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SYNTHESIS OF COMPLEX CARBOHYDRATES IN GOLGI REGION

SYNTHESIS OF COMPLEX CARBOHYDRATES IN THE GOLGI REGION OF SECRETORY CELLS

BY

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To Raymond

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INTRODUCTION

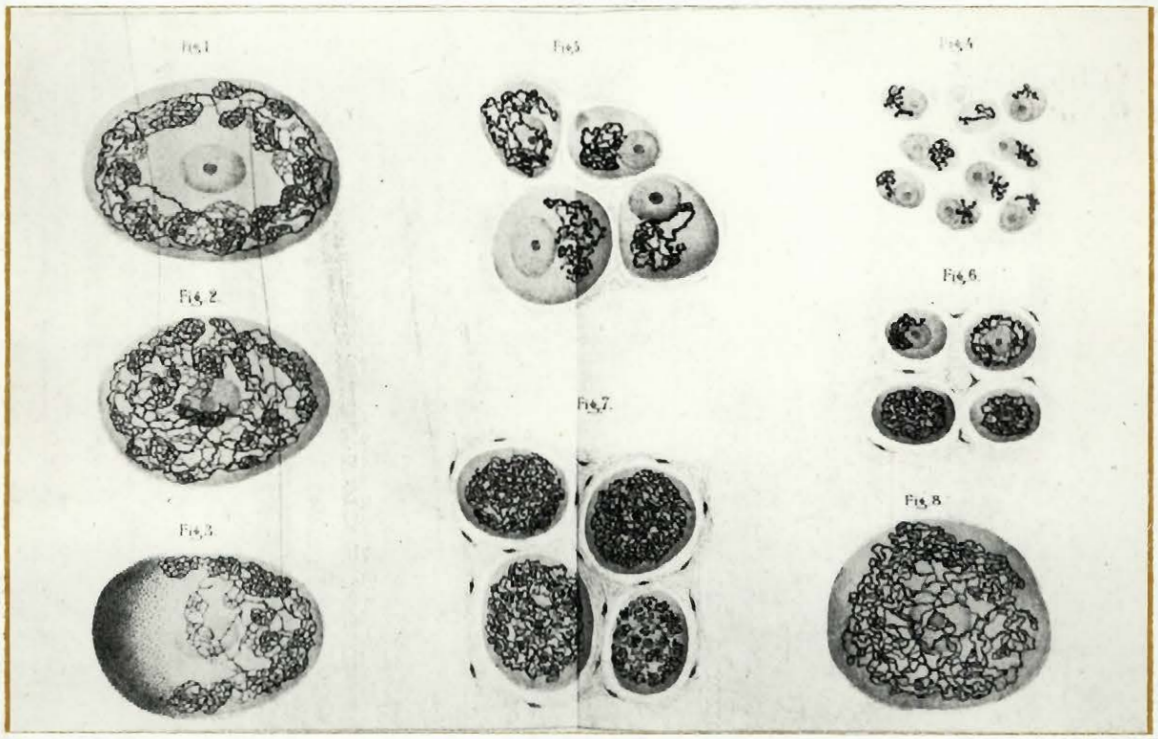
Beginnings: the Golgi apparatus

In 1891, 7 years before Camillo Golgi discovered his famous apparatus, Ramon y Cajal experienced one of his "most deplorable disappointments". While manipulating heavy metal impregnation techniques, he happened to immerse pieces of rat cerebrum in a potassium bichromate-gold chloride sclution, and saw a selective reduction of gold salt in an intracellular apparatus, then unknown. Why did he not repeat the procedure and publish a description? "Well, the confounded reaction never appeared again!" (Ramon y Cajal, 1937).

It was not until 1898 that "l'appareil reticulaire" within the cytoplasm of Purkinje cells was described by Camillo Golgi, eminent professor of histology and pathology at Pavia. The long osmium-silver impregnation technique with which he demonstrated the apparatus was one of many similar methods developed by Golgi and his Spanish contemporary, Ramon y Cajal, to deposit reduced metals on nerve cells and their processes. Why, under certain conditions, this apparatus should reduce metal salts is to this day unknown. Nevertheless, Golgi was able to control the capricious phenomenon well enough to repeat his feat and to publish his now-famous findings (Text Fig. A).

The experience of Ramon y Cajal was only the first of a

Camillo Golgi ....



.... and his drawings.

[A]

(Reproduced from Bertarelli, 1950)

long series of disappointments and rivalries which were to color and complicate the history of the Golgi apparatus.

In the years that followed Golgi's discovery, he and Ramon y Cajal exchanged and modified their procedures until they were able to preserve and impregnate the Golgi apparatus fairly reproducibly. Under the conservative direction of Golgi, members of his Pavia school described the details of the blackened structure in many cells, but carefully refrained from speculating on its function. Others in Spain and Germany followed suit and as a result, the earliest lore of the Golgi apparatus is characterized by serene objectivity.

Essentially, the search was on for a cytoplasmic area that would reduce osmium and/or silver salts. The findings varied from the perinuclear net that Golgi had demonstrated in Purkinje cells to small juxtanuclear nets in other vertebrate cells, and multiple spheroid or disc-shaped bodies (termed "dictyosomes") in the cells of invertebrates, plants and protozoa. In the 40 years following its christening, over 2,200 papers appeared describing the Golgi apparatus in the vast majority of cells, seen by impregnation with osmium, silver, gold, copper or iron (Hirsch, 1939). In most of these, the apparatus was described as a net of anastomosing plates and threads. Others, beginning with Hirschler (1918), believed the connecting threads to be artifacts of over-impregnation; the real apparatus was a system of lamellae

(or "dictyosomes") either arranged as discrete bodies or loosely integrated.

Attempted interpretations

Ramon y Cajal (1914) was the first to seriously speculate about the function of the Golgi apparatus on the basis of its appearance in secretory cells in various physiological states. In unstimulated pancreatic acinar cells, he observed a small compact apparatus flattened above the nucleus: "fase de reposo?" he speculates. In stimulated pancreatic cells, the apparatus was hypertrophied and fragmented among the zymogen granules, resembling the continually extended Golgi apparatus of those active secretors, the intestinal goblet cells. The change and the resemblance suggested to Ramon y Cajal that the Golgi apparatus takes part in the process of secretion by effecting the formation of secretion granules.

This interpretation was extended to the mucous cells of Triton by Nassanow (1923, 1924), to other gland cells by Bowen (1926) and later to many other secretory cells. Hirsch (1939) reviewed and illustrated the appearance of secretion granules within the lamellae of the Golgi apparatus, as best seen in the light microscope.

In non-secretory cells, however, the function of the Golgi apparatus was not so clear. Attributed to this structure were the formation of the acrosome in spermatids (Bowen, 1929), absorbtion of extracellular materials such as the vital dyes

trypan blue and neutral red (reviewed by Bourne, 1951), and regulation of water balance (Nassanow, 1924). Clues were sought in its chemical composition; in various cells, it was said to contain lipid (Baker, 1954), ascorbic acid (Giroud et al, 1934) and alkaline phosphatase (Emmel, 1945).

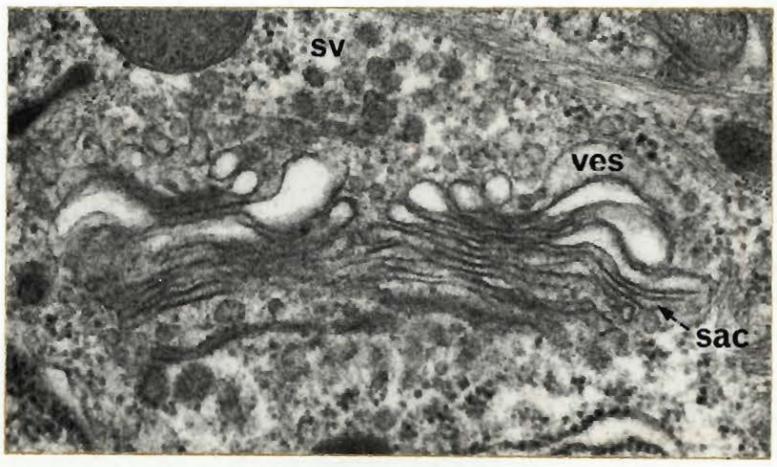
The uncertainty was compounded in 1927 when Walker and Allen claimed that the fixed, impregnated Golgi apparatus is an artifact. Whether its counterpart in the living cells consists of neutral red vacuoles, mitochondria, myelin figures or other materials was the subject of a controversy whose fires were fed by the limitations of light microscopy and those of early electron microscopy as well (see Palade and Claude, 1949). On the other hand, many authors believed that the fixed Golgi apparatus corresponded to a real and distinct organelle in the living cell. The voluminous literature produced in this heat was periodically reviewed (Macklin and Macklin, 1932; Kirkman and Severinghaus, 1938; Hirsch, 1939; Hibbard, 1945; Benseley, 1951; Bourne, 1951).

New light on the apparatus: the Golgi complex

Since the life of the controversy depended on a general lack of precise information about the nature of the apparatus, it was abruptly dissipated as information from electron microscopy appeared, beginning with the work of Dalton (1951) and others in the early 1950's, which established the reality of the apparatus. Its ultrastructural characteristics were

described in cells of vertebrates (Sjöstrand and Hanzon, 1954; Rhoden, 1954; Dalton and Felix, 1954, 1956; Haguenau, 1958; Palay, 1958), invertebrates (Afzelius, 1956), plants (Hodge et al, 1956), metazoa and protozoa (Grasse, 1957; Grasse and Carasso, 1957).

In these widely-differing cells, the Golgi apparatus was recognizable in the electron microscope as a variation on a theme (Text Fig. B). The most constant components were flattened cisternae or saccules (sac), composed of unit membranes 60-70 A thick. They were arranged in parallel array to form stacks, and many such stacks were present in a single cell. In close association with the saccules were found very small dense vesicles (sv) and large vesicles (ves) of varying size. The total complement of stacked saccules, with their associated vesicles was termed the Golgi complex of the cell.



[B]

Was this, then, the osmium or silver-blackened apparatus that had mystified histologists since the time of Golgi and Ramon y Cajal? An examination of osmium-impregnated tissues in the electron microscope revealed black precipitates either in the flattened saccules, the nearby vesicles, or both (Dalton and Felix, 1956). Apparently, each black lamella, dictyosome, or discrete body of the light microscopists' "Golgi apparatus" corresponded roughly to one stack of saccules of the electron microscopists' "Golgi complex".

Interpretations revised

The structural complexities revealed by the electron microscope dispelled some postulated Golgi functions but opened the door to new interpretations.

For example, after administration of large quantities of lipid into the lumen of the intestine, accumulations of lipid droplets were detected in intestinal columnar cells, particularly in the large vesicles of the Golgi complex (Weiss, 1955; Palay and Karlin, 1959). The Golgi complex was thus considered a "way station" for pinocytotic vesicles which were transporting fat across the epithelium. Later studies indicated that the vesicles near the Golgi complex were perhaps not unique in this respect, for fat droplets were seen in the smooth-surfaced endoplasmic reticulum throughout the cell (Ladman et al, 1963; Cardell et al, 1965). Furthermore, pinocytosis may not be a normal fat transport mechanism

(Cardell et al, 1965).

In other cells (particularly those of liver), dense enzyme-containing granules identified as lysosomes were found to arise in the Golgi complex (Novikoff et al, 1962).

In spermatids, Burgos and Fawcett (1955)and Clermont (1956) showed that the Golgi complex was clearly involved in the formation of the acrosome, as Leblond (1950) and Leblond and Clermont (1952) had demonstrated in the light microscope.

Focus on the secretory process

In secretory cells, the observations of light microscopists were similarly reinforced. Here, the large Golgi vesicles, and often the saccules as well, contained material identical to that seen in membrane-bound mucigen granules elsewhere in the cytoplasm. From the bottom of the stack to the top (that is, from one "face" to the other in the direction of the arrow, Text Fig. B) the lumens of the saccules were progressively enlarged until the entire saccule, or peripheral part of a saccule, resembled the nearby vesicles. Thus there seemed to be a progressive transformation of saccules into vesicles. It was concluded that the Golgi saccules give rise to Golgi vesicles which are then released from the Golgi complex as secretion granules. In addition, it was presumed that the loss of Golgi saccules from one face of the stack would be compensated by the formation of new saccules at the opposite face (Grasse, 1957; Haguenau, 1958; Palay, 1958). Of the several

hundred publications that have documented the formation of secretion granules in the Golgi complex (see Hirsch, 1963), only a few are cited here (Farquhar and Wellings, 1957; Dalton, 1961; Frei and Sheldon, 1961; Hirsch, 1961; Ziegel and Dalton, 1962).

In many of these publications, the ultrastructural appearances suggested other functions besides the formation of secretion granules, which nonetheless were correlates of the secretory process. Light microscopists (see Kirkman and Severinghaus, 1938) had conjectured that the Golgi apparatus concentrates secretory material; in the electron microscope, such concentration was suggested by a progressive increase in the electron density of the content of Golgi saccules and vesicles. Somehow, it was thought, the Golgi complex must remove solvent (water) to effect the concentration, and must thus be involved in the cell's "water metabolism". In addition, the ultimate fate of the Golgi membranes was traced. Specifically, the Golgi-derived membranes of secretion granules were seen to fuse with, and become incorporated into the plasma membrane, as their contents were released (Palade, 1959). Analogously, in the mitotic telophase of plant cells, Golgi vesicles containing secretory material were seen to congregate on a plane equidistant from the daughter nuclei and to fuse, forming the new cell membranes (Whaley and Mollenhauer, 1963). Apparently, the Golgi complex was involved in the cell's "membrane metabolism"

(Fawcett, 1962).

Briefly, all of these conceptions of the dynamics of the Golgi complex were derived from study of static electron micrographs. Indeed, the images of this membrane system strongly suggested motion, but such evidence of dynamic phenomena was purely indirect, and the speed with which they occur was anyone's guess.

When the technique of radioautography provided documentation of the secretory process in time, the Golgi complex was again found to play a role, although not an initiating one. The protein-secreting pancreatic acinar cells provided a model for radioautographic studies in the light microscope (Warshawsky et al, 1963) and electron microscope (Caro, 1961; Caro and Palade, 1964; Van Heyningen, 1964). They demonstrated that secretory proteins were synthesized in the rough-surfaced endoplasmic reticulum (presumably in association with ribosomes) and soon appeared in the cisternae of this reticulum. Very rapidly, labeled protein somehow passed into the Golgi complex where it appeared to be concentrated. Within the next 10 to 30 minutes, the labeled protein began to appear in membrane-bound secretion granules. As was expected, the granules with their cargo of labeled protein then left the Golgi region to migrate to the cell surface and release their contents. The striking aspect of these findings was the rapidity with which the Golgi complex segregated labeled protein for export.

Electron microscope radioautography revealed a comparable sequence of events in several other protein-secreting cells such as thyroid follicular cells (Nadler et al, 1964), chondroblasts (Revel and Hay, 1963), fibroblasts (Ross and Benditt, 1965), mammary gland cells (Wellings and Philp, 1965), and plasma cells (Clark and Helmreich, 1965). From these studies of protein secretion, it was clear that the Golgi complex plays an intermediate role of "concentrator" and "packager".

But how do the saccules of the Golgi complex accomplish this concentration and packaging? Is the secretory material chemically altered in the process? In the hope of answering these questions, attempts had already been made to detect the presence of enzymes in this membrane system; these might provide clues of metabolic processes occurring there. To this end, the large Golgi complex of rat epididymal cells was isolated by centrifugation on a sucrose density gradient. In the "Golgi fraction" thus obtained, significantly high concentrations of phospholipid and alkaline phosphatase were found, but tests for many other enzymes were negative (Schneider et al, 1953; Kuff and Dalton, 1959). With the development of histochemical techniques for locating enzymes with the electron microscope, Novikoff and his associates detected nucleoside diphosphatases in the Golgi complex of many cells, and thiamine pyrophosphatase in the flattened Golgi saccules of almost all cells examined (Novikoff et al, 1961, 1962; Goldfischer et al, 1964). Since

the normal function of these enzymes in metabolism is not clear, their presence did not shed light on the function of the Golgi complex.

In summary, since no one had succeeded in demonstrating metabolic processes in the Golgi complex, it was generally believed that secretory proteins were not chemically changed during the process of concentration and packaging. Neither enzyme analysis nor radioautography had indicated synthetic activity in the Golgi complex.

Formulation of the problem

To better understand the process of secretion, it seemed reasonable to begin by taking into account the precise nature of the secretion product. The products of most secretory cells are not pure proteins; rather they consist of proteins associated with carbohydrates. Such carbohydrate-protein complexes are members of a broad class of substances, the "complex carbohydrates". (Glycogen and glycolipids are also complex carbohydrates, but they are not found in secretory materials.)

For example, mucous secretions consist of carbohydrate-protein complexes whose carbohydrate moiety may comprise 70-80% of the molecular weight (Tsuiki and Pigman, 1960; Spiro, 1963). Many serous secretions, once believed to consist purely of proteins, contain relatively small carbohydrate moieties (Mandel and Ellison, 1963; Plummer and Hirs, 1963). Similarly, many exocrine secretions (Leblond et al, 1957) endocrine

secretions, blood group substances and plasma proteins (Spiro, 1963) as well as some connective tissue secretions (Glegg et al, 1954; Dorfman, 1963) are carbohydrate-protein complexes.

In these "complex carbohydrates", the various monosaccharides of the carbohydrate moiety are linked to each other, and to the protein moiety, via glycosidic ester bonds; this implies that synthetic processes must be involved in their formation. Indeed, biochemists have devoted much attention to the synthesis of complex carbohydrates. They have shown that all the necessary monosaccharides - hexoses, hexosamines, sialic acids, fucose and hexuronic acids - may be formed by enzymatic conversions of glucose, without breakdown or rearrangement of the 6-carbon chain (summarized by Dorfman, 1963; Spiro, 1963). In the course of these conversions, each monosaccharide is linked to a nucleotide to form a "nucleotide-sugar", which in turn is capable of donating its monosaccharide for the synthesis of complex carbohydrates (Leloir and Cardini, 1960; Spiro, 1963; Strominger, 1964). In some cases, the carbohydrate moiety is then sulfated (Dorfman, 1963). Either during or after its synthesis, the carbohydrate moiety is linked to the completed protein to form a carbohydrate-protein complex.

Electron microscope radioautography had established the rough-surfaced endoplasmic reticulum as the site of synthesis of the protein moiety, but the intracellular site of synthesis of the carbohydrate moiety was unknown. To determine this site

was the objective of the research reported here.

Implication of the Golgi complex

Before these experiments were launched, however, some important clues had been provided by earlier investigations. While staining intestinal tissue with the periodic acid-Schiff technique, a method which selectively stains certain complex carbohydrates (Leblond et al, 1957), Gersh (1949) had observed that the Golgi region of intestinal columnar cells was stained. Leblond (1950) systematically applied this method to all tissues of the rat, and detected stained material in the Golgi region of several cell types. These results were confirmed by Arzac and Flores (1952). Later, the colloidal iron method for detection of acidic carbohydrates (Mowry, 1963) was found to stain the Golgi region of a wide variety of normal and malignant cells (Badinez et al, 1962). These results indicated that the Golgi region of at least some cell types contains complex carbohydrate. Since glycogen and glycolipid had been removed from these sections by enzymatic digestion and solvents, the stained material was identified as carbohydrate-protein complexes.

In addition, radioautographic evidence had intimated that the Golgi region had something to do with complex carbohydrates for secretion. It was known that radioactive sulfate, when injected into rats, is incorporated into the secretion products of certain mucous cells, such as the goblet cells of intestine

(Belanger, 1954; Dziewiatkowsky, 1956). By examining radioautographs of the tissues one hour after injection of ^{35}S-sulfate, Jennings and Florey (1956) localized the uptake in a supranuclear area which they assumed to be the Golgi region. They concluded that the complex carbohydrate of mucus is sulfated in the Golgi complex. Recently, electron microscope radioautography of goblet cells confirmed that the Golgi saccules are the site of sulfation (Lane et al, 1964).

Was the entire carbohydrate moiety synthesized in the Golgi complex? Or was it synthesized elsewhere, and simply concentrated and sulfated there? Resolution of the problem was attempted by using the technique of radioautography.

Plan of the experiments, and resulting contributions

As summarized above, glucose had been established as the precursor of all the various monosaccharides required for the synthesis of complex carbohydrates. Indeed, Kumamoto (1956) had shown, by radioautography of rat tissues after injection of labeled glucose, that radioactivity was incorporated into the secretion products of a variety of mucous cells, presumably into the complex carbohydrates of mucus. Her work gave no indication of the site of synthesis, however.

To determine the intracellular site at which glucose label is incorporated into complex carbohydrates, ^{3}H-glucose was injected into rats. The tissues were collected and fixed shortly (5-15 minutes) after injection, and at various later

times.

> The radioautographs thus obtained revealed that complex carbohydrates (specifically, carbohydrate-protein complexes) are synthesized in the Golgi region of a variety of secretory cells.

These initial findings, however, were obtained from paraffin sections observed in the light microscope, so that the exact site of synthesis could not be ascertained. Was the glucose label incorporated into complex carbohydrates in the Golgi complex, or in some nearby organelle? If in the Golgi complex, in what component? And then, how did the labeled material leave the complex to be secreted?

These questions could only be answered by a detailed study of a representative secretory cell. Several factors indicated that the goblet cell of rat colon was the cell of choice: First, biochemical studies had revealed that the colonic mucosa of sheep secretes an abundant carbohydrate-protein complex whose carbohydrate moiety contains hexoses, hexosamines, sialic acid and fucose, as well as sulfate residues (Kent and Marsden, 1963). By incubating the mucosa with labeled glucose, Draper and Kent (1963) found that the label appeared in all the various monosaccharides of the carbohydrate moiety, while the protein moiety was not labeled. Secondly, our light microscope radioautographs had shown uptake of large amounts of glucose label in colonic goblet cells.

Thus, the carbohydrate-protein complex which Draper and Kent labeled was most likely a product of colonic goblet cells. Thirdly, the goblet cells of colon are numerous, and are endowed with an extensive Golgi complex (Florey, 1960; Shearman and Muir, 1960). Indeed, after injection of labeled glucose, the goblet cells of rat colon lent themselves well to a radioautographic study in the electron microscope. The study yielded a series of original observations:

> The exact site of synthesis of complex carbohydrate for secretion was ascertained. Direct evidence was obtained concerning the dynamic behavior of the Golgi complex, including the formation of secretory granules and the replacement of Golgi saccules. A hitherto undescribed mode of secretion was observed. Finally, several aspects of the functioning of goblet cells were elucidated.

Although the majority of glucose in goblet cells may be used for the synthesis of complex carbohydrates, this is not the case in all cells. Some glucose is broken down in glycolysis; its metabolites may then be used in the synthesis of other macromolecules such as proteins and nucleic acids. In this light, it seemed useful to trace the uptake of another monosaccharide - galactose - which is also a precursor of complex carbohydrates but is not readily broken down. Accordingly, the behavior of labeled galactose was compared to that of labeled glucose. From these radioautographs, further observ-

ations were made:

- The site of synthesis of complex carbohydrate in mucous cells was confirmed.
- The site of synthesis of complex carbohydrate in a number of non-mucous epithelial cells was demonstrated. (This had not been possible with the glucose precursor.)
- In a variety of cell types, a differential uptake of glucose and galactose was found.

Finally, the incorporation of the monosaccharide glucosamine, used by biochemists as a specific precursor of complex carbohydrates, was studied by radioautography.

- A radioautographic survey revealed the distribution of glucosamine incorporation in rat tissues.
- The site of synthesis of complex carbohydrate in the colonic goblet cell was confirmed.

MATERIALS AND METHODS

Radioautographic experiments were performed by administration of radioactive precursors to white rats of purebred strains (Sprague-Dawley and Fischer) as summarized in Table I. Radioautographs of the tissues were prepared for examination in the light and electron microscopes.

Administration of radioactive precursors

In most experiments, the labeled substance was injected systemically. At suitable time intervals thereafter, the animals were anesthetized with ether and their tissues were collected for fixation. Each time interval indicated in the right hand column of Table I represents one animal injected and sacrificed. By intraperitoneal injections, the uptake of ^{3}H-1-D-glucose was compared to that of ^{3}H-6-D-glucose (experiments 1 and 2). The uptake and subsequent migration of ^{3}H-6-D-glucose label was investigated, first with a low dose injected subcutaneously (exp. 7) and then with a high dose, injected intraperitoneally (exp. 8). In addition, the distribution of ^{3}H-6-D-glucose label was compared to that of other precursors, ^{3}H-1-D-galactose, ^{14}C-1-D-glucosamine and ^{35}S-sulfate, after intraperitoneal injections (exps. 9-13). The incorporation of ^{3}H-4,5-D,L-leucine in the colon was examined after intravenous injection (exp. 14).

In further experiments designed to expose the colonic epithelium of 100-gram rats to large quantities of labeled precursors, the radioactive material was administered either to anesthetized rats by local injection into the lumen of the colon (exps. 3-5, 15), or to a segment of colon by incubation at 37° C in 1 ml Krebs-Ringer Bicarbonate (Paul, 1959) with added ^{3}H-6-D-glucose (exp. 6). By local injection, the incorporation of ^{3}H-1-D-glucose (exp. 5), ^{3}H-6-D-glucose (exps. 3 and 4) and ^{3}H-4,5-D,L-leucine (exp. 15) were compared. In the above experiments, only the colon was fixed.

PARAFFIN SECTIONS: Preparation and Radioautography

Histological techniques

A few tissues were impregnated with zinc iodide - osmium tetroxide according to Maillet (1959) to produce deposits of reduced osmium in some (or all) flattened Golgi saccules (Dalton and Felix, 1956; Friend and Murray, 1965). They were embedded in paraffin, sectioned at 3 microns and examined in the light microscope without further staining or radioautography, to determine the intracellular location of the Golgi complex.

Tissues from each experimental animal were fixed in neutral buffered formalin (Pearse, 1961) for 24-48 hours at 4° C, rinsed in 70% ethanol, embedded in paraffin and sectioned at 4 microns. In experiment 7, a segment of colon was fixed in Carnoy fluid (Pearse, 1961) for 24 hours and was processed

with the formalin-fixed tissues. A few sections from each experiment were stained with hematoxylin and eosin for histology and radioautography.

Histochemical techniques

Deparaffinized sections (some of which were subjected to the enzymatic treatments described below) were stained by methods to detect carbohydrates. The periodic acid (PA)-Schiff technique for the detection of the vicinal hydroxyl groups of glycogen and glycoproteins (Leblond et al, 1957; Pearse, 1961) was applied either without counterstain or with hematoxylin counterstain. The colloidal iron method for detection of the carboxylic and sulfuric acid groups of carbohydrates (Mowry, 1958, 1963) was followed by counterstaining with the Feulgen reaction for nuclear DNA (Pearse, 1961). Feulgen counterstaining enhanced the definition of the colloidal iron stain and prevented the partial desensitization of the emulsion produced when colloidal iron alone was used to pre-stain radioautographs. A few paraffin sections were stained with toluidine blue at neutral pH to detect metachromatic, highly acidic carbohydrates such as chondroitin sulfate (Pearse, 1961).

Enzymatic treatments

Alpha amylase digestion was performed on deparaffinized sections from all glucose and galactose experiments, by immersion in filtered saliva at 60° C for 10 or 20 minutes. This procedure removed cytoplasmic PA-Schiff-positive material

(which was then assumed to be glycogen) from liver, muscle, and several other tissues. Other enzymatic treatments were carried out only on ^{3}H-glucose-labeled tissues from experiment 8.

