DETECTION OF COMPLEX CARBOHYDRATES IN THE GOLGI APPARATUS OF RAT CELLS

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ABSTRACT

Two methods used for the electron microscopic detection of glycoproteins were applied to a variety of cell types in the rat; one involved successive treatment of sections with periodic acid, chromic acid, and silver methenamine; and the other, a brief treatment with a chromic acid–phosphotungstic acid mixture. The results obtained with the two methods were identical and, whenever the comparison was possible, similar to those obtained with the periodic acid–Schiff technique of light microscopy. In secretory as well as in nonsecretory cells, parts of the Golgi apparatus are stained. The last saccule on one side of each Golgi stack is strongly reactive (mature face), and the last saccule on the other side shows little or no reactivity (immature face); a gradient of reactivity occurs in between these saccules. The more likely explanation of the increase in staining intensity is that carbohydrate is synthesized and accumulates in saccules as they migrate toward the mature face. In many secretory cells, the mature face is associated with strongly stained secretory granules. Other structures stained are: (*1*) small vesicles, dense and multivesicular bodies, at least some of which are presumed to be lysosomal in nature; (*2*) cell coat; and (*3*) basement membrane. The evidence suggests that the Golgi saccules provide glycoproteins not only for secretion, but also for the needs of the lysosomal system as well as for incorporation into the cell coat and perhaps basement membrane.

INTRODUCTION

Those carbohydrate macromolecules which are oxidized by periodic acid (PA) may be detected in histological sections by the PA-Schiff technique. Most of the authors who have used this technique agree that the reactive carbohydrates are widely distributed in organs and tissues and are particularly abundant in secretory material (1, 2). Reactions have been reported to occur in the Golgi region of many cells, particularly in kidney (3), intestinal epithelium (1, 4–7), thyroid (8), and pituitary (9) as well as in spermatids (1, 2, 10). However, in most of these cells, the localization of reactive carbohydrate within the Golgi region lacked precision.

It became important, therefore, to locate the sites of periodic acid–reactive carbohydrates in the electron microscope. Alkaline silver solutions may replace the Schiff reagent as an electron stain so as to detect the aldehydic groups produced by periodic acid oxidation (11–20). Applying such a technique to formalin-fixed thyroids, van Heyningen in this Department observed that the follicular colloid and several types of cytoplasmic globules which were stained with the Schiff reagent in the light microscope were also stained with silver methenamine in the electron microscope (21).

Later, the technique with some improvements

(22) was applied to a study of the carbohydrate coat of cells. In the course of this work, a stained Golgi apparatus was occasionally seen (23). Thiéry stained this organelle in goblet cells by using a thiosemicarbazide-silver proteinate technique (24). Other authors utilized colloidal iron or thorium to detect acidic carbohydrates and found Golgi reactions again in goblet cells (25) as well as in columnar cells of intestine (26) and in chondrocytes (27). Finally, Pease, using phosphotungstic acid (28), came to the conclusion that "in intestinal goblet cells, Golgi vacuoles show a specific reaction, contrary to what is usually observed . . . in cells of other types." The question then arose whether the presence of carbohydrate reactions in the Golgi apparatus

FIGURES 1–5 *Small intestine of the rat.* PA-CrA-silver (except Fig. 4, taken from a PA-silver–stained section).

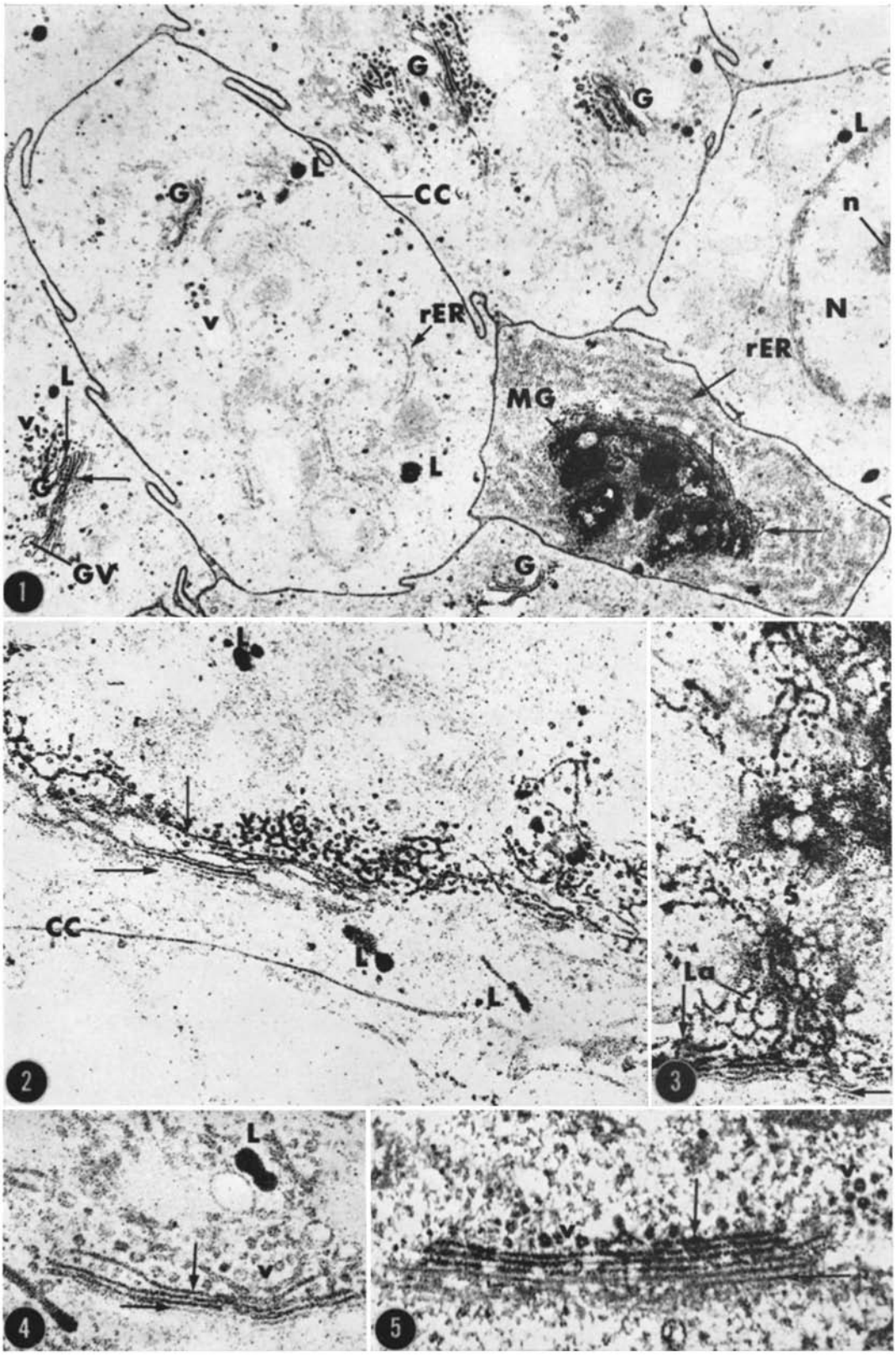
FIGURE 1 Cross-section through the Golgi region of columnar cells lining a duodenal villus. Several columnar cells and a heavily stained goblet cell (lower right) are separated by a sharp line (*CC*), wavy in places. This line is attributed to the fusion of the stained "cell coats" found on the outer surface of adjacent cells. The columnar cell at right shows the nonspecifically stained nucleus (*N*) and nucleolus (*n*). Within some of the columnar cells one or several stained Golgi stacks (*G*) may be seen. In most of the stacks, it is possible to identify an immature face with saccules that are little or not stained (horizontal arrow in the cell at lower left) and a mature face with saccules that are strongly stained and are associated with vesicles (vertical arrow). At the edge of the saccules, the Golgi vacuoles are outlined by a stained line (*GV*). Finally, the columnar cells contain a few intensely stained dense bodies, presumed to be lysosomal in nature (*L*) as well as stained small vesicles (*v*). In contrast, the cisterns of rough endoplasmic reticulum (*rER*) show only a slight nonspecific staining of the ribosomes; their content is unreactive. In the goblet cell at lower right, the group of mucous globules is intensely stained (*MG*). On one side, the stained Golgi saccules are distinguishable, with the immature face at the horizontal arrow and the intensely stained mature face at the vertical arrow. A special feature of this cell type is the presence of a weakly stained content in the cisterns of rER. × 18,000.

