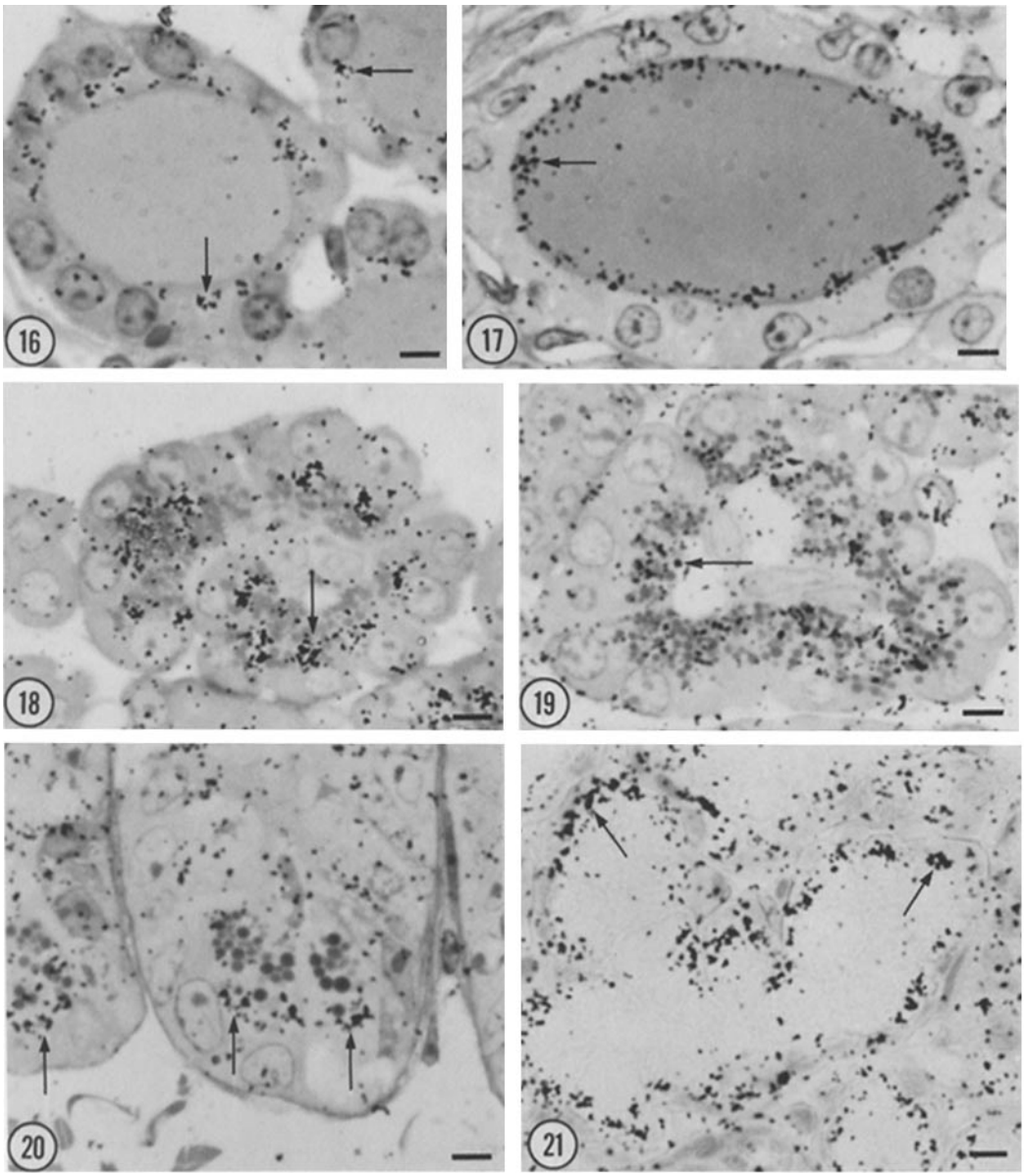


FIGURES 14 and 15 Electron microscope radioautographs of kidney proximal convoluted tubule cells from animals injected with [^{3}H] *N*-acetylmannosamine. Stained with uranyl acetate and lead citrate.

