

SYNTHESIS OF THE CARBOHYDRATE OF MUCUS
IN THE GOLGI COMPLEX AS SHOWN BY ELECTRON
MICROSCOPE RADIOAUTOGRAPHY OF GOBLET CELLS
FROM RATS INJECTED WITH GLUCOSE-H³

MARIAN NEUTRA and C. P. LEBLOND

From the Department of Anatomy, McGill University, Montreal, Canada

ABSTRACT

It is known that colonic goblet cells utilize glucose to synthesize the carbohydrate portion of mucus glycoprotein. To determine the intracellular site of this synthesis, glucose-H³ was injected into 10-g rats. At 5, 20, 40 min, 1, 1½, and 4 hr after injection, segments of colon were fixed and prepared for electron microscope radioautography. By 5 min after injection, label had been incorporated into substances present in the flattened saccules of the Golgi complex. At 20 min, both Golgi saccules and nearby mucigen granules were labeled. By 40 min, mucigen granules carried almost all detectable radioactivity. Between 1 and 4 hr, these labeled granules migrated from the supranuclear region to the apical membrane; here, they were extruded singly, retaining their limiting membrane. The evidence indicates that the Golgi saccule is the site where complex carbohydrate is synthesized and is added to immigrant protein to form the complete glycoprotein of mucus. The Golgi saccule, distended by this material, becomes mucigen granules. It is roughly estimated that one saccule is released by each Golgi stack every 2 to 4 min: a conclusion implying continuous renewal of Golgi stacks. It appears that the Golgi synthesis, intracellular migration, and release of mucus glycoprotein occur continually throughout the life of the goblet cell.

INTRODUCTION

In 1914, long before the existence of the Golgi complex was fully accepted, Ramon y Cajal identified it as the site of formation of mucigen granules in goblet cells (40). Since then, the electron microscope has amply demonstrated that the Golgi complex is indeed involved in secretion. In particular, electron microscope radioautography has shown that the secretory proteins formed in the ergastoplasm migrate to the Golgi complex, where they are segregated into secretion granules or vesicles (6, 32, 41, 43, 53).

The products of most secretory cells, however, are not pure proteins; rather, they consist of

protein associated with carbohydrate. The presence of carbohydrate is well known in mucous secretions (50) and has recently been demonstrated in pancreatic (39), parotid (28), and other exocrine secretions (25) as well as in connective tissue products (25), hormonal factors, plasma proteins, etc. (50). Two main types of complex carbohydrates¹ have been recognized in secretory

¹The term "complex carbohydrates" refers to glycoproteins and mucopolysaccharides, as well as to glycogen and glycolipids. The last, soluble in organic solvents, are largely removed from tissues in histological processing.

materials: long chains loosely bound to protein to form the "mucopolysaccharides" (glycosaminoglycans) predominating in connective tissue secretions, and shorter chains firmly bound to protein to form the "glycoproteins" predominating in epithelial secretions.

It was not known, however, in what part of the secretory cell the synthesis of carbohydrate moieties takes place. After Bélanger (3) had found that the goblet cells of intestine took up sulfate-S³⁵ into their mucus, Jennings and Florey (20) localized the uptake in a supranuclear zone which they believed to be the Golgi region; they inferred that the Golgi complex was the site of sulfation of the carbohydrates of mucus. Indeed, recent electron microscope studies of the goblet cells of rat colon pinpointed the flattened Golgi saccules or even the membranes of these saccules as the sulfation site (24).

The next step was to determine where the carbohydrate moiety itself is formed. Since glucose may be converted to all the monosaccharides taking part in the synthesis of complex carbohydrates (8, 50), we injected glucose labeled with tritium into albino rats, and examined a variety of cells by radioautography (36). At 5 to 15 min after injection, the label was found in the Golgi region of secretory cells, and at later times it was in the secretion product. The Golgi reaction was heavy in cells elaborating a carbohydrate-rich "mucous" secretion (particularly in the goblet cells of colon) and light in those producing a carbohydrate-poor "serous" secretion. It was concluded that in both cases glucose was used for the synthesis of complex carbohydrate in the Golgi region. Since the results were the same whether glucose was labeled in the 1 or 6 position, it was likely that the entire 6-carbon chain of glucose was a building block in this synthesis (37).

This work, done with the light microscope, did not reveal the exact site of synthesis within the Golgi region, nor the manner in which secretory material left this region. It was therefore decided to investigate the problem with the electron microscope. The goblet cells of the rat colon were chosen for this study. Biochemical work *in vitro* had shown that the mucous material secreted by the colonic mucosa contains a glycoprotein whose monosaccharide components are derived from glucose (9). Presumably, this glycoprotein was produced by goblet cells. Indeed, after injection of labeled glucose, the goblet

cells of rat colon lent themselves well to a radioautographic study in the electron microscope.

MATERIALS AND METHODS

Four-day-old (9 to 10 g) male Sherman rats were selected, which were in the act of nursing and whose stomachs were full of milk. Each was given an intraperitoneal injection of 1 mc of glucose-6-H³ (Radiochemical Centre, Amersham, England, specific activity 1300 mc/mole). At various time intervals after injection (5, 20, 40 min and 1.5, 4 hr) the rats were anesthetized with ether, and 2 segments of colon were removed. One segment was fixed in neutral buffered formalin pH 7.0 (35) at 4°C for 24 hr, embedded in paraffin, and sectioned at 4 μ for the enzymatic digestions described at the end of this section. The other segment was fixed for 1 hr in cold isotonic 2.5% glutaraldehyde in phosphate buffer (19), rinsed for 30 min in 4 changes of buffer, postfixed in isotonic Veronal-buffered 1% osmium tetroxide for 1.5 hr (54), and embedded in Epon. Half μ sections were mounted on glass slides with a wire loop and coated by dipping in liquid Ilford L-4 emulsion. After exposure and development, a drop of filtered 1% toluidine blue was placed on the slide, allowed to stand at room temperature for 5 min, and rinsed off with distilled water. After drying, a cover slip was mounted.

Electron microscope sections, cut from the same tissues blocks as the $\frac{1}{2}$ μ sections, were prepared in two ways. Some were placed on Formvar-coated grids (41) and dipped in Gevaert 307 emulsion. After development, the grids were allowed to dry for about 20 min, then were removed from the slides and stained for 15 to 20 min with drops of lead hydroxide (21).

Some sections (4 hr interval) were mounted on celloidin-coated slides, coated with emulsion and, after development, stripped and mounted on grids (44). This method, followed by prolonged staining through the celloidin with lead citrate (42), yielded better preparations than the former technique.

In addition, one 100 g rat received an injection of 0.5 mc of glucose-H³ into the lumen of the colon. A segment of colon at the site of injection was removed 5 min later, fixed in Veronal-buffered 1% osmium tetroxide (55), embedded in methacrylate, sectioned, and coated with Ilford L-4 emulsion (41).

Enzymatic digestions were performed on deparaffinized 4 μ sections, prior to radioautography (NTB2 emulsion, reference 23). Sections were treated with *alpha amylase* by immersion in saliva for 10 min at 60°C. This procedure removed cytoplasmic periodic acid (PA)-Schiff-positive material (assumed to be glycogen) from liver, muscle, and a few other tissues. For *hyaluronidase* treatment (Calbiochem, Los Angeles, California, from bovine testis, activity 380

IU/mg), sections were incubated in a 0.05% enzyme solution in phosphate buffer at pH 5.5 for 4 hr at 37°C, while controls were incubated in buffer alone. After thorough rinsing, they were stained with colloidal iron-Feulgen before radioautography. Following this treatment, toluidine blue-stained sections of cartilage and connective tissue showed complete loss of metachromasia. Digestion with *beta glucuronidase* (Calbiochem, from bovine liver, activity 360 IU/mg) was done according to Fullmer (13). Sections were oxidized in peracetic acid for 30 min at room temperature, then digested in a solution of 15 mg of beta glucuronidase in 50 ml of acetate buffer pH 4.5 for 4 hr at 37°C. Three types of control sections were prepared: some were treated with peracetic acid alone, some were incubated in buffer alone, and others were incubated in the enzyme solution without prior peracetic acid oxidation. Only the acid-enzyme sequence abolished the colloidal iron staining of goblet cell mucus.