FIGURE 2 Golgi region of a columnar cell of the duodenal epithelium. Starting from the base, the picture shows the cell coat (*CC*), dense bodies (*L*), and the prominent Golgi apparatus. In this and many of the subsequent micrographs, a horizontal arrow indicates the little or not stained saccules of the immature face of the Golgi apparatus; there are more and more intensely stained saccules up to the mature face, which is indicated here and elsewhere by a vertical arrow. At the right of this arrow, the obliquely cut edges of the saccules show a lacy arrangement, within which intensely stained, small vesicles appear (*v*). × 24,000.

FIGURE 3 Section which in places is parallel to the surface of a Golgi stack. Same preparation as Fig. 2. At the base of the figure, a Golgi stack with a staining gradient is seen in cross-section. Above, the saccules of the mature face run parallel to the plane of the section and show solid staining in places (*S*). The passage from solid staining to a lacy arrangement (*La*) is interpreted as a transformation of the edges of reactive saccules into anastomosing branches. × 30,000.

FIGURE 4 Cross-section of a Golgi stack in a duodenal columnar cell. PA-silver. There is a gradation from the barely stained saccules of the immature face (horizontal arrow) to the stained ones of the mature face. The vesicles (*v*) seen next to the mature face (vertical arrow) have an unstained core. × 30,000.

FIGURE 5 Cross-section of a Golgi stack, from the same material as Figs. 2 and 3, but counterstained with uranyl and lead. The Golgi saccules of the immature face are not stained with silver, but they are shown by the counterstain. On the mature face (vertical arrow), silver stains material present within the lumen of the saccules as well as the vesicles (*v*), whose core remains unstained. × 33,000.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was restricted to certain cell types or was a general phenomenon. Furthermore, since there were nonspecific reactions in which chromatin and the ribosomes of rough endoplasmic reticulum took up silver in the absence of periodic acid treatment (21, 22), light Golgi reactions might not be distinguished from the stained rough endoplasmic reticulum. An attempt was then made to eliminate these nonspecific reactions.

Mowry, working with the light microscope, had shown that the insertion of a chromic acid (CrA) step between periodic acid oxidation and silver methenamine improved results (29). When we applied this technique to the electron microscope the nonspecific staining of ribosomes and nuclei was greatly diminished (30). This periodic acid–chromic acid-silver (PA-CrA–silver) technique was, therefore, applied to a study of the Golgi apparatus in a number of cell types.

Meanwhile Marinozzi (31) and Pease (28) had shown that phosphotungstic acid may stain carbohydrates in sections of tissues embedded in hydrophilic resins. Marinozzi's technique was modified and used at pH 0.3 instead of pH 2.7, and a substantial increase in contrast and specificity attained (32). The low pH was obtained by addition of hydrochloric or chromic acid. The chromic acid–phosphotungstic acid (chromic acid–PTA) technique was also used to investigate the Golgi apparatus in many cell types.

METHODS

Tissues obtained from young or adult rats were fixed by immersion in or perfusion with a 2.5% glutaraldehyde solution containing 0.1 M Sörensen or Millonig buffer.

For the straight *PA-silver technique* used at the outset of this investigation (Figs. 4, 9), tissues were embedded in Epon and the sections, which were rinsed after each step, were successively floated over solutions of periodic acid, silver methenamine, and sodium thiosulfate; then the sections were mounted on formvar-coated grids as described in detail elsewhere (22). Control sections were floated over silver methenamine without prior passage over periodic acid.

The *PA-CrA–silver technique* (Figs. 1–3, 5–8, 10–13) differed in four ways from PA-silver: Vestopal was used as embedding medium instead of Epon; the sections were mounted on uncoated stainless steel grids immediately after sectioning and then dipped in periodic acid; after periodic acid treatment, the grids were taken through chromic acid before the silver methenamine treatment; and the final thiosulfate

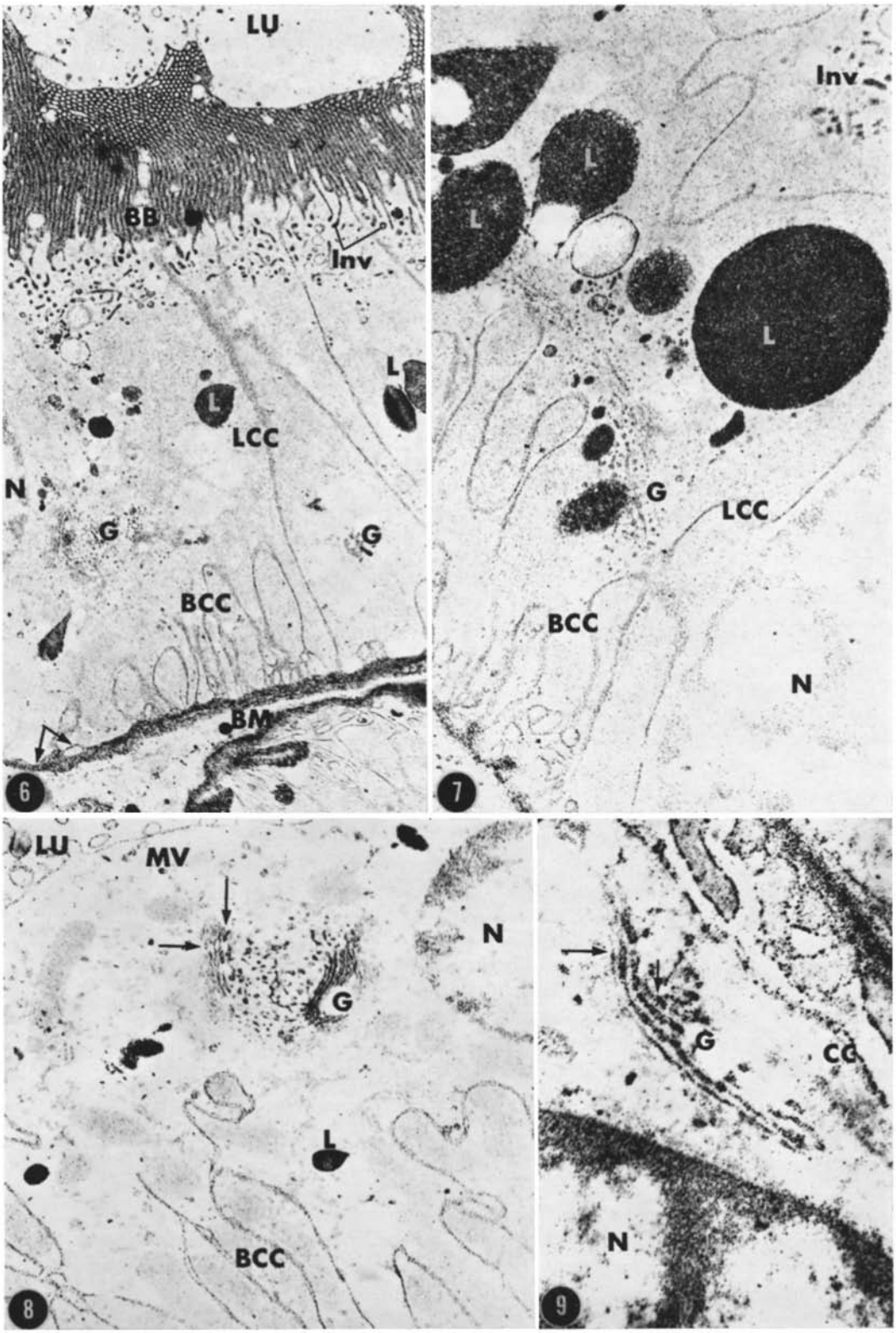
FIGURES 6–9 *Kidney.* PA-CrA–silver (except Fig. 9, taken from a PA-silver–stained section). In some of the figures, the immature face of the Golgi apparatus is indicated by a horizontal arrow, whereas the mature face is indicated by a vertical arrow.

FIGURE 6 Proximal convoluted tubule. Next to the lumen (*LU*) the microvilli of the brush border (*BB*) have an intensely stained coating. Cell coat material also lines the surface of the pitlike invaginations of the apical membrane (*Inv*), the lateral cell surface (*LCC*) and the basal infoldings of the plasma membrane (*BCC*). Finally, the basement membrane is stained (*BM*). The two arrows at lower left point to places where the basal cell coat is a thin line distinguishable from the basement membrane. Elsewhere, the two structures are not clearly separated. Within the cell, the edge of the nonspecifically stained nucleus may be distinguished (*N*) and nearby, a specifically stained Golgi region (*G*) and dense bodies (*L*). × 10,000.

FIGURE 7 Proximal convoluted tubule. The stack of Golgi saccules curves across the figure in association with small vesicles and dense bodies. Some of the latter reach a particularly large size. (Labels as in Fig. 6). × 20,000.