For hyaluronidase treatment (Calbiochem., bovine testicular, activity 380 IU/mg) sections were incubated in a 0.05% enzyme solution in phosphate buffer pH 5.5 for 4 hours at 37° C, while controls were incubated in buffer without enzyme. After thorough rinsing, they were stained with colloidal iron-Feulgen for radioautography, or with toluidine blue for histology. The toluidine blue metachromasia of cartilage matrix was completely abolished in the enzyme-treated sections. The metachromatic material extracted by the enzyme was assumed to be chondroitin sulfuric acids A or C, or hyaluronic acid (Pearse, 1961).

Beta glucuronidase (Calbiochem., bovine liver, activity 360 IU/mg) was used according to the method of Fullmer (1960). Sections were oxidized in peracetic acid for 30 minutes at room temperature, then incubated in 50 ml acetate buffer pH 4.5 containing 15 mg beta glucuronidase, for 4 hours at 37° C. Three types of control sections were prepared: some were treated with peracetic acid alone, others were incubated in buffer without enzyme, and still others were incubated in buffered enzyme solution, without prior peracetic acid oxidation. After rinsing, the sections were stained with colloidal iron-Feulgen for histology and radioautography. Only the acid-

enzyme sequence abolished the colloidal iron staining of mucous cells.

Radioautographic methods

Each group of sections for radioautography usually included an unstained section, and sections pre-stained with hematoxylin-eosin, PA-Schiff, or colloidal iron-Feulgen. In all glucose and galactose experiments, an amylase-treated section was included. In experiment 8, sections treated with hyaluronidase or beta glucuronidase were included as well. All of these were coated by dipping in liquid Kodak NTB2 emulsion (Kopriwa and Leblond, 1962). After exposure, development and mounting of coverslips, they were examined with the light microscope.

Assessment of radioautographic reactions

It was not possible to make quantitative assessments of the radioautographic reactions, for several reasons. First, there is variation in glucose metabolism among individuals: in our experiments, this was reflected in differing reaction intensities observed after equal doses of ^{3}H-glucose. Second, the specific activities (Table I) and the overall metabolism of the various precursors varied greatly, so that the amounts of label available to a given tissue were not comparable. Third, the radioautographic reactions often were confined to a small area of cytoplasm, and appeared as dense clumps of silver grains in which the individual grains were obscured.

Thus, the reactions over various tissues after administration of a single precursor were only very roughly quantitated; in a given tissue, the reactions after different precursors were compared qualitatively.

In enzyme studies, however, the adjacent sections used were not subject to animal or precursor variability. Thus, when individual grains were visible, the reactions were qualitatively assessed by grain counting. Counts were made in representative tissues and were expressed as silver grains per unit area, or per cell. By comparing counts over enzyme-treated sections with those over controls, the percentage of radioactive material extracted by the enzyme was calculated.

PLASTIC SECTIONS: Preparation and Radioautography

Fixation and embedding

For improved resolution of radioautographs in the light microscope, or for localization of label in the electron microscope, colon and a few other tissues were fixed by electron microscope techniques and were embedded in plastic media.

In the series of ^{3}H-glucose injected rats (exp. 8), colon and pancreas were excised and fixed in isotonic, phosphate-buffered 2.5% glutaraldehyde (Hommes et al, 1966) at 4° C for 1 hour, rinsed for 30 minutes in 4 changes of buffer, postfixed in isotonic veronal-buffered 1% osmium

tetroxide (Warshawsky, 1965) at 4° C for 1.5 hours, dehydrated in graded alcohols and embedded in Epon (Luft, 1961). After injection of ^{14}C-glucosamine (exp. 11) or ^{3}H-galactose (exp. 10), colon and liver were similarly fixed and embedded. To remove glycogen from ^{3}H-galactose-labeled liver, a modification was introduced: small blocks of glutaraldehyde-fixed liver were incubated at 37° C in a phosphate-buffered 1% solution of alpha amylase (Worthington Biochemical Corp., hog pancreas, twice crystallized, activity 712 U/mg) for 2 hours before postfixation and embedding. Controls were incubated in buffer without enzyme (Coimbra, 1965).

Segments of colon from the rat injected locally with ^{3}H-glucose (exp. 4) and from the series of rats injected intravenously with ^{3}H-leucine (exp. 14) were fixed in veronal-buffered 1% osmium tetroxide (Zetterqvist, 1956). Similar segments from the ^{35}S-sulfate injected rats (exp. 12) were fixed in glutaraldehyde and postfixed in osmium tetroxide as described above. These tissues were embedded in methacrylate.

Half-micron sections

For light microscope radioautography, half-micron sections of all plastic-embedded tissues were placed with a wire loop on glass slides and were coated by dipping in liquid Ilford L4 emulsion (1 part emulsion in 3 parts distilled water) or in undiluted Kodak NTB2 emulsion. After exposure and development (10 minutes in D 170), a drop of filtered 1% toluidine blue

was placed on the slide, allowed to stand at room temperature for 5 minutes, and rinsed off with distilled water. After drying, a coverslip was mounted.

Ultrathin sections

For electron microscopy, ultrathin sections (gold-silver interference colors, 700-1000 A; Pease, 1964) were cut from the same blocks as the half-micron sections. Sections of colon and liver for morphological studies were placed directly on copper grids, stained with lead hydroxide (Karnovsky, 1961) and/or uranyl acetate (Pease, 1964), and were observed and photographed in a Siemens Elmiskop I.

Sections for radioautography were prepared in two ways: some (exps. 4, 8) were placed on formvar-coated grids (Revel and Hay, 1963) and were dipped in melted emulsion. After development, the grids were allowed to dry for about 20 minutes, then were removed from the slides and stained for 15-20 minutes with drops of lead hydroxide. Other sections (exp. 8: 4-hour interval, and exp. 11) were mounted on celloidin-coated glass slides, coated with emulsion and after development, were stripped from the slides and mounted on grids (Salpeter and Bachmann, 1964). This method, followed by prolonged staining through the celloidin with lead citrate (Reynolds, 1963) yielded better preparations than the former technique.

Whatever the method of preparation, the sections were coated either with Ilford L4 emulsion (1 part emulsion in 5

parts water) or with Gevaert 307 emulsion (2 parts emulsion in 1 part water). After exposure ranging from 3 weeks to several months, they were developed for 2 minutes in D19B at 18° C, poststained as described above, and finally examined and photographed in a Siemens Elmiskop I.

OBSERVATIONS PART ONE:

Summary of results obtained from radioautographs of paraffin sections

Soon after administration of ^{3}H-glucose, ^{3}H-galactose or ^{35}S-sulfate, radioautographs of a variety of secretory cells revealed that most of the radioactivity was localized in a small cytoplasmic area.

I. Localized radioactivity

Uptake of labeled precursors

At 5, 10 or 15 minutes after either ^{3}H-6-glucose or ^{3}H-1-glucose administration (systemic, local or *in vitro*, exps. 1-9) radioautographic reactions appeared over all types of mucus-secreting cells. The great majority of silver grains were confined to a supranuclear region, a position occupied by the Golgi complex (Table II). Such localized supranuclear reactions were taken to indicate the presence of radioactivity in the Golgi region, or "Golgi-localized label". When high doses of ^{3}H-glucose were administered systemically (exp. 2), locally (exps. 3-5) or in vitro (exp. 6), the Golgi-localized label in mucous cells was intense and in addition, a reaction often appeared over non-mucous epithelial cells, in a supranuclear area again corresponding to the Golgi region. In chondrocytes, clusters of silver grains appeared over the paranuclear cytoplasm; since the Golgi complex is similarly

located, this was also considered to be Golgi-localized label.

At 10 minutes after injection of ^{3}H-galactose (exp. 10) radioautographic reactions appeared over some (but not all) types of mucous cells, over several other types of epithelial cells, and over chondrocytes. In each case, the silver grains were localized over a cytoplasmic region occupied by the Golgi complex, and were assumed to indicate Golgi-localized label (Table II).

Similarly, 5 or 10 minutes after ^{35}S-sulfate injection (exps. 12 and 13) silver grains were located over the Golgi region of some (but not all) types of mucous cells, and of chondrocytes (Table II) as previous authors had observed (Jennings and Florey, 1956).

From these observations it was concluded that in the Golgi region, the labels from ^{3}H-l- or ^{3}H-6-glucose, ^{3}H-l-galactose and ^{35}S-sulfate were incorporated into substances retained during histological processing. A comparison was then made of the relative amounts of Golgi-localized radioactivity (i.e. density of silver grains) in various cells after injection of a single precursor; these were roughly quantitated as + (light) to ++++ (intense), and were recorded in a single column of Table II. In a given cell type, however, the amounts of radioactivity present after injection of different precursors were not quantitatively comparable (see Materials and Methods, Assessment of radioautographic reactions). Thus,

although similar doses were given (exps. 9 and 10) the results were qualitatively compared.

Migration of labeled substances

The radioactivity derived from ^{3}H-glucose, ^{3}H-galactose and ^{35}S-sulfate was located in the Golgi region only at the early time intervals. Later, radioautographic reactions were observed over the apical (or peripheral) cytoplasm, progressively farther from the Golgi region and nearer to the secretory surface of the cell. Finally, reactions appeared over the extracellular secretory products. Although this apparent migration of radioactive material was observed in all the cell types listed in Table III, the speed of migration varied considerably. This speed was roughly estimated on the basis of radioautographs obtained 5 minutes to 4 hours after ^{3}H-glucose injection in 10-gram rats (exps. 7 and 8), or 10 and 30 minutes after ^{3}H-glucose or ^{3}H-galactose injection in 20-gram rats (exps. 9 and 10). Although some variation was seen among cells of a given type, the Golgi-localized label in mucous cells began to appear outside the cells at 3 or 4 hours after injection (except in Brunner's glands, where label appeared in the lumens after 1 or 2 hours). In non-mucous epithelial cells and chondrocytes, migration of Golgi-localized radioactivity (most clearly followed after ^{3}H-galactose injection) was much more rapid (Table III).

Effect of enzymatic treatments on localized label

To identify the labeled substances seen first in the Golgi region and later in secretion products, the reduction of radioautographic reactions by specific enzymes was assessed, either subjectively or by counting of silver grains. The effects of the enzymes on ^{3}H-glucose labeled substances in representative secretory cells are summarized in Table IV. Radioactive material which was removed by an enzymatic treatment was assumed to be the specific substrate of that enzyme.

Although salivary alpha amylase did remove labeled material (thus identified as glycogen) from the cytoplasm of some cells, it removed no ^{3}H-glucose label from the Golgi region or the secretory material of any cell observed. Nor did alpha amylase remove the Golgi-localized label seen after ^{3}H-galactose injection.

Hyaluronidase had no effect on the reactions over epithelial cells, but did reduce the reactions over connective tissue cells and matrix. The radioactive substances removed were thus identified as acid mucopolysaccharide (specifically, hyaluronic acid or chondroitin sulfuric acids A or C).

Inversely, the sequence peracetic acid-beta glucuronidase did not affect the radioactive substances in connective tissue cells and matrix, but drastically reduced the localized reactions over mucous epithelial cells.

II Scattered radioactivity

Distribution of scattered label

Although the great majority of ^{3}H-glucose label in mucous cells was localized in the Golgi region or secretory material, a small amount of label was scattered throughout the cytoplasm, outside the Golgi region and mucigen. In mucous cells, the intense Golgi-localized reactions were easily distinguished from the light scattered reactions. In non-mucous epithelial cells, however, particularly after high doses of ^{3}H-glucose, this scattered label often made it impossible to clearly distinguish Golgi-localized label. The amount of scattered label in all cells increased at late time intervals (Table V, control).

In most cells, ^{3}H-galactose label was limited at first to the Golgi region. Only in cells of muscle, liver, and colonic epithelium was scattered label observed (Table V, control).

Effect of enzymatic treatments on scattered label

Hyaluronidase, or the sequence peracetic acid beta glucuronidase had no effect on the scattered label. The amount of this label extracted from a few cell types by amylase digestion is summarized in Table V. Amylase extracted large quantities of cytoplasmic ^{3}H-glucose-labeled material from striated muscle, but little or none from intestinal goblet and columnar cells, liver, pancreas and other cells. That is, most of the ^{3}H-glucose scatter remained in these cells after extraction of

glycogen. However, amylase digestion extracted all the scattered ^{3}H-galactose-labeled material (except for a small amount in muscle fibers, Table V).

Thus, in amylase-digested sections, ^{3}H-galactose radioactivity was limited to the Golgi region at 10 minutes after injection, permitting detection of small amounts of Golgi-localized label in non-mucous epithelial cells (Table II).

III. Uptake of ^{14}C-1-D-glucosamine

After injection of ^{14}C-glucosamine, radioautographs of paraffin sections showed that the tissues had incorporated widely varying amounts of radioactivity (Figs. 1 and 2). The distributions of label at the 10- and 30-minute intervals were similar; (the heavier label at 10 minutes was due to the higher specific activity of the precursor, exp. 11). Since the radioautographic resolution of the ^{14}C label was poor in 4-micron paraffin sections, the distribution of label in various tissues was evaluated in unstained radioautographs at low magnification (Figs. 1 and 2, Table VI).

OBSERVATIONS PART TWO:

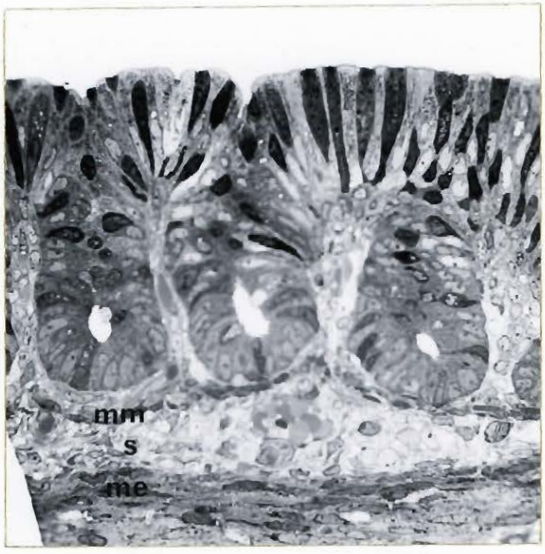
Results obtained in selected cell types

MUCOUS CELLS

I. GOBLET CELLS OF COLON

A. STRUCTURE

The organization of the colon of 10- or 20-gram rats is best seen in half-micron sections, stained with toluidine blue (Text Fig. C). Within the muscularis externa (me) are the submucosal connective tissue (s), the muscularis mucosae (mm) and the mucosa. A simple columnar epithelium lines the lumenal surface and dips into crypts, 3 of which are seen in Text Figure C. The goblet cells are distinguished by the dark staining of their cytoplasm with toluidine blue. They are abundant, both in the crypts where they are short (20-25 μ) and on the surface where they are narrow and tall (40-45 μ).



[C]

The surface goblet cells, because of their abundance and size, are readily located in the electron microscope (Fig. 3). Their ultrastructural organization is relatively constant, and has been represented schematically in Figure 4. 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. The supranuclear region is dominated by an extensive Golgi complex. In longitudinal section, the Golgi complex appears U-shaped (Figs. 3 and 4, left) and in a transverse section through the supranuclear region, it describes a ring (Figs. 4, lower right, and 5). 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 within 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-bound saccules (also called 'flattened vesicles' or 'cisternae') arranged in several stacks. In longitudinal section, up to 5 stacks may be seen (Fig. 3) and in cross section, as many as 4 (Fig. 5). Although the stacks usually seem to be separated by cytoplasm (Fig. 5, c) adjacent

stacks may be partially continuous (Fig. 6, con). These continuations suggest that some or all of the stacks may be interconnected on a plane above or below the plane of section.

Each stack consists of 7 to 12, usually 10, saccules (Figs. 5, 6 and 18). In the descriptions that follow, the saccules on the outer aspect of the Golgi cup (nearer the lateral cell membrane or nucleus) are termed "peripheral", and those nearer the central mucigen granules are termed "central". The peripheral saccules are generally so flattened that their lumens are virtual (Figs. 5, 6 and 18, ps). In some stacks, however, the outermost saccule (Fig. 18, arrows) or saccules (Figs. 5 and 6, arrows) have visible lumens and adjacent to them, the cisternae of endoplasmic reticulum often show local loss of ribosomes (Figs. 5 and 6, rER). In all stacks, the central saccules (cs) are irregularly distended. A gradual increase in the size of the lumens is seen from peripheral to central saccules. 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 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 granules, as proposed by previous authors (Bierring, 1962; Florey, 1960; Freeman, 1962; Shearman and Muir, 1960).

Within a goblet cell, central and apical mucigen granules appear to be of a similar size. Although their membranes sometimes are broken, as others have described (Freeman, 1962; Shearman and Muir, 1960) they more often are intact, even next to the apical surface. In this region (Figs. 7, 8 and 29) a thin layer of cytoplasm is insinuated between the mucigen granules and the apical cell membrane. Occasionally, one or more clefts are seen across this layer, through which single, membrane-bound mucigen granules seem to be passing into the lumen (Figs. 7 and 29). When the plane of section passes to one side of the cleft (Fig. 8) the granule appears to be escaping under a cytoplasmic "bridge". Indeed, free granules are found in the lumen; these may have a broken membrane and their contents may be partly released (Fig. 29, upper left).

In the goblet cells of the crypts, all of the elements described above are present, but their arrangement is modified by the presence of great numbers of mucigen granules. In cells high in the crypts (near the lumenal surface, Fig. 9) the nucleus and basal cytoplasm seem to be squeezed toward the basement membrane by the tightly-packed mucigen granules above. The Golgi complex does not form a large U, but usually occupies a smaller region, just above and lateral to the nucleus. In cells deep in the crypts (Fig. 10) the nucleus and part of the supranuclear region may be pressed against the basement membrane while the Golgi stacks are insinuated among the mucigen granules, along

with some rough-surfaced endoplasmic reticulum and mitochondria. When examining radioautographs of crypt goblet cells in the light microscope, these structural variations must be considered.

B. RADIOAUTOGRAPHIC RESULTS: ^{3}H-GLUCOSE

Light microscopy

Five to 15 minutes after administration of ^{3}H-glucose, an intense radioautographic reaction appeared over the Golgi region of colonic goblet cells. This localized uptake was repeatedly observed, regardless of the position of the tritium label (carbon 1 or 6) the route of administration (subcutaneous, intraperitoneal, local, or in vitro) and the animals' age and weight (10, 20 or 100 grams). The uptake and subsequent migration of label was most clearly seen in radioautographs of half-micron sections of colon from 10-gram rats, after intraperitoneal injection of ^{3}H-glucose (exp. 8, Figs. 12-17).

In longitudinal sections of surface goblet cells taken 5 minutes after injection (Figs. 12 and 14) a U-shaped band of silver grains was observed above the nucleus, corresponding in shape and extent to the Golgi complex cut as in Figures 3 and 4 (left). In transverse section (Fig. 13, a) a ring of silver grains was seen around the central mucigen, corresponding to the Golgi complex cut as in Figures 4 (lower right) and 5.

Longitudinal sections of goblet cells from a rat sacrificed at 20 minutes after injection (Fig. 15) again showed a U-shaped

band of silver grains which was, however, denser and wider than at 5 minutes. At 40 minutes and 1 hour, there was still an intense radioautographic reaction, but now the silver grains formed a solid mass over the supranuclear region. By 1.5 hours (Fig. 16) the mass occupied both the supranuclear region and the lower part of the apical mucigen. Finally, at 4 hours (Fig. 17) 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 the mucus present in the lumen. In the goblet cells of the crypts, the labeled material usually had not reached the apical cell surface by 4 hours after injection. Rather, it appeared at intermediate levels of the apical region, separated from the lumen by variable amounts of unlabeled mucigen.

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

Thus it was clear that the ^{3}H-glucose label had been incorporated in the Golgi region into substances which were destined for secretion. But were these substances restricted at first 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 minutes after intra-

peritoneal or local injection of ^{3}H-glucose showed silver grains over the stacks of Golgi saccules (Fig. 19) where they were distributed fairly evenly, with no detectable preference for peripheral or central saccules. At this time, the mucigen granules, rough-surfaced endoplasmic reticulum and other organelles were not significantly labeled.

At 20 minutes (Figs. 20 and 21) silver grains were still seen over Golgi saccules throughout the stack, but some now appeared over the nearby mucigen granules as well. Because the width of a silver grain was several times that of a flattened Golgi saccule, the silver grains overlay both membranes and lumens, so that the radioactive source could have been in either or both. In the case of the mucigen granules, however, the grains were usually over the contents rather than over the limiting membrane.

By 40 minutes (Figs. 22 and 23) the bulk of the radioactive material was in the central mucigen granules, while the Golgi saccules were almost completely without label. By 1 or 1.5 hours (Figs. 24, 25 and 26), this localization had changed little, although some labeled granules were found farther from the Golgi complex. Labeled and unlabeled granules were found lying side by side, their limiting membranes intact.

The position of these labeled mucigen granules at 4 hours varied considerably from cell to cell, as predicted from the study of the half-micron sections. Usually, the most apical

granules were heavily labeled, with a few lightly labeled just below (Fig. 27). In the apex of some cells, a "front" of heavily-labeled granules was seen (Fig. 28). The U-shape of the front in longitudinal section was reminiscent of the U-shape of the Golgi complex. Thus, the labeled mucigen granules near the lateral membrane had reached the apical surface of the cell, while those in the central region were in a lower position. At the apex of goblet cells, both on the surface (Figs. 28, 30 and 31) and in the crypts (Fig. 32) the occasional labeled granule was seen in the process of being extruded through a cleft in the apical cytoplasm. Finally, labeled material was seen in the lumen, near the epithelial cells.

Effect of fixation on release of mucigen

From the radioautographs of glutaraldehyde-fixed colon studied in both light and electron microscopes, it seemed that the rate of migration of labeled material varied among goblet cells. Nevertheless, most of the surface goblet cells had begun to release labeled mucigen at about 4 hours after injection of the labeled precursor (Table III). In this process, their apical membranes were not disrupted (Figs. 11 and 29). Similarly, in paraffin sections of colon fixed in neutral buffered formalin 3 hours after injection of ^{3}H-glucose, the apical membranes of surface goblet cells usually appeared intact; at this time, the radioactive mucigen was within the cells (Figs. 35 and 36). But when an adjacent segment of colon from the same

animal was fixed in Carnoy, the apical membranes of surface goblet cells were disrupted and most of the labeled mucigen had streamed into the lumen (Figs. 33 and 34).

Effect of enzymatic treatments on staining properties and localized radioactivity

When histochemical methods for carbohydrates are applied to paraffin sections of rat colon, the mucigen of goblet cells is intensely stained. Thus, it is colored bright magenta by the PA-Schiff technique (Fig. 35), turquoise blue by the colloidal iron method (Fig. 37) and by Alcian blue (Spicer, 1960), and metachromatically (violet) by toluidine blue (Spicer, 1960). Loss of these staining properties after enzymatic treatments would indicate specific removal of carbohydrate-containing material. Concomitant reduction of the radioautographic reaction would suggest that the carbohydrate material removed was radioactive.

Neither alpha amylase nor testicular hyaluronidase reduced the intensity of staining or the localized radioactivity of goblet cells. In contrast, the sequence peracetic acid-beta glucuronidase, reported to remove PA-Schiff-stained material from goblet cells (Fullmer, 1960) removed colloidal iron-stained material as well; simultaneously, it extracted much radioactivity from the Golgi region at early times and from the mucigen at later times after injection (Figs. 37-40).

Scattered label after ^{3}H-glucose injection

Although the great majority of ^{3}H-glucose label was localized in the Golgi region or mucigen of goblet cells, a light scatter of label appeared over the rest of the cytoplasm, especially after high doses of ^{3}H-glucose (Figs. 12-17). At late time intervals, when the mucigen was intensely labeled, this scatter was enhanced and appeared in electron micrographs as random grains over various organelles (Figs. 25 and 26, sc). In paraffin sections, scattered label was only slightly reduced by alpha amylase, and was not reduced by hyaluronidase or beta glucuronidase treatments (Fig. 40).

C. RADIOAUTOGRAPHIC RESULTS: OTHER PRECURSORS

^{3}H-galactose

When the goblet cells of colon were examined 10 minutes after injection of ^{3}H-galactose, a radioautographic reaction appeared over the Golgi region (Fig. 44); this reaction was enhanced at 30 minutes (Figs. 45, 46 and 49). In both paraffin and half-micron Epon sections, some silver grains were scattered in the basal cytoplasm of goblet cells (Figs. 45 and 46). When paraffin sections were treated with alpha amylase, however, this scattered label was completely removed, leaving only the Golgi-localized label (Figs. 42 and 43).

^{14}C-glucosamine

At 10 and 30 minutes after ^{14}C-glucosamine injection, radioautographs of half-micron Epon sections revealed the

presence of label in the Golgi region of colonic goblet cells, both on the surface (Fig. 48) and in the crypts (Figs. 47 and 50). Since the radioautographic resolution of the ^{14}C label was not as precise as that of ^{3}H, and since in crypt goblet cells the Golgi complex is intimately associated with other organelles (Fig. 10), the localization of label was further examined by radioautography of EM sections. At 10 minutes after injection (Fig. 51) most of the silver grains observed were indeed located over the flattened saccules of the Golgi complex. At 30 minutes, they were over both the saccules and the nearby mucigen granules (with a few grains over other organelles).

^{35}S-sulfate

Similarly, 5 to 15 minutes after ^{35}S-sulfate injection (Fig. 53) radioactive material was localized in the Golgi region of goblet cells, as reported by previous authors (Jennings and Florey, 1956; Godman and Lane, 1964). At the one hour interval (Fig. 54) it was detected throughout the supranuclear region and at 3 hours (Fig. 55) in the apical mucigen.

^{3}H-leucine

In contrast, radioautographs of colon at 6 and 20 minutes after ^{3}H-leucine injection revealed label widely distributed over the epithelium (Figs. 56 and 57). The reaction over goblet cells was not localized in the Golgi region; rather, it was particularly heavy in the basal and perinuclear cytoplasm, corresponding to the distribution of the rough-surfaced endoplasmic reticulum (Figs. 3 and 4).

II. GOBLET CELLS OF DUODENUM

A. STRUCTURE

On the duodenal villi of young rats, the goblet cells are scattered among the more numerous columnar cells (Fig. 58). The base of the goblet cell is regularly occupied by the nucleus, and the apical third is distended by a dense accumulation of mucigen. The supranuclear region contains a central column of mucigen granules extending from the nucleus to the apical mucigen; this column is surrounded by cytoplasm containing the Golgi complex (McNabb, 1964). The mucigen of goblet cells of the small intestine, like that of large intestine, is stained by histochemical methods for carbohydrates such as PA-Schiff (Fig. 58), colloidal iron (Fig. 69) and Alcian blue, and is metachromatic (Spicer, 1960).

B. RADIOAUTOGRAPHIC RESULTS

The radioautographic results were similar in goblet cells throughout the small intestine. At 5, 10 or 15 minutes after administration of ^{3}H-glucose (labeled either at carbon 1 or 6) to 10-, 20- or 100-gram rats, a radioautographic reaction appeared over duodenal goblet cells, localized in the Golgi region (Figs. 59-61). The extent of the labeled area depended on the height of the column of supranuclear mucigen granules (Fig. 61), while the intensity of the reaction depended on the dose of precursor given, and the route of administration. In all cases, however, the label was localized in the Golgi region.

These cells also incorporated ^{35}S-sulfate in their Golgi region, as reported by others (Jennings and Florey, 1956).

In contrast, goblet cells of small intestine incorporated no detectable ^{3}H-galactose label at 10 (Fig. 62) or 30 minutes after injection. Half-micron sections of duodenum, 10 minutes after ^{14}C-glucosamine indicated that the goblet cells had incorporated relatively little label.

The migration of ^{3}H-glucose-labeled material in duodenal goblet cells was traced from the Golgi region to the apical cell surface (Figs. 63-68). Labeled material was present in the supranuclear region at 5, 20 and 40 minutes after injection. At 40 minutes and later, it appeared in the apical region, although its position in various cells varied considerably (Fig. 67). In most cells, the label had reached the apical cell surface by 4 hours after injection (Fig. 68).