RESULTS

Structure of the Goblet Cells of Rat Colon

In 10-g rats, the goblet cells of the colon are readily distinguished by the dark staining of their cytoplasm with toluidine blue (Fig. 4). They are abundant, both in the crypts where they are short (15 to 20 μ) and on the surface where they are narrow and tall (40 to 45 μ).

The surface goblet cells, because of their abundance and size, are readily located in the electron microscope (Fig. 1). The basally placed nucleus is surrounded by dense cytoplasm containing regular, ribosome-studded cisternae of endoplasmic reticulum with the occasional mitochondrion. Such cytoplasm extends along the periphery of the cell, up to the apical membrane (Fig. 3, and reference 34).

The supranuclear region is dominated by an extensive Golgi complex. In a longitudinal section the Golgi complex appears U-shaped (Fig. 1), and in a transverse section through the supranuclear region it describes a ring (Fig. 2). In three dimensions, the Golgi complex is a cuplike structure whose base lies just above the nucleus, and whose walls extend up the sides of the cell. The space from the Golgi "cup" to the apical cell membrane is filled with mucigen granules. For descriptive purposes, the mucigen granules located within the Golgi cup are called "central granules," and those lying above the brim of the cup, "apical granules."

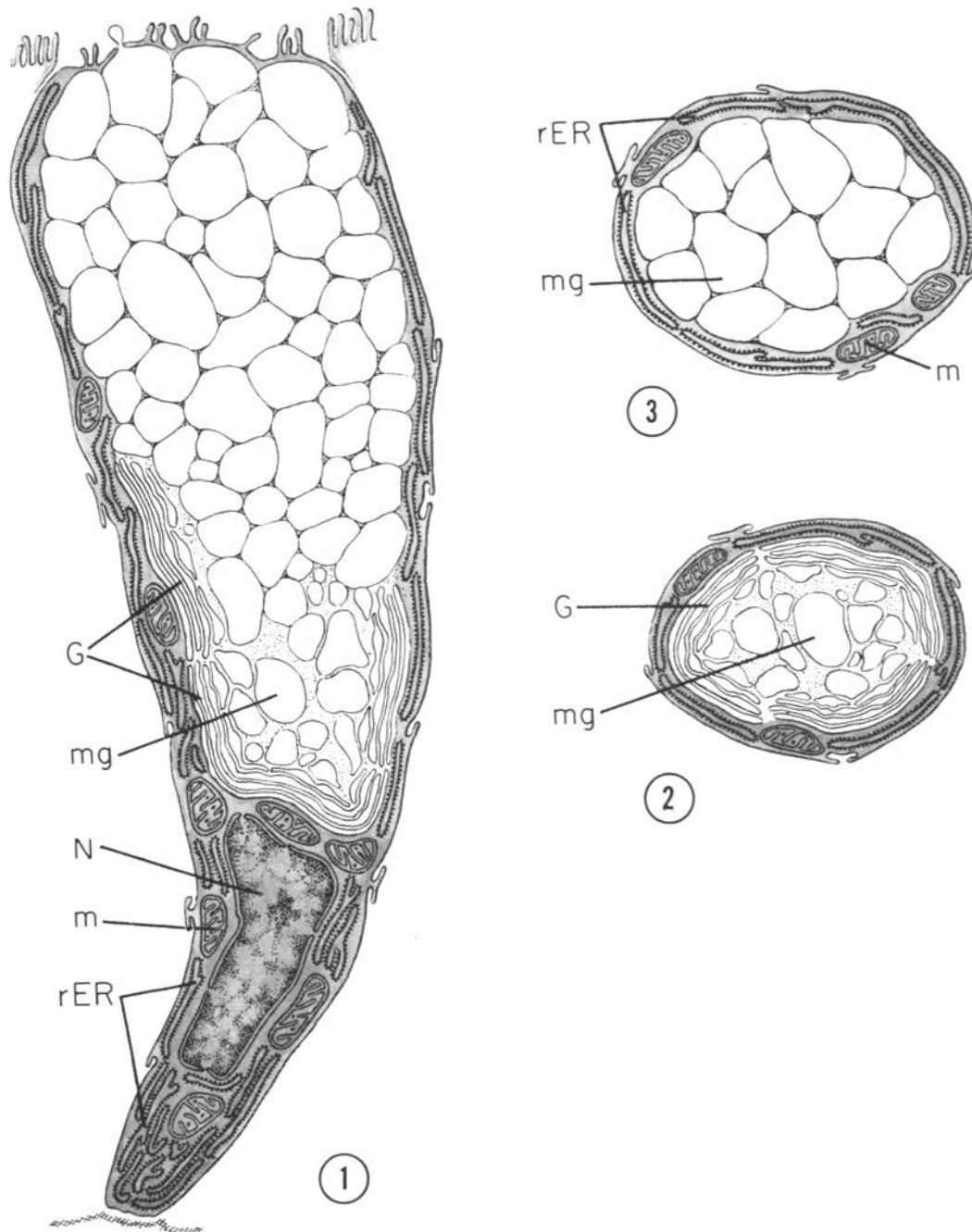
The Golgi complex in section appears to be

composed of flattened membrane-bounded saccules, also called "flattened vesicles" or "cisternae." These saccules are arranged in several stacks, or "dictyosomes" (three stacks are seen in Fig. 1). Each stack consists of 7 to 12, usually 10, saccules (Fig. 11). The saccules on the outer aspect of the Golgi cup (nearer the cell membrane or nucleus) are termed "peripheral," and those near the central mucigen are termed "central." With the possible exception of the most peripheral saccule which may have a visible lumen (Fig. 11, small arrows), the peripheral saccules (*ps*) are usually so flattened that their lumens are virtual. However, as the central saccules are approached, the lumens gradually increase in size. The most central saccules (*cs*) are irregularly distended. The most distended portions of the central saccules approach the size of the nearby mucigen granules, and are limited by a similar membrane. Furthermore, the material in the lumens of these saccules appears light and finely fibrillar, like the content of the mucigen granules. Hence, there seems to be a gradual morphological transition from flat Golgi saccule to spheroidal mucigen granule, as already proposed by previous authors (4, 10, 11, 47).

Within a goblet cell, the membranes of central and apical mucigen granules usually remain intact. On occasion, however, coalescence of some granules has been observed. At the apex, a thin layer of cytoplasm insinuates itself between the mucigen granules and the apical cell membrane. Occasionally, one or several clefts are seen across this layer, through which a membrane-bounded mucigen granule is seen passing into the lumen (Fig. 18). Indeed, free granules are found in the lumen; these may have a broken membrane and their content may be partly released (Fig. 18, upper left).

Radioautographic Results

LIGHT MICROSCOPY: Five min after an intraperitoneal injection of glucose- H^3 into a 10 g rat, a radioautographic reaction appeared over the goblet cells of the colon. In longitudinal sections of surface goblet cells (Figs. 5 and 7), a U-shaped band of silver grains was observed above the nucleus, corresponding in shape and extent to the Golgi complex cut as in Fig. 1. In transverse sections (Fig. 6 *a*) a ring of silver grains was seen around the central mucigen, corresponding to the Golgi complex cut as in Fig. 2. In addition, a



FIGURES 1 to 3 Semischematic drawings based on electron micrographs of surface goblet cells of colon of 10-g rats.

FIGURE 1 In longitudinal section, the Golgi complex (*G*) forms a U above the nucleus (*N*). It is composed of several stacks of saccules. Each stack includes 7 to 12 saccules (only 4 of which are depicted here). There seem to be transitions between the most central saccules and the loosely packed mucigen granules (*mg*) which occupy the central portion of the supranuclear area. Above, closely packed mucigen granules occupy the cell apex (*m*, mitochondria; *rER*, rough-surfaced endoplasmic reticulum or ergastoplasm).