FIGURE 8 Distal convoluted tubule. The Golgi apparatus is surrounded by the nonspecifically stained nucleus at right (*N*), the lumen at top (*LU*) with the stained coat of the stubby microvilli (*MV*), and the basal infoldings also with a stained coat (*BCC*). The immature face of the Golgi apparatus (*G*) is on its convex surface and the mature face, on its concave surface, where stained vesicles are also present. × 20,000.

FIGURE 9 Podocyte, stained with PA-silver. This section which was prepared without the chromic acid step shows an intense but nonspecific staining of the nucleus (*N*). There is specific staining of cell coat (*CC*) and Golgi apparatus (*G*). × 36,000.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rinse was omitted. Thus, the grids passed successively through periodic acid (20 min), water (30 min), 10% chromic acid (5 min), 1% sodium bisulfite (1 min), water (30 min), then silver methenamine at 60°C (30 min) and water again, before being dried for examination in a Siemens Elmiskop II. The intensity of staining could be modified by the method of varying the duration of treatment with silver methenamine from 25 to 40 min. In a few cases, the grids carrying stained sections were floated on 1% osmium tetroxide for 10 min and counterstained with uranyl and lead salts for 15 min each. This technique, which has not yet been thoroughly investigated, frequently obscured weak silver reactions, and was only illustrated in one figure (Fig. 5).

The *chromic acid–PTA technique* (Figs. 14–29) made use of tissues embedded in glycol-methacrylate (33). The sections were floated for two min on a solution containing 1% phosphotungstic acid in 10% chromic acid, briefly rinsed on distilled water, and mounted on formvar-coated grids (32).

RESULTS

PA-CrA–Silver (and PA-Silver)

Cell nuclei are stained in control sections taken only through silver methenamine and, therefore, nuclear staining is considered to be nonspecific. After pretreatment with periodic acid in the PA-silver technique, the nonspecific staining of nuclei by the silver solution persists (Fig. 9). When both periodic acid and chromic acid are used in the PA-CrA–silver technique, the nuclear staining is markedly reduced (Figs. 6, 8, *N*).

In the *intestinal epithelium*, reactivity of the Golgi apparatus of columnar and goblet cells was observed in sections stained with PA-CrA–silver (Fig. 1) as well as with PA-silver (Fig. 4), but not in control sections stained with silver methenamine alone. The Golgi stacks of columnar cells appear asymmetric because of a gradient in their staining reaction (Figs. 1, 2, 4). The saccules on one side of the stack are unstained, and those on the other side are intensely stained. In this and other cell types, the unstained side will be called the "immature face" and is indicated in the photographs by horizontal arrows, whereas the intensely stained side will be called the "mature face" and is indicated by vertical arrows. The difference is particularly clear after counterstaining with uranyl acetate and lead (Fig. 5), when the saccules of the immature face have few or no silver grains in their lumen, while those of the mature face contain much silver. The intermediate saccules show a gradual or fairly sudden increase in staining intensity. Furthermore, at the mature face, stained vesicles with a diameter of about 0.06 μ have an unstained core (Figs. 4, 5). A few such vesicles are scattered throughout the cytoplasm (Fig. 1) and sometimes reach the cell surface, where they appear to contact the cell membrane.

The last saccule on the mature face, when examined in sections cut parallel or slightly oblique, appears solid in the center and fenestrated at the periphery (Fig. 3, *S*), where thin branches spread out to form a lacy pattern (Fig. 3, *La*). Stained vesicles are often seen within these branches (Fig. 2).

In addition to Golgi saccules and vesicles, other bodies, which are spherical or oval, stain deeply (Figs. 1, 2, 4, *L*). While these bodies are often associated with the Golgi region (Fig. 4), they may be found elsewhere in the cytoplasm. They will be referred to as "dense bodies." Furthermore, stained vesicles may be seen within multivesicular bodies, the matrix of which may be stained or not. No staining is observed in the cisterns of rough endoplasmic reticulum (Fig. 1). Finally, sharp reactive lines are found between cells (Fig. 1). These lines have been attributed to the fusion of the carbohydrate-rich cell coats of adjacent cells (34).

At the right of Fig. 1, an intensely stained group of mucigen globules appears within a goblet cell. The group of globules is associated with stacks of stained Golgi saccules (Fig. 1). It is usually possible to distinguish an immature face which is located on the convex side of the stack and where saccules are weakly stained and a mature face which is on the concave side and where saccules are intensely stained. Around the Golgi region of this cell, the cisterns of rough endoplasmic reticulum contain weakly stained material—an uncommon feature.

In the *kidney*, the cells of the proximal convoluted tubules (Figs. 6, 7) show a staining of Golgi stacks. These stacks are associated with stained vesicles and sometimes dense bodies (which in these cells may reach a particularly large size; Fig. 7). Cell coat material occurs at the surface of microvilli, on the lining of the pit-like invaginations observed between the bases of the microvilli, on the lateral plasma membranes, and on the infoldings of the basal plasma membrane (Fig. 6).

In the cells of distal convoluted tubules (Fig. 8) the Golgi saccules are arranged in a semicircle, with the mature face on the concave surface and the immature face on the convex surface of the stack. Stained saccules are also seen in other kidney cells, for instance, in the podocytes of the glomerular tuft (Fig. 9). It may be seen in this figure that the staining of a Golgi saccule may vary along its length (horizontal arrow).

In the *testis*, staining of the Golgi apparatus has been observed in interstitial cells as well as in the cells of seminiferous tubules. Spermatocytes have a roughly spherical Golgi region composed of several Golgi stacks (Fig. 10, *G*). Their mature face is oriented toward the center of the Golgi region and the immature face, toward the outside. The immature face is weakly stained and is associated with a hazily stained area, which may be seen below the horizontal arrow at center left in Fig. 10. The Golgi region of spermatids (Fig. 11) seems to contain a single stack of saccules, with a hazy staining next to the immature face which itself is weakly stained. Stained vesicles with an unstained core are next to the mature face (Fig. 11). These vesicles have been seen fusing with the intensely stained headcap (*HC*). Similar vesicles are thinly scattered in the cytoplasm and are often close to the cell membrane (Fig. 11, *v*). In younger spermatids, the acrosomic granules—the precursors of the headcap (10)—are also intensely stained. These structures seem to arise from fusion of vesicles at the mature face.

In the *thyroid gland* (Fig. 12) the Golgi apparatus is less sharply defined than in the intestine and testis. The mature face displays stained vesicles and dense bodies, some of which are similar to the stained "apical" vesicles found at the cell apex along the colloid border (Fig. 12, *AV* and reference 24). Colloid droplets, when present, are also stained (21).

The acinar cell of the *pancreas* (Fig. 13) contains Golgi stacks with a gradient of staining intensity. Facing the mature face are small vesicles with an unstained core and zymogen granules which are outlined by a reactive shell. However, the zymogen granules located at a distance from the Golgi region do not have such a reactive shell. It may be presumed, therefore, that this reaction characterizes zymogen granules undergoing maturation.

Chromic Acid–PTA Technique

When this technique was compared with the PA-CrA–silver technique, the texture of the reactions differed but the results were essentially similar. The only notable exceptions were glycogen and, to a lesser extent, mucigen granules of goblet cells which were retained and stained with PA-CrA–silver but absent with the chromic acid–PTA technique. These components were extracted during preparation of the glycol methacrylate–embedded material. With the chromic acid–PTA technique, high magnifications may often be reached without loss of clarity (e.g. 100,000 in Fig. 15).

In the *intestinal epithelium* and *thyroid follicular cells*, which are not depicted with this technique, the saccules of the Golgi apparatus exhibit the same differences in reactivity from the immature face to the mature face as with the PA-CrA–silver technique. In the follicular cells of the thyroid, apical vesicles, dense bodies and the colloid again react intensely.

The Golgi stacks of acinar cells in the *pancreas* are reactive. Unstained saccules are at the immature face (labeled *4* in Fig. 15) and a reaction appears gradually as shown by saccules labeled *3*, *2*, and *1*. As with the PA-CrA–silver techniques, there is a reaction on the rim of the zymogen granules in the Golgi region; and dense bodies as well as small vesicles are stained (Fig. 14).

The cells of the *anterior pituitary* show a Golgi reaction, with stained granules often associated with the mature face (Fig. 16).

In the cells of *kidney* tubules and glomerulus, it is apparent that the chromic acid-PTA technique stains not only the Golgi stacks (Figs. 17–19) but also dense bodies and vesicles as well as cell coat and basement membrane, thus again emphasizing the same structures as the PA-CrA–silver technique (Fig. 7 vs. 17).