The effects of enzymatic treatments paralleled those observed in colonic goblet cells. Of the three enzymes used, only the sequence peracetic acid-beta glucuronidase was effective in removing the colloidal iron-stained mucigen and its associated radioactivity (Figs. 69-72).

Scattered label, not associated with secretory material, appeared throughout the epithelium at 40 minutes and later after injection of high doses of ^{3}H-glucose (exp. 8, Figs. 65-68). In goblet cells, it was seen in the basal cytoplasm. None of the enzymes used, including alpha amylase (Figs. 65-68)

reduced this scatter. After ^{3}H-galactose injection, however, no scattered label was detected (Fig. 62).

III. OTHER TYPES OF MUCOUS CELLS

The estimated intensities of the radioautographic reactions summarized in Table II indicate that all types of mucous cells observed incorporated ^{3}H-glucose label in their Golgi region; for example, the surface mucous cells of stomach were heavily labeled (Fig. 74). In certain of these types, a considerable amount of ^{3}H-galactose label was incorporated as well (Brunner's gland, sublingual gland, and goblet cells of colon). In other types, however, little or no ^{3}H-galactose label was detected, even after long exposure (mucous cells of tracheal glands, surface of stomach, Fig. 75; submaxillary gland, and duodenum). Similarly, ^{35}S-sulfate incorporation was observed in some mucous cell types but not in others, as previously reported (Jennings and Florey, 1956). No correlation between uptake of galactose and sulfate could be found.

The effects of enzyme treatment on mucous cells were similar to those observed in goblet cells. Thus, only the sequence peracetic acid-beta glucuronidase removed radioactive and colloidal iron-stained material from the surface mucous cells of stomach (Figs. 76-79). In this case, however, peracetic acid alone extracted some labeled material (Fig. 78).

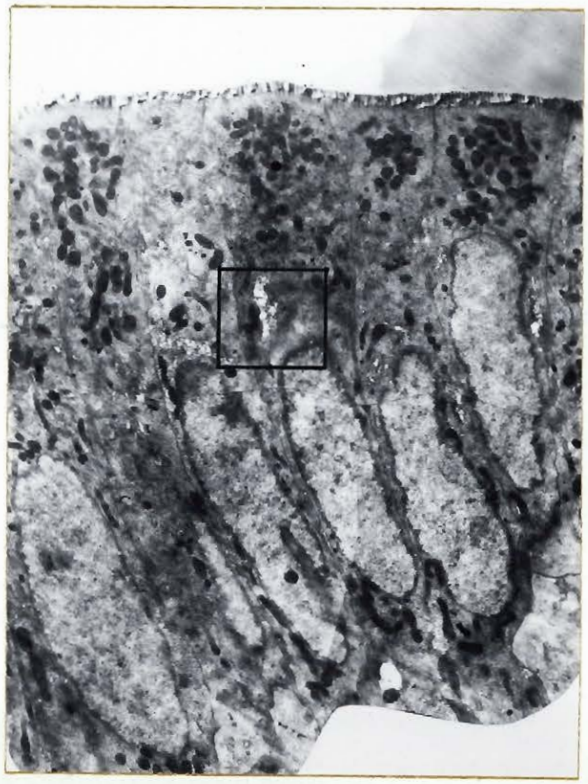
VARIOUS EPITHELIAL CELLS

I. COLUMNAR CELLS OF INTESTINE

Uniform columnar cells comprise much of the surface epithelium of small and large intestine. In both locations, they are morphologically and histochemically similar. Since the radioautographic observations of duodenal and colonic surface columnar cells were generally similar as well, they will be presented together.

A. STRUCTURE

In longitudinal section, these tall, regular cells are rectangular, with oval nuclei and light-staining cytoplasm; their striated apical border is evident in half-micron, toluidine blue-stained sections (Text Fig. C). In the electron microscope, this border is seen to consist of uniform, closely-packed microvilli (Text Fig. D).



[D]

On closer inspection (Fig. 80) the microvilli are covered by a layer of fuzzy material and at their tips this fuzzy coat is continuous with a thicker layer of fine, filamentous material, as others have described (McNabb, 1964; Ito, 1965). Above and lateral to each columnar cell nucleus, small vertically-oriented Golgi saccules are seen (Text Fig. D). At higher magnification (Fig. 81) each element of the Golgi complex is seen to consist of a stack of flattened saccules.

When paraffin sections of intestinal epithelium are prepared by the Maillet technique (Fig. 87) the Golgi elements are stained black, and can be located in the light microscope as small lamellae (each corresponding to one stack of saccules). In addition, these lamellae are stained by histochemical methods for carbohydrates such as colloidal iron (Figs. 86 and 88) and PA-Schiff (Fig. 58). Material on the striated border which has been identified as the fuzzy, filamentous coat (Ito, 1965) is also stained by these methods (Figs. 58, 86 and 88).

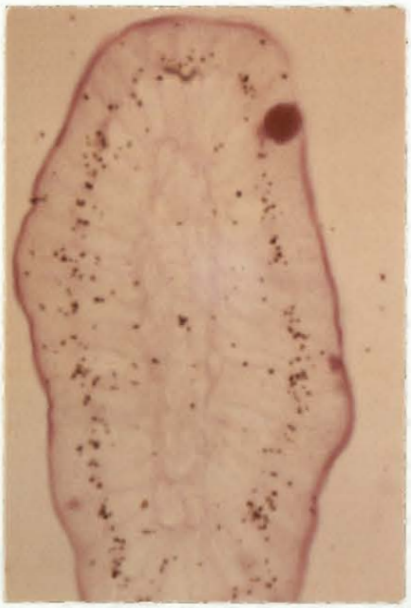
B. RADIOAUTOGRAPHIC RESULTS

Low or moderate systemic doses of ^{3}H-l- or ^{3}H-6-glucose (exps. 1, 7 and 9) produced no detectable labeling of duodenal columnar cells (Figs. 59 and 90) and variable labeling of colonic columnar cells (Fig. 41). However, 5 to 15 minutes after high systemic doses (exp. 2), local injection (exps. 3-5) or administration *in vitro* (exp. 6), radioactive material was detected in the Golgi region (Figs. 82-85). Such high doses

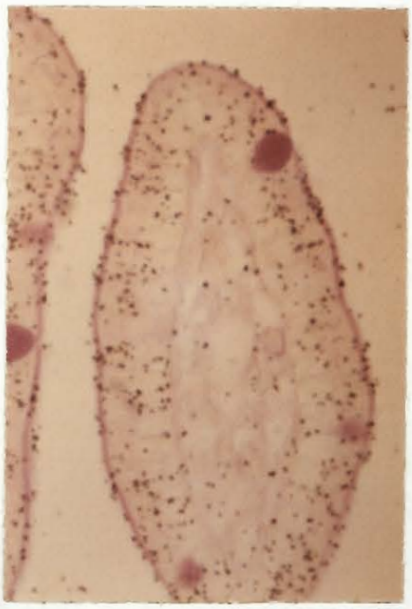
also resulted in a general scatter of label throughout the cytoplasm of columnar cells (Figs. 41, 83 and 85) which in some cases obscured any Golgi-localized label (Figs. 15-17). Because of this scatter, it was impossible to follow the migration of the Golgi-labeled material at later time intervals. Neither hyaluronidase nor beta-glucuronidase extracted labeled material from these cells (Figs. 37-40, 69-72). Similarly, after removal of glycogen by amylase, most of the scattered label and all of the Golgi-localized label remained (Fig. 41, Tables IV and V).

When ^{3}H-leucine was administered parenterally (exp. 14) radioactivity was detected in columnar cells, scattered throughout the cytoplasm (Figs. 56 and 57). When injected locally into the lumen of the duodenum, a similar scatter was seen. A local injection of ^{3}H-leucine into the colon, however, was poorly absorbed: most radioactivity remained in the lumen (exp. 15, Fig. 86).

Moderate doses of ^{3}H-galactose (exp. 10) were sufficient to produce considerable labeling of duodenal columnar cells. At 10 minutes after injection, the galactose-labeled material was restricted to the Golgi region, with no scatter in the rest of the cytoplasm (Figs. 62, 89 and 91; Text Fig. E). At 30 minutes after injection, most of the label was located in the apical cytoplasm and along the apical cell surface, while a smaller amount remained in the Golgi region (Fig. 92; Text Fig. F).



[E]



[F]

Colonic columnar cells showed a lighter Golgi-localized label, along with some scattered label in other parts of the cytoplasm. Unlike the ^{3}H-glucose scatter, all of the scatter produced by ^{3}H-galactose was extracted with amylase and was thus identified as glycogen (Table V; compare Figs. 42 and 43 with 41).

II. EPITHELIAL CELLS OF EPIDIDYMIS AND KIDNEY

The cuboidal epithelial cells which line the epididymal tubules in the 20-gram rat showed little or no radioactivity after injection of ^{3}H-glucose (exp. 9). Ten minutes after ^{3}H-galactose injection, however, silver grains were seen over the supranuclear cytoplasm (Fig. 93), a region occupied by the Golgi complex (Bloom and Fawcett, 1962). At 30 minutes (Fig. 94) most of the silver grains were associated with the apical cell border and with material in the lumen.

In the kidney, most ^{3}H-galactose label was incorporated in the epithelial cells of the proximal convoluted tubules (Fig. 95). Here, it was detected at 10 minutes after injection as small clumps of silver grains near the nuclei, while the PA-Schiff-stained brush border was not labeled. At 30 minutes (Fig. 96), ^{3}H-galactose-labeled material was present not only in the cytoplasm but in the brush border as well.

III. PANCREATIC ACINAR CELLS

In sections of pancreatic acini of 10- or 20-gram rats (Fig. 97), small centroacinar cells are often seen near the lumen. The Maillet technique (Fig. 98) demonstrates that the Golgi region of acinar cells lies above the nucleus, separated from the lumen by apical cytoplasm. The colloidal iron method (Fig. 99) indicates the presence of acidic carbohydrates in the supranuclear Golgi region, but not in the rest of the cytoplasm. In addition, the border of the lumen is intensely stained.

In radioautographs of pancreas, 10 minutes after ^{3}H-galactose injection, small but distinct accumulations of silver grains appeared over the supranuclear region of some acinar cells (Fig. 100). All centroacinar cells and cells of small ducts incorporated ^{3}H-galactose label as well; at 30 minutes after injection, this label was not distinguishable from label in the apical cytoplasm of acinar cells. Consequently, migration of the Golgi-labeled material was not clearly detected.

Soon after administration of high doses of ^{3}H-glucose, a scattered reaction appeared over acinar and centroacinar cells (Fig. 101). Although at 20 and 40 minutes a few acinar cells were labeled in the supranuclear area (Figs. 102 and 103), much label was detected in the basal cytoplasm.

Uptake of ^{14}C-glucosamine in pancreatic acinar cells was

detected in paraffin sections (Figs. 1 and 2), but its localization was not clear. In half-micron sections, appreciable amounts of radioactivity were detected only in centroacinar cells at 10 and 30 minutes after injection (Figs. 104 and 105).

IV. PARENCHYMAL CELLS OF LIVER

The PA-Schiff technique revealed the presence of glycogen in the liver cells of all rats observed.

In 10- and 20-gram rats, after high (exp. 8) or moderate (exp. 9) doses of ^{3}H-glucose, the radioautographic reactions over liver cells were relatively weak, and did not seem to be concentrated over the PA-Schiff-stained glycogen areas of the cytoplasm (Fig. 106: in photographs, the magenta stain appears black, masking the silver grains). After extraction of glycogen with amylase (Fig. 107) the radioautographic reactions were scarcely or not reduced, and the amylase-resistant radioactive material was scattered throughout the liver cell cytoplasm.

Ten minutes after injection of a moderate dose of ^{3}H-galactose (exp. 10), a heavy radioautographic reaction appeared over the cytoplasm of liver cells, mainly concentrated over PA-Schiff-stained glycogen areas (Fig. 108: again not demonstrable in photographs). Extraction of glycogen greatly reduced the reaction (Fig. 109). The remaining amylase-resistant radioactivity appeared as clusters of silver grains which on closer inspection (Fig. 110) were located either adjacent to nuclei, at cell interfaces or midway between sinusoids. Finer resolution was obtained in radioautographs of half-micron sections of amylase-digested liver (Figs. 111 and 112); here, the radioactive material was confined to the cytoplasm adjacent to bile canaliculi.

The radioactivity in liver cells at 10 minutes after ^{14}C-glucosamine injection was not as strictly localized as the galactose label. Nevertheless, a large proportion of the silver grains were located near bile canaliculi (Fig. 113).

The cytoplasm near canaliculi, when examined with the electron microscope, was seen to contain the Golgi complex, along with rough- and smooth-surfaced endoplasmic reticulum and mitochondria. The endoplasmic reticulum and mitochondria, however, were also distributed throughout the cell while the Golgi complex was confined to the pericanalicular area, as others had observed (Novikoff, 1959; Dougherty, 1964; Bruni and Porter, 1965).

CONNECTIVE TISSUE CELLS

I. CHONDROCYTES AND MATRIX OF CARTILAGE

The cartilage matrix and chondrocytes of 10-, 20- and 100-gram rats exhibited similar staining properties, whether in the trachea or the epiphyseal plate of femur. Although chondrocytes of epiphyseal plate incorporated greater amounts of labeled precursors, the uptake and migration of label and the effects of enzymatic treatments were similar in tracheal and epiphyseal cartilages from all the rats examined.

Staining properties

In chondrocytes, the Maillet technique demonstrated a large Golgi complex, usually placed near or around the nucleus (Fig. 115). The cytoplasm of chondrocytes was consistently stained by the colloidal iron method, while the surrounding matrix was irregularly and often lightly stained (Figs. 116-118). The glycogen in chondrocytes was brilliantly stained by the PA-Schiff technique (Figs. 120 and 122) and after it was extracted, both chondrocytes and matrix retained a light PA-Schiff stain (Figs. 121 and 123). Finally, it is well known that both chondrocyte cytoplasm and matrix show strong metachromasia when stained with toluidine blue (Text Fig. G).

Radioautographic results

Ten minutes after injection of ^{35}S-sulfate (Fig. 116), a radioautographic reaction appeared over a paranuclear region corresponding to the position of the Golgi complex. By 1 and

3 hours after injection, labeled material was present in both cells and matrix.

Similarly, 5 or 10 minutes after injection of ^{3}H-glucose (Fig. 117), ^{3}H-galactose (Fig. 118) or ^{14}C-glucosamine (Fig. 119), radioautographs revealed labeled material in a paranuclear position. Although the labeled material was confined to chondrocytes at 5, 10, 15 and 20 minutes after injection, it was present in both cells and matrix at 30 minutes and later. The relative amounts of radioactivity present at various time intervals could not be ascertained from these radioautographs, however, for great variations were seen in various regions of cartilage.

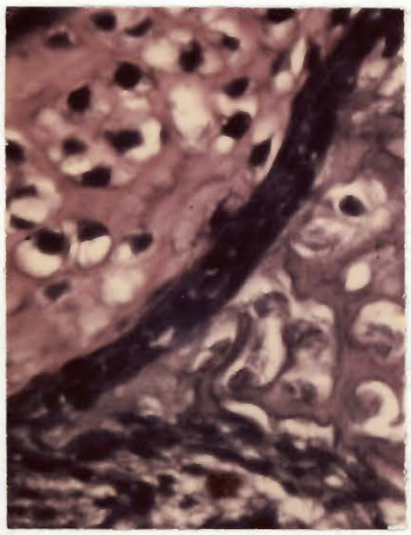
Effects of enzymatic treatments

The sequence peracetic acid-beta glucuronidase, which had removed labeled mucigen from epithelial cells, had no effect on cartilage (or on other connective tissues, Table IV). Salivary alpha amylase succeeded in removing the glycogen from the cytoplasm of chondrocytes, but none of the ^{3}H-glucose or ^{3}H-galactose radioactivity was associated with this glycogen. The radioactive material was amylase-resistant, in chondrocytes of both tracheal (Fig. 121) and epiphyseal cartilage (Fig. 123).

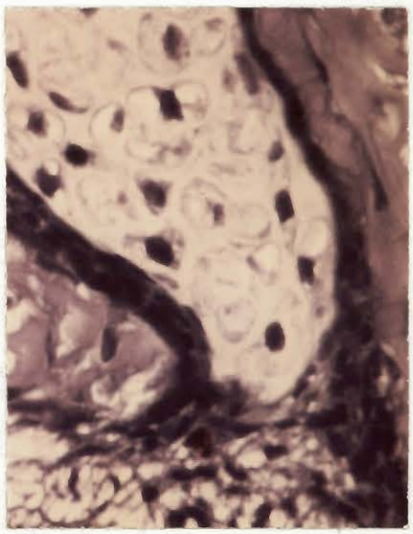
Treatment with testicular hyaluronidase altered the staining properties of cartilage and extracted labeled material as well. Thus, in toluidine blue-stained control sections (Text Fig. G), matrix and chondrocytes were metachromatic

(presumably due to the presence of chondroitin sulfuric acid) as were neighboring mast cells (due to their content of heparin sulfate). In hyaluronidase-treated sections (Text Fig. H), the metachromasia of cartilage was abolished but that of mast cells was intact.

[G]



[H]



Along with the metachromatic material, ^{3}H-glucose label was extracted from cells and matrix, but the amount of radioactive material extracted varied with the time interval (Table VII). At five minutes after injection, the labeled material

present in the chondrocyte cytoplasm was not hyaluronidase-labile. At 20 minutes, 10% was labile (Figs. 124 and 125) and at later times, roughly half the labeled material in cells and matrix was labile (Figs. 126-129). A similar phenomenon was observed in the connective tissue of incisor tooth pulp; that is, no radioactive material was extracted at early time intervals but as time passed, the material became progressively more labile (Table VII). Thus, labeled material in both cartilage and tooth pulp at first was resistant to the action of hyaluronidase, but later was vulnerable.

DISCUSSION

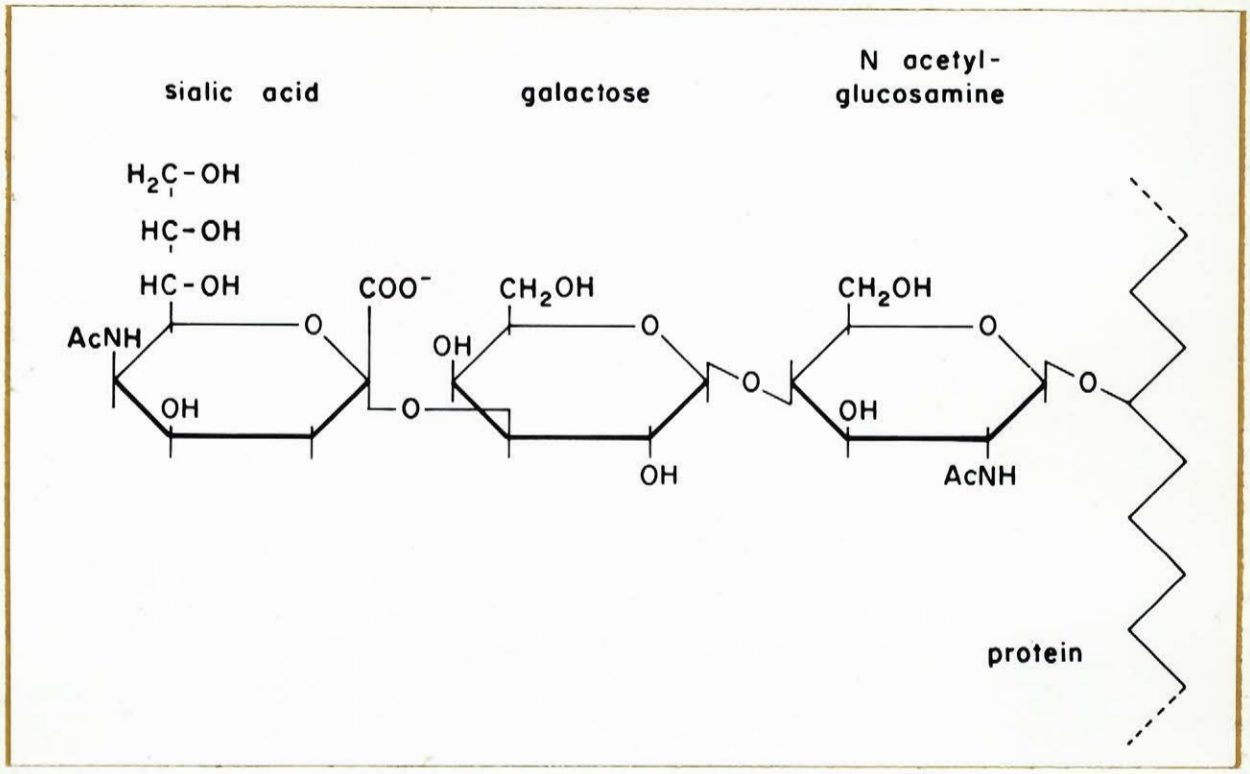
I. THEORETICAL BASIS OF EXPERIMENTS

Nature of complex carbohydrates

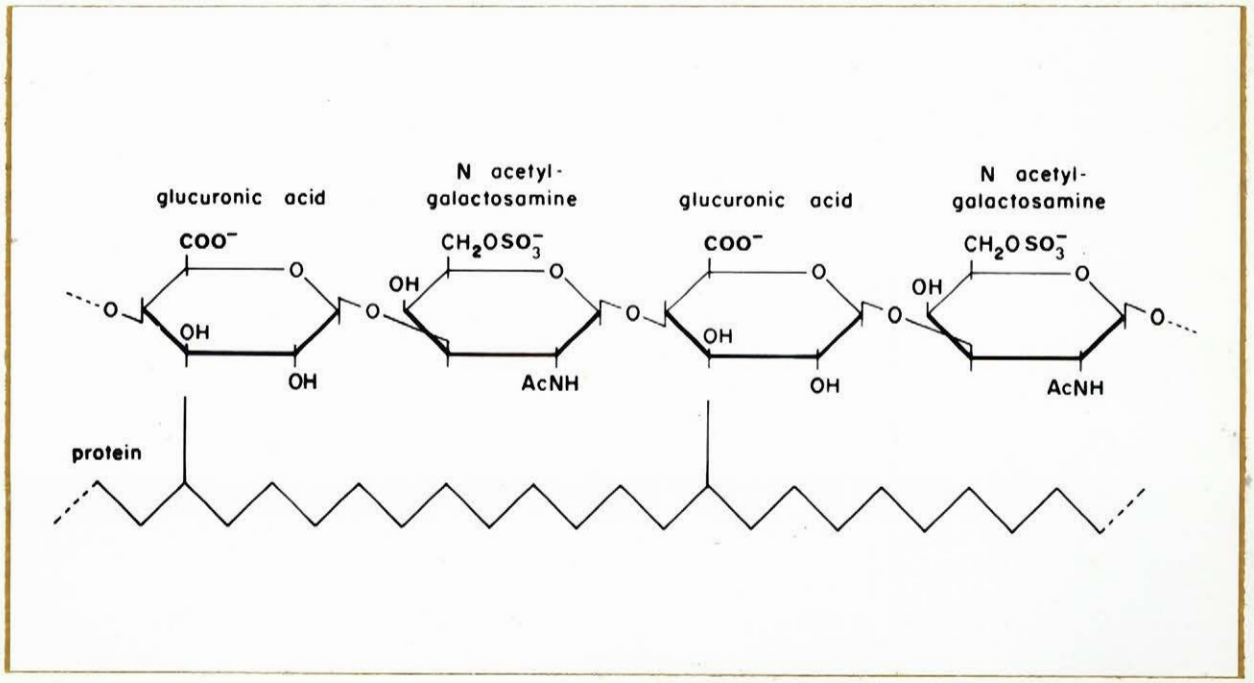
The carbohydrate-protein complexes found in secretory materials are of two chemically-distinct types known as "glycoproteins" and "mucopolysaccharides".

A glycoprotein consists of a backbone protein molecule to which relatively short heterosaccharide chains are linked as prosthetic groups (Text Fig. I). Although the composition, size and number of the carbohydrate-prosthetic groups are unique to each glycoprotein, certain features are common to all. At one end of the carbohydrate chain is a hexosamine residue which is firmly attached (by a strong glycosidic ester linkage) to the protein moiety at a specific amino acid site. At the other end, which is free, a sialic acid or fucose residue is found. The rest of the chain is composed of variable numbers of hexosamines (sometimes sulfated) and hexoses, but no hexuronic acids are present. If there are sufficient numbers of sialic acid and sulfate groups, the glycoprotein is acidic.

A mucopolysaccharide, in contrast, consists of a long, linear heterosaccharide chain which is loosely bound (by weak glycosidic ester linkages) to a protein at many points along its length, so that the carbohydrate and protein moieties may be imagined lying parallel to each other (Text Fig. J). The



[I] A portion of a GLYCOPROTEIN (hypothetical) showing one carbohydrate chain.



[J] A portion of a MUCOPOLYSACCHARIDE (chondroitin sulfate).

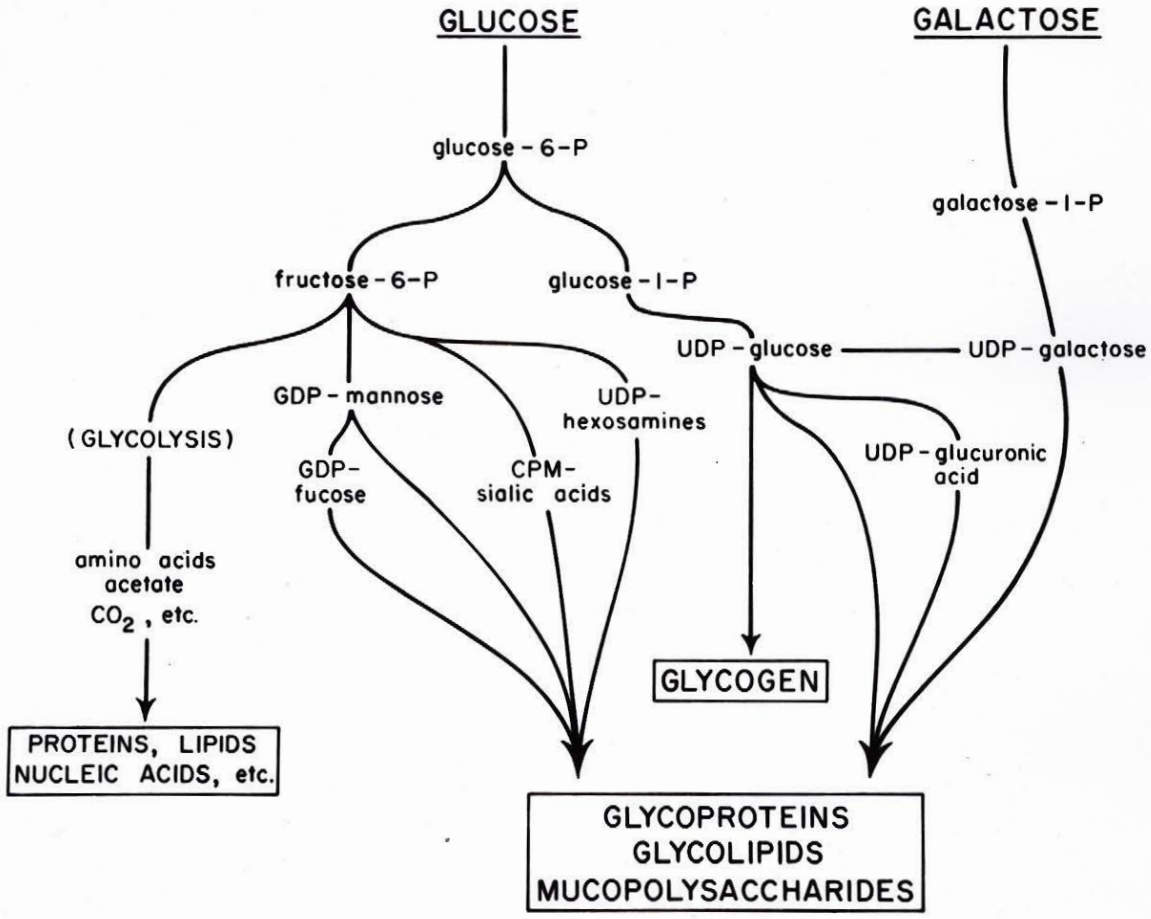
carbohydrate chain is composed of two alternating monosaccharides, usually hexosamine and hexuronic acid; there is no sialic acid or fucose. The hexosamines often carry sulfate groups, and these, along with the carboxyl groups of the hexuronic acids, render many mucopolysaccharides highly acidic.