FIGURE 2 Transverse section through the supranuclear region. The Golgi complex (*G*) forms a ring around mucigen granules (*mg*). The Golgi complex in turn is rimmed by a narrow margin of cytoplasm.

FIGURE 3 Transverse section above the Golgi complex. The group of apical mucigen granules (*mg*) is rimmed by cytoplasm. Rough endoplasmic reticulum (*rER*) and mitochondria (*m*) may be distinguished.

light, scattered reaction was distributed over basal and lateral cytoplasm (Figs. 5 and 7).

Longitudinal sections of goblet cells from a rat sacrificed 20 min after injection (Fig. 8) again showed a U-shaped band of silver grains, which was, however, denser and wider than at 5 min. At 40 min and 1 hr, there was still an intense radioautographic reaction, but now the grains formed a solid mass over the supranuclear region. By 1½ hr (Fig. 9), the mass occupied both the supranuclear region and the lower part of the apical mucigen. Finally, at 4 hr (Fig. 10), while a few goblet cells had radioactive material distributed throughout the group of mucigen granules, most cells were labeled only in the apical region. Label was occasionally seen in mucus present in the lumen close to the cell apex.

On no occasion was an unlabeled goblet cell observed in the crypts or on the surface of the colon.

From these observations, it was concluded that glucose- H^3 was incorporated in the Golgi region into substances retained during histological processing. But were these substances restricted to the Golgi complex, and, if so, to the saccules or to some other component? And then, how did they leave the Golgi saccules to become mucigen granules?

ELECTRON MICROSCOPY: Radioautographs of goblet cells at 5 min after intraperitoneal or local injection of glucose- H^3 showed silver grains over the stacks of Golgi saccules (Fig. 12), where they were distributed fairly evenly, with no detectable preference for central or for peripheral saccules. At this time, there was no reaction over mucigen granules. Nor were the rough surfaced endoplasmic reticulum and the other organelles significantly labeled.

At 20 min, silver grains were still seen over Golgi saccules throughout the stack, but some grains now appeared over the nearby mucigen granules as well (Figs. 13 and 14). The size of the silver grains was such that in the Golgi region they overlay both membrane and content of saccules, so that the source could have been in either or both. In the case of mucigen granules, however, silver grains were usually not over the limiting membrane, but over the content.

By 40 min, the bulk of the radioactive material was in the central mucigen granules, while the Golgi saccules were almost completely without label (Fig. 15). By 1 or 1½ hr (Fig. 16), this

localization had changed little, although some labeled granules were found farther from the Golgi complex.

The position of the labeled mucigen granules at 4 hr varied considerably from cell to cell, as predicted from the study of the ½ μ sections. Usually, the most apical granules were heavily labeled, with a few lightly labeled just below (Fig. 17). In both crypt and surface goblet cells an occasional labeled granule was seen in the process of being extruded through a cleft of the apical cytoplasm (Fig. 19). Finally, labeled mucus was on occasion seen in the lumen next to the epithelial surface.

ENZYMATIC TREATMENTS: *Alpha amylase* did not influence the intensity of either the early Golgi reaction or the mucigen reaction observed at later times. However, the reaction scattered over the rest of the cells (Figs. 5 to 10) was slightly but distinctly decreased. *Hyaluronidase* had no effect. When sections had been treated by the sequence *peracetic acid-beta glucuronidase*, the mucus was no longer stained with colloidal iron and the radioautographic reactions over Golgi complex and mucigen were largely removed, although the reaction scattered over the rest of the cytoplasm and over the rest of the tissue persisted (Figs. 20 and 21).

DISCUSSION

Nature of the Material Labeled in the Golgi Region of Goblet Cells after Glucose- H^3 Injection

Radioautographic reactions were observed over the rat colon 5 min after injection of glucose- H^3 (Figs. 5 and following). Glucose is known to diffuse into tissues rapidly; for example, 85 to 90% of an intravenous dose of glucose was cleared from the blood of rats, mice, and dogs within 5 min (1, 2, 51). The glucose present as free monosaccharide in the cells of colon would be washed out during fixation and processing, as would other monosaccharides and nucleotide sugars derived from it. But those monosaccharides that were incorporated into complex carbohydrates or other large molecules would be retained in the sections so that their label would be recognized in radioautographs.

Indeed, the 5-min radioautographs showed that label was retained in the saccules of the Golgi

complex (Figs. 5 to 7, and 12). At 40 min and later, label was in mucigen granules (Figs. 9, 10, and 15 to 17). At 4 hr, some label appeared in the mucus seen outside the cells. It was concluded that a component of mucus had been synthesized from glucose- H^3 in the Golgi saccules.

Enzymatic treatments gave some information about the nature of this labeled mucus component. Digestion of paraffin sections with alpha amylase or hyaluronidase failed to remove any label from the Golgi region or from mucigen. Hence, the labeled material was neither glycogen nor the hyaluronidase-labile mucopolysaccharides, hyaluronic acid and chondroitin sulfate A or C. In contrast, the sequence peracetic acid-beta glucuronidase (reported to specifically remove epithelial glycoproteins, reference 13) did extract much radioactivity from the Golgi region at early

times and from mucigen at late times after injection (Figs. 20 and 21). This evidence suggested that at least the labeled material that was extracted consisted of glycoprotein.

The mucus material at the surface of sheep colonic mucosa was shown by Kent and Marsden to contain a large glycoprotein fraction, in which they identified the monosaccharides hexosamine, galactose, fucose, and sialic acid, as well as sulfate residues (22). When Draper and Kent incubated sheep colonic mucosa with glucose- $1-C^{14}$, the label appeared in all the monosaccharides of the glycoprotein, whereas the protein moiety was not labeled (9). In our experiments, after either local or systemic administration of labeled glucose, nearly all the radioactivity of the colonic mucosa was in the goblet cells (Figs. 5 to 10). Therefore, it was assumed that the glycoprotein investigated

FIGURE 4 Colon of 10 g rat ($\frac{1}{2} \mu$ Epon section toluidine blue stain) including epithelium, muscularis mucosae (*mm*), submucosa (*s*), and inner edge of muscularis externa (*m*). The tall columnar surface epithelium is composed of deeply stained goblet cells (*gob*) and lighter columnar cells (*c*). Both cell types are shorter in the epithelium of the crypts. $\times 390$.

FIGURES 5 to 10 Radioautographs of $\frac{1}{2} \mu$ Epon sections of surface epithelium of colon of 10 g rat, at various times after injection of glucose- H^3 . Toluidine blue stain. $\times 1300$.

FIGURE 5 Interval of 5 min. In the goblet cell, a U-shaped band of grains appears between the nucleus (*N*) and the mucigen (*M*), corresponding to the position of the Golgi complex (*G*). Light reaction is scattered over the rest of the cytoplasm of the goblet cells, as well as over columnar cells. (3 month exposure).