FIGURE 14 Proximal tubule cell from a mouse sacrificed 10 min after injection. Exposed 4 mo. In this particular cell, nearly all of the silver grains are associated with the two Golgi stacks (*G*). The luminal microvillous border (*mv*) is unlabeled. × 12,000. Bar, 1 μm.

FIGURE 15 Proximal tubule cell from a rat sacrificed 24 h after injection. Exposed 8 mo. A substantial reaction now occurs over the microvillous border (*mv*). In addition, numerous silver grains are associated with lysosomes (*L*). One grain is found over a lateral membrane (*lm*). × 12,200. Bar, 0.5 μm.

intervals. This phenomenon was particularly evident in cell types usually considered as nonsecretory, such as duodenal villus columnar cells (Fig. 7) as well as kidney proximal and distal tubule cells (Fig. 15). This migration of labeled glycoproteins to the plasma membrane was similar to that previously observed in the same cell types after injection of [^{3}H]fucose (3, 6, 10, 11, 14). It is likely that the cell surface labeling represents membrane glycoproteins which have been demonstrated by



FIGURES 16 and 17 Light microscope radioautographs of Epon sections of thyroid tissue from animals injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5 μm.

FIGURE 16 Thyroid follicles from a mouse sacrificed 10 min after injection. Exposed 10 mo. The follicular cells exhibit a reaction in which the silver grains are localized to form paranuclear grain clusters (arrows).

FIGURE 17 Thyroid follicle from a rat sacrificed 4 h after injection. Exposed 2 mo. Almost no silver grains are seen over the follicular cells, but a heavy reaction occurs over the peripheral portion of the luminal colloid (arrow).

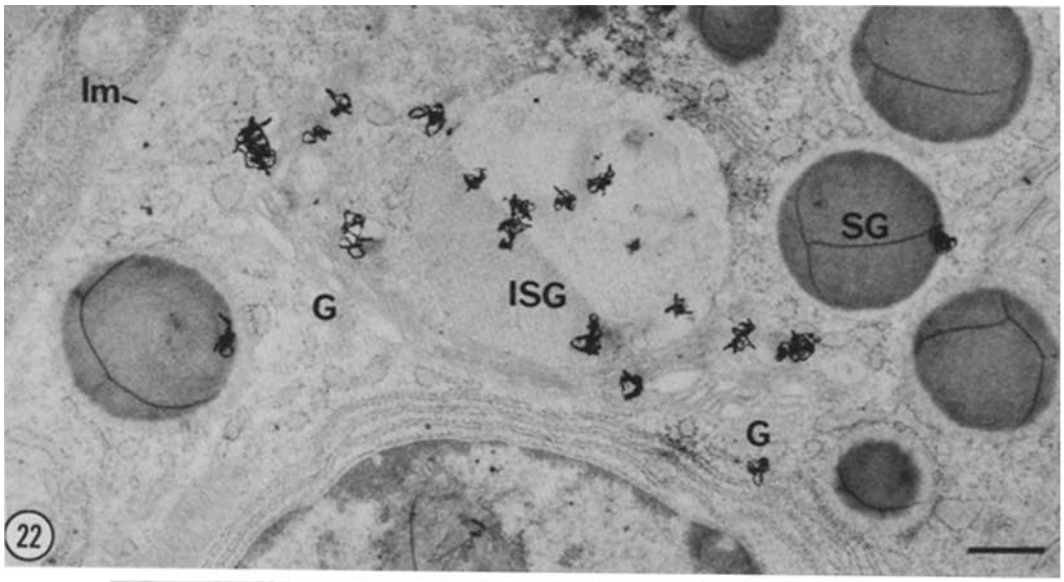
FIGURES 18 and 19 Light microscope radioautographs of Epon sections of pancreatic acini from rats injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5 μm.