Particularly prominent are the Golgi reactions in the endothelial cells of *capillaries*, as illustrated in Figs. 22 and 23, in which the mature face is seen to be close to the nucleus. Stained vesicles (*v*) seem to arise from the last saccule on this face (Fig. 23).

Golgi reactions may be seen in a wide variety of other cells, either in the form of discrete reactions as in blood eosinophils (Fig. 20) and lymphocytes (Fig. 21), or in a horseshoe arrangement as in reticular cells (Fig. 24) and spermatids (Fig. 26), or as rather large flat stacks, as in nerve

cells (Fig. 25), pancreatic intercalated duct cells (Fig. 27), rod cells of the retina (Fig. 28) and epididymal epithelium (Fig. 29). The last two pictures show multivesicular bodies containing vesicles with an unstained core. The matrix of the bodies also seems to be stained.

A list of the cells in which a Golgi reaction was observed with either the PA-CrA–silver technique or the chromic acid–PTA technique or both (Table I) reveals the widespread distribution of this reaction in rat cells.

DISCUSSION

Validity of the Technique

The *PA-Schiff technique* detects 1,2-glycol and alpha-amino-alcohol groups. In theory, these groups might occur in many substances, but in vitro tests were usually negative with proteins and mucopolysaccharides and strongly positive with glycogen and glycoproteins (35). Indeed, extracts from a number of PA-Schiff–reactive sites contained glycoproteins; their treatment with periodic acid eliminated glucose, fucose, as well as most galactose residues (36). It was concluded that, after glycogen had been extracted by alpha-amylase from a tissue section, the periodic acid–Schiff technique was specific for glycoproteins (36, 37).

When the *PA-silver technique* was used by various authors (11–20) to examine the distribution of periodic acid–reactive carbohydrates in the electron microscope, the results were not as satisfactory as one might wish (38), partly because the widespread use of osmium tetroxide for fixation interfered with the specificity of the method and produced a heavy silver background. After formalin (21) and especially glutaraldehyde fixation (22, 34), the results improved. The distribution of the reactions was, for the most part, similar to

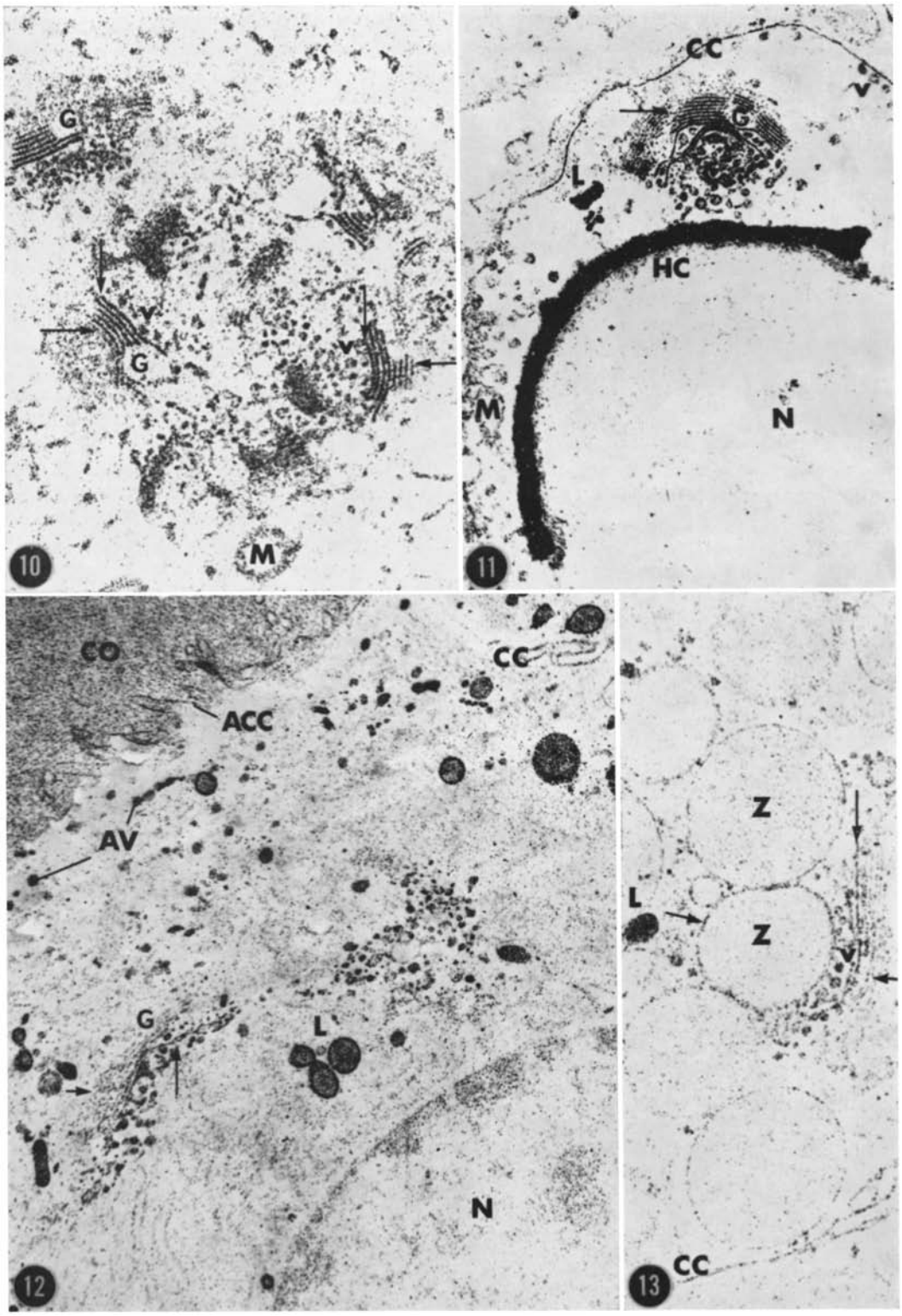
FIGURES 10–13 *Various epithelia.* PA-CrA–silver. The immature face of the Golgi apparatus is indicated by a horizontal arrow and the mature face by a vertical arrow.

FIGURE 10 Golgi region of spermatocyte, seminiferous epithelium. Several Golgi stacks (*G*) are arranged in a group occupying most of the picture. The individual stacks have an immature face oriented outside the group and a mature face oriented inwards. Vesicles (*v*) are seen at the mature face. A diffuse greying is seen outside the immature face (as shown under the horizontal arrow at center left). At *M*, a mitochondrion with weak staining of the cristae is observed. (The cristae are located at the periphery of these mitochondria.) × 30,000.

FIGURE 11 Golgi region of spermatid and headcap, seminiferous epithelium. From the top down, it is possible to see the cell coat (*CC*), a diffuse greying (region under *CC*), the convex immature face, the neatly stacked saccules with maximum staining at the concave mature face, stained vesicles, and the intensely stained headcap (*HC*). At *M*, a weakly stained mitochondrion. At upper right, a stained vesicle (*v*) is located against the cell membrane. × 20,000.

FIGURE 12 Follicular cell of thyroid gland. Most of the field is occupied by a follicular cell, with a Golgi stack at lower left (*G*). At upper left, the colloid (*CO*) is stained uniformly. The limit between cell and colloid, with its microvilli, is indicated by a line staining more intensely than the colloid itself; it is the apical cell coat (*ACC*). At upper right, part of the lateral cell coat (*CC*) is visible. At lower right, the nonspecifically stained nucleus (*N*) is seen. The Golgi apparatus shows the weakly stained immature face and the more reactive mature face with many small stained bodies. Some of these bodies are similar to the apical vesicles (*AV*) found at the cell apex next to the colloid. A few dense bodies (*L*) are scattered in the cell. × 20,000.

FIGURE 13 Acinar cell of pancreas. At right, the Golgi apparatus shows the unstained immature face and the stained mature face. At the mature face, small vesicles with an unstained core (*v*) are seen. In addition, the zymogen granules (*Z*) located close to the Golgi apparatus show a stained rim (oblique arrow). Stained dense bodies (*L*) are also present. × 30,000.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that of the PA-Schiff technique, except that nuclei, nucleoli, ribosomes, and a few other structures were stained nonspecifically by the silver reagent (24), and that glycogen was not stained. Furthermore, when paraffin sections of glutaraldehyde-fixed tissues were acetylated (39) before periodic acid oxidation and silver methenamine treatment, the same inhibition of reactive sites was observed as with PA-Schiff (A. Rambourg and W. Hernandez, unpublished). Similarly, when oxidized sections were treated with chlorous acid to block aldehydic groups (40), staining with methenamine silver or Schiff was abolished (A. Rambourg and W. Hernandez, unpublished).