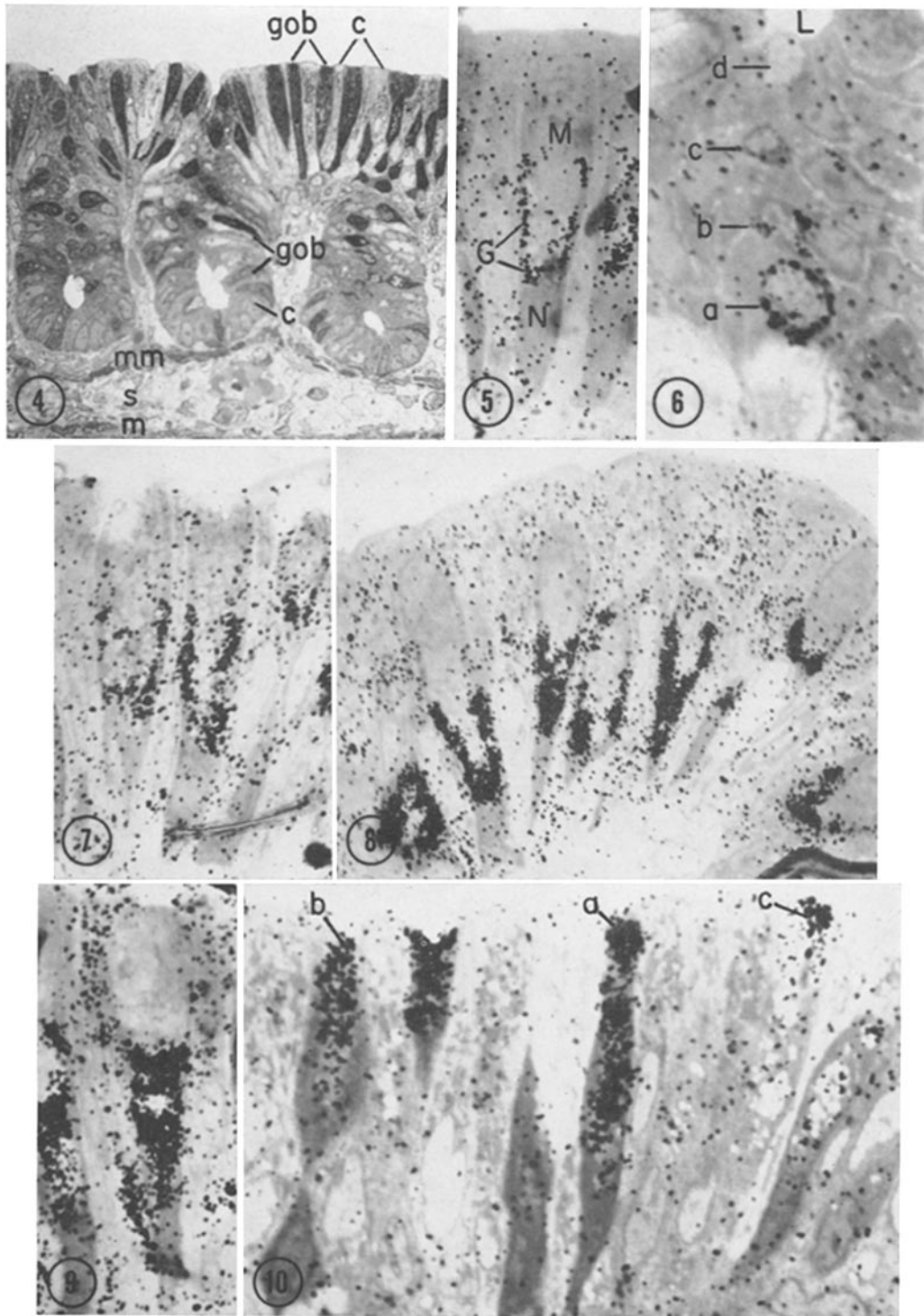
FIGURE 6 Interval of 5 min. Four goblet cells have been transversely sectioned at progressively higher levels, from supranuclear region (*a*) through intermediate levels (*b*, *c*, *d*) to lumen (*L*). The supranuclear reaction at (*a*) describes a ring around the central mucigen, as does the Golgi complex. At a slightly higher plane (*b*), only part of a ring is present, while above (*c*, *d*) no reaction is seen. (3 month exposure).

FIGURE 7 Interval of 5 min. Longer exposure ($7\frac{1}{2}$ months) enhances the Golgi complex-localized reaction but does not alter its localization.

FIGURE 8 Interval of 20 min. Several goblet cells, longitudinally or obliquely sectioned, are present. The band of grains is denser and thicker than that seen at 5 min, but it shows the same localization. ($7\frac{1}{2}$ month exposure).

FIGURE 9 Interval of $1\frac{1}{2}$ hr. The intense radioautographic reaction covers the supranuclear area and the lower edge of the apical mucigen. (3 month exposure).

FIGURE 10 Interval of 4 hr. Individual variations in the reactions of several goblet cells are seen. Although one goblet (*a*) has radioactive material distributed throughout its mucigen, most show a reaction over the apical portion (*b*). One has mucigen labeled at the uppermost edge only (*c*). (3 wk exposure).



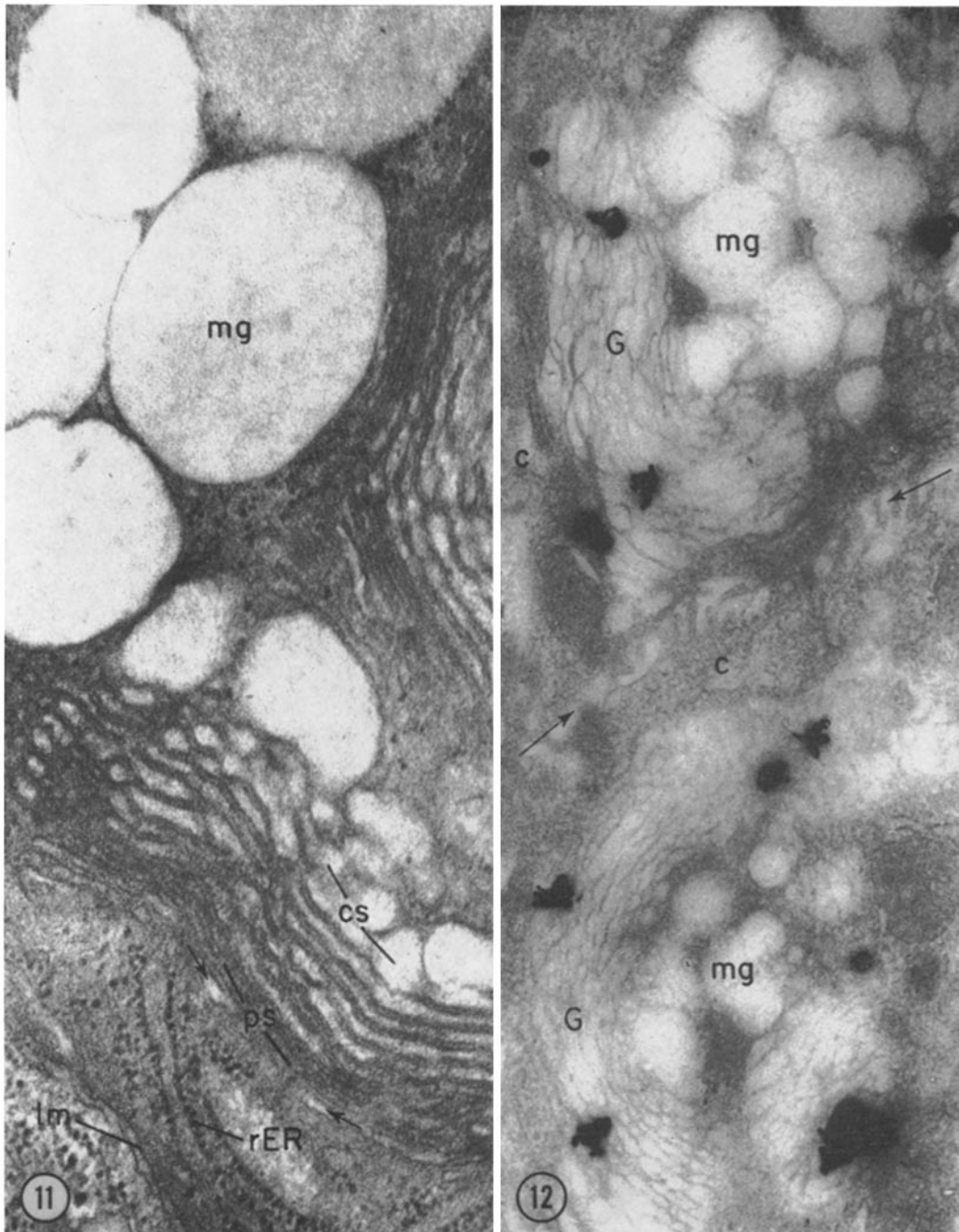
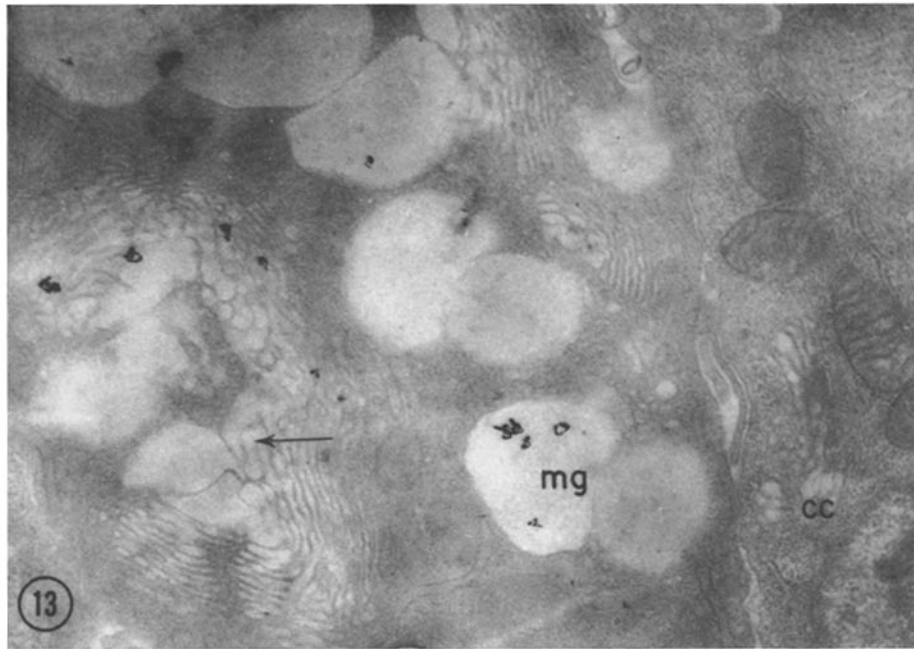