The glycoproteins and mucopolysaccharides are chemically distinct from other complex carbohydrates. The polysaccharide glycogen is a long-chain polymer of glucose and is not protein-bound, while the carbohydrate-lipid complexes, glycolipids, have special structures which will not be discussed here. Of the complex carbohydrates, only glycoproteins and mucopolysaccharides have been found in cell secretions. Those of epithelial cells are generally glycoprotein in nature (Spiro, 1963) while those of connective tissue cells are primarily mucopolysaccharide, but often contain glycoprotein as well (Dorfman, 1963).

Synthesis of complex carbohydrates

Formation of the building blocks

Beginning with the work of Leloir and his colleagues in the early 1950's, much light has been shed on the precursors required for the synthesis of complex carbohydrates (Leloir and Cardini, 1960; Dorfman, 1963; Spiro, 1963; Strominger, 1964; Ginsburg, 1964). Glucose, the only monosaccharide abundant in the bloodstream, is converted (without breakdown or rearrangement of its 6-carbon chain) to various monosaccharides (Text Fig. K). To be utilized in synthesis, however, mono-


GLUCOSE
GALACTOSE
glucose - 6-P
galactose - 1-P
fructose - 6-P
glucose - 1-P
UDP - glucose
UDP - galactose
GDP - mannose
UDP-
hexosamines
(GLYCOLYSIS)
GDP-
fucose
CPM-
sialic acids
UDP - glucuronic
acid
amino acids
acetate
CO_2 , etc.
GLYCOGEN
PROTEINS, LIPIDS
NUCLEIC ACIDS, etc.
GLYCOPROTEINS
GLYCOLIPIDS
MUCOPOLYSACCHARIDES

[K]

saccharides must first be linked via diphosphate bonds to nucleotides (such as uridine, guanosine, or cytidine) to form "nucleotide sugars": a common example is uridine diphosphate-glucose (UDP-glucose). In this "active" form, further conversions may occur to provide additional nucleotide sugars. For example, some UDP-glucose is oxidized to UDP-glucuronic acid, UDP-glucosamine is epimerized to UDP-galactosamine, and GDP-mannose is reduced to GDP-fucose (Text Fig. K). All the resulting nucleotide sugars are necessary participants as "glycosyl donors" in the synthesis of complex carbohydrates.

Our experiments were designed to supply secretory cells with radioactive monosaccharides, in the hope that these would be transformed into labeled nucleotide sugars and would then be used in the synthesis of complex carbohydrates. But is this actually the fate of injected monosaccharides?

Fate of injected precursors

GLUCOSE: Injected glucose is rapidly cleared from the bloodstream by mixing with the extravascular glucose pool. For example, within 5 minutes after an intravenous injection into rats, mice and dogs, at least 85-90% was distributed to the tissues (Baker et al, 1955; Steele et al, 1956; Baker and Heubotter, 1963). Although intraperitoneal injections result in somewhat longer availability, it is probable that a large part of our ^{3}H-glucose was distributed to the tissues by the early time intervals (5, 20 minutes). Once in the tissues,

what metabolic pathways would the ^{3}H-glucose follow?

The rat has a free glucose pool of about 35 mg per 100 grams body weight (gbw) (Depocas, 1959) so our maximum dose of 1.4 mg/100 gbw should not have altered normal metabolism. A portion of the body glucose is metabolized via glycolytic pathways and the Krebs cycle to give rise to amino acids, acetate, glycerol, CO_2 and other small molecules which in turn may be used for the synthesis of nucleic acids, proteins, lipids, etc. (Text Fig. K). The alternative synthetic pathways involve the conversion of glucose, as outlined above, to various nucleotide sugars which are used for the synthesis of all types of complex carbohydrates: glycoproteins, mucopolysaccharides, glycogens and glycolipids. The total amounts of glucose allocated to glycolytic and synthetic pathways have not been adequately measured (Katz and Wood, 1960). In any case, each cell uses glucose to its own ends: while glucose is rapidly broken down to give rise to amino acids in liver and brain (Gaitonde et al, 1965), it is largely allocated to glycoprotein synthesis in the colonic mucosa (Draper and Kent, 1963).

GALACTOSE: Although little is known about the body pool size and clearance rate of galactose, it is probable that our dose of 5.45 mg/100 gbw was distributed as rapidly as glucose. Of that which remained in the bloodstream long enough to reach the kidneys, about 60% would be excreted (Davson, 1961).

Once within the cells, all the galactose that enters metabolism is first converted to UDP-galactose (Kalckar, 1958). In this form it is not subject to glycolysis, but is an "active glycosyl donor" capable of adding galactosyl residues to monosaccharides (Watkins and Hassid, 1961), to the carbohydrate moiety of glycoproteins (Krauss and Sarcione, 1964; Sarcione, 1964; McGuire et al, 1965; Kalckar, 1965) and to cerebral glycolipids (Cleland and Kennedy, 1960).

The conversion of UDP-galactose to UDP-glucose has been detected only in liver cells (Guyton, 1961); it seems that most cells do not have the necessary epimerase. In liver, the UDP-glucose thus formed may be used for the synthesis of glycogen, but it does not directly enter glycolytic pathways (Leloir and Cardini, 1960; Kohn et al, 1963).

GLUCOSAMINE: Like other monosaccharides, glucosamine is rapidly distributed to the tissues. In rats, 95% of an intravenous dose had left the bloodstream within 10 minutes (Boas and Foley, 1955). Kohn et al (1962) outlined the metabolic fates of ^{14}C-glucosamine: four hours after injection, they found considerable quantities excreted in the urine, but the labeled retained in the liver was present only as the hexosamine and sialic acid residues of glycoprotein. No label appeared in hexoses, amino acids, glycogen or CO_2. Subsequent work has established that injected glucosamine gives rise only to UDP-hexosamines and CMP-sialic acids (Text Fig. K) which

are then utilized in the synthesis of glycoproteins (Sarcione, 1962; Del Giacco and Maley, 1964; Athineos et al, 1964) and mucopolysaccharides (Capps and Shetlar, 1963; White et al, 1965).

The uptake of ^{14}C-glucosamine into the glycoproteins and mucopolysaccharides of various rat tissues was surveyed by Robinson et al (1964) at 30, 60 and 90 minutes after injection. At 30 minutes, the highest concentrations of label were found in lung, liver, small intestine and kidney (in descending order). To identify the tissue elements responsible for this uptake, rat tissues were visually surveyed by radioautography at 10 and 30 minutes after injection of ^{14}C-glucosamine. Our results (Figs. 1 and 2; Table VI) were not entirely consistent with those of Robinson et al. The highest concentrations of label were found in small intestine, with somewhat lesser amounts in liver, kidney and large intestine, while lung was only lightly labeled.

The discrepancies may well have been due to the age difference between Robinson's (375 gm) rats and ours (25 gm). Furthermore, it should be pointed out that the large amounts of glucosamine employed in our experiments far exceeded a "tracer dose". According to estimations by Spiro (1959) a 25-gram rat has a body glucosamine pool of about 4.7 mg in liver and 10.8 mg in serum. Since injections of 6.0 mg (10 minute interval) and 70.1 mg (30 minute interval) undoubtedly

altered the pool size and may have altered the metabolism of glucosamine, the radioautographic results must be interpreted with caution.

In summary, the available evidence indicates that ^{3}H-glucose could serve as precursor of the entire carbohydrate moiety of complex carbohydrates but, especially in certain tissues, would also be broken down in glycolysis. ^{3}H-galactose would not be broken down, but would supply galactosyl residues for glycoprotein and glycolipid synthesis (and in liver, glucosyl residues for glycogen synthesis). ^{14}C-glucosamine would provide the hexosamine and sialic acid residues of glycoproteins and mucopolysaccharides. Of the other precursors, ^{35}S-sulfate is used only for the sulfation of complex carbohydrates (Pasternak and Kent, 1958) while radioautography after ^{3}H-leucine injection is a reliable technique for detecting sites of protein synthesis (Droz and Warshawsky, 1963).

Intracellular sites of synthesis

A nucleotide sugar donates its monosaccharide to a growing oligosaccharide chain. In the variety of systems studied, this addition or polymerization is catalized by enzymes variously called "transglycosylases", "transferases" or "synthetases".

That which catalyses the addition of glucose to a glycogen molecule has been found in soluble form in the cytoplasm of liver cells (Luck, 1961). In contrast, those which link mono-

saccharides together to form the carbohydrate moiety of mucopolysaccharides and glycoproteins have consistently been found in the microsomal or "particulate" fraction of animal cells and bacteria (Markovitz and Dorfman, 1962; Silbert, 1963; Perlman et al, 1964). Their function does not depend upon RNA, and they are believed to be membrane-bound (Markovitz and Dorfman, 1962; Sarcione, 1963; Kent, 1964). So far, no one has isolated an enzyme that will link carbohydrate to protein.

Since 1963, several biochemists have attempted to elucidate the exact sequence of events involved in glycoprotein synthesis and the intracellular sites at which they occur. To determine these sites, they have relied on the isolation of subcellular fractions by ultracentrifugation, before or after administration of radioactive galactose or glucosamine. In general, they agree that the synthesis of the protein moiety is completed before the carbohydrate moiety is added (Sarcione, 1964; Eylar and Cook, 1965; O'Brien et al, 1965). Around the addition of the carbohydrate moiety, however, revolves a controversy in which three theories are current:

1) The carbohydrate moiety (consisting of multiple oligosaccharide chains) is completely synthesized and then is added to the completed protein.

This view was engendered by the finding, in colostrum, of a nucleotide-trisaccharide whose monosaccharide sequence was typical of glycoproteins (Jourdian et al, 1961). From ascites

carcinoma cells, Eylar and Cook (1965) isolated a "post-microsomal membrane fraction" which contained enzymes capable of catalyzing the synthesis of oligosaccharides from UDP-galactose and UDP-glucosamine. The authors suggested that these smooth membranes may belong to the Golgi complex, and speculated that Golgi-synthesized oligosaccharides would migrate to the rough-surfaced endoplasmic reticulum to join the completed protein. They failed to demonstrate the presence of carbohydrate in their "rough membrane fraction", however.

2) The carbohydrate moiety is built onto the completed protein, one monosaccharide at a time.

McGuire et al (1965) removed two terminal monosaccharides (sialic acid and galactose) from a serum glycoprotein to obtain an "incompletely glycosylated" glycoprotein. In the presence of UDP-galactose and a "galactosyl transferase" from colostrum, galactosyl residues were replaced onto the carbohydrate moiety. Sarcione (1964) compared the kinetics of incorporation of leucine and galactose into serum glycoprotein, from 3 to 15 minutes after administration to liver cells. His results indicated that this monosaccharide is added to a completed protein at a membrane site, perhaps on the membranes of the endoplasmic reticulum.

3) The proximal monosaccharide, glucosamine, is incorporated at specific sites on the growing peptide (while it is still on

the ribosome), and the rest of the carbohydrate moiety is added later, as the protein migrates through the endoplasmic reticulum.

Molnar et al (1965) detected glucosamine incorporation both in a "ribosome" fraction and in "microsomal membrane" fractions, whereas sialic acid was incorporated only in the latter. In contradiction, however, neither Sarcione and Sokal (1964) nor Sinohara and Sky-Peck (1965) were able to detect uptake of glucosamine or glucose in any RNA fraction.

Briefly, most of these studies indicated that the carbohydrate moiety is synthesized and/or added to the protein moiety in association with some intracellular membrane system. Unfortunately, because of the artificial disruption and imperfect separation of these membranes by ultracentrifugation, biochemists have not determined whether the crucial membranes belong to the rough-surfaced endoplasmic reticulum, the smooth-surfaced endoplasmic reticulum or the Golgi complex. To detect the site of synthesis without disrupting cell ultrastructure, electron microscope (EM) radioautography seemed the ideal tool.

Potentials of radioautography

Before the radioautographs obtained after injection of labeled monosaccharides could be interpreted, the potentials of the technique were clearly defined. Free monosaccharides and free nucleotide sugars present in the cells at the time of sacrifice would be washed out during fixation and processing.

Thus, the sites of monosaccharide conversion and formation of nucleotide sugars would not be detected. Similarly, short oligosaccharide chains which were not protein-bound would be lost, so that we could not determine whether the carbohydrate moiety is added to protein as a completed oligosaccharide chain, or one monosaccharide at a time. But, once bound to protein (as glycoprotein or mucopolysaccharide) the monosaccharides would be retained by fixation, and their label could be detected by radioautography.

Although labeled monosaccharides could also be incorporated into glycolipids and glycogen, lipids are largely removed by the organic solvents of histological processing, and glycogen may be extracted with amylase.

Thus, at very short times after administration of labeled monosaccharide precursors, the radioactivity in tissue sections should be located at the site of synthesis of glycoprotein and mucopolysaccharide.

The resolution obtainable with EM radioautography (about 0.1 μ; Caro, 1962) would permit localization of labeled material in a specific organelle only if its area in section were sufficiently large. In the colonic goblet cell, the organelles known to be involved in secretion of its glycoprotein product (rough-surfaced endoplasmic reticulum, Golgi complex and secretion granules) are clearly separated into large areas of cytoplasm (Figs. 3-6). Therefore this cell was selected as a representative glycoprotein-synthesizing cell to be studied by EM radioautography.

II. GOBLET CELLS OF COLON

Site of synthesis

Light microscope radioautographs of rat colon taken 5 minutes after injection of labeled glucose showed that the great majority of the radioactivity retained was present in the Golgi region of goblet cells (Figs. 12-14). EM radioautography revealed that this label was specifically associated with the saccules of the Golgi complex (Fig. 19). To be retained in the sections, the ^{3}H-glucose label must have been taken up into macromolecules in the course of their synthesis. Precisely what was the nature of the substances synthesized in the Golgi complex?

The migration and subsequent release of the labeled material indicated that it was a component of the goblet cells' secretion product, mucus (Figs. 16, 17, 20-30). Although the exact composition of goblet cell secretion is not known (Horowitz, 1963), histochemical techniques have shown that rat colonic goblet cell mucigen contains glycoproteins and acidic carbohydrates.

Enzymatic treatments of paraffin sections provided some information: since alpha-amylase and hyaluronidase failed to remove any label from the Golgi region or from mucigen (Fig. 41) the labeled material was neither glycogen nor the hyaluronidase-labile mucopolysaccharides. In contrast, the sequence peracetic acid-beta glucuronidase (reported to specifically

remove epithelial glycoproteins from sections; Fullmer, 1960) did extract much radioactive, stainable material from the Golgi region at early times and from the mucigen at later times after injection (Fig. 40). Although the specificity of this treatment is not established, the results suggested that the labeled material might be glycoprotein in nature.

The strongest evidence was provided by the experiments of Draper and Kent (1963). They found that sheep colonic mucosa in vitro incorporated large quantities of ^{14}C-glucose and converted the great majority of it to other ^{14}C-monosaccharides - galactose, hexosamine, fucose and sialic acid - for synthesis of the carbohydrate moiety of mucus glycoprotein. Since our radioautographs had shown that nearly all the label in rat colonic mucosa was present in goblet cells (Figs. 12-17), it was probable that the glycoprotein investigated by Draper and Kent was actually a product of goblet cells. It followed that the radioautographic reaction seen over the Golgi saccules at 5 minutes after injection was due to various ^{3}H-monosaccharides (derived from ^{3}H-glucose) that had been incorporated in the synthesis of the carbohydrate moiety of glycoprotein. Since such incorporation would occur without breakdown of the 6-carbon chain, the identical localization of ^{3}H-1- and ^{3}H-6-glucose labels was consistent with this conclusion.

Thus, radioautographs of colonic goblet cells provided a demonstration that the saccules of the Golgi complex are the site where monosaccharides are linked together and/or linked

to protein to form glycoprotein. Here, then, would be the membrane site postulated by biochemists. Finally, sulfation of the carbohydrate moiety would occur in the Golgi saccules as well (Fig. 53; Lane et al, 1964).

Since from biochemical evidence, one would expect galactose to be incorporated, without breakdown, into the carbohydrate moiety of the glycoprotein, the early localization of ^{3}H-galactose label (Figs. 44-46) confirmed the Golgi complex as the site of synthesis.

Further support was provided by the incorporation of ^{14}C-glucosamine label (presumably as hexosamine or sialic acid) into substances synthesized in the Golgi saccules (Figs. 47, 48, 51, 52). In EM radioautographs of goblet cells, no significant reaction was seen over the rough-surfaced endoplasmic reticulum. However, the sensitivity of this technique is low with ^{3}H label but even lower with ^{14}C (Pelc et al, 1961) so that if a relatively small amount of glucosamine were incorporated in association with ribosomes (as suggested by Molnar et al, 1965) it would not have been detected.

Functioning of the Golgi complex

Radioactivity was detected in Golgi saccules at 5 and 20 minutes after injection (Figs. 19-21) but no longer at 40 minutes (Figs. 22, 23). Presumably by 40 minutes the level of labeled precursors had fallen too low to provide detectable amounts of radioactivity to the synthetic processes in Golgi

saccules, and these processes were continuing with the usual unlabeled raw materials.

The disappearance of label from the saccules was associated with its appearance in nearby mucigen granules. Since no connections were seen between successive saccules of a stack, it is unlikely that the labeled material diffused from peripheral to central saccules and from there, into mucigen granules. It is more reasonable to assume that labeled mucigen granules arose from the expansion and release of the most central saccule, which in turn had come from a more peripheral position in the stack. This conclusion is consistent with the gradual morphological transition from peripheral to central saccules and from the latter to mucigen granules, as observed here (Figs. 5, 6, 18) and by others (Palay, 1958; Florey, 1960; Shearman and Muir, 1960; Bierring, 1962; Freeman, 1962).

Since at all time intervals, 7-12 saccules were present in each Golgi stack, the transformation of labeled central saccules into labeled mucigen granules must have been compensated by formation of new, unlabeled peripheral saccules. Indeed, on the basis of its ultrastructure, Grassé (1957) had concluded that the Golgi complex is functionally polarized: the vesiculation and loss of saccules from one "face" of the stack with the compensatory formation of new saccules at the other would call for a stepwise displacement of saccules through the stack. Mollenhauer and Whaley (1963) likewise

distinguished the "forming face" from the "maturing face" where vesicles of secretory material appear to be released. Grassé's view has been amply supported by ultrastructural studies of the Golgi complex in various cells (Grimstone, 1961; Bruni and Porter, 1964; Mollenhauer, 1965), by the preferential localization of enzymes in the saccules of one face only (Novikoff and Shin, 1964) and by the presence of osmium-reducing substances only in saccules of the "forming face" (Friend and Murray, 1964).

The delicacy and fenestrations of saccules at the "forming face" suggest that they are newly-formed (Hodge et al, 1956; Mollenhauer, 1965) but the mechanism of their formation is poorly understood. In cells of pancreas, liver and Brunner's gland, this saccule seems to be formed by fusion of many small vesicles, similar to those seen near the stack (sv in Text Fig. B). These vesicles in turn appear to arise as "buds" from local, ribosome-free areas of the nearby rough-surfaced endoplasmic reticulum. Since it is known that secretory protein must somehow pass from the latter into the Golgi complex, it has been suggested that the small vesicles carry newly-synthesized protein from the rough-surfaced endoplasmic reticulum, and fuse to provide the Golgi complex with new, protein-containing saccules (Ziegel and Dalton, 1962; Bruni and Porter, 1964; Novikoff and Shin, 1964; Friend, 1965). At least in pancreas, there is radioautographic evidence to

support this view (Jamieson and Palade, 1966). In colonic goblet cells the most peripheral saccules were indeed the shortest and most delicate of the stack, and the nearby cisternae of rough-surfaced endoplasmic reticulum often showed local loss of ribosomes, but few small vesicles and no clear "buds" were seen (Fig. 6).

By whatever means the new Golgi saccules are formed in the goblet cell, the radioautographic results provided direct evidence that Golgi saccules are transformed into mucigen granules and are replaced by new saccules. Furthermore, from these results it was possible, for the first time, to estimate the rate of transformation and replacement. Since label was present in saccules at all levels of the stack at 20 minutes but in none of them at 40 minutes, those labeled saccules present at the 20-minute interval must have been replaced by unlabeled saccules before the 40-minute interval. Perhaps, then, in as little as 20 minutes (or at least within 40 minutes) an entire Golgi stack is renewed. With an average of 10 saccules per stack, it is conjectured that a central saccule is released in the form of one or more mucigen granules every 2 to 4 minutes.

Fate of mucigen granules

After release from the Golgi stack, mucigen granules appeared to be displaced upward by the addition of new granules

from below. In previous EM studies of the colonic goblet cell, it has been reported that mucigen granules coalesce by breaking of membranes and fusion of contents. (Shearman and Muir, 1960; Freeman, 1962). In the present studies, the membranes of some granules seemed to be broken, particularly in crypt goblet cells (Figs. 9, 10). In most cases, however, the membranes remained intact as long as the granules were inside the cell, and the granules did not increase in size as they moved from the Golgi region to the apical cell surface (Figs. 3, 26). The presence of heavily-labeled and unlabeled granules side by side (Figs. 24-26) also suggests that the granules did not usually merge while migrating through the cell.

Upon reaching the apical surface, the granules seemed to be released singly, through small gaps in the apical cytoplasmic layer (Figs. 7, 8, 29-31). In the course of its exit, the intact membrane of the granule was in close contact with the plasma membrane lining the cytoplasmic gap or cleft (Fig. 29). The exact relationship of these two membranes was not clear, however, so that it was not determined whether the granule membrane remains intact during the entire process of extrusion, and whether the integrity of the plasma membrane is subsequently restored. Because such images appeared in longitudinal sections (perpendicular to the cell surface) they do not seem to be artifacts of section.

To date, no comparable images of the release of secretion

granules have been reported (Kurosumi, 1961). Although a somewhat similar image appeared in a recent EM study of Paneth cell secretion, the authors made no specific comment (Staley and Trier, 1965). In zymogenic secretory cells, the secretion products seem to be released by fusion of the granule membrane with the apical cell membrane. As both membranes break at the point of fusion, the granule's contents are released and its membrane is incorporated into the plasma membrane (Palade et al, 1961; Ito and Winchester, 1963; Staley and Trier, 1965). EM studies of the goblet cells of small intestine have produced images of deep, mucus-filled pits in the apical cytoplasm, which communicate with the lumen via channels between microvilli. In the light of findings in zymogenic cells, the author interpreted this as a stage in the release of the contents of a large apical mucigen granule, just after the granule and plasma membranes had fused and broken open (Trier, 1963). But his conclusion was derived only from cells sectioned tangentially to the apical cell surface: in such a section, a shallow surface depression may appear as a deep pit.

From similar tangential sections at the surfaces of tracheal goblet cells, Rhoden and Dalhamn (1956) showed large clusters of mucigen granules lying free in a membrane-bound "crater" in the cell apex. They concluded that the mucigen granules had been extruded "en masse", leaving a gaping hole in the cytoplasm, but they failed to demonstrate this in

longitudinal section, and seemed not to consider that a tangential section across an irregular surface may show islands of apparently-free cytoplasm. Since 1956, several authors describing the EM structure of colonic goblet cells have shown masses of mucigen granules bulging into the lumen (Shearman and Muir, 1960; Hollman and Haguenau, 1961; Freeman, 1962). In these and other publications, it was assumed that the apical membrane would eventually break under the pressure of the growing mass of mucigen granules and the entire contents of the goblet would flow out into the lumen (Palay, 1958; Bierring, 1962; Hollman, 1963, 1965). It is curious that this assumption was made with confidence when the ultrastructural evidence was tenuous. In the light of previous ideas on the functioning of goblet cells, however, it is quite understandable.

Functioning of goblet cells

It is commonly thought that goblet cell function is cyclic. The gradual accumulation of mucigen in the apical cytoplasm and the subsequent discharge of the mucigen mass have been termed a "secretory cycle". This concept has deep roots: as early as 1899, von Ebner described the goblet cell in the "discharging condition", with mucus exuding through a "stoma" on the apical cell surface. Chlopkow (1928) anthropomorphically stated that the cell is evacuated through a sphincter which opens periodically under the pressure of the mucigen mass. It has long been disputed whether goblet cells go through one

or several such "secretory cycles" (Clara, 1926; Macklin and Macklin, 1932; Hollman and Haguenau, 1961). Since the chief and goblet cells of rat colon arise from mitosis in the crypts, glide up the walls to the surface and are finally sloughed off into the lumen about 4-6 days after they arose (Messier and Leblond, 1960), a single secretory cycle has been postulated by Shearman and Muir (1960). These authors suggest that goblet cells synthesize and accumulate 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 cycles, electron microscopists have distinguished "undischarged, full" goblet cells from "discharged, exhausted" cells (Palay, 1958; Florey, 1960; Freeman, 1962).

Significantly, the only direct evidence of goblet cell function available did not supprt the secretory cycle concept. By radioautography after injection of ^{35}S-sulfate, Jennings and Florey (1956) demonstrated that mucigen was formed continually in all goblet cells, regardless of whether they were "young" or "mature", on the surface or in the crypts. From the fate of the ^{35}S-labeled mucigen in time, they suggested that the formation and the gradual discharge of mucus occur simultaneously.

Similarly, the radioautographic results presented here are inconsistent with the existence of secretory cycles in

the goblet cells of rat colon. First, 5 to 15 minutes after injection of ^{3}H-glucose in 10-, 20- or 100-gram rats, radioautographic reactions appeared over the Golgi region of all goblet cells, both in the crypts and on the surface. From this, it was tentatively concluded that all goblet cells are continuously active in synthesizing the complex carbohydrate of mucus.

Secondly, radioautographs of colon between 20 minutes and 4 hours after injection demonstrated that, in all goblet cells, the radioactive material came out of the Golgi region in mucigen granules which then migrated toward the apical surface (Figs. 12-27). Although the rate of migration varied from cell to cell (Fig. 17), migration appeared to be occurring in all and the labeled granules often moved as a "wave" toward the apical membrane (Fig. 28). This implies continual addition of new, unlabeled granules below the wave and continual loss of unlabeled granules above.

Thirdly, the manner in which 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 (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 (Figs. 33-36). Isotonic glutaraldehyde, a fixative which minimizes swelling (Malamed and

Weissman, 1965), produced no discharge either (Fig. 11) and in the electron microscope, apical membranes of crypt and surface goblet cells did not seem to be torn off (Figs. 7, 8, 29). Thus in the normal rat, massive emptying of surface goblet cells appears to be an artifact of fixation. Indeed, EM observations of crypt and surface goblet cells rather suggest that mucigen granules are released singly, at different points along the apical membrane (Figs. 7, 29). Such images of local extrusion were frequently observed; at the surface of some cells, several of them were seen in one EM section (Fig. 7). Serial sections might well reveal their presence in all cells.

The sequence of events from synthesis to extrusion was observed in goblet cells with relatively few mucigen granules as well as in cells distended by many of them (although in the latter, migration was generaly slower). It is probable that not only the rate of migration but also the rates of synthesis and extrusion vary among individual cells.

Nevertheless, in all goblet cells of the rat colon, the synthesis, intracellular migration and 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.