The *PA-CrA–silver technique* furnished the same over-all pattern as PA-silver, but the nonspecific staining of ribosomes and nuclei was minimized (Figs. 6, 8). This technique also caused glycogen to react intensely, suggesting that chromic acid oxidation of glycogen produced aldehydic groups which reacted with silver methenamine, whereas those groups produced by periodic acid alone did not. In a light microscope comparison, the distribution of the PA-Schiff and PA-CrA–silver reactions was found to be strikingly similar (A. Rambourg and W. Hernandez, unpublished). Even though comparison with the electron microscope was less readily done, it was clear that the structures stained by PA-Schiff, e.g. mucus (1, 2), basement membrane (1, 2), cell coat (41), and Golgi apparatus (1–10), were also stained with PA-CrA–silver, as shown respectively in Figs. 1, 6, 7, and 5, among others. Since the two techniques stained the same structures, it was concluded that PA-CrA–silver, like PA-Schiff, detected carbohydrate macromolecules oxidized by periodic acid, that is, glycogen and glycoproteins.[1]

When the *chromic acid–PTA technique* was compared with the PA-CrA–silver technique, the results were again identical, as may be seen by comparing the preparations of pancreas (Figs. 13 and 14) and kidney tubules (Figs. 7 and 17). The specificity of the chromic acid–PTA technique was examined on sections mounted on carbon-coated grids. These sections were acetylated for 12 hr at 37°C or methylated for 4 hr at 60°C before staining (A. Rambourg, unpublished). Methylation was ineffective, but acetylation abolished the reaction. The reactivity of acetylated sections was restored by a 45-min treatment with 1% potassium hydroxide at room temperature. It was concluded that hydroxyl groups, presumably those of glycoproteins, were involved in the reaction. A further test of this hypothesis was carried out by the addition of a few drops of the chromic acid–phosphotungstic acid reagent to solutions of various substances (alcohols, amino acids, histones, DNA, glycogen, starch, heparin, chondroitin sulfate, orosomucoid . . .) and it was found that only certain alcohols, glycogen, and glycoproteins interacted with phosphotungstic acid at pH 0.3 (A. Rambourg, unpublished).

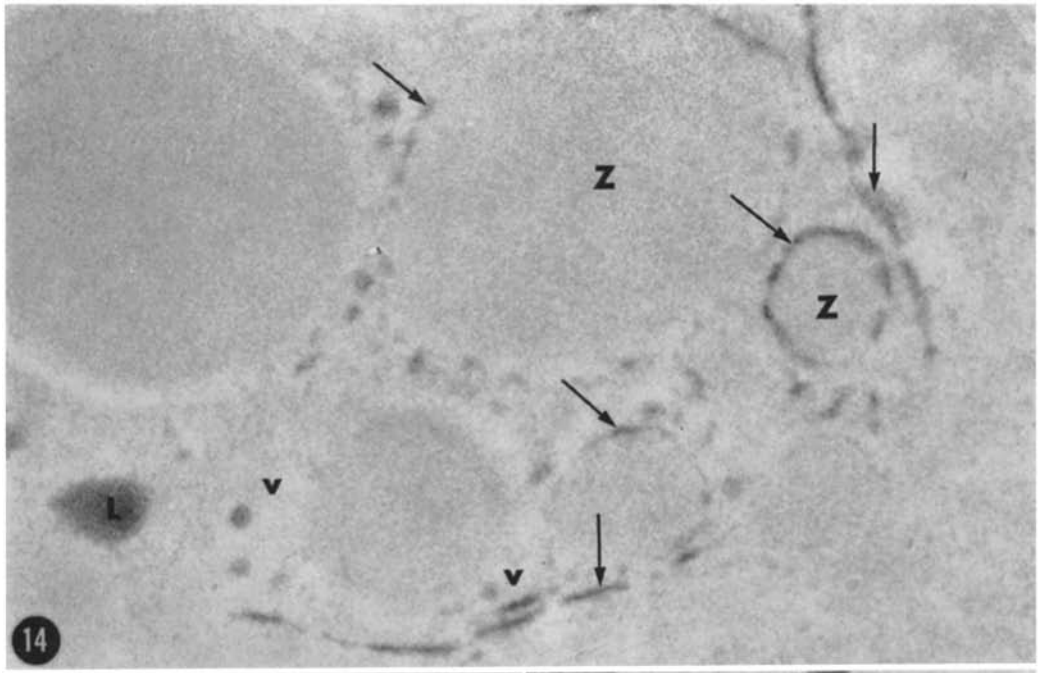
[1] The possibility that PA-Schiff–reactive glycolipids are involved in the Golgi apparatus has to be considered, since there are indications that lipids occur in this organelle (59). However, there is good evidence that lipids are almost completely extracted from glutaraldehyde-fixed tissues by the absolute alcohol and propylene oxide used in processing (60, 61).

FIGURES 14–16 *Glandular epithelia.* Chromic acid-PTA.

FIGURE 14 Acinar cell of pancreas. As in Fig. 13, the Golgi saccules on the mature face (vertical arrow) are filled with stained material. Staining is again seen in small vesicles (*v*) and dense bodies (*L*). The zymogen granules (*Z*) located close to Golgi saccules are rimmed by stained material (oblique arrows). The zymogen granules farther away (top left) do not show this reaction. × 50,000.

FIGURE 15 Acinar cell of pancreas. On the immature face (horizontal arrow), the Golgi saccules labeled *3* and *4* are little or not stained. The saccules labeled *1* and *2* which are next to the mature face are stained. The rough endoplasmic reticulum (*rER*) is unstained. × 100,000.

FIGURE 16 Pituitary gland, anterior lobe. Next to secretion granules of various types (*g*) and small vesicles (*v*), the Golgi saccules at the mature face (vertical arrow) contain stained material, while on the opposite side of the stack those saccules of the immature face (horizontal arrow) do not. × 48,000.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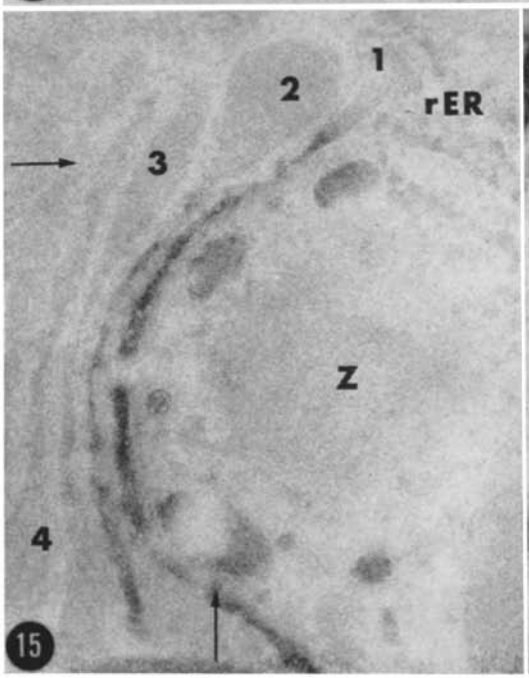
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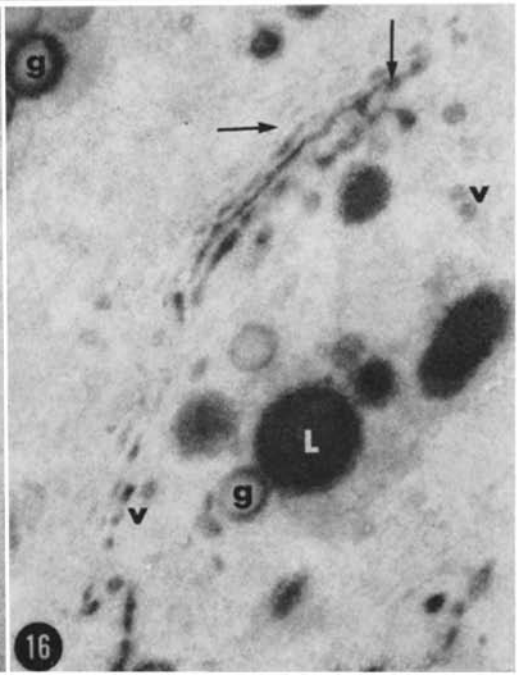
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Since the reactive alcohols tested were of too low molecular weight to be retained during processing and glycogen was known to be lost, the reactive substances of tissue sections should be glycoproteins.

Staining of Golgi Apparatus

The reactivity of the Golgi apparatus was demonstrated not only by the 29 figures of this article, but also by the list of cells in which this organelle was stained (Table I). Presumably, the Golgi apparatus would also be stained in the cells which have not been examined, so that this organelle would contain glycoproteins in all cells of the rat.