FIGURE 11 Goblet cell of colon of 10 g rat. In the lower half of the micrograph is a stack of Golgi saccules, separated from the lateral cell membrane (*lm*) by cytoplasm containing cisternae of rough endoplasmic reticulum (*rER*) and a few free ribosomes. The peripheral saccules (*ps*) are flat and have no discernible lumen (with the exception of the outermost saccule, small arrows). When examined from left to right, the saccules become progressively dilated until the most central saccule (*cs*) is expanded into several small ovoid structures which resemble the fully formed mucigen granules seen nearby (*mg*). $\times 60,000$.

FIGURE 12 Radioautograph at 5 min after local injection of glucose- H^3 , showing a transverse section through the supranuclear region of two goblet cells (arrows indicate intercellular space). Silver grains overlie the stacks of Golgi saccules (*G*). Neither the central mucigen granules (*mg*) nor the peripheral cytoplasm (*c*) are labeled. (Ilford L-4 emulsion, 4 month exposure). $\times 21,000$.



FIGURES 13 and 14 Radioautographs of rat colonic goblet cells at the 20 min interval. These and the following micrographs were obtained after an intraperitoneal injection of glucose- H^3 . (Gevaert 307 emulsion, $2\frac{1}{2}$ month exposure).

FIGURE 13 Supranuclear region of a goblet cell in longitudinal section, next to a columnar cell (*cc*). In the oddly oriented Golgi complex, the arrow runs from flat peripheral saccule to dilated central saccule. Some silver grains are seen over the Golgi saccules, and others over nearby mucigen granules (*mg*). $\times 30,000$.

FIGURE 14 Goblet cell at high magnification, with columnar cell (*cc*) below. (The dark area at upper left is the grid.) The label is again seen at all levels of the Golgi stack, and in an adjacent mucigen granule (*mg*) where it is associated with the content rather than with the limiting membrane. $\times 56,000$.

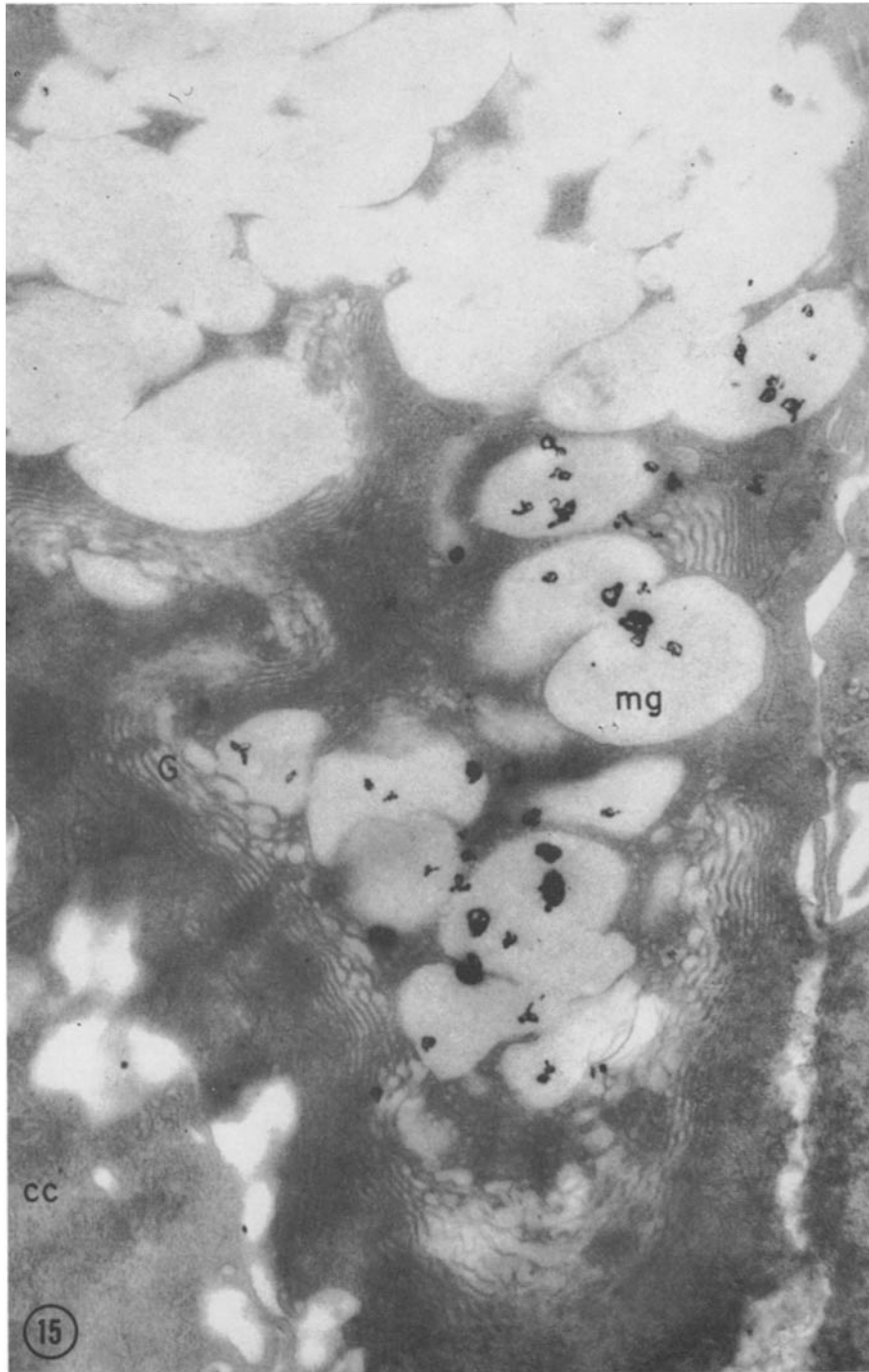


FIGURE 15 Longitudinal section of a goblet cell, 40 min after glucose- H^3 injection. Columnar cells (*cc*) are on both sides. The Golgi complex (*G*) is not labeled. Silver grains now lie over the central mucigen granules (*mg*) but not over the apical mucigen granules at the top. (Gevaert 307 emulsion, $2\frac{1}{2}$ month exposure.) $\times 24,000$.