Significance of scattered radioactivity

The great majority of ^{3}H-glucose label in goblet cells was associated with secretory material, first in the Golgi complex and later in mucigen granules. Indeed, colonic mucosa allocates most of its glucose to the synthesis of a glycoprotein secretion product (Draper and Kent, 1963). Because of its low sensitivity (Pelc et al, 1962) the EM radioautographic technique usually detected only the large quantities of label present in the secretion product. But in the more sensitive light microscope radioautographs, a light scattered reaction appeared over the basal and lateral cytoplasm (Figs. 12-17). Was this scatter indicative of complex carbohydrate synthesis outside the Golgi region?

As summarized above, not all glucose is allocated to complex carbohydrate synthesis: some is broken down in glycolysis, and the resulting metabolites (which in this case would be labeled) may then be used in the synthesis of proteins, nucleic acids, lipids, etc. Since radioautography can only detect the large, non-lipid molecules which are retained in histological sections, the scattered label could *a priori* be due to complex carbohydrates (glycoproteins, mucopolysaccharides or glycogen), to proteins, or to nucleic acids. The identity of the scattered label was thus determined by a process of elimination.

If the scattered cytoplasmic label were due to uptake of

intact ^{3}H-glucose into glycogen, it should be extracted by alpha amylase. In fact, this enzyme removed small amounts of label so that a fraction of the scatter, but only a small one, consisted of glycogen. What about the rest? If labeled mucopolysaccharides (hyaluronic acid or chondroitin sulfates A or C) were responsible for the scattered reaction, hyaluronidase treatment should remove it ... but such was not the case. Similarly, the procedure that supposedly extracts epithelial glycoproteins, peracetic acid-beta glucuronidase, did not affect the scattered label (even though it extracted most of the radioactive mucigen, Fig. 40).

If the cytoplasmic scatter remaining after removal of glycogen were due to labeled nucleic acids, a comparable scatter should be observed soon after injection of an RNA-DNA precursor such as ^{3}H-cytidine. After such injection, however, radioactivity in goblet cells was restricted to the nucleus (Fig. 20 in Amano et al, 1965). Finally, if the scatter were caused by labeled proteins, a similar distribution of label should be seen after injection of a protein precursor such as ^{3}H-leucine. Soon after this was injected, radioactivity was present in the basal and lateral cytoplasm of goblet cells (Figs. 56, 57); this pattern was not unlike the pattern of label seen after ^{3}H-glucose injection (Figs. 12-15). Thus it seemed likely that the scatter was due to uptake of ^{3}H-amino acids (from breakdown of ^{3}H-glucose) into protein synthesis.

If this conclusion were valid, then no scatter should appear after injection of a labeled monosaccharide which is not broken down to produce amino acids, such as ^{3}H-galactose. Radioautographs of colonic goblet cells labeled with ^{3}H-galactose did reveal, besides the Golgi-localized label, radioactivity in the basal cytoplasm (Figs. 45, 46); however, the basal label was completely removed by alpha amylase, and was thus identified as glycogen* (Figs. 42, 43). Hence with the doses of ^{3}H-galactose employed here, no synthesis of glycoprotein was detected outside the Golgi region. It followed that the scattered label seen after ^{3}H-glucose injection was not indicative of glycoprotein synthesis, but was probably due to synthesis of protein.

To conclude, in radioautographs of colonic goblet cells obtained soon after injection of ^{3}H-glucose, ^{3}H-galactose or ^{3}H-glucosamine, only the Golgi region showed signs of synthesis of complex carbohydrates for secretion.

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* According to biochemical studies, galactose may be converted and utilized for glycogen synthesis only in liver (Guyton, 1961). These radioautographs, along with those of striated muscle (Table V), provide evidence that such conversion may occur in certain extra-hepatic tissues as well.

III. ASPECTS OF SECRETION IN OTHER CELL TYPES

Mucous cells

Like the colonic goblet cells, all cells which produce mucous secretions were heavily labeled after ^{3}H-glucose injection, first in their Golgi region and then in their mucigen. All these cells are known to produce carbohydrate-protein complexes with a relatively large carbohydrate moiety. Indeed, their labeled secretions were invulnerable to amylase (Figs. 63-68) and hyaluronidase, but were extracted by peracetic acid-beta glucuronidase (Figs. 72, 79). Although their secretory materials migrated at variable rates (Table III) it seemed safe to draw certain analogies among them. Thus, from the results obtained in colonic goblet cells, it was concluded that all these cell types had incorporated ^{3}H-glucose in their Golgi complex to synthesize complex carbohydrates for secretion.

It may seem paradoxical that no glucosyl residues have been found in most of the mucous secretions analyzed so far (Leblond et al, 1957; Tsuiki and Pigman, 1960; Kent, 1962; Horowitz and Hollander, 1962; Kent and Mrasden, 1963; Gottschalk, 1963). As summarized in Text Fig. K, however, glucose may be converted to form all the monosaccharide components of complex carbohydrates, and through these conversions, glucose label could appear in newly-formed mucus.

Although the exact compositions of the mucus secreted by

individual cell types is largely unknown (Horowitz, 1963) there is biochemical evidence, histochemical evidence (Spicer, 1960) and radioautographic evidence using ^{35}S-sulfate (Belanger, 1954; Dziewiatkowski, 1956; Jennings and Florey, 1956) that the secretion product(s) of each cell type are unique. Could their compositions be elucidated on a cellular level by radioautography using labeled monosaccharides?

Sublingual mucus is known to contain about 75% carbohydrate (Tsuiki and Pigman, 1960) while submaxillary mucus contains about 40% (Blix, 1963). Indeed, after ^{3}H-glucose injection, radioautographic reactions were much heavier over sublingual than over submaxillary gland. Since the entire carbohydrate moiety could be formed from glucose, one might think that the intensity of the radioautographic reaction would directly reflect the size of the carbohydrate moiety and would be an index applicable to all mucous cells. The reaction intensity, however, is equally dependent on the rate of synthesis, so such an index would not be valid.

The labeled glucose and galactose employed differed in specific activities (Table I) and in overall metabolism (see "Fate of injected precursors"), so that incorporation of the two precursors could be compared only roughly. Nevertheless, in examining their radioautographic distributions in various tissues, clear differences emerged (Table II). In contrast to the uptake of glucose label by mucous cells of all types

(Figs. 59-61, 74) the galactose label was incorporated by only certain types of mucous cells. Thus, it appeared in mucous cells of colon (Figs. 42, 46) but not in those of duodenum (Fig. 62) or stomach (Fig. 75). Since in most cells galactose is not converted to other monosaccharides (Guyton, 1961) it might be thought that those cells which incorporated much galactose produced a mucus rich in galactosyl residues, while others had little or no galactose in their mucus. In almost all types of mucus analyzed, however, galactosyl residues have been found (Leblond et al, 1957; Tsuiki and Pigman, 1960; Kent, 1962; Horowitz and Hollander, 1962; Kent and Marsden, 1963; Gottschalk, 1963). While a higher dose of ^{3}H-galactose might have permitted detection of uptake in more cell types, it may be that some cells do not have the enzymatic equipment to make use of free galactose, and instead obtain their galactosyl residues by conversion of glucose. Thus, the composition of mucous secretions was not elucidated by these radioautographic results.

In any case, whenever ^{3}H-galactose was taken up in mucous cells, it was first localized in the Golgi region. Here again, the Golgi reactions were not due to labeled glycogen since they were not decreased by amylase treatment; presumably, they were due to ^{3}H-galactosyl residues taken up into mucous glycoprotein.

Columnar cells of intestine

Intestinal columnar cells, long believed to be purely absorbtive in function, were only recently admitted to the ranks of secretory cells. EM radioautography revealed that, 15 minutes after administration *in vitro* of ^{3}H-glucose, ^{35}S-sulfate or ^{3}H-acetate, the labels were incorporated into substances in the cytoplasm of cat intestinal columnar cells. By one hour, the radioactive substances appeared in the layer of filamentous or fuzzy material coating the surface of the microvilli (Ito, 1965a). Thus established as a secretion product of the columnar cell, this fuzzy coat received much attention. With light- and electron microscopes, the coat was seen stained by PA-Schiff, colloidal iron, colloidal thorium and Alcian blue (Ito, 1965b; Curran et al, 1965; Spicer, 1965; Wetzel et al, 1965); from this, we could assume that it contains glycoproteins and acidic carbohydrate groups. In addition, the enzyme alkaline phosphatase, long recognized on the surface of columnar cells (Emmel, 1945; Moog and Werner, 1952) was located in the densest part of the fuzzy coat, immediately adjacent to the plasma membrane (Fig. 80, f, left; Ito, 1965b). This enzyme is itself a glycoprotein (Portmann et al, 1960).

The radioautographic evidence did not reveal the exact intracellular site of synthesis of surface coat material, but clues were provided when material with similar properties was detected in the Golgi complex. Thus, alkaline phosphatase was

found in the Golgi region of columnar cells (Emmel, 1945; Fredericsson, 1956) and material in this region was stained by PA-Schiff (Fig. 58; Gersh, 1949; Leblond, 1950; Rambourg et al, 1966), colloidal iron (Figs. 86, 88; Badinez et al, 1962) and Alcian blue (Spicer, 1965). With the electron microscope, colloidal iron-stained material was seen within the flattened Golgi saccules (Wetzel et al, 1965). We wondered then, if the complex carbohydrate of the surface coat was synthesized in this organelle.

Indeed, 5-15 minutes after administration of large doses of ^{3}H-glucose or moderate doses of ^{3}H-galactose, a clearcut uptake of label was observed in the Golgi region of columnar cells (Figs. 82-85, 89, 91). At later time intervals after ^{3}H-glucose injection, heavy scattered label appeared over the entire cytoplasm so that the reactions could no longer be related to a specific part of the cell. (The resemblance of the ^{3}H-glucose scatter to that seen after ^{3}H-leucine injection (Figs. 56, 57) suggested that much ^{3}H-glucose had been broken down to provide labeled amino acids for protein synthesis).

After ^{3}H-galactose, however, there was no scattered label: the radioactive material restricted to the Golgi region at 10 minutes was seen at 30 minutes in the apical cytoplasm and surface coat (Figs. 91, 92; Text Figs. E, F). Thus, it appeared that Golgi-synthesized material migrates through the apical cytoplasm to the surface. It followed that a component

of the surface coat is synthesized in the Golgi complex. Presumably, this component is the carbohydrate moiety of carbohydrate-protein complexes. Since at least in man, the mucosa of small intestine does not convert galactose to other monosaccharides (Oockerman and Lundborg, 1965) the carbohydrate moiety would be rich in galactose residues. This is consistent with Kalckar's recent proposal that galactose is an important constituent of the complex carbohydrates present at cell surfaces (Kalckar, 1965).

The colonic columnar cells incorporated less galactose label than those of duodenum (Figs. 42, 62), perhaps reflecting differences in the composition or the rate of production of their surface coats. In any case, since all columnar cells showed synthetic activity in the Golgi region and subsequent migration of material to the surface, it seems that these cells are continually adding material to their apical surface. Yet the thickness of the coat seems to be constant: this addition must therefore be balanced by a continual loss of coat material into the lumen. Such renewal may ensure a continual supply of surface enzymes (Moog, 1965) and a rapid replacement of this protective layer in case of damage (Ito, 1965a).

Epithelial cells of kidney and epididymis

In these cells, as in intestinal columnar cells, ^{3}H-galactose label was seen at 10 minutes in the Golgi region and at 30 minutes in the apical cytoplasm and on the apical surface (Figs. 93-96). These observations imply secretory activity in both cell types.

The proximal convoluted tubule cells of kidney may be secreting several substances. A PA-Schiff-positive surface coat containing alkaline phosphatase is present on the apical brush border (Moog and Werner, 1952), and at least in intestinal columnar cells, there is evidence that this glycoprotein enzyme is synthesized in the Golgi region (Moog, 1965). Evidence obtained from bat kidney suggests that the enzyme ATPase, associated with the tubule cell surfaces, may arise in the Golgi region as well (Rosenbaum and Melman, 1964). In addition, the specific glycoproteins of urine (Dische, 1963) are known to originate in the epithelial cells of the proximal convoluted tubule (Keutel, 1965). Granules containing PA-Schiff-positive material are present in the supranuclear region of these cells (Tessanow, 1965) suggesting the production of some glycoprotein in the Golgi region.

Thus the ^{3}H-galactose-labeled substance, presumably glycoprotein in nature, may have been secreted onto the apical cell surface to renew the enzyme-containing surface coat, or it may have been released into the lumen as urinary glyco-

protein.

Little is known about the secretion product(s) of the epididymal epithelial cells. Material on their apical surface is stained by PA-Schiff (Leblond, 1950) and colloidal iron (Fig. 93) indicating the presence of glycoproteins and acidic carbohydrates. At least a part of the PA-Schiff-reactive glycoproteins may be the enzyme alkaline phosphatase, which has been found in abundance on the apical surface (Allen, 1964).

These cells are endowed with a large Golgi complex (Dalton and Felix, 1956) which is intensely stained by the PA-Schiff technique (Leblond, 1950). In a "Golgi fraction" isolated from these cells by ultracentrifugation, high concentrations of alkaline phosphatase were found (Kuff and Dalton, 1959).

From such scant and fragmentary evidence, it can only be suggested that here, as in the kidney tubule, the galactose-labeled material may serve to renew the apical surface coat or may be released into the lumen.

Pancreatic acinar cells

Although it is generally thought that "serous" secretions are purely protein in nature, recent studies have revealed that they often consist of glycoproteins which are relatively poor in carbohydrate (Mandel and Ellison, 1963). In particular, the serous secretion of pancreatic acinar cells contains at least one glycoprotein, the enzyme ribonuclease, which contains 2% carbohydrate (Plummer and Hirs, 1963). Furthermore, blood group substances (which are glycoproteins; Spiro, 1963; Kalckar, 1965) have been detected by immunofluorescence in the zymogen granules of normal pancreatic acinar cells (Szulman, 1960) and in secretion products in the ducts (Kent, 1963).

In a histochemical study, Munger (1964) detected small amounts of PA-Schiff-positive material in the zymogen granules of human pancreas, but no such complex carbohydrate was detected in rat pancreatic acinar cells (Leblond, 1950). When histochemical methods were applied to pancreas of the young rats used in our experiments, a light PA-Schiff staining of zymogen granules suggested the presence of some glycoprotein. Strangely, the colloidal iron method demonstrated acidic carbohydrates not in zymogen granules, but rather in the Golgi region and on the apical cell surface (Fig. 99).

Radioautographs at 10 minutes after ^{3}H-galactose injection showed a small but distinct accumulation of label in

the Golgi region, with little or no scatter in the rest of the cell (Fig. 100). (After ^{3}H-glucose injection, the scatter of label (Figs. 101-103) suggested that these cells had broken down much of their ^{3}H-glucose to provide amino acids for protein synthesis.)

The ^{3}H-galactose results suggested that a small amount of complex carbohydrate is synthesized in the Golgi region of pancreatic acinar cells. Perhaps this carbohydrate is added there to proteins coming from their own site of synthesis, the ergastoplasm, as they are "packaged" into zymogen granules (Caro and Palade, 1964; Van Heyningen, 1964).

The centroacinar and small duct cells consistently incorporated ^{3}H-galactose and ^{14}C-glucosamine (Figs. 100, 104, 105) and yet these cells contain few organelles (Porter and Bonneville, 1963) and do not show signs of secretory activity. In cases of cystic fibrosis, small duct cells may transform into mucous cells (Shackleford, 1965) to produce abnormal glycoproteins (Johansen, 1963), but their normal synthetic activities are not known.

Liver parenchymal cells

Biochemical studies indicate that glucose, galactose and glucosamine have distinctly different fates in the liver (see "Fate of injected precursors"). All the galactose that enters into liver cell metabolism is changed to UDP-galactose, which may directly supply galactosyl residues for the synthesis of plasma glycoproteins (Sarcione, 1964) or may be converted to UDP-glucose to provide glucosyl residues for glycogen synthesis (Leloir and Cardini, 1960). Indeed, ^{3}H-galactose injection produced considerable amounts of labeled glycogen throughout the cytoplasm of liver cells. After amylase extraction of this glycogen, the remaining label was located in a pericanalicular region (Figs. 110-112) corresponding to the position of the Golgi complex (Fig. 114). Recently, EM radioautography has confirmed that ^{3}H-galactose is incorporated into macromolecular substances in the Golgi saccules, within 5 minutes after its injection (Droz, 1966).

It is known that plasma glycoproteins are synthesized in liver cells. The protein moiety is formed in the rough-surfaced endoplasmic reticulum (Peters, 1962) and the carbohydrate moiety is synthesized and/or added to the protein at some unidentified membrane site (Sarcione, 1964). In an EM study of rat liver cells, Bruni and Porter (1965) provided morphological evidence that plasma glycoproteins pass from the rough-surfaced endoplasmic reticulum through the Golgi complex in

the course of their secretion. The incorporation of ^{3}H-galactose in the Golgi complex suggests that this is the site where synthesis of the carbohydrate moiety occurs.

Liver cells allocate some glucose to the synthesis of glycogen and glycoprotein (Sarcione, 1962) and some to glycolytic pathways for production of amino acids (Gaitonde et al, 1965). The multiplicity of its fate could account for the lack of localization of ^{3}H-glucose label in the liver cell (Fig. 107).

Glucosamine is well established as a specific precursor of the carbohydrate moiety of plasma glycoproteins (Sarcione and Sokal, 1964; Sinohara and Sky-Peck, 1965; Molnar et al, 1965). The localization of most ^{14}C-glucosamine label in the Golgi region of liver cells (Fig. 113) lends support to the conclusion drawn above. As for the label detected in nuclei and in other parts of the cell, further work is needed to determine its significance.

Chondrocytes

Cartilage matrix is composed of collagen and the sulfated acid mucopolysaccharide, chondroitin sulfate (Text Fig. J) both of which are synthesized in chondrocytes (Thorp and Dorfman, 1963; Campo and Dziewiatkowski, 1963). In addition, glycoprotein is present in the matrix (Glegg et al, 1954; Herring and Kent, 1958; Anderson, 1962). At least in bovine cartilage, the glycoprotein is linked via its galactose-rich oligosaccharide side chains to the mucopolysaccharide (Roden and Armand, 1966).

It is established that collagen and chondroitin sulfate are produced in the same cells; furthermore, their syntheses seem to be closely related (Prockop et al, 1964; de la Haba and Holtzer, 1965). EM radioautographic studies have shown that proteins (assumed to be collagen subunits) are synthesized in the rough-surfaced endoplasmic reticulum, migrate to the Golgi complex to be segregated into secretion granules and finally, are discharged from the cell (Revel and Hay, 1963). With the EM, chondroitin sulfate has been identified as hyaluronidase-labile material stained by colloidal thorium, and thus has been located in the vesicles of the Golgi complex as well as in cytoplasmic secretion granules (Revel, 1964). EM radioautography after injection of ^{35}S-sulfate revealed that this mucopolysaccharide is sulfated in association with the saccules and vesicles of the Golgi complex (Godman and

Lane, 1964; Fewer et al, 1964). There is evidence, however, that sulfation occurs after the synthesis of the polysaccharide is complete (Dorfman, 1963; Mathews and Hinds, 1963). In fact, the enzymes that mediate sulfation are separable from those which mediate polysaccharide synthesis (Perlman et al, 1964). Was chondroitin sulfate synthesized elsewhere and then sulfated in the Golgi complex, or was the mucopolysaccharide synthesized there as well?

The early uptake of ^{3}H-glucose in the Golgi region of chondrocytes (Fig. 117) indicated that the synthesis of complex carbohydrate does indeed occur in this location. Since amylase failed to remove the labeled material (Figs. 120-123) it was not glycogen. By 30-40 minutes after injection, the material began to appear in the matrix, indicating that ^{3}H-glucose had been utilized for the synthesis of some matrix component(s).

In cartilage, as in other tissues, glucose may be converted to other monosaccharides for the synthesis of mucopolysaccharides (Text Fig. K; Lucy et al, 1961; Deiss et al, 1962). If the glucose-labeled material in our sections were the mucopolysaccharide, chondroitin sulfate, it would be removed from the sections by the enzyme hyaluronidase (Pearse, 1961). Indeed, one hour and later after ^{3}H-glucose injection, hyaluronidase removed 42-68% of the labeled material (Figs. 126-129; Table VII) so that a substantial part was identified as chondroitin sulfate. But what about the rest?

Since the metachromasia of cartilage depends on the presence of the acidic mucopolysaccharide, chondroitin sulfate, the complete loss of metachromasia after hyaluronidase treatment (Text Figs. G, H) would indicate that all the chondroitin sulfate had been removed. In this case, the labeled material remaining after enzyme treatment could be a non-metachromatic matrix component, such as glycoprotein. It is known, however, that metachromasia may be prevented when basic proteins or other cations are attached to the acidic groups of the mucopolysaccharide (French and Benditt, 1953; Spicer, 1963). Indeed, the variable staining of matrix by colloidal iron (Figs. 116-118; Conklin, 1963) showed that many of the acidic groups in the matrix are not available for dyebinding. Thus, the labeled material present after enzyme treatment could be a hyaluronidase-resistant chondroitin sulfate whose acidic groups are masked, preventing metachromasia.

At the early times after injection, the labeled material in chondrocytes was particularly resistant to hyaluronidase (Figs. 124, 125; Table VII). To interpret this phenomenon, it was presumed that ^{3}H-glucose could have been taken up into both chondroitin sulfate and protein. If we further assume that hyaluronidase was effective in removing all chondroitin sulfate from sections, then most of the intracellular label at early times (5, 20 minutes) must have consisted of glyco-

protein. Such a situation could result from a loss of nascent chondroitin sulfate in the course of fixation with retention of nascent glycoprotein. Or, we could assume that nascent chondroitin sulfate was retained in sections but at early times was hyaluronidase-resistant. In this case, nascent chondroitin sulfate must have been somehow protected from the action of the enzyme; this would occur soon after its synthesis, that is when the mucopolysaccharide was still in the Golgi complex.

^{3}H-galactose label was also incorporated into the Golgi region of chondrocytes for the synthesis of a component of matrix (Fig. 118), and the secretion of this component paralleled that labeled with ^{3}H-glucose. In most cells, galactose is not converted to other monosaccharides (Text Fig. K; Guyton, 1961) and indeed, no ^{3}H-galactose label appeared in the glycogen of chondrocytes (Figs. 120-123). Chondroitin sulfate contains no galactosyl residues but the glycoprotein of cartilage matrix is rich in galactosyl residues (Roden and Armand, 1966). Thus, it may be that the ^{3}H-galactose-labeled material synthesized in the Golgi region was matrix glycoprotein.

It is known that ^{14}C-glucosamine may be directly incorporated into mucopolysaccharides at the site of connective tissue formation (White et al, 1965) and may also be incorporated in the synthesis of glycoproteins (see "Fate of in-

jected precursors"). Thus the incorporation of ^{14}C-glucosamine label in chondrocytes (Fig. 119) could have indicated synthesis of either or both the glycoprotein and the mucopolysaccharide components of matrix.

The labeling of all chondrocytes by ^{3}H-glucose, ^{3}H-galactose and ^{14}C-glucosamine, along with the effect of hyaluronidase on the glucose label, implies that the glycoprotein and mucopolysaccharide of cartilage matrix, as well as the collagen, may be synthesized simultaneously in a single cell. Because of the intimate relation of the two types of complex carbohydrate in the matrix, it is suggested that their syntheses may be closely related in the Golgi complex. In addition, the secretory proteins passing through this organelle would consist not only of collagen protein (as suggested by Revel and Hay, 1963) but also of the protein moiety of glycoprotein.

IV. PROBLEMS NOT RESOLVED

In the introduction to this thesis, an old and common question was resurrected: what is the role of the Golgi complex? For the various secretory cells discussed here, some answers to the question have been offered. Between the lines of this discussion, however, the question has been replicating in more elaborate forms: if the Golgi complex harbors enzymes responsible for the synthesis of complex carbohydrates for secretion, what is its role in non-secretory cells?

To begin an answer, it must first be stressed that the line between secretory and non-secretory cells has become increasingly blurred. Evidence is rapidly accumulating that free cells (protozoa, tumor cells, immature blood cells) synthesize and secrete complex carbohydrate to provide a layer of specific carbohydrate-protein complexes on their surfaces (Gasic and Gasic, 1962; Marshall and Nachmias, 1964; Kalckar, 1965; Eylar and Matiole, 1965; Eylar and Cook, 1965). There is evidence that a step in the synthesis of these surface materials takes place in the Golgi complex (Marcus, 1962; Gasic and Gasic, 1962; Eylar and Cook, 1965).

Similarly, epithelial cells are endowed with (or endow themselves with) a carbohydrate-containing coat which covers their entire surface (Rambourg et al, 1966). As was illustrated here, the Golgi complex synthesizes complex carbohydrates for export to the thick apical portion of the surface coat; smaller

amounts may well be sent to the basal and lateral parts. In addition, at least some epithelial cells synthesize and secrete a component of their own basement membrane (Hay and Revel, 1963; Pierce, 1964).

The Golgi complex in plant cells is concerned with the secretion of surface coats as well, as EM studies have suggested (Whaley and Mollenhauer, 1963). By EM radioautography it was recently shown that ^{3}H-glucose was incorporated in the Golgi complex of root tip cells for the synthesis of a complex carbohydrate (probably pectin) which is then secreted into the cell wall (Northcote and Pickett-Heaps, 1966).

In certain cells, complex carbohydrate material is segregated into membrane-bound granules in the Golgi region (as if for secretion) but the granules never actually leave the cell. This is true of the acrosome of spermatids (Burgos and Fawcett, 1956), the neurosecretion of neurons (Sharrer and Brown, 1961) and the specific granules of leucocytes (Bainton and Farquhar, 1965).

Thus, in a broad sense, it seems that most or all cells may have a secretory function, and that the complex carbohydrate portion of their secretion product may be synthesized in the Golgi complex.

But even if this is true, the Golgi complex is riddled with biochemical enigmas. The synthesis of specific complex carbohydrates must be genetically determined, but virtually

nothing is known about how nucleotide sequences control the monosaccharide sequences of complex carbohydrates. Are the "transferases" synthesized on nuclear DNA or RNA, or on cytoplasmic ribosomes? And then, how do they arrive on the Golgi membranes? The presence of the enzymes thiamine pyrophosphatase and nucleoside diphosphatase in the Golgi complex of almost all cells (Goldfischer et al, 1964) suggests a common function; do these enzymes play a role in complex carbohydrate synthesis or in some other Golgi-localized process?

Recent EM findings hint at the complexity of this organelle's function. For example, in Sciara salivary gland cells, three different types of Golgi stacks appear to be simultaneously producing 3 distinct types of secretion granules (Phillips and Swift, 1965). As neutrophil myelocytes mature, their Janus of a Golgi complex halts the production of azurophilic granules from one "face" of the stack, and starts producing neutrophil granules - from the opposite face! (Bainton and Farquhar, 1965). Such observations, along with the discovery of rodlike "intercisternal elements" between Golgi saccules (Turner and Whaley, 1965) leave questions about the control of Golgi form and function wide open. In the perspective of these questions, it seems certain that we will continue to wonder at, speculate about, and investigate the Golgi complex for some time to come.

SUMMARY AND CONCLUSIONS

The products of most secretory cells consist of carbohydrate-protein complexes known as "glycoproteins" and "mucopolysaccharides". The protein moiety was known to be synthesized in the rough-surfaced endoplasmic reticulum, but the intracellular site at which the carbohydrate moiety is synthesized and/or linked to protein was not established. Since glucose may be converted to supply all the various monosaccharide building blocks for complex carbohydrate synthesis, ^{3}H-glucose was injected into rats, and their tissues were fixed at various times, from 5 minutes to 4 hours later.

Radioautographs of a variety of secretory cells revealed that very soon after injection the label was incorporated in the Golgi region and later, it appeared in the secretion product. Extraction of the glucose-labeled material by specific enzyme treatment (peracetic acid-beta glucuronidase or hyaluronidase) along with other evidence, demonstrated that at least some of the Golgi-synthesized substance in mucous cells was the glycoprotein of mucus, while that in chondrocytes was, at least in part, the mucopolysaccharide of cartilage matrix.