On one side of the apparatus, the saccules were strongly stained. It soon became apparent that the stained side was associated with secretion granules (Figs. 13, 14) and stained vesicles (Figs. 4, 23) and corresponded to the side described as "mature face" (42). On the other hand, the unstained side showed no productive activity and corresponded to the area where new saccules are thought to be produced, that is, the "forming" (42) or "immature" face. The saccules that were piled up between the two faces showed a more or less gradual increase in staining intensity as the mature face was approached. Examination of preparations such as Fig. 15 and of counterstained sections such as that illustrated in Fig. 5 revealed that the content of the saccules was stained rather than their membrane. Hence, the saccules contained glycoprotein; and the glycoprotein content increased from the immature to the mature face. A staining gradient of Golgi saccules was also observed in the columnar cells of the small intestine after staining of acid groups with colloidal iron (25, 26), a fact suggesting that the glycoprotein carried acid groups, presumably as sialic acid residues.

Staining of Intracellular Structures

What is the role of glycoprotein in the Golgi complex? Many *secretory materials* such as goblet cell mucus (Fig. 1), secretory granules of some anterior pituitary cells (Fig. 16), or the content of apical vesicles as well as the luminal colloid in the thyroid (Fig. 12) were stained with our two carbohydrate techniques. These observations were in line with the glycoprotein nature of mucus, of some pituitary hormones (FSH, TSH), and of thyroglobulin respectively (36). At least in the case of goblet cell mucus, there was good evidence of its formation in the Golgi apparatus. First, routine EM staining showed gradual transitions between the top Golgi saccule and nearby mucigen globules (43–45). Secondly, radioautography showed that the label of ^{3}H-glucose was taken up, immediately after injection, by all Golgi saccules whatever their level in the stack. At a later interval, the label was located only in mucigen globules. These two lines of evidence indicated (46) that Golgi saccules are the site of synthesis of the carbohydrate groups of mucus.

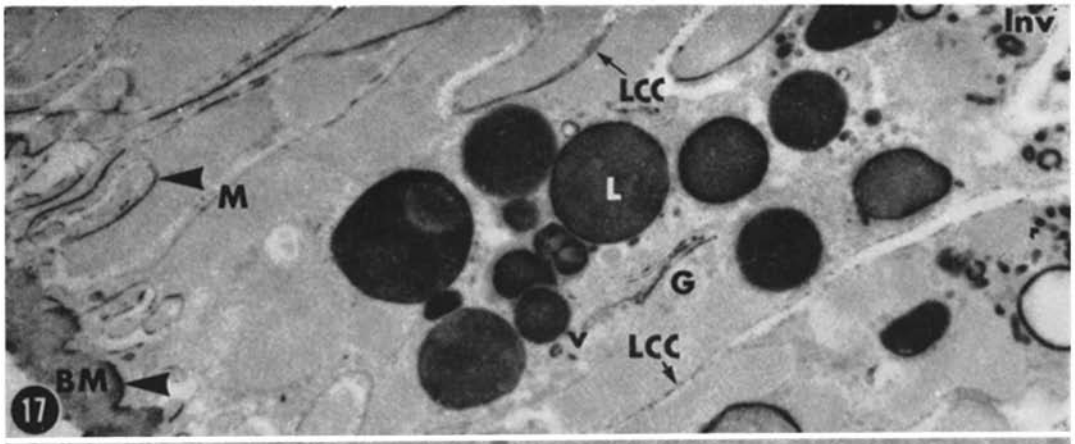
Since the number of saccules in a Golgi stack remained constant (46), the release of the most

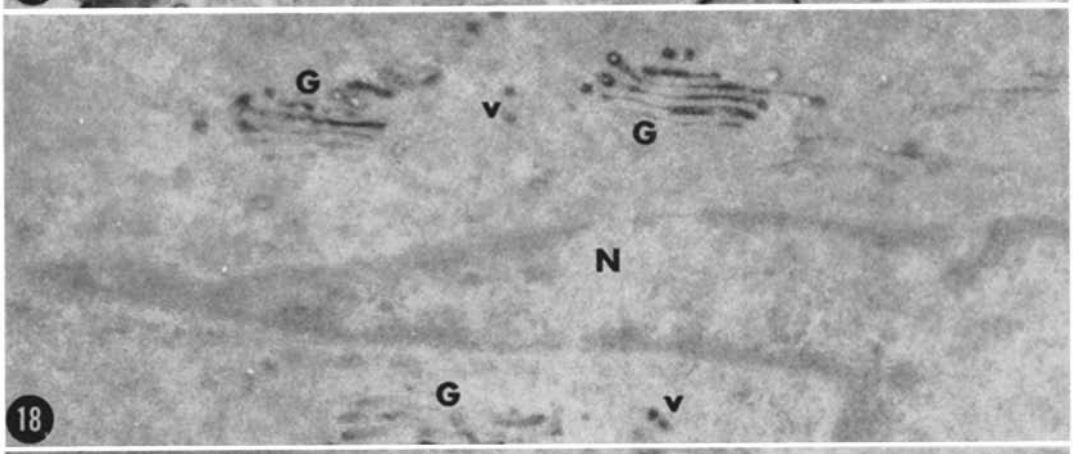
FIGURES 17–19 *Kidney.* Chromic acid–PTA.

FIGURE 17 Proximal convoluted tubule. The basement membrane (*BM*) is stained at lower left; cell coat material is seen along infoldings of the basal plasma membrane as dark loops (upper arrowhead) which may be followed along the surface of the basement membrane (lower arrowhead). Cell coat may also be observed in the lateral intercellular spaces (*LCC*) as well as along the membrane invaginations (*Inv*) from the apical surface. At center, small vesicles (*v*) and dense bodies (*L*) are concentrated around a stack of Golgi saccules (*G*). *M*, mitochondria. × 16,000.

FIGURE 18 Podocyte. Three Golgi stacks (*G*) are seen above and below the nucleus (*N*). The mature face of the stacks is away from the nucleus. A few vesicles (*v*) are scattered in the cytoplasm. × 42,000.

FIGURE 19 Distal convoluted tubule. The stained material of the cell coat fills up a basal membrane infolding crossing the field at left (*BCC*). Dense bodies are stained at center (*L*). At right, Golgi saccules (*G*) may be observed whose staining increases toward the upper right corner of the picture. Small vesicles (*v*) may also be seen, one of which at left center is near the basal cell coat. *M*, mitochondria. × 28,000.


Inv
LCC
M
L
G
V
LCC
BM
17


G
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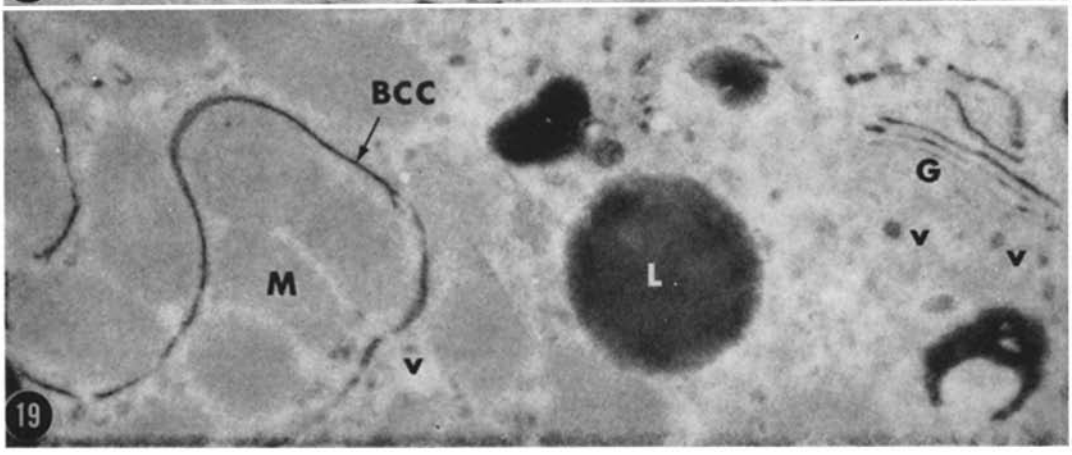
18


BCC
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19

mature saccule in the form of a mucigen globule had to be compensated for by the formation of a new saccule, presumably by fusion of pale vesicles at the immature face. As this process was repeated, the new saccules migrated in the direction of the mature face. At the same time, all saccules were synthesizing carbohydrate, so that they contained increasing amounts of it as they approached the mature face. This was reflected in the staining gradient from immature to mature face.