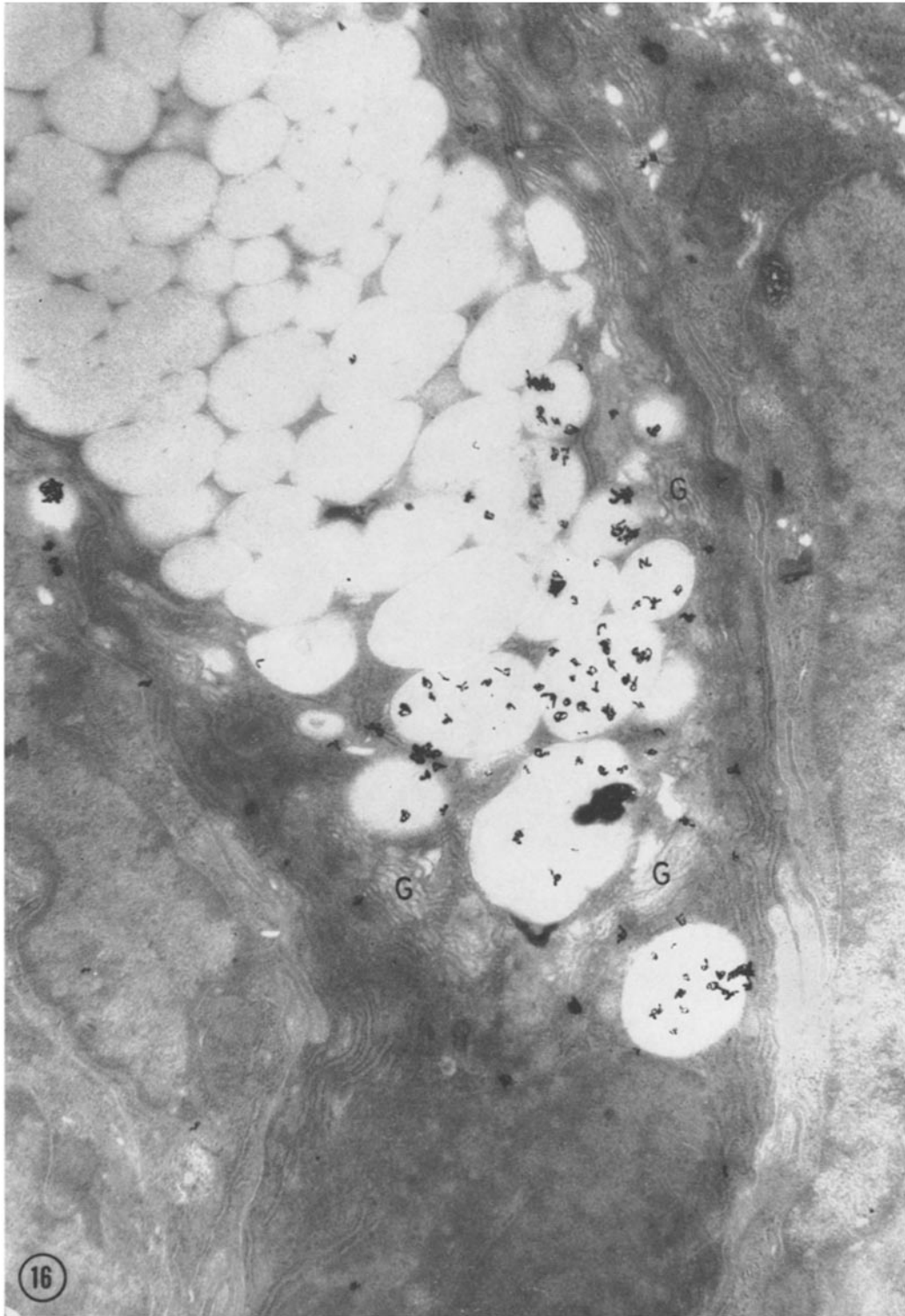


FIGURE 16 Longitudinal section of a goblet cell $1\frac{1}{2}$ hr after glucose- H^3 injection. Discretely labeled mucigen granules are located mainly in the supranuclear region. The Golgi stacks (G) are little or not labeled. (Gevaert 307 emulsion, $2\frac{1}{2}$ month exposure.) $\times 14,000$.

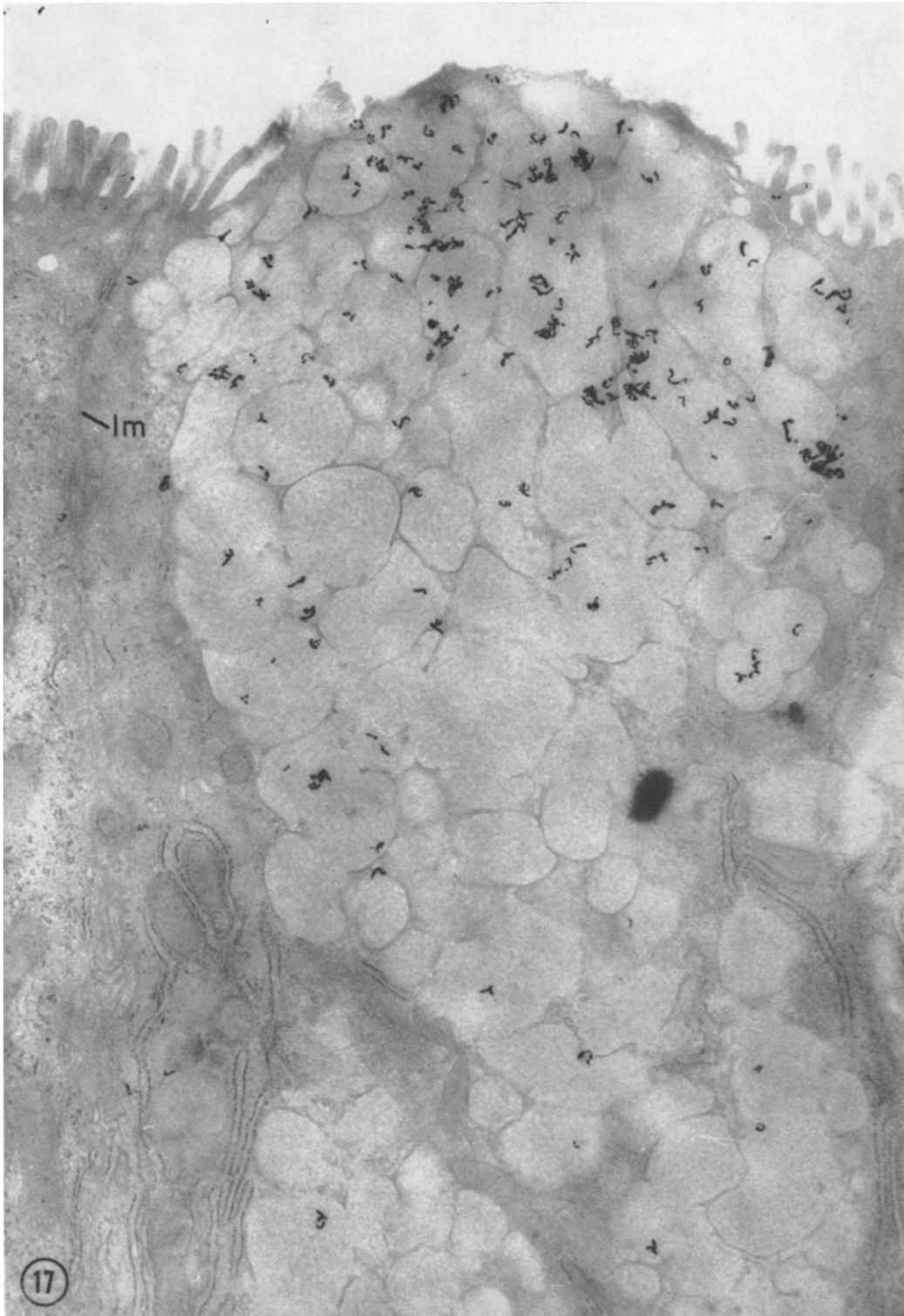


FIGURE 17 Longitudinal section of a goblet cell 4 hr after glucose- H^3 injection. The heavily labeled mucigen granules are commonly found near the apical cell membrane, with a "trail" of more lightly labeled granules below. (A layer of cytoplasm along the lateral cell membrane, *lm*, is seen extending up to the apical membrane.) (Gevaert 307 emulsion, celloidin stripping technique (44), 1 month exposure.) $\times 16,500$.