To determine with more precision the intracellular site of synthesis, the goblet cells of colon (which utilize large amounts of glucose for the synthesis of the carbohydrate moiety of mucus glycoprotein) were further studied with electron

microscope radioautography. By 5 minutes after injection of ^{3}H-glucose, label was incorporated into substances present in the flattened saccules of the Golgi complex. At 20 minutes, both saccules and nearby mucigen granules were labeled and by 40 minutes, mucigen granules carried almost all detectable radioactivity. Between 1 and 4 hours, these labeled granules migrated from the supranuclear region to the apical cell membrane; here, they appeared to be extruded singly, retaining their limiting membranes.

From these results, it was concluded that the Golgi saccule is the site where the carbohydrate moiety is synthesized and/or added to immigrant protein to form glycoprotein for secretion. This conclusion was reinforced by the observation that ^{3}H-galactose and ^{14}C-glucosamine, both of which are building blocks of complex carbohydrates, were also incorporated in the Golgi complex of colonic goblet cells. The conclusion was considered valid for other secretory cells as well.

Further conclusions were drawn concerning the secretory process in colonic goblet cells in particular: it was estimated that one saccule is released from each stack every 2 to 4 minutes - a conclusion implying continuous renewal of Golgi stacks. Various types of evidence indicated that the Golgi synthesis, intracellular migration and release of mucus glycoprotein occur continuously, throughout the life of the

goblet cell.

In a variety of secretory cells, the distributions of ^{3}H-glucose and ^{3}H-galactose labels were compared. While glucose label appeared in the Golgi region of all mucous cells, galactose label was incorporated in the Golgi region of some (but not all) mucous cells. Label from both ^{3}H-glucose and ^{3}H-galactose, as well as ^{14}C-glucosamine, appeared first in the Golgi region of chondrocytes and later in cartilage matrix.

After amylase extraction of glycogen, the Golgi-localized reactions after either ^{3}H-glucose or ^{3}H-galactose were not diminished. In the cytoplasm outside the Golgi region, there remained a scatter of ^{3}H-glucose label, but no ^{3}H-galactose label. As a result, Golgi-localized radioactivity was more readily detected with ^{3}H-galactose, and was observed in non-mucous epithelial cells of kidney, epididymis, pancreas, liver and intestine. In the last case at least, the Golgi-synthesized material was secreted as a surface coat.

It is concluded that the carbohydrate moiety of glycoproteins and mucopolysaccharides is synthesized and/or linked to protein in the Golgi complex of secretory cells.

BIBLIOGRAPHY

AFZELIUS, B.A., Exp. Cell Res. 11: 67, 1956.

ALLEN, J.M., J. Histochem. Cytochem. 11: 529, 1962.

AMANO, M., LEBLOND, C.P. and NADLER, N.J., Exp. Cell Res. 38: 314, 1965.

ANDERSON, A.J., Biochem. J. 82: 372, 1962.

ARZAC, J.P. and FLORES, L.G., Stain Technol. 27: 9, 1952.

ATHINEOS, E., KUKRAL, J.C. and WINZLER, R.J., Arch. Biochem. Biophys. 106: 338, 1964

BADINEZ, O., GASIC, G., LOEBEL, F. and BAYDAK, T., Nature 193: 704, 1962.

BAINTON, D.F. and FARQUHAR, M.G., J. Cell Biol. (Proc.) 27: 6A, 1965.

BAKER, J.R., Quart. J. Microscop. Sci. 95: 383, 1954.

BAKER, N., SHIPLEY, R.A., INCEFY, G., CLARK, R. and SKINNER, S.M., Fed. Proc. 14: 17, 1955.

BAKER, N. and HEUBOTTER, R., Fed. Proc. 22: 357, 1963.

BELANGER, L.F., Anat. Rec. 118: 755, 1954.

BENSLEY, R.R., Exp. Cell Res. 2: 1, 1951.

BERTARELLI, E., Camillo Golgi ed il Suo Tempo (Istituto Sieroterapico Milanese S. Belfanti, Milano, 1950).

BIERRING, F., Arch. Path. Microbiol. Scand. 54: 241, 1962.

BLIX, G., Ann. N.Y. Acad. Sci. 106: 164, 1963.

BLOOM, W. and FAWCETT, D.W. in A Textbook of Histology (Philadelphia & London, W.B. Saunders Co., 1962) p. 572.

BOAS, N.F. and FOLEY, J.B., Proc. Soc. Exp. Biol. Med. 88: 445, 1955

BOURNE, G.H., in Cytology and Cell Physiology (Clarendon Press, Oxford, 1951) p. 249.

BOWEN, R.H., Anat. Rec. 32: 151, 1926.

BOWEN, R.H., Quart. Rev. Biol. 4: 299, 484, 1929.

BRUNI, C. and PORTER, K.R., Am. J. Path. 46: 691, 1965.

BURGOS, M.H. and FAWCETT, D.W., J. Biophys. Biochem. Cytol. 1: 4, 1955.

CAMPO, R.D. and DZIEWIATKOWSKI, D.D., J. Cell Biol. 18: 19, 1963.

CAPPS, J.C. and SHETLAR, M.R., Proc. Exp. Biol. Med. 114: 118, 1963.

CARDELL, R.R., BADENHAUSEN, S. and PORTER, K.R., J. Cell Biol. (Proc.) 27: 120A, 1965.

CARO, L.G., J. Biophys. Biochem. Cytol. 10: 37, 1961.

CARO, L.G., J. Cell Biol. 15: 189, 1962.

CARO, L.G. and PALADE, G.E., J. Cell Biol. 20: 473, 1964.

CHLOPKOW, A., Zeitschr. f. Zellforsch. u. Mikr. Anat. 7: 502, 1928.

CLARA, M., Zeitschr. f. Mikr.-Anat. Forsch. 6: 256, 1926.

CLARK, S.L. and HELMREICH, E., Anat. Rec. (Proc.) 151: 336, 1965.

CLELAND, W.W. and KENNEDY, E.P., J. Biol. Chem. 235: 45, 1960.

CLERMONT, Y., J. Biophys. Biochem. Cytol. (Suppl.) 2: 119, 1956.

COIMBRA, A. and LEBLOND, C.P., J. Cell Biol. (submitted) 1966.

CONKLIN, J.L., Am. J. Anat. 112: 259, 1963.

CURRAN, R.C., CLARK, A.E. and LOVELL, D., J. Anat. 99: 427, 1965.

DALTON, A.J., Nature 168: 244, 1951.

DALTON, A.J., Zeitschr. f. Zellforsch. 36: 522, 1952.

DALTON, A.J. and FELIX, M.D., J. Biophys. Biochem. Cytol. (Suppl.) 2: 79, 1956.

DALTON, A.J., in The Cell. J. Brachet and A.E. Mirsky, eds. (Academic Press, New York, 1961) vol. 2, p. 603.

DAVSON, H., in A Textbook of General Physiology (Little, Brown & Co., Boston, 1961) p. 433.

DEISS, W.P., HOLMES, L.B. and JOHNSTON, C.C., J. Biol. Chem. 237: 3555, 1962.

DE LA HABA, G. and HOLTZER, H., Science 149: 1263, 1965.

DELGIACCO, R. and MALEY, F., J. Biol. Chem. 239: PC2400, 1964.

DEPOCAS, F., Can. J. Biochem. Physiol. 37: 285, 1959.

DISCHE, Z., Ann. N.Y. Acad. Sci. 106: 259, 1963.

DORFMAN, A., J. Histochem. Cytochem. 11: 2, 1963.

DOUGHERTY, W.J., J. Cell Biol. (Proc.) 23: 25A, 1964.

DRAPER, P. and KENT, P.W., Biochem. J. 86: 248, 1963.

DROZ, B. and WARSHAWSKY, H., J. Histochem. Cytochem. 11: 426, 1963.

DROZ, B., C.R. Acad. Sci. (in press), 1966.

DZIEWIATKOWSKI, D.D., J. Biophys. Biochem. Cytol. 2: 29, 1956.

EMMEL, V.M., Anat. Rec. 91: 39, 1945.

EYLAR, E.H. and MATIOLI, G.T., Science 147: 869, 1965.

EYLAR, E.H. and COOK, G.M.W., Proc. Nat. Acad. Sci. U.S.A. 54: 1678, 1965.

FARQUHAR, M.G. and WELLINGS, S.R., J. Biophys. Biochem. Cytol. 3: 319, 1957.

FAWCETT, D.W., Circulation 26: 1105, 1962.

FEWER, D., THREADGOLD, J. and SHELDON, H., J. Ultrastruct. Res. 11: 166, 1964.

FLOREY, H.W., Quart. J. Exp. Physiol. 45: 329, 1960.

FREDERICSSON, B., Exp. Cell Res. 10: 63, 1956.

FREEMAN, J.A., Anat. Rec. 144: 341, 1962.

FREI, J.V. and SHELDON, H., J. Biophys. Biochem. Cytol. 11: 719, 1961.

FRENCH, J.E. and BENDITT, E.D., J. Histochem. Cytochem. 1: 321, 1953.

FRIEND, D.S., J. Cell Biol. 25: 563, 1965.

FRIEND, D.S. and MURRAY, M.J., Amer. J. Anat. 117: 135, 1965.

FULLMER, H.M., J. Histochem. Cytochem. 8: 113, 1960.

GAITONDE, M.K., DAHL, D.R. and ELLIOTT, K.A.C., Biochem. J. 94: 345, 1965.

GASIC, G. and GASIC, T., Nature 196: 170, 1962.

GERSH, I., Arch. Path. 47: 99, 1949.

GINSBURG, V., Advances in Enzymology 35: 35, 1964.

GIROUD, A., LEBLOND, C.P. and RABINOWICZ, M., C.R. Soc. Biol. 115: 1088, 1934.

GLEGG, R.E., EIDINGER, D. and LEBLOND, C.P., Science 120: 839, 1954.

GODMAN, G.C. and LANE, N., J. Cell Biol. 21: 353, 1964.

GOLDFISCHER, S., ESSNER, E. and NOVIKOFF, A., J. Histochem. Cytochem. 12: 72, 1964.

GOLGI, C., Arch. Ital. Biol. 30: 60, 1898.

GOTTSCHALK, A., Ann. N.Y. Acad. Sci. 106: 168, 1963.

GRASSE, P.-P., C.R. Acad. Sci. (Paris) 245: 1278, 1957.

GRASSE, P.-P. and CARASSO, N. Nature 179: 31, 1957.

GRIMSTONE, A.V., Biol. Rev. 36: 97, 1961.

GUYTON, A.C., in A Textbook of Medical Physiology (W.B. Saunders, Philadelphia, 1961) p. 875.

HAGUENAU, F., Internat. Rev. Cytol. 7: 425, 1958.

HAY, E.D. and REVEL, J.-P., Develop. Biol. 7: 152, 1963.

HERRING, G.M. and KENT, P.W., Brit. J. Exp. Path. 39: 631, 1958.

HIBBARD, H., Quart. Rev. Biol. 20: 1, 1945.

HIRSCH, G.C., Protoplasma-Monographien (Bornträger, Berlin, 1939) vol. 18.

HIRSCH, G.C., in Biological Structure and Function, T.W. Goodwin and O. Lindberg, eds. (Academic Press, New York, 1961) vol. 1.

HIRSCH, G.C., in Intracellular Membrane Structure, S. Seno and E.V. Cowdry, eds. (Japan Soc. for Cell Biol., Okayama, 1963).

HIRSCHLER, J., Arch. f. Mikr. Anat. 91, 1918.

HODGE, A.J., McLEAN, J.D. and MERCER, F.V., J. Biophys. Biochem. Cytol. 2: 597. 1956.

HOLLMAN, K.H. and HAGUENAU, F., C.R. Soc. Biol. 155: 1780, 1961.

HOLLMAN, K.H., Ann. N.Y. Acad. Sci. 106: 545, 1963.

HOLLMAN, K.H., Zeitschr. f. Zellforsch. 68: 502, 1965.

HOMMES, O.R., WARSHAWSKY, H. and LEBLOND, C.P., Stain Technol. (submitted), 1966.

HOROWITZ, M.I. and HOLLANDER, F., Fed. Proc. 21: A-262, 1962.

HOROWITZ, M.I., Ann. N.Y. Acad. Sci. 106: 278, 1963.

ITO, S. and WINCHESTER, R.J., J. Cell Biol. 16: 541, 1963.

ITO, S., Anat. Rec. (Proc.) 151: 489, 1965a.

ITO, S., J. Cell Biol. 27: 475, 1965b.

JAMIESON, J.D. and PALADE, G.E., Proc. Nat. Acad. Sci. U.S.A. 55: 424, 1966.

JENNINGS, M.A. and FLOREY, H.W., Quart. J. Exp. Physiol. 41: 131, 1956.

JOHANSEN, P.G., Biochem. J. 87: 63, 1963.

JOURDIAN, G.W., SHIMIZU, F. and ROSEMAN, S., Fed. Proc. 20: 161, 1961.

KALCKAR, H.M., Advances in Enzymology 20: 111, 1958.

KALCKAR, H.M., Science 150: 305, 1965.

KARNOVSKY, M.J., J. Biophys. Biochem. Cytol. 11: 729, 1961.

KATZ, J. and WOOD, H.G., J. Biol. Chem. 235: 2165, 1960.

KENT, P.W., Gastroenterology 43: 292, 1962.

KENT, P.W. and MARSDEN, J.C., Biochem. J. 87: 38P, 1963.

KENT, P.W., Biochem. J. 91: 2P, 1964.

KENT, S.P., J. Histochem. Cytochem. 11: 273, 1963.

KEUTEL, H.J., J. Histochem. Cytochem. 13: 155, 1965.

KIRKMAN, H. and SEVERINGHAUS, A.E., Anat. Rev. 70: 413, 557; 71: 79, 1938.

KOHN, B.D., HOFFMAN, R.C. and KOHN, P., J. Biol. Chem. 238: 1193, 1963.

KOHN, P., WINZLER, R.J., and HOFFMAN, R.C., J. Biol. Chem. 237: 304, 1962.

KOPRIWA, B.M. and LEBLOND, C.P., J. Histochem. Cytochem. 10: 269, 1962.

KRAUSS, S. and SARCIONE, E.J., Biochim. Biophys. Acta 90: 301, 1964.

KUFF, L. and DALTON, A.J., in Subcellular Particles, T. Hayashi, ed. (Ronald Press, New York, 1959) p. 114.

KUMAMOTO, Y., Ph.D. Thesis, Dept. of Anatomy, McGill University, 1956.

KUROSUMI, K., Int. Rev. Cytol. 11: 1, 1961.

LADMAN, A.J., PADYKULA, H.A. and STRAUSS, E.W., Amer. J. Anat. 112: 389, 1963.

LANE, N., CARO, L., OTERO-VILARDEBO, L.R. and GODMAN, G.C., J. Cell Biol. 21: 339, 1964.

LEBLOND, C.P., Amer. J. Anat. 86: 1, 1950.

LEBLOND, C.P. and CLERMONT, Y., Amer. J. Anat. 90: 167, 1952.

LEBLOND, C.P., GLEGG, R.E. and EIDINGER, D., J. Histochem. Cytochem. 5: 445, 1957.

LELOIR, L.F. and CARDINI, C.E., in The Enzymes, P.D. Boyer, H. Lardy and K. Myrback, eds. (Academic Press, New York, 1960) vol. 2, p. 39.

LUCK, D.J.L., J. Biophys. Biochem. Cytol. 10: 195, 1961.

LUCY, J.A., WEBB, M. and BIGGERS, J.D., Biochim. Biophys. Acta 54: 259, 1961.

LUFT, J.H., J. Biophys. Biochem. Cytol. 9: 409, 1961.

MACKLIN, C.C. and MACKLIN, M.T., in Special Cytology, E.V. Cowdry, ed. (Hoeber Inc., New York, 1932) p. 231.

MAILLET, M., C.R. Soc. Biol. 153: 939, 1959.

MALAMED, S. and WEISSMAN, J., Proc. 8th Internat. Congress of Anatomists, Wiesbaden; 74, 1965.

MANDEL, I.D. and ELLISON, S.A., Ann. N.Y. Acad. Sci. 106: 271, 1963.

MARCUS, P.I., Cold Spring Harbor Symposia on Quantitative Biology 27: 351, 1962.

MARKOVITZ, A. and DORFMAN, A., J. Biol. Chem. 237: 273, 1062.

MARSHALL, J.M. and NACHMIAS, V.T., J. Histochem. Cytochem. 13: 92, 1965.

MATHEWS, M.B. and HINDS, L. de C., Biochim. Biophys. Acta 74: 198, 1963.

McGUIRE, E.T., JOURDIAN, G.W., CARLSON, D.M. and ROSEMAN, S., J. Biol. Chem. 240: PC 4112, 1965.

McNABB, J., M.Sc. Thesis, Dept. of Anatomy, McGill University, Montreal, Canada, 1964.

MESSIER, B. and LEBLOND, C.P., Amer. J. Anat. 106: 247, 1960.

MOLLENHAUER, H.H. and WHALEY, W.G., J. Cell Biol. 17: 222, 1963.

MOLLENHAUER, H.H., J. Cell Biol. 24: 504, 1965.

MOLNAR, J., ROBINSON, G.B. and WINZLER, R.J., J. Biol. Chem. 240: 1882, 1965.

MOOG, F. and WENGER, E.L., Amer. J. Anat. 90: 339, 1950.

MOOG, F., Advances in Enzyme Regulation 3: 221, 1965.

MOWRY, R.W., Lab. Invest. 7: 566, 1958.

MOWRY, R.W., Ann. N.Y. Acad. Sci. 106: 402, 1963.

MUNGER, B.L., Amer. J. Anat. 115: 411, 1964.

NADLER, N.J., YOUNG, B.A., LEBLOND, C.P. and MITMAKER, B., Endocrinology 74: 333, 1964.

NASSANOW, D.N., Arch. Mikroskop. Anat. u. Entwicklungsmech. 97: 136, 1923.

NASSANOW, D.N., Arch. Mikroskop. Anat. u. Entwicklungsmech. 103: 437, 1924.

NORTHCOTE, D.H. and PICKETT-HEAPS, J.D., Biochem. J. 98: 159, 1966.

NOVIKOFF, A.B., J. Histochem. Cytochem. 7: 240, 1959.

NOVIKOFF, A.B., GOLDFISCHER, S. and ESSNER, E., J. Histochem. Cytochem. 9: 459, 1961.

NOVIKOFF, A.B. and GOLDFISCHER, S., Proc. Nat. Acad. Sci. U.S.A. 47: 802, 1961.

NOVIKOFF, A.B., ESSNER, E., GOLDFISCHER, S. and HEUS, M. in Interpretation of Ultrastructure (Academic Press, New York, 1962) vol. 1, p. 149.

NOVIKOFF, A.B. and SHIN, W.Y., J. Microscopie 3: 187, 1964.

O'BRIEN, P.J., CANADY, M.R. and NEUFELD, E.F., Fed. Proc. 24: 231, 1965.

OCKERMAN, P.A. and LUNDBORG, H., Biochim. Biophys. Acta 105: 34, 1965.

PALADE, G.E. and CLAUDE, A., J. Morphol. 85: 35, 1949.

PALADE, G.E. in Subcellular Particles, T. Hayashi, ed. (Ronald Press, New York, 1959) p. 64.

PALADE, G., SIEKEVITZ, P. and CARO, L. in The Exocrine Pancreas (Little, Brown & Co., Boston, 1961) p. 23.

PALAY, S.L. in Frontiers in Cytology (Yale Univ. Press, New Haven, 1958) p. 305.

PALAY, S.L. and KARLIN, L.J., J. Biophys. Biochem. Cytol. 5: 373, 1959.

PASTERNAK, C.A. and KENT, P.W., Biochem. J. 68: 452, 1958.

PAUL, J. in Cell and Tissue Culture (E. and S. Livingstone Ltd., Edinburgh, 1959) p. 69.

PEARSE, A.G.E. in Histochemistry, Theoretical and Applied (Churchill Ltd., London, 1960): fixation, pp. 787, 788; PA-Schiff technique, pp. 231, 832; Feulgen reaction, p. 193; toluidine blue, p. 251; hyaluronidase, p. 614.

PEASE, D.C. in Histological Techniques for Electron Microscopy (Academic Press, New York, 1964): sections, p. 184; uranyl acetate, p. 234.

PELC, S.R., COOMBES, J.D. and BUDD, G.C., Exp. Cell Res. 24: 192, 1961.

PERLMAN, R.L., TESLER, A. and DORFMAN, A., J. Biol. Chem. 239: 3623, 1964.

PETERS, T., J. Biol. Chem. 237, 1181, 1962.

PHILLIPS, D.M. and SWIFT, H., J. Cell Biol. 27: 395, 1965.

PIERCE, G.B., J. Cell Biol. (Proc.) 23: 74A, 1964.

PLUMMER, T.H. and HIRS, C.H.W., J. Biol. Chem. 238: 1396, 1963.

PORTER, K.R. and BONNEVILLE, M.A. in An Introduction to the Fine Structure of Cells and Tissues (Lea & Febriger, Philadelphia, 1963) plate 7.

PORTMANN, P., ROSSIER, R. and CHARDONNENS, H., Helv. Physiol. Pharm. Acta 18: 414, 1960.

PROCKOP, D.J., PETTENGILL, O. and HOLTZER, H., Biochim. Biophys. Acta 83: 189, 1964.

RAMBOURG, A., NEUTRA, M. and LEBLOND, C.P., Anat. Rec. (in press), 1966.

RAMON Y CAJAL, S., Trabajos del Laboratorio de Investigaciones Biologicas 12: 127, 1914.

RAMON Y CAJAL, S., Memoirs of the American Philosophical Society 8 (pt. 2): 571 (footnote), 1937.

REVEL, J.-P. and HAY, E.D., Zeitschr. f. Zellforsch. 61: 110, 1963.

REVEL, J.-P., J. de Microscopie 3: 535, 1964.

REYNOLDS, E.S., J. Cell Biol. 17: 208, 1963.

RHODEN, J., Stockholm: Karolinska Institute, 1954.

RHODEN, J. and DALHAMN, T., Zeitschr. f. Zellforsch. 44: 345, 1956.

ROBINSON, G.B., MOLNAR, J. and WINZLER, R.J., J. Biol. Chem. 239: 1134, 1964.

RODEN, L. and ARMAND, G., J. Biol. Chem. 241: 65, 1966.

ROSENBAUM, R.M. and MELMAN, A., J. Cell Biol. 21: 325, 1964.

ROSS, R. and BENDITT, E.P., J. Cell Biol. 27: 83, 1965

SARCIONE, E.J., Biochemistry 1: 1132, 1962.

SARCIONE, E.J., Arch. Biochim. Biophys. 100: 516, 1963.

SARCIONE, E.J., J. Biol. Chem. 239: 1686, 1964.

SARCIONE, E.J. and SOKAL, J.E., Fed. Proc. 23: 273, 1964.

SCHARRER, E. and BROWN, S., Zeitschr. f. Zellforsch. 54: 530, 1961.

SCHNEIDER, W.C., DALTON, A.J., KUFF, E.L. and FELIX, M.D., Nature 172: 161, 1953.

SHACKLEFORD, J.M., personal communication, 1965.

SHEARMAN, D. and MUIR, A., Quart. J. Exp. Physiol. 45: 337, 1960.

SILBERT, J.E., J. Biol. Chem. 238: 3542, 1963.

SINOHARA, H. and SKY-PECK, H.H., Biochim. Biophys. Acta 101: 90, 1965.

SJÖSTRAND, F.S. and HANZON, V., Exp. Cell Res. 7: 393, 1954.

SPICER, S.S., J. Histochem. Cytochem. 8: 18, 1960.

SPICER, S.S., Exp. Cell Res. 28: 480, 1962.

SPICER, S.S., J. Histochem. Cytochem. 13: 211, 1965.

SPIRO, R.G., J. Biol. Chem. 234: 742, 1959.

SPIRO, R.G., New Engl. J. Med. 269: 566, 1963.

STALEY, M.W. and TRIER, J.S., Amer. J. Anat. 117: 365, 1965.

STEELE, R., WALL, J.S., de BODO, R.C. and ALTSZULER, N., Am. J. Physiol. 187: 15, 1956.

STROMINGER, J.L., Biophys. J. pt. 2 (Suppl.): 139, 1964.

SZULMAN, A.E., J. Exptl. Med. 111: 785, 1960.

TESSANOW, W., Acta Histochem. 21: 312, 1965.

THORP, F.K. and DORFMAN, A., J. Cell Biol. 18: 13, 1963.

TRIER, J.S., J. Cell Biol. 18: 599, 1963.

TSUIKI, S. and PIGMAN, W., Arch. Oral Biol. 2: 1, 1960.

TURNER, F.R. and WHALEY, W.G., Science 147: 1303, 1965.

VAN HEYNINGEN, H.E., Anat. Rec. 148: 485, 1964.

VON EBNER, A. in Handbuch der Gewebelehre des Menschens, Koelliker (W. Engelmann, Leipzig, 1899) vol. 3, p. 174.

WALKER, C. and ALLEN, M., Proc. Roy. Soc. Biol. 101: 468, 1927.

WARSHAWSKY, H., LEBLOND, C.P. and DROZ, B., J. Cell Biol. 16: 1, 1963.

WARSHAWSKY, H., personal communication, 1965.

WATKINS, W.M. and HASSID, W.Z., Biochem. Biophys. Res. Comm. 5: 260, 1961.

WEISS, J.M., J. Exptl. Med. 102: 775, 1955.

WELLINGS, S.R. and PHILP, J.R., Zeitschr. f. Zellforsch. 61: 871, 1964.

WETZEL, M.G., WETZEL, B.K. and SPICER, S.S., J. Histochem. Cytochem. (Proc.) 13: 702, 1965.

WHALEY, W.G. and MOLLENHAUER, H.H., J. Cell Biol. 17: 216, 1963.

WHITE, B.N., SHETLAR, M.R., SHURLEY, H.M. and SCHILLING, J.A., Biochim. Biophys. Acta 101: 97, 1965.

ZEIGEL, R.F. and DALTON, A.J., J. Cell Biol. 15: 45, 1962.

ZETTERQVIST, H., quoted by D.C. PEASE in Histological Techniques for Electron Microscopy (Academic Press, New York, 1964) p. 38.

TABLE I

SUMMARY OF RADIOAUTOGRAPHIC EXPERIMENTS

Radioactive materials	Specific activity (mc/mM)	Dose μc per gram body weight	Body weight (grams)	Route of administration*	Time elapsing between injection and sacrifice
Glucose: uptake					
1) ^{3}H-1-glucose+	222	4	100	IP	15 min, 3 hrs
2) ^{3}H-6-glucose†	250	60	110	IP	15 min
3) "	1300	50 total	100	local	5 min
4) "	1300	500 total	100	local	5 min
5) ^{3}H-1-glucose+	222	? total	100	local	5 min
6) ^{3}H-6-glucose†	1300	100 total	250	in vitro	5,15 min
Glucose: migration					
7) ^{3}H-6-glucose†	1300	5	10	SC	5,15,30,45 min 1,2,3 hrs
8) "	1300	100	10	IP	5,20,40 min 1,1.5,4 hrs
Comparison of precursors					
9) ^{3}H-6-glucose†	1300	25	20	IP	10,30 min
10) ^{3}H-1-galactose+	83	25	20	IP	10,30 min
11) ^{14}C-1-glucosamine†	36.3	45	20	IP	10 min
"	2.78	45	20	IP	30 min
12) ^{35}S-sulfate**	carrier-free	2	10	IP	10,30 min 1,3 hrs
13) "	"	2	100	IP	5,15 min 1,3 hrs
14) ^{3}H-4,5-leucine+	5450	35	50	IV	2,6,10,20,30 min
15) "	5450	50 total	100	local	5,15,30 min

* IP, intraperitoneal; SC, subcutaneous; IV, intravenous; local, into lumen of intestine; in vitro, in 1 ml medium.