FIGURE 18 10 min after injection. Exposed 6 mo. The acinar cells exhibit a fairly heavy reaction in which the grains are localized to a supranuclear (Golgi) region (arrow).

FIGURE 19 24 h after injection. Exposed 3 mo. The supranuclear region of the acinar cells is now unlabeled, and the reaction is located over the zymogen granules in the apical half of the cytoplasm (arrow).

FIGURE 20 Light microscope radioautograph of an Epon section of crypts of Lieberkuhn from a mouse sacrificed 10 min after injection of [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. Exposed 5 mo. The Paneth cells at the base of the crypts exhibit a fairly strong reaction in which the silver grains are localized to the supranuclear region (arrows). The secretion granules in the apical portion of the cytoplasm are unlabeled. × 1,000. Bar, 5 μm.

FIGURE 21 Light microscope radioautograph of a paraffin section of a sublingual gland mucous acinus from a rat sacrificed 10 min after injection of [^{3}H]*N*-acetylmannosamine. Exposed 6 mo. The acinar cells exhibit a heavy reaction in which the silver grains are localized to the supranuclear (Golgi) region (arrows). No reaction occurs over the mucus-filled apical cytoplasm. × 900. Bar, 5 μm.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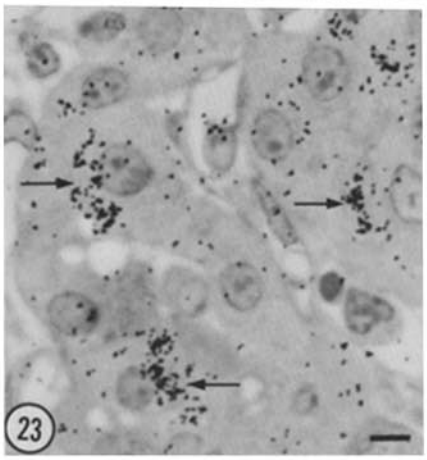
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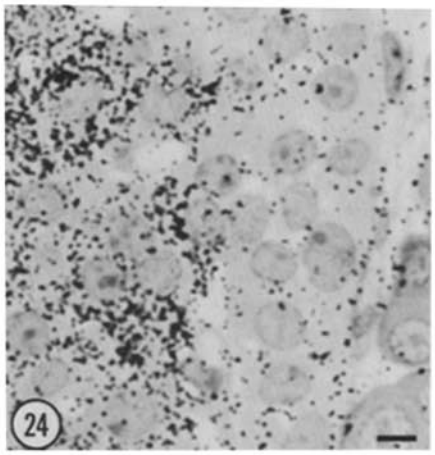
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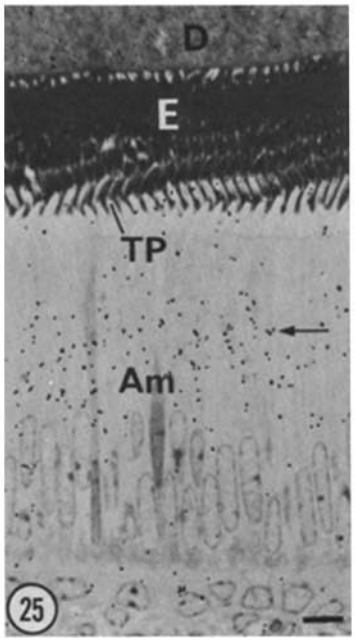
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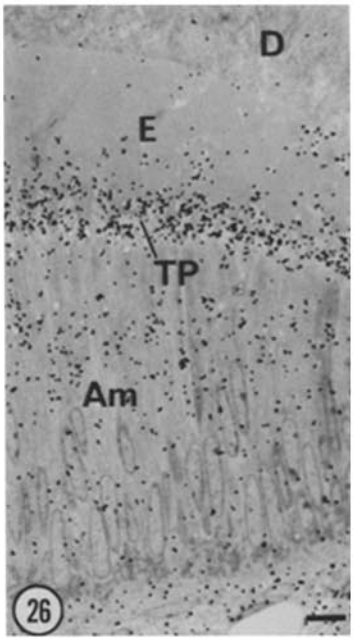
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histochemical and biochemical techniques in the plasma membrane of virtually all cell types (5).