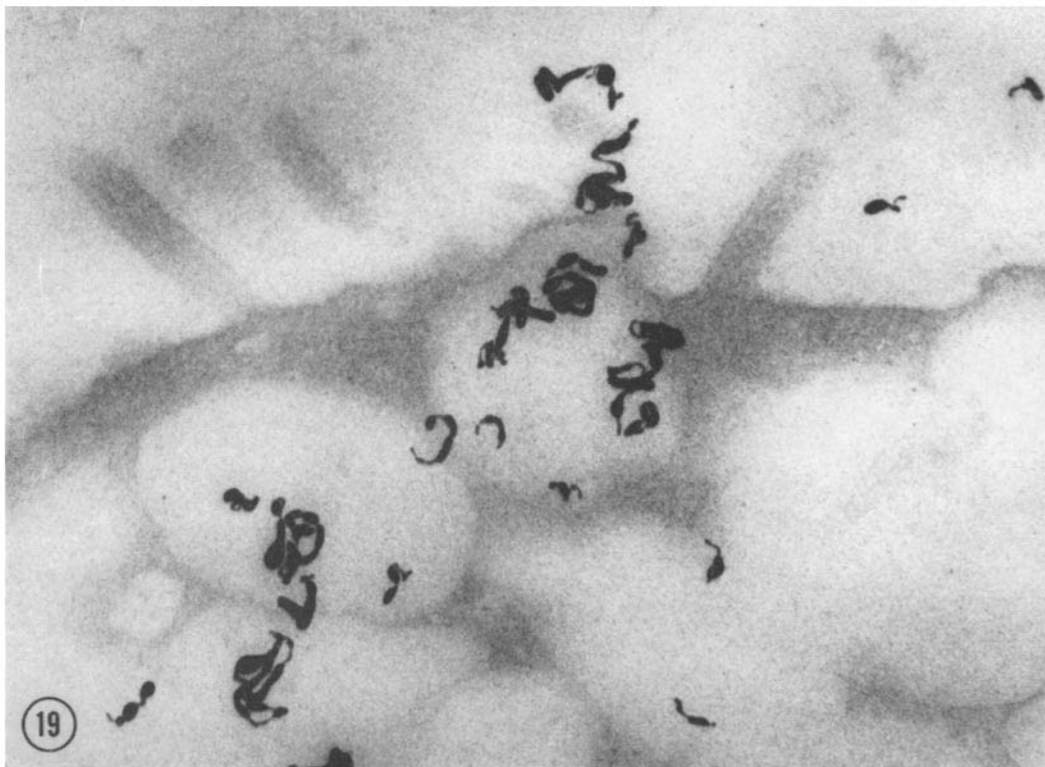
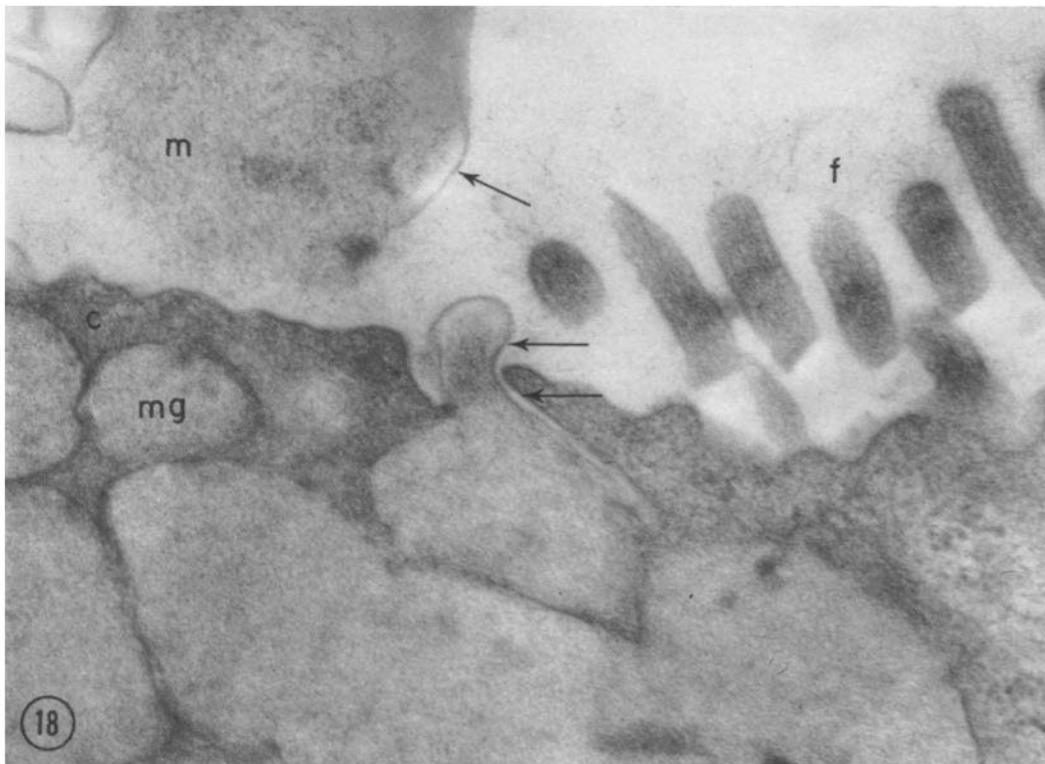
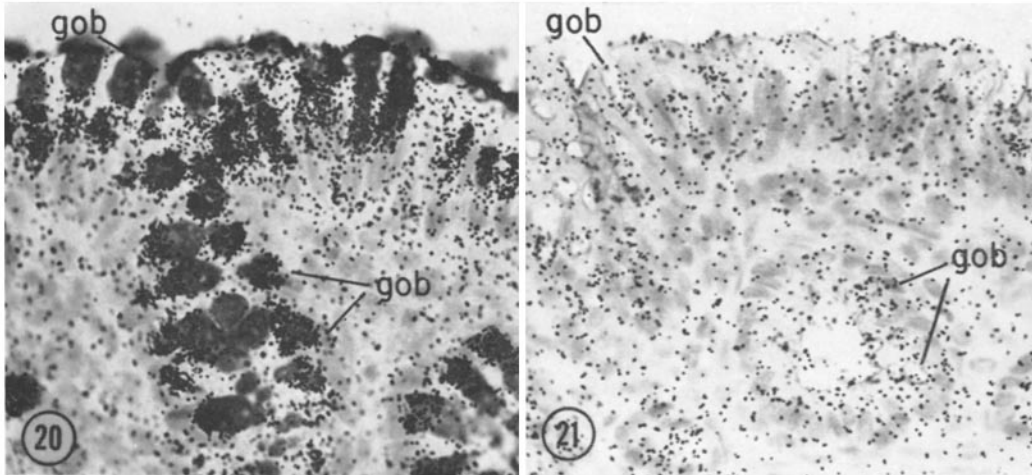


FIGURE 18 The apical surface of a goblet cell at high magnification. The uppermost mucigen granules (*mg*) are separated from the lumen by a narrow rim of cytoplasm (*c*). A few microvilli with associated "fuzz" (*f*) are present. In the center of the picture, a mucigen granule is being extruded through a cleft in the apical cytoplasm. In the lumen a granule has broken open, releasing its mucus (*m*). The small arrows indicate, from the top down, the unit membranes which limit part of the free, broken granule, the granule in passage, and the cytoplasmic cleft. $\times 65,000$.

FIGURE 19 Radioautograph, 4 hr after glucose- H^3 injection, of an area comparable to that of Fig. 18. A radioactive mucigen granule is being extruded. Thus, 4 hr after the labeling of its contents in the Golgi region, this intact granule has completed its migration through the cell. (Gevaert 307 emulsion, 1 month exposure.) $\times 65,000$.