Since many other secretory materials were known to consist of glycoprotein (47), the synthesis of the carbohydrate moiety in Golgi saccules could account for the staining of the Golgi apparatus in many other secretory cells, for instance, in thyroid cells (Fig. 12).

When secretory materials contained protein with little or no carbohydrate, as in the case of pancreatic enzymes, gastric pepsin, and some pituitary hormones such as STH or ACTH (47), the matrix of secretory granules was not stained but, for unexplained reasons, the edge of these granules was stained (as long as they were in the Golgi region), as was seen in the acinar cells of pancreas (Figs. 13, 14), chief cells of gastric glands, and some pituitary cells. However, in spite of such minimal staining of secretory materials, Golgi saccules were quite reactive (Fig. 15).

The majority of the cells illustrating Golgi staining in this article had *no known secretory activity*. The question arose as to why reactive material was accumulated in the Golgi saccules of

TABLE I

Listing of Cells in which a Reactive Golgi Apparatus was Observed

SURFACE EPITHELIA: esophagus, *small* and *large intestine*; bladder; *vascular endothelia.*

GLAND CELLS: *goblet cells* (*duodenum*, jejunum, rectum), chief cells, *mucous neck cells*, and parietal cells in glands of the stomach body, argentaffin cells; Paneth cells,* *acinar cells, and duct cells of acinar pancreas; hepatocytes*; mucous cells of lateral nasal gland.*

Six cell types of anterior pituitary; *follicular cells* and light cells of thyroid gland.*

GENITAL ORGANS: oocytes, theca interna, and interstitial cells of ovary; fallopian tube epithelium. Spermatocytes,* *spermatids*, Sertoli cells,* spermatogonia*; Leydig cells*; *epididymal epithelium.*

HARD TISSUES: odontoblasts*; ameloblasts*; osteoclasts.

NERVOUS SYSTEM: neurons; glial and Schwann cells; retinal rods.

EXCRETORY SYSTEM: *podocytes: cells of proximal and distal convoluted tubules.*

OTHER CELLS: *fibrocytes*; lymphocytes; neutrophile and eosinophile granulocytes; reticular cells (spleen, lymph node, thymus); Kupffer cells.*

Note: All cells listed were examined after staining with the chromic acid–PTA technique, except those with an asterisk (*), which were only investigated with the PA-CrA–silver technique. The cells in italics were observed with both techniques.

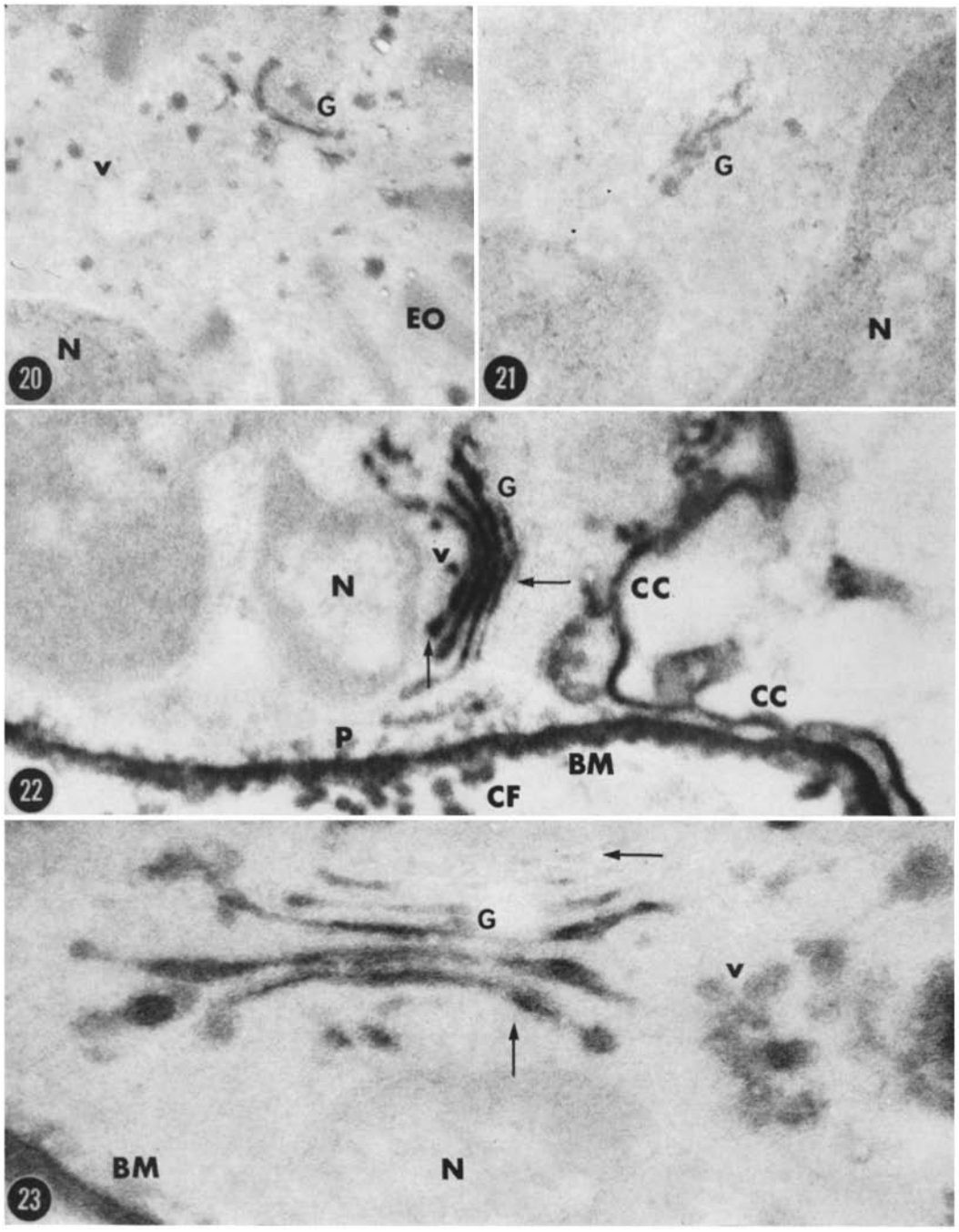
FIGURES 20–23 *Blood cells and endothelial cells.* Chromic acid–PTA.

FIGURE 20 Eosinophile. A small Golgi apparatus is seen at upper center (*G*). The definite reactivity of small vesicles (*v*) contrasts with the weak staining of eosinophilic granules (*EO*). *N*, nucleus. × 21,000.

FIGURE 21 Lymphocyte. A discrete Golgi apparatus (*G*) is seen in the concavity of the nucleus (*N*). × 28,000.

FIGURE 22 Blood capillary, thyroid gland. At the base of the figure, collagen fibers (*CF*) and basement membrane (*BM*) are seen. Both are stained. On the upper side of the basement membrane, stained vesicles may be pinocytotic (*P*). At right, the apical surface of the endothelial cells is outlined by the stained cell coat (*CC*). At center, staining of Golgi saccules (*G*) increases from right to left, where the mature face shows stained small vesicles (*v*). × 56,000.

FIGURE 23 Golgi apparatus of an endothelial cell, capillary of thyroid gland. As in Fig. 21, the staining of the Golgi saccules (*G*) increases from the top to reach a maximum in the saccule of the mature face next to the nucleus (*N*). Stained vesicles (*v*) seem to bulge from the ends of the stained saccules. *BM*, basement membrane. × 11,400.


G
v
EO
N
20
G
N
21
G
v
N
CC
CC
P
BM
CF
22
G
v
BM
N
23

such cells. The problem was examined by the consideration of which materials other than secretions were reactive. Vesicles about 0.06 μ in diameter were stained in columnar cells of small intestine (Figs. 4, 5), acinar cells of pancreas (Figs. 13, 14), kidney cells (Figs. 17, 19), endothelium of capillaries (Figs. 22, 23), and perhaps all cells (Figs. 11, 16, 24, 25, 27–29). They abounded at the mature face of Golgi stacks (Fig. 4) where they seemed to originate (Fig. 23). Hence, some of the reactive material arising in Golgi saccules may be used to build up stained vesicles. The evidence was similar, though less clear, in the case of *dense bodies*. These bodies were strongly stained spheres of varying size (0.1–1.5 μ), which either stained uniformly throughout, or showed irregularities in staining, shape, and content, and occasionally displayed an intensely stained edge (Figs. 7, 17). Such bodies were seen in columnar cells of intestine (Figs. 1, 2), kidney (Fig. 17), thyroid (Fig. 12), pancreas (Figs. 13, 14), and may, again, be present in all cells. They were often associated with the Golgi region (Fig. 8), particularly in the concavity of the mature face (Figs. 24, 26). Their reactive material would then seem to come from Golgi saccules, either because the dense bodies arose within the saccules or because the glycoprotein was carried from saccules to dense bodies by vesicles. A similar situation may hold with the stained *multivesicular bodies* often found in the Golgi region (Figs. 28, 29). Perhaps the incorporation of stained vesicles into dense bodies may, in fact, change some of them into multivesicular bodies.