In the case of duodenal villus columnar cells, the proportion of silver grains observed over the lateral plasma membrane after [^{3}H]*N*-acetylmannosamine injection was relatively less than that observed after [^{3}H]fucose injection, i.e., at 4 h after [^{3}H]*N*-acetylmannosamine injection, only 15% of the total grains occurred over the lateral plasma membrane compared to 46% over the microvillus border (Table II), whereas at 4 h after [^{3}H]fucose injection, 35% of the total grains occurred over the lateral plasma membrane compared to 33% over the microvillous border (Fig. 38 of reference 6). These results suggest that different populations of glycoproteins migrate from the Golgi apparatus to lateral and apical regions of the plasma membrane.

Migration of label to the plasma membrane was also observed in some secretory cells, i.e., hepatocytes, as described in the previous paper (9). Such plasma membrane reaction could be visualized in hepatocytes, since the secretion products of this cell are released into the blood and are thus rapidly carried away from the cell. The prewash with lactated Ringer's solution before perfusion fixation would have removed the blood from the circulatory system; thus radioautographic reaction occurring over the plasma membrane could be attributed to labeled plasma membrane molecules (5). In the case of many other secretory cell types, the interpretation of reaction at the cell surface was more difficult. Thus, at cell surfaces where the secretion product of a cell accumulated, plasma membrane reaction could not easily be differentiated from reaction over the adjacent secretion product, i.e., the apical surfaces of thyroid follicular cells (Fig. 17), ameloblasts (Fig. 26), odontoblasts or osteoblasts, and the entire surface of chondroblasts and fibroblasts. Similarly, at secretory cell surfaces which bordered on connective tissue, it was difficult to differentiate plasma membrane reaction from reaction over components of adjacent connective tissue or basal lamina, i.e., the basal surfaces of thyroid follicular cells (Fig. 17), pancreatic acinar cells (Fig. 19) and many others. Finally, in intestinal goblet cells (Fig. 6), lateral membrane reaction could not be differentiated from reaction over the adjacent lateral membrane of columnar cells.

MIGRATION TO LYSOSOMES: In most of the cell types examined in the electron microscope, some label appeared at later time intervals in lysosome-like dense bodies. In tissues incubated for acid phosphatase activity (as described in the preceding article [9]) these bodies stained positively, indicating their lysosomal nature. Such labeling of lysosomes in hepatocytes and Kupffer cells was described in the preceding paper (9). In duodenal villous columnar cells, many lysosomal bodies were labeled at 4 h after injection (Fig. 7) although they accounted for only 2% of the total grains. This situation was similar to that observed in the same cell type after [^{3}H]fucose injection (4, 10). In kidney proximal and distal tubule cells, lysosomes also became highly labeled (Fig. 15), a phenomenon previously observed after [^{3}H]fucose injection (11). As discussed in the preceding article, label observed in lysosomes at late time intervals after [^{3}H]*N*-acetylmannosamine may have migrated directly from the Golgi apparatus or may have come from the plasma membrane or material from outside of the cells. Such label could represent lysosomal hydrolases or glycoproteins of the lysosomal limiting membrane (9). Ginsel et al. (10), on the other hand, have suggested that the label in the lysosomal bodies in intestinal columnar cells may represent cell-coat glycoproteins, with the lysosomes playing a crinophagic role in the regulation of transport or secretion of cell-coat material.