FIGURES 20 and 21 Radioautographs of rat colon, $1\frac{1}{2}$ hr after injection of glucose- H^3 . They have been treated as indicated below, stained with colloidal iron-Feulgen, and radioautographed. (Kodak NTB2 emulsion, 9 day exposure.) $\times 600$.

FIGURE 20 Control section incubated in buffered beta glucuronidase (without peracetic acid pretreatment). The goblet cells (*gob*) on the surface and in the crypts are heavily labeled, and their mucigen is stained by colloidal iron. When compared to sections incubated in buffer without enzyme, no difference was seen; thus, the enzyme alone had no effect on the labeled material.

FIGURE 21 Section adjacent to that in Fig. 20, treated with peracetic acid prior to incubation in buffered beta glucuronidase. The stainable mucigen has been removed from the goblet cells (*gob*) and the radioautographic reaction is drastically reduced. The reaction over connective tissue is unchanged.

by Draper and Kent was synthesized in goblet cells and was in fact the characteristic component of the mucus elaborated by these cells. The radioactivity we detected in Golgi saccules and mucigen granules of rat goblet cells would be due to the uptake of glucose- H^3 label into such a glycoprotein.²

The sequence of events involved in the uptake of glucose into glycoprotein has been partly elucidated. Glucose is converted to various monosaccharides without breakdown of the 6-carbon chain, and linked to nucleotides to form nucleotide sugars such as uridine diphosphate galactose and guanidine diphosphate mannose; these nucleotide sugars in turn donate their monosaccharides for the synthesis of complex carbohydrates (26, 50, 52). Little is known about the enzymes (synthetases and transferases) which link monosaccharides together, beyond the fact that these enzymes are found in the microsomal fraction of animal cells and bacteria (48) but their

² The nature of the material producing the reaction scattered outside Golgi region and mucigen is examined in the next article.

function does not depend upon the presence of RNA (29, 45). Even less is known about the enzymes that link carbohydrate chains to protein. The evidence indicates that, after the protein moiety has been synthesized in association with ribosomes, the carbohydrate moiety (either as an entire chain or one monosaccharide at a time) is added in association with membranous structures (16, 46, 49); but the intracellular location of the membranes involved has not been established.

Our early radioautographs provided a demonstration that the saccules of the Golgi complex are the site where glucose and other monosaccharides become associated to form complex carbohydrate. Here would be the membrane site postulated by biochemists where this carbohydrate combines with protein to form the glycoprotein of mucus. Here also, the sulfate of the carbohydrate moiety (22) would be added (24).

Behavior of Golgi Saccules

A significant Golgi reaction was present at 5 and 20 min (Figs. 12 to 14), but no longer at 40 min (Fig. 15). Presumably, by 40 min, the level

of labeled precursors had become too low to provide significant radioactivity to Golgi saccules.

The disappearance of label from the saccules at 40 min was associated with its appearance in nearby mucigen granules. Since no connections were seen between the successive saccules of a stack, it is unlikely that the labeled material diffused from peripheral into central saccules and, from there, into mucigen granules. It is more reasonable to assume that labeled mucigen granules arose from the expansion of the most central saccules, which in turn had come from a more peripheral position in the stack. This conclusion is in line with the gradual morphological transition from peripheral to central saccules and from the latter to mucigen granules (Fig. 11, and references 4, 10, 11, 34, 47). In fact, since at all time intervals, 7 to 12 saccules were present in each Golgi stack, the transformation of a central saccule into mucigen granules has to be compensated by formation of a new peripheral saccule. Grassé and other authors, working with various animal and plant cells, have already made observations suggesting that continuous formation of peripheral saccules balances the transformation of central saccules into secretion granules (5, 12, 14, 15, 31, 33). Our radioautographic observations not only gave direct evidence in support of this concept, but also provided crude estimates of the turnover time of Golgi stacks: since label was distributed over the stack of saccules at 20 but not at 40 min, those saccules labeled at the 20 min interval must have been replaced by unlabeled saccules before the 40 min interval. Perhaps then, an entire Golgi stack was renewed in as little as 20 min (or at least within 40 min). With an average of 10 saccules per stack, it is conjectured that a saccule is released in the form of mucigen granules as often as every 2 to 4 min.

Fate of Mucigen Granules

After release from the Golgi stack, mucigen granules appeared to be displaced upward by the continual addition of new granules from below (Figs. 9, 10, 16, 17). It has been reported that mucigen granules coalesce by breaking of membranes and fusion of contents (11, 47). This seldom happens in the goblet cells of the rat colon; usually the size of the mucigen granules did not seem to change as they moved from the Golgi region to the apical cell surface; and indeed, the membranes of the granules usually remained intact as long as

they were inside the cells. The presence of labeled and unlabeled granules side by side (e.g. Fig. 16) also suggested that most mucigen granules did not merge with one another while migrating across the cell.

Finally, mucigen granules were released to the outside through gaps in the apical cytoplasmic layer (Figs. 18 and 19) and, once outside, their membrane broke, freeing the content as mucus.

Functioning of Goblet Cells

A current view of the function of goblet cells is that they gradually accumulate mucigen granules and then discharge them to the outside "by rupture of the apical cell membrane so that the mass of the mucus escapes through the break" (17). The sequence of mucus production and discharge would constitute a "secretory cycle." It has long been disputed whether goblet cells go through one or several such cycles (7, 17, 18). Since the columnar and goblet cells of the rat colon arise from mitosis in crypts, glide up the walls to the surface, and are finally sloughed off into the lumen about 4 to 6 days after they arose (30), a single secretory cycle has been recently postulated by Shearman and Muir (47). These authors suggested that goblet cells synthesize mucigen while in the crypts and, once having attained a surface position, discharge the entire mass of mucigen into the lumen. In line with the concept of secretory cycle, electron microscopists have distinguished "undischarged, full" goblet cells from "discharged, exhausted" cells (10, 11).

Our findings do not support the existence of a secretory cycle in the goblet cells of the colon of 10-g and 100-g rats. First, observations made 5 min after injection of glucose- H^3 revealed that a radioautographic reaction appeared over all goblet cells, whether in the crypts or on the surface. Unpublished experiments done in the morning or the afternoon, including one rat fasted for 18 hr, gave the same results. We tentatively conclude that all goblet cells are continually active in synthesizing the carbohydrates of mucus.

Secondly, radioautographs of colon between 20 min and 4 hr after injection demonstrated that, in all goblet cells, the labeled material came out of the Golgi region in the form of mucigen granules, which then migrated toward the free surface. While the rate of migration varied from cell to cell, migration appeared to be occurring in all (Fig. 10).

Thirdly, the manner in which the mucigen is released may have been misjudged by some authors as a result of harsh fixation procedures. Thus, we found that a segment of young rat colon fixed in Carnoy's (as in the experiments of Shearman and Muir) showed massive discharge of mucus from surface goblet cells, whereas no discharge occurred in an adjacent segment fixed in neutral buffered formalin (unpublished). Isotonic glutaraldehyde, a fixative which minimizes swelling (27), produced no discharge either (Fig. 4); and the electron microscope showed that the apical membranes of crypt and surface goblet cells were not torn off. Indeed, electron microscope observations of crypt and surface goblet cells rather suggest that mucigen granules are released singly, at different points along the apical membrane (Figs. 18 and 19). Such pictures of localized extrusion were frequently observed; at the surface of some cells, several of them were seen in one electron microscope section. Serial sections might well reveal their presence in all cells. Occasionally several mucigen granules are excreted through a single gap. Exceptionally large groups of granules were expelled together. In any case, the evidence

suggests that excretion of mucigen granules is taking place in all goblet cells.

The sequence of events from synthesis to extrusion was observed in goblet cells with few mucigen granules as well as in cells distended by many of them. In the latter, there seemed to be a slowing down of the migration. It is probable that not only the rate of migration but also the rate of synthesis and extrusion varies among individual cells.

Nevertheless, in all goblet cells of the rat colon, the *Golgi synthesis*, the *intracellular migration*, and the *release* of mucus seem to occur *continually* during the 4 to 6 days of the cell's life, that is, until the entire cell is sloughed off into the lumen.

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