The presence of acid phosphatase and other hydrolases in some Golgi saccules, as well as in small vesicles, dense bodies, and multivesicular bodies suggested that these structures be considered part of the lysosomal system (48, 49). Indeed, Fishman et al. (50, 51) demonstrated that one of the lysosomal enzymes, beta-glucuronidase, is a glycoprotein. *N*-acetyl-β-glucosaminidase seems to be another lysosomal enzyme which is a glycoprotein (52). Such glycoprotein enzymes would arise in Golgi saccules and be passed on to dense and multivesicular bodies (53). In this regard, there is evidence that, on incubation of fragments of liver and spinal ganglion with ^{3}H-galactose, the label which at early times was concentrated in the Golgi region appeared later in small vesicles, dense and multivesicular bodies (A. Rambourg and B. Droz, unpublished), as if the Golgi apparatus provided glycoprotein for these bodies. Since at least some of these bodies

FIGURES 24–29 *Varia*. Chromic acid-PTA. The immature face of the Golgi apparatus is indicated by a horizontal arrow and the mature face by a vertical arrow.

FIGURE 24 Reticular cell. The immature face makes up the convex surface of the Golgi apparatus (*G*), while the mature face on the concave surface is associated with a few reactive bodies. × 35,000.

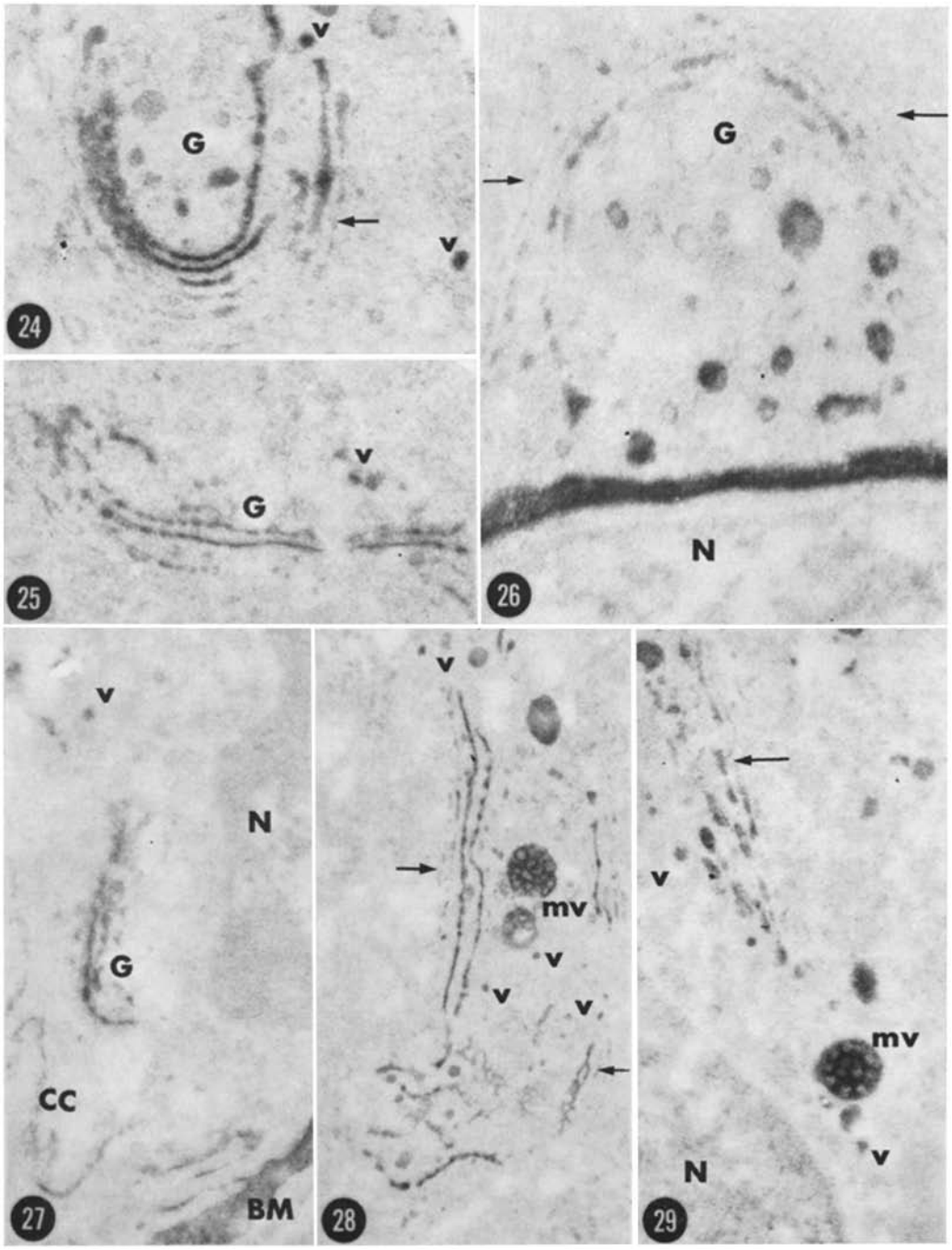
FIGURE 25 Neuron. A few Golgi saccules (*G*) and small vesicles (*v*) are depicted. × 35,000.

FIGURE 26 Spermatid. As in Fig. 24, the mature face makes up the concave surface of the Golgi stack (*G*). Vesicles are seen between this surface and the intensely stained headcap, which appears here as a horizontal band covering the nucleus (*N*). × 35,000.

FIGURE 27 Epithelial cell of a pancreatic intercalated duct. A Golgi stack (*G*) can be distinguished at center left. In the lower half of the picture, the wavy line corresponds to the cell coat (*CC*), whereas the dark band visible at lower right is the basement membrane (*BM*). Stained vesicles (*v*) can be seen at upper left. *N*, nucleus. × 32,000.

FIGURE 28 Rod cell of retina. A clear-cut Golgi stack is seen at left, and two discrete stacks at right. Next to the mature face (center), small vesicles (*v*) and two multivesicular bodies (*mv*) which seem to contain vesicles with an unstained core can be seen. The network at lower center probably results from an oblique section through the edge of a stained Golgi saccule. × 24,000.

FIGURE 29 Epididymis. At upper left, a Golgi stack with small vesicles (*v*). Below a, multivesicular body (*mv*). *N*, nucleus; *v*, vesicles. × 25,000.


v
G
v
24
G
v
25
G
N
26
v
N
G
CC
BM
27
v
mv
v
v
v
28
v
mv
N
v
29

are part of the lysosomal system, the Golgi apparatus may be involved in the maintenance of this system.

Staining of Extracellular Structures

That all cell surfaces are covered by a carbohydrate-rich *cell coat* (34) was fully confirmed here (Figs. 1, 6, 17). There is little information on the origin of the cell coat, although in intestinal columnar cells it was shown that, after injection of labeled amino acids and sugars, the label appeared first in the cytoplasm and later at the apical cell surface (54). Following ^{3}H-galactose injection, the label again appeared in the cytoplasm where it was concentrated in the Golgi region and, at later intervals, it was on the cell surface (55). The recent work with ^{3}H-galactose in vitro mentioned above (A. Rambourg and B. Droz, unpublished) also showed that labeled small vesicles approached the cell membrane. Since some small vesicles were often seen to be close to the plasma membrane, it is tempting to speculate that they ferry material from the mature face of the Golgi apparatus to the cell surface, where this material would constitute the cell coat.

As for the *basement membrane* (Figs. 6, 16, 21, 22), there is some evidence that its substance comes from adjacent epithelial cells (56). Finally, the *collagen fibers*, which were stained with the two techniques used in the present work (Fig. 22), are known to arise from fibroblasts (57, 58), in which collagen precursors can be traced from ribosomes through the Golgi apparatus (57, 58), where, perhaps, they took up the carbohydrate component responsible for their staining (although the possibility that the Golgi apparatus may be bypassed has been mentioned, 58).

From the foregoing, we conclude that the Golgi apparatus not only elaborates glycoprotein as the secretory material in many actively secreting cells, but also may elaborate a carbohydrate moiety for the lysosomal system within cells and, as the need arises, for the cell coat, basement membrane, and collagen fibers outside cells.

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