+ New England Nuclear Corporation, Boston

† Radiochemical Centre, Amersham

** Charles E. Frosst and Co., Montreal

TABLE II

INCORPORATION OF COMPLEX CARBOHYDRATE PRECURSORS

IN THE GOLGI REGION OF 10-20 GRAM RATS

Tissue and Cell	Radioautographic reaction in Golgi region, 5-15 minutes after injection of		
	^{3}H-GLUCOSE	^{3}H-GALACTOSE	^{35}S-SULFATE
Small intestine: goblet	+++	-	+++
columnar	±	++	-
Large intestine: goblet	++++	++	+++
columnar	±	+	-
Brunner's gland: mucous	++++	+++	-
Stomach: surface mucous	++++	±	++
mucous neck	+	-	-
Salivary glands:			
sublingual (mucous)	+++	++	-
submaxillary (mucous)	+	-	-
Trachea: epithelial mucous	++	+	+
mucous gland	+++	-	+++
serous gland	±	-	-
Pancreas: acinar	±	+	-
Liver: parenchymal	-	+	-
Epididymis: epithelial	±	++	-
Kidney: proximal tubule	-	++	-
Chondrocytes in trachea	+	+	++
in knee joint	+++	+++	+++

± in the 'glucose' column indicates a reaction seen only after high dose in 100-gram rat (Table I, experiments 2-5).

TABLE III

MIGRATION OF LABELED SECRETORY MATERIAL

Cells showing a reaction in the Golgi region at 5-15 min. after injection of ^{3}H-glucose or -galactose*	Approximate time at which label first appeared	
	in cell apex (or peripheral cytoplasm)	in extracellular secretory products
Mucus-secreting cells: (^{3}H-glucose label)		
duodenal goblet	40 min. - 1 hr.	3-4 hr.
colonic goblet	1 hr.	3-4 hr.
Brunner's gland	45 min.	1-2 hr.
stomach: surface mucous	40 min.	4 + hr.
sublingual gland	1 hr.	4 + hr.
submaxillary gland	45 min.	3 hr.
trachea: epithelial mucous	1 hr.	4 hr.
mucous glands	1 hr.	3 hr.
Non-mucous epithelial cells: (^{3}H-galactose label)*		
duodenal columnar	*	30 min.
colonic columnar	*	30 min.
epididymis: epithelial	*	30 min.
kidney: proximal tubule	*	30 min.
Chondrocytes: (^{3}H-glucose and -galactose label)	15-20 min.	30-40 min.

* Galactose-labeled tissues were observed only at 10 and 30 minutes after injection.

TABLE IV

REMOVAL OF ^{3}H-GLUCOSE-LABELED MATERIAL FROM THE GOLGI REGION OR SECRETORY MATERIAL OF REPRESENTATIVE CELLS, BY ENZYMATIC DIGESTION

Tissue or Cell	Salivary amylase	Testicular hyaluronidase	Peracetic acid--glucuronidase
Secretory epithelia:			
colonic goblet	no effect	no effect	removal
duodenal goblet	"	"	removal
intestinal columnar	"	"	no effect
stomach surface mucous	"	"	removal
sublingual mucous	"	"	removal
Connective tissues:			
cartilage (trachea)	"	removal	no effect
loose connective tissue (tooth pulp)	"	removal	"

TABLE V

EFFECT OF AMYLASE DIGESTION ON SCATTERED LABEL IN CELLS OF 10- AND 20-GRAM RATS 30-40 MINUTES AFTER INJECTION OF ^{3}H-GLUCOSE OR ^{3}H-GALACTOSE

Tissue and cell	Scattered label present in cytoplasm			
	control		amylase-digested	
	glucose	galactose	glucose	galactose
Striated muscle	++++	++++	++	+
Liver: parenchymal	++	+++	++	-
Colon: goblet	++	++	+	-
columnar	++	++	+	-
Duodenum: goblet	++	-	+	-
columnar	+	-	+	-
Pancreas: acinar	+	-	+	-

TABLE VI

APPROXIMATE INTENSITY OF RADIOAUTOGRAPHIC REACTIONS IN VARIOUS TISSUES OF 20-GRAM RATS AT 10 AND 30 MINUTES AFTER INJECTION OF ^{14}C-GLUCOSAMINE

Radioautographic reactions*	Tissue or organ	Within tissue, radioactivity concentrated in:
Intense	small intestine	epithelium: columnar cells
	large intestine	epithelium: goblet cells
Heavy	liver	cells near portal space
	kidney	proximal convoluted tubules
	tongue (30 min)	epithelium and muscle fibers
Moderate	pancreas	small ducts, islets
	sublingual gland	mucous cells
	epiphyseal cartilage	chondrocytes
Light	tracheal cartilage	chondrocytes
	lung	
	submaxillary gland	
	stomach	surface epithelium
Negligible	myocardium	
	testis, epididymis	
	spleen, thymus	

* The intense labeling of the peritoneal surfaces of abdominal and pelvic organs, due to direct absorption of the intraperitoneally-injected precursor, was disregarded.

TABLE VII

PERCENT REDUCTION IN COUNT OF SILVER GRAINS

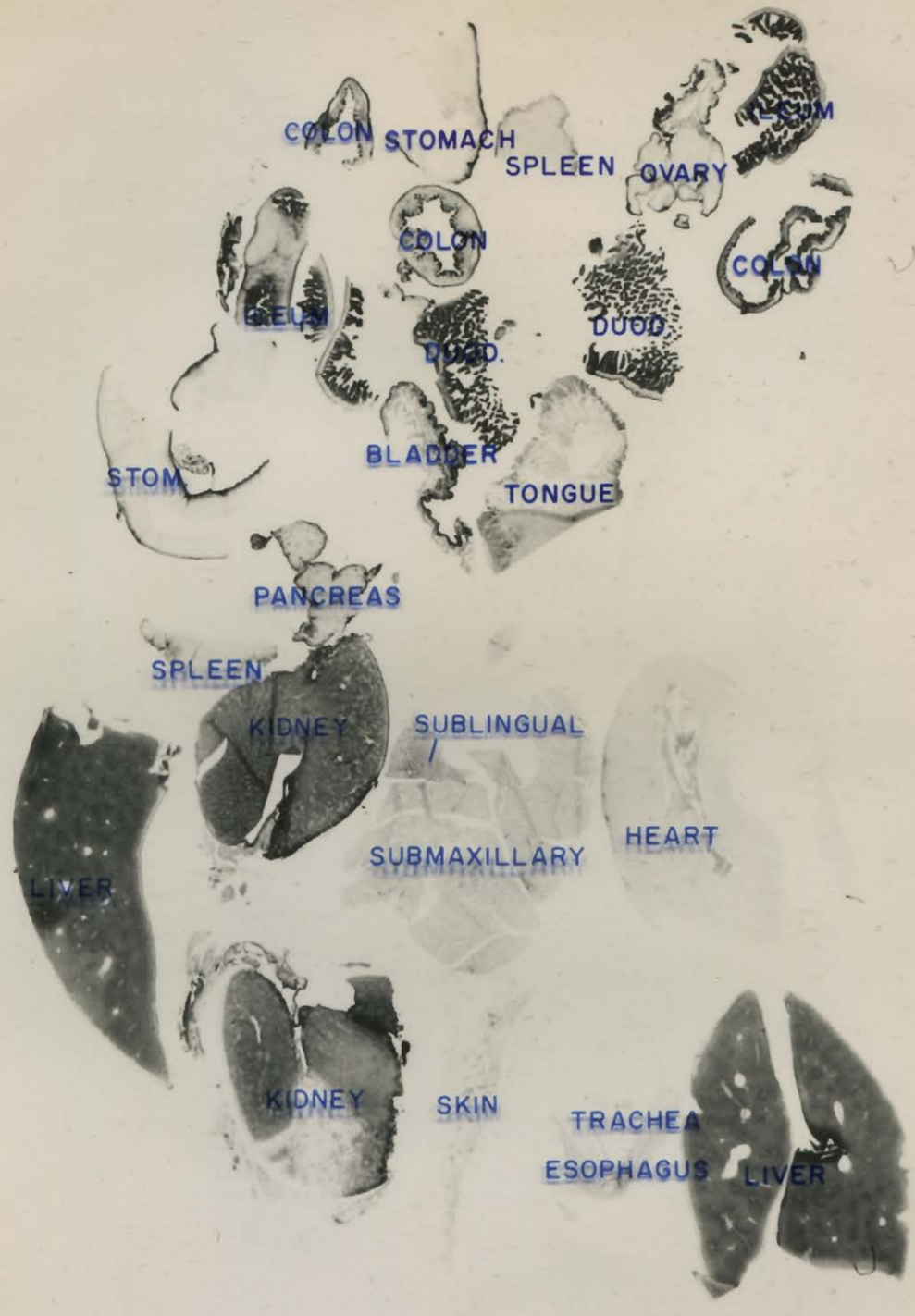
AFTER DIGESTION WITH TESTICULAR HYALURONIDASE

Time after injection	Chondrocyte cytoplasm	Matrix	Pulp of tooth (loose connective tissue)
5 minutes	0	*	0
20 "	8-10	*	0
40 "	37	50	12
1 hour	47	57	32
1.5 hours	44	58	38
4 "	68	42	74

* Labeled material first appeared outside the cells at 40 minutes after injection

Fig. 1 Unstained radioautograph of a variety of rat tissues, 10 minutes after injection of ^{14}C-glucosamine. (Exposed 4 weeks)

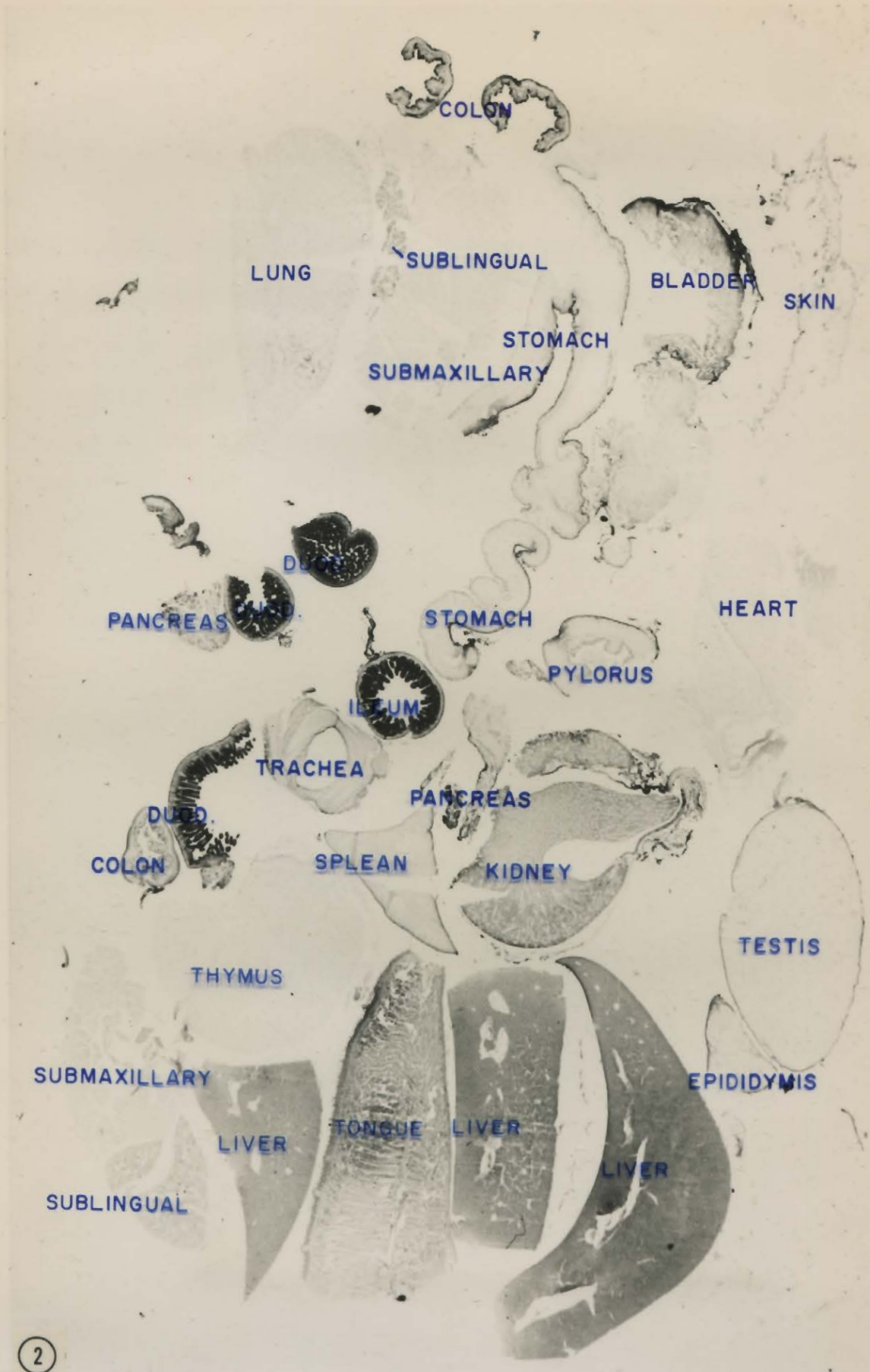
The darkest and clearest images in the photographic emulsion are produced by the tissues which contain the greatest concentrations of radioactivity: intestinal epithelium, liver, and kidney. Other tissues have incorporated smaller amounts of label.


COLON
STOMACH
SPLEEN
OVARY
ILEUM
COLON
COLON
ILEUM
DUOD.
DUOD.
BLADDER
STOM.
TONGUE
PANCREAS
SPLEEN
KIDNEY
SUBLINGUAL
HEART
SUBMAXILLARY
LIVER
KIDNEY
SKIN
TRACHEA
ESOPHAGUS
LIVER

①

Fig. 2 Unstained radioautograph of a variety of rat tissues, 30 minutes after injection of ^{14}C-glucosamine. (Exposed 4 weeks)

Epithelia of small and large intestine contain high concentrations of radioactive material; liver, kidney and tongue contain considerable quantities as well.


COLON
LUNG
SUBLINGUAL
BLADDER
SKIN
STOMACH
SUBMAXILLARY
PANCREAS
STOMACH
HEART
PYLORUS
TRACHEA
PANCREAS
COLON
SPLEAN
KIDNEY
TESTIS
THYMUS
SUBMAXILLARY
EPIDIDYMIS
LIVER
LIVER
LIVER
SUBLINGUAL

(2)

Fig. 3 Longitudinal section through a surface goblet cell in colon of 10-gram rat, with adjacent columnar cells (cc), as drawn in Fig. 4, left. 8250 X

Above the basal nucleus (N) 4 or 5 stacks of Golgi saccules (G) form a U around a group of central mucigen granules (mg). Similar mucigen granules fill the cell apex. The dense cytoplasm around the nucleus and near the lateral membranes contains rough-surfaced endoplasmic reticulum (rER) and mitochondria (m).

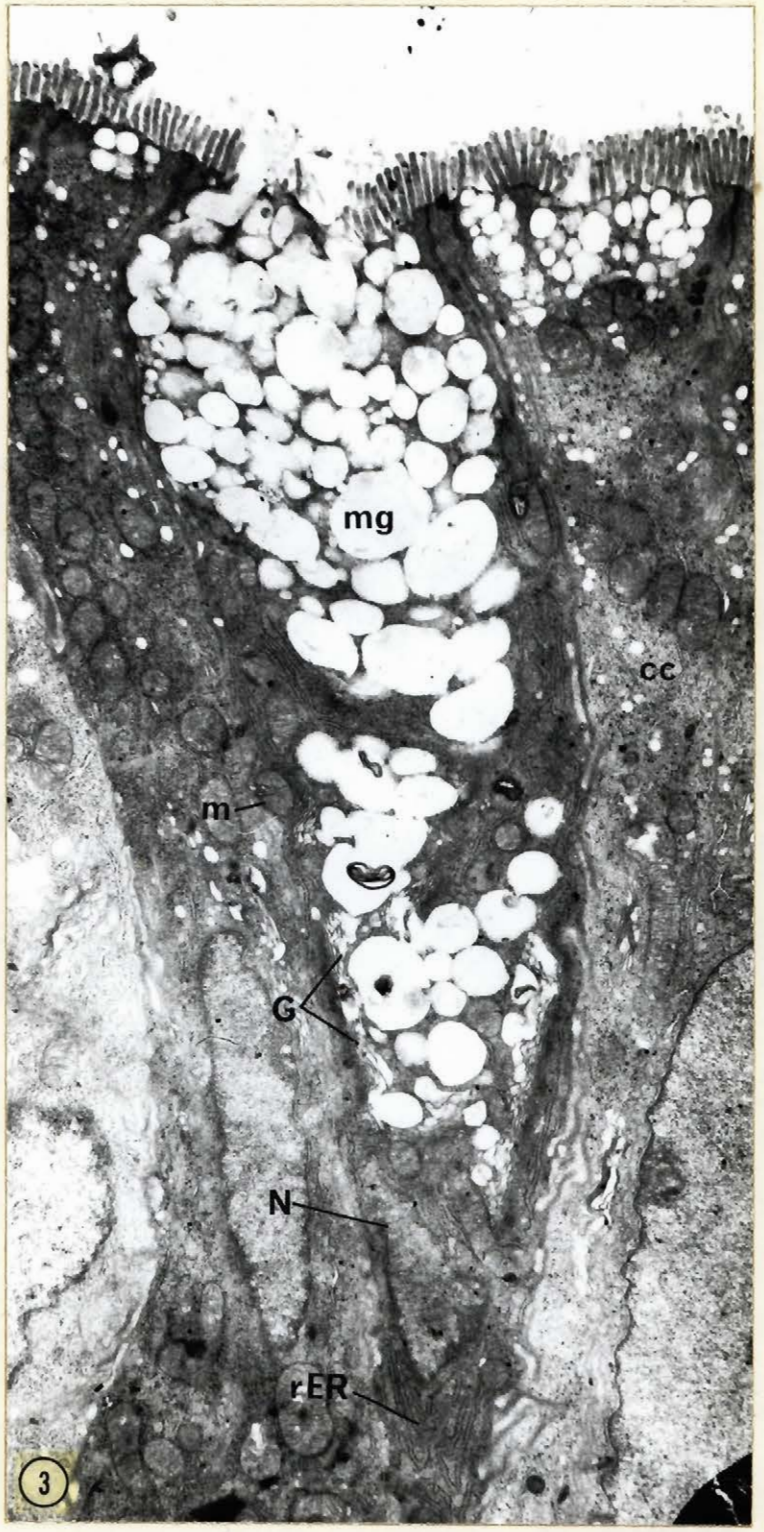
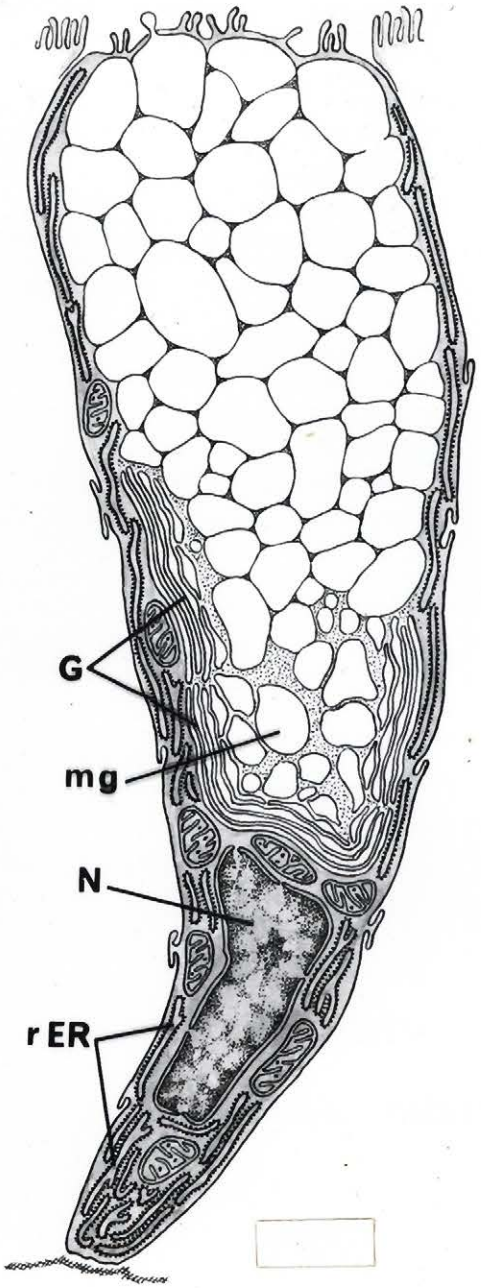

mg
cc
m
G
N
rER
3

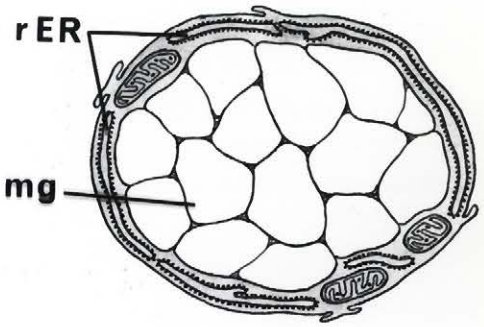
Fig. 4 Semi-schematic drawings based on electron micrographs of surface goblet cells in colon of 10 or 20-gram rats.

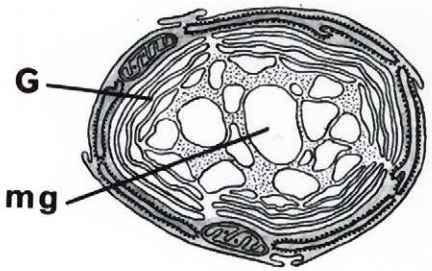
Left: In longitudinal section, the Golgi complex (G) forms a U above the nucleus (N). Each of its several stacks is composed of 7-12 saccules (only 4 of which are depicted here). There seem to be transitions between distended central saccules and the mucigen granules which occupy the central portion of the supranuclear region ("central granules"). Above, closely-packed "apical granules" occupy the cell apex. (rER, rough-surfaced endoplasmic reticulum).

Lower right: Transverse section through the supranuclear region. The Golgi complex (G) forms a ring around the central mucigen granules (mg).

Upper right: Transverse section above the Golgi complex. The group of apical mucigen granules (mg) is rimmed by a narrow margin of cytoplasm with rough-surfaced endoplasmic reticulum (rER) and mitochondria.







4

Fig. 5 Cross section through the supranuclear region of a surface goblet cell in colon of 10-gram rat, as depicted schematically in Fig. 4, lower right. 27500 X

Three or four Golgi stacks, each consisting of 10 saccules, form a ring around a few central mucigen granules. Between the lateral cell membrane (LM) and the Golgi complex is a layer of cytoplasm with cisternae of rough-surfaced endoplasmic reticulum and mitochondria. The outer-most saccules (arrows) are distended, while the other peripheral saccules (ps) are flat with no discernable lumens. The central saccules (cs) are progressively more widely distended, forming transitions between flat Golgi saccule and spheroidal mucigen granule. (c, cytoplasm).

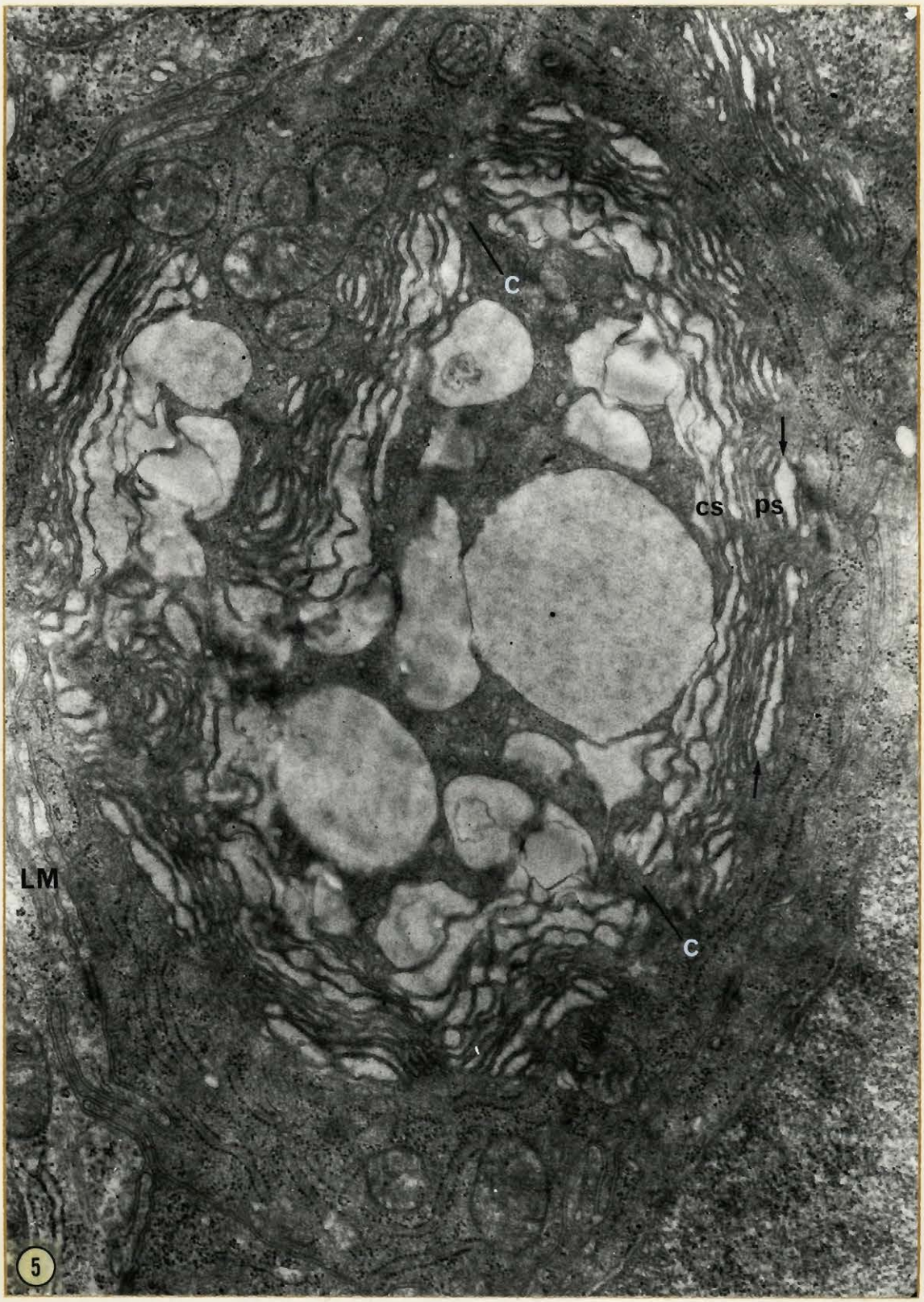
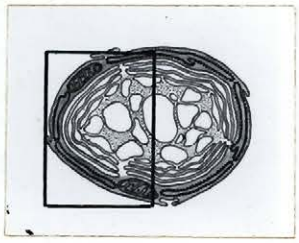

C
cs
ps
LM
C
5

Fig. 6 Part of a transverse section through the supranuclear region of a goblet cell, as illustrated schematically. 48000X



Two adjacent Golgi stacks meet at a right angle; in the angle, their central saccules are continuous (con). The lumens of the outermost saccules are wide (arrows), and the nearby cisternae of endoplasmic reticulum (rER) appear to have lost their ribosomes in the region nearest the Golgi complex. The content of the distended central saccules (cs) has the same density as the content of the mucigen granules (mg).