Comparison of Radioautographic Reactions Observed after [^{3}H]N-acetylmannosamine Administration with Those Previously Observed after [^{3}H]Fucose Administration

As seen in Table I, the pattern of incorporation of label observed in the present study after [^{3}H]*N*-acetylmannosamine injection into young rats was similar in many respects to that previously observed after [^{3}H]fucose injection (6). It may be seen however, that the reactions observed after [^{3}H]*N*-acetylmannosamine (sp act: 2.2 Ci/mmol) injection were much weaker than those observed after injection of an equal dose of [^{3}H]fucose (sp act: 4.3 Ci/mmol), i.e., in Table I, the radioautographs of animals injected with [^{3}H]*N*-acetylmannosamine were exposed for 6 mo, whereas those of animals injected with

FIGURE 22 Electron microscope radioautograph of the supranuclear region of a Paneth cell from a mouse sacrificed 10 min after [^{3}H]*N*-acetylmannosamine injection. Stained with uranyl acetate and lead citrate. Exposed 8 mo. Several of the silver grains are associated with the Golgi stack (*G*), while many others overlie an immature secretion granule (*ISG*). Two grains are seen over the edges of mature secretion granules (*SG*). (*lm*, lateral plasma membrane). × 20,000. Bar, 0.5 μm.

FIGURES 23 and 24 Light microscope radioautographs of Epon sections of pancreatic islet of Langerhans tissue from rats injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5 μm.

FIGURE 23 10 min after injection. Exposed 6 mo. Some of the islet cells exhibit heavy reactions in which the silver grains are localized to a large paranuclear Golgi region (arrows); the remainder of the cytoplasm is unlabeled. Other islet cells in this photograph exhibit no reaction.

FIGURE 24 24 h after injection. Exposed 3 mo. Central islet cells (to the left of the figure) exhibit a heavy diffuse reaction over their cytoplasm, but those at the periphery (to the right) are much less labeled.

FIGURES 25 and 26 Light microscope radioautographs of Epon sections of ameloblasts from the teeth of rats injected with [^{3}H]*N*-acetylmannosamine. Stained with iron hematoxylin. × 1,000. Bar, 5 μm.

FIGURE 25 30 min after injection. Exposed for 3 mo. The ameloblasts (*Am*) exhibit a light reaction in which most of the silver grains are localized to the elongated supranuclear region which contains the Golgi apparatus (arrow). A few grains also occur over the distal Tomes' processes (*TP*). No reaction is seen over the enamel (*E*) or the dentin (*D*) at the top of the figure.

FIGURE 26 4 h after injection. Exposed for 3 mo. Reaction remains over the supranuclear region of the ameloblasts (Am), but now in addition a heavy band of reaction occurs over the Tomes' processes (*TP*). Some grains may be associated with the adjacent enamel (*E*). No reaction is seen over the dentin (*D*).

[^{3}H]fucose were exposed for only 1 mo. At 2 min after [^{3}H]*N*-acetylmannosamine injection, the radioautographic reactions were extremely weak and only appeared in paraffin sections after long exposure times.

The relative intensity of reaction over different cell types after [^{3}H]*N*-acetylmannosamine injection also differed from that observed after [^{3}H]fucose injection (Table I). Thus, in many simple epithelial cells, and all stratified epithelial cells, no significant reaction was observed after [^{3}H]*N*-acetylmannosamine injection, whereas substantial reactions had appeared in these cell types after [^{3}H]fucose injection. In secretory cells the differences were not as striking but in chondroblasts, no reaction was seen after [^{3}H]-*N*-acetylmannosamine while a fairly heavy reaction had occurred after [^{3}H]fucose. Only in occasional cell types did the reverse pattern occur, i.e., in some kidney proximal tubule cells and in fibrocartilage cells, a heavier reaction (relative to that seen over duodenal villous columnar cells) was observed after [^{3}H]*N*-acetylmannosamine than after [^{3}H]fucose. Similarly in osteoclasts the reaction observed after [^{3}H]*N*-acetylmannosamine was more intense than that seen after [^{3}H]fucose.

Finally, in at least one cell type the intracellular distribution of reaction after [^{3}H]*N*-acetylmannosamine injection differed from that after [^{3}H]fucose injection. This was discussed earlier in this article in the case of duodenal villous columnar cells, in which the reaction observed over the lateral plasma membrane at 4 h after [^{3}H]*N*-acetylmannosamine injection was relatively much weaker than that observed at 4 h after [^{3}H]-fucose injection.

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