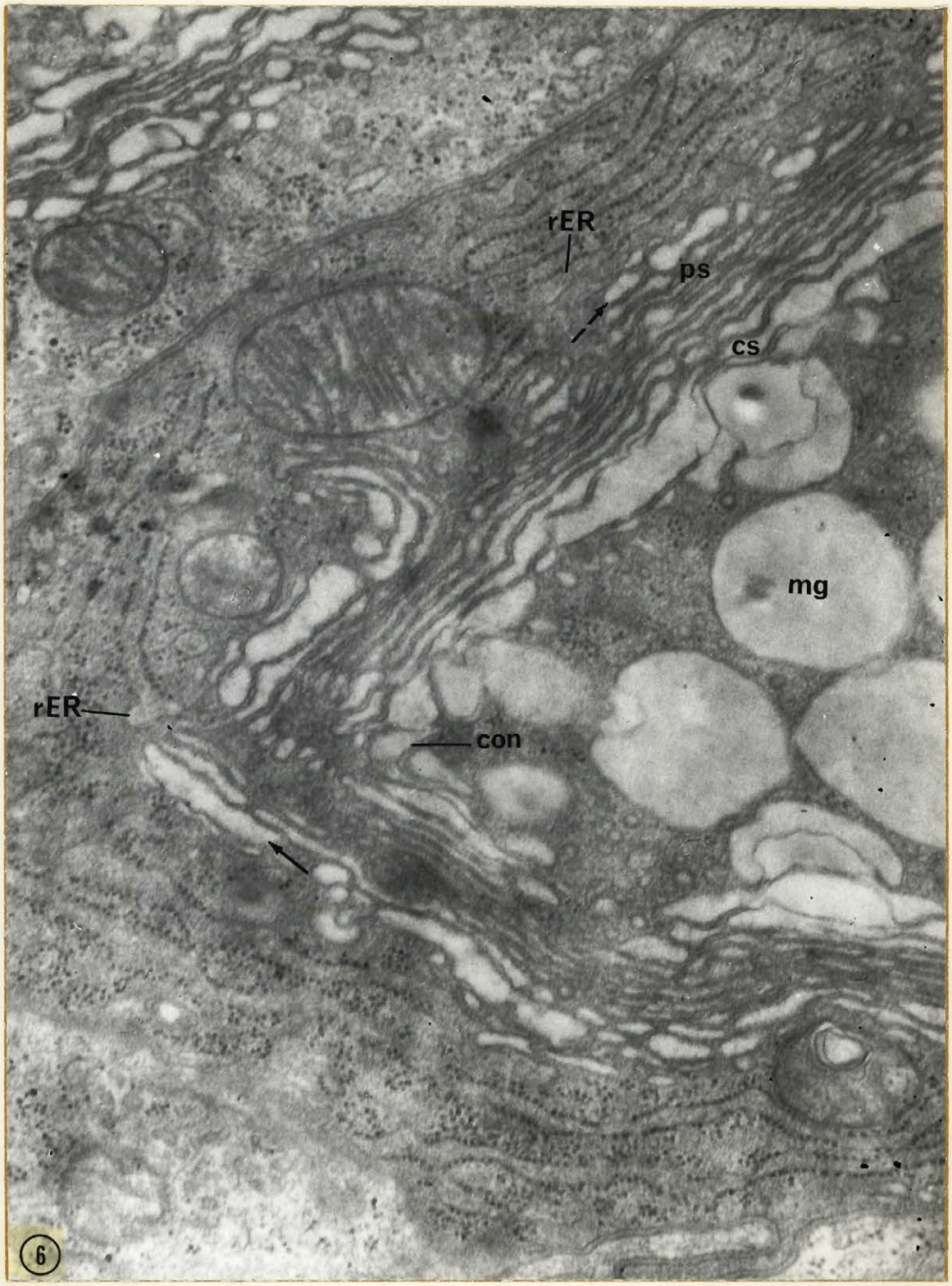

rER
ps
cs
mg
rER
con
6

Fig. 7 The apical surface of a longitudinally-sectioned goblet cell in colon of 10-gram rat. 21000 X

An irregular layer of dense cytoplasm (c) with a few short microvilli separates the mass of apical mucigen granules from the lumen. Through separate clefts in this layer, 3 membrane-bound mucigen granules are seen bulging into the lumen (arrows) presumably in the course of being released from the cell.

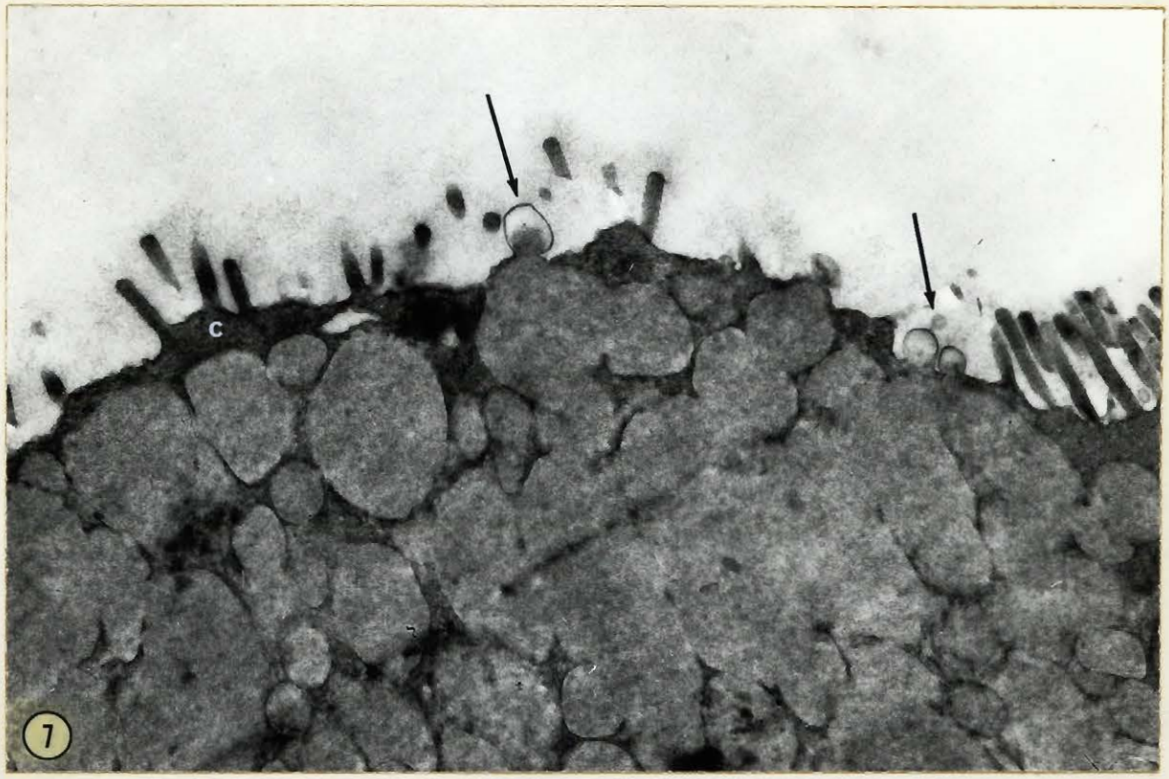

C
7

Fig. 8 The apical surface of the colonic goblet cell near a lateral cell membrane (LM). 42000 X

The section passes just to one side of a cleft in the apical cytoplasm (c), but twice traverses the mucigen granule (mg), which is passing through the cleft into the lumen. The fibrillar mucigen seems to be flowing under a cytoplasmic bridge; in the lumen it is still membrane-bound (arrow).

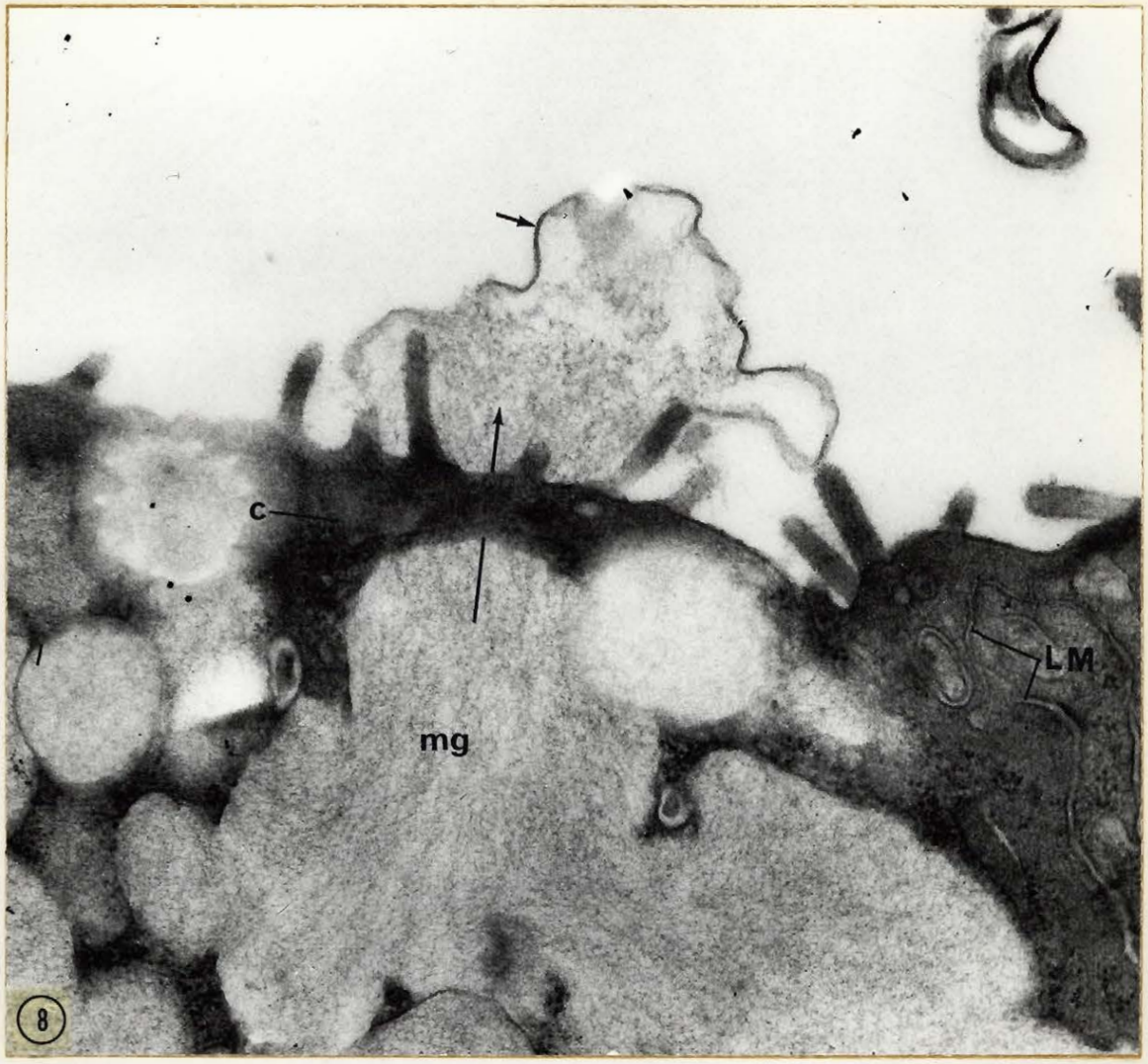

c
mg
LM
8

Fig. 9 A colonic goblet cell near the mouth of a crypt. 10000 X

The lateral membrane (LM) of the short goblet cell covers its cytoplasmic interdigitations with the neighbouring columnar cell. The nucleus of the goblet cell seems to be distorted by the closely packed mucigen granules (mg) which distend the cell apex. The Golgi complex (G) is limited to a small region above the nucleus (N).

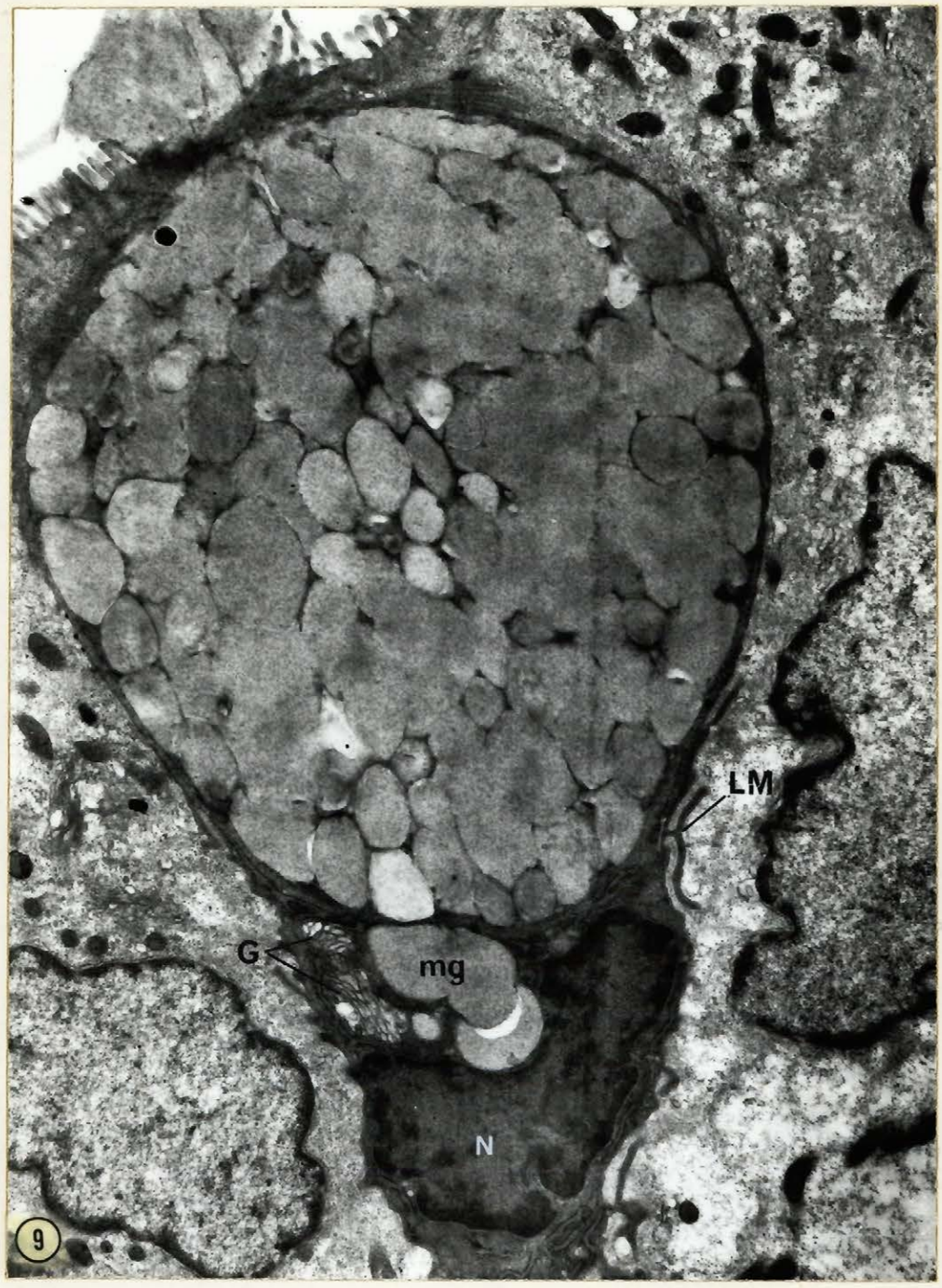

LM
G
mg
N
9

Fig. 10 A goblet cell deep in a colonic crypt of a 20-gram rat, with several neighbouring columnar cells. 10500 X

The small compressed nucleus (N) is confined to a small area on the basement membrane, while tightly-packed mucigen granules fill the rest of the cell. The stacks of saccules comprising the Golgi complex (G) are insinuated among the mucigen granules, along with some cisternae of rough-surfaced endoplasmic reticulum.

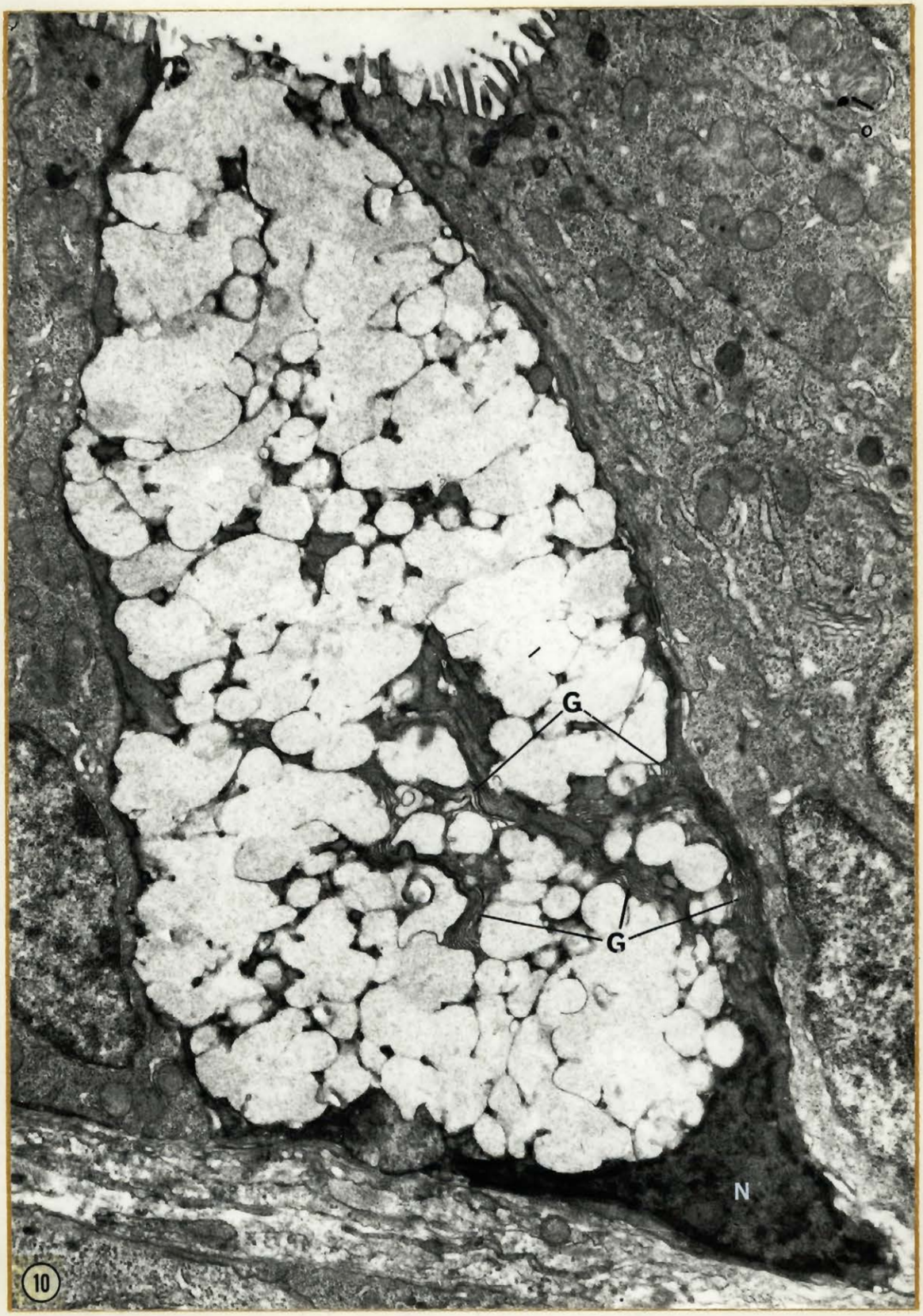

G
G
N
10

Fig. 11 Colon of 10 gram rat 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. (Half-μ Epon section, toluidine blue stain). 390 X

Figs. 12-17 Radioautographs of surface epithelium in colon of 10 gram rat, at various times after injection of ^{3}H-glucose. (Half-micron Epon sections, Ilford L-4 emulsion, toluidine blue stain). 1300 X

Fig.12 Five minute interval. 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. (Exposed 3 months).

Fig.13 Five minute interval. 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. (Exposed 3 months).

Fig.14 Five minute interval. Longer exposure (7 1/2 months) enhances the Golgi-localized reaction but does not alter its location.

Fig. 15 Twenty minute interval. Several goblet cells, longitudinally or obliquely sectioned, are present. The band of grains is denser and thicker than that seen at 5 minutes, but it shows the same localization. (Exposed 7.5 months).

Fig. 16 One and a half hour interval. The intense radioautographic reaction covers the supranuclear area and the lower edge of the apical mucigen. (Exposed 3 months).

Fig. 17 Four hour interval. 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). (Exposed 3 weeks).

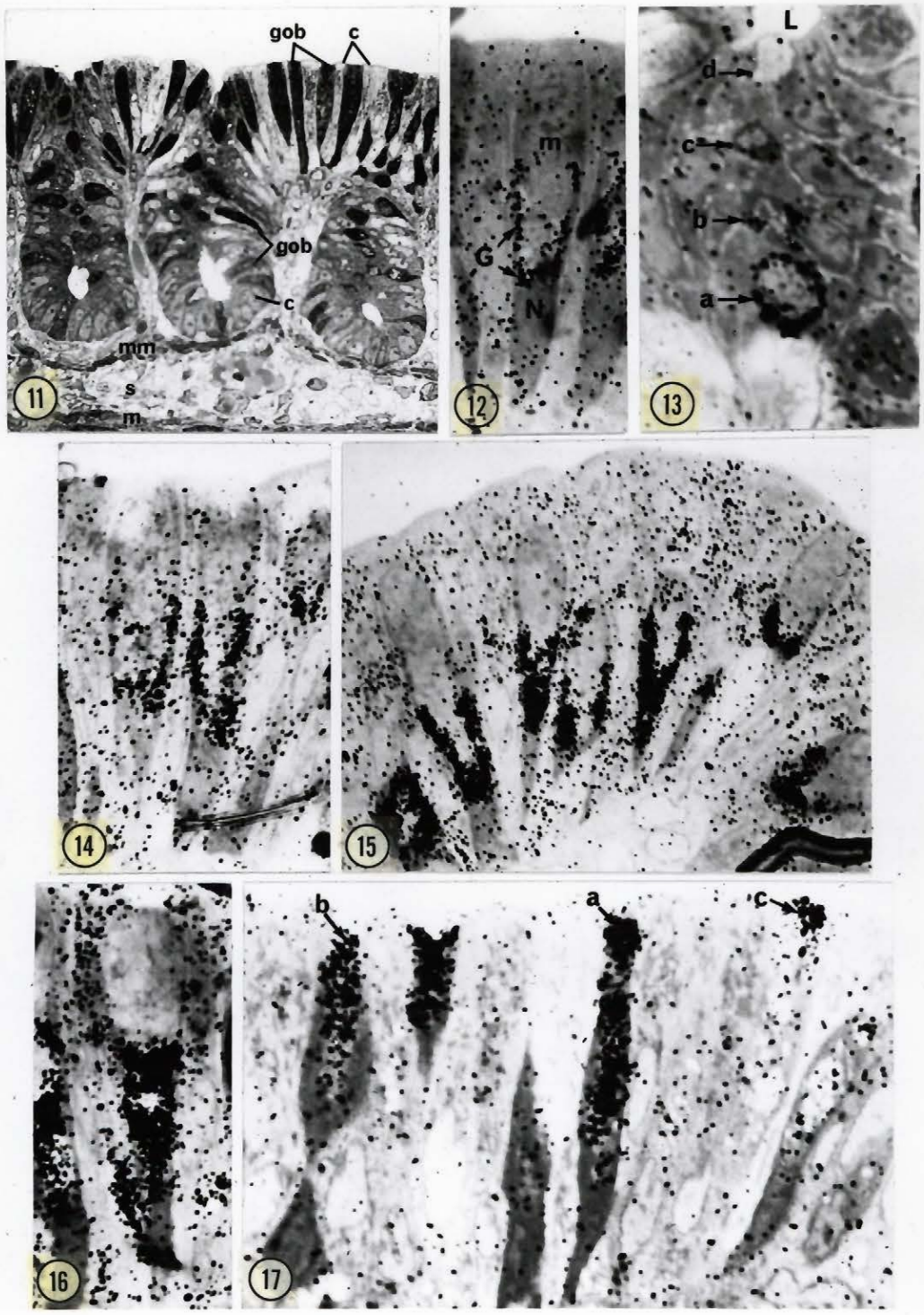
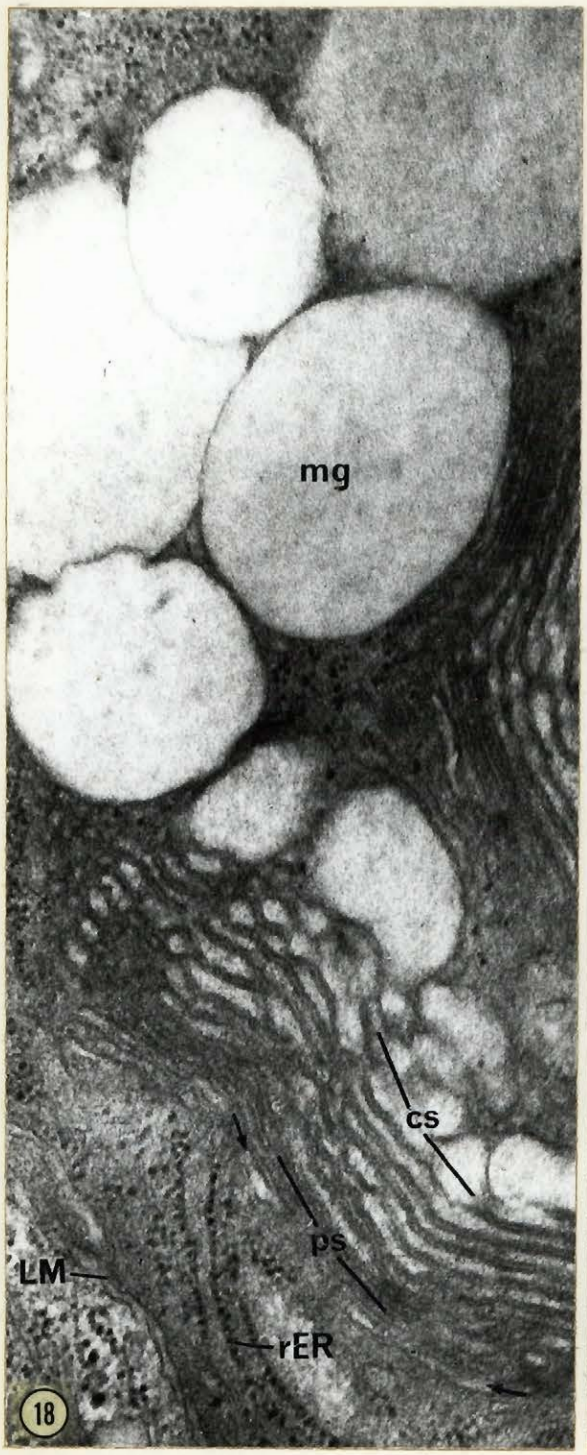

gob
c
gob
c
mm
s
m
11
m
G
N
12
L
d
c
b
a
13
14
15
16
b
a
c
17

Fig. 18 Goblet cell in colon of 10 gram rat. 60,000 X

In the lower half of the picture is a stack of Golgi saccules, separated from the lateral cell membrane (LM) by cytoplasm containing cisternae of rough-surfaced endoplasmic reticulum (rER) and a few free ribosomes. The peripheral saccules (ps) are flat and have no discernable 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).

Fig. 19 Radioautograph at 5 minutes after local injection of ^{3}H-glucose, showing a transverse section through the supranuclear region of two goblet cells (arrows indicate intercellular space). (Ilford L-4 emulsion, exposed 4 months) 21,000 X

Silver grains overlie the stacks of Golgi saccules (G). Neither the central mucigen granules (mg) nor the peripheral cytoplasm (c) are labeled.


mg
cs
ps
LM
rER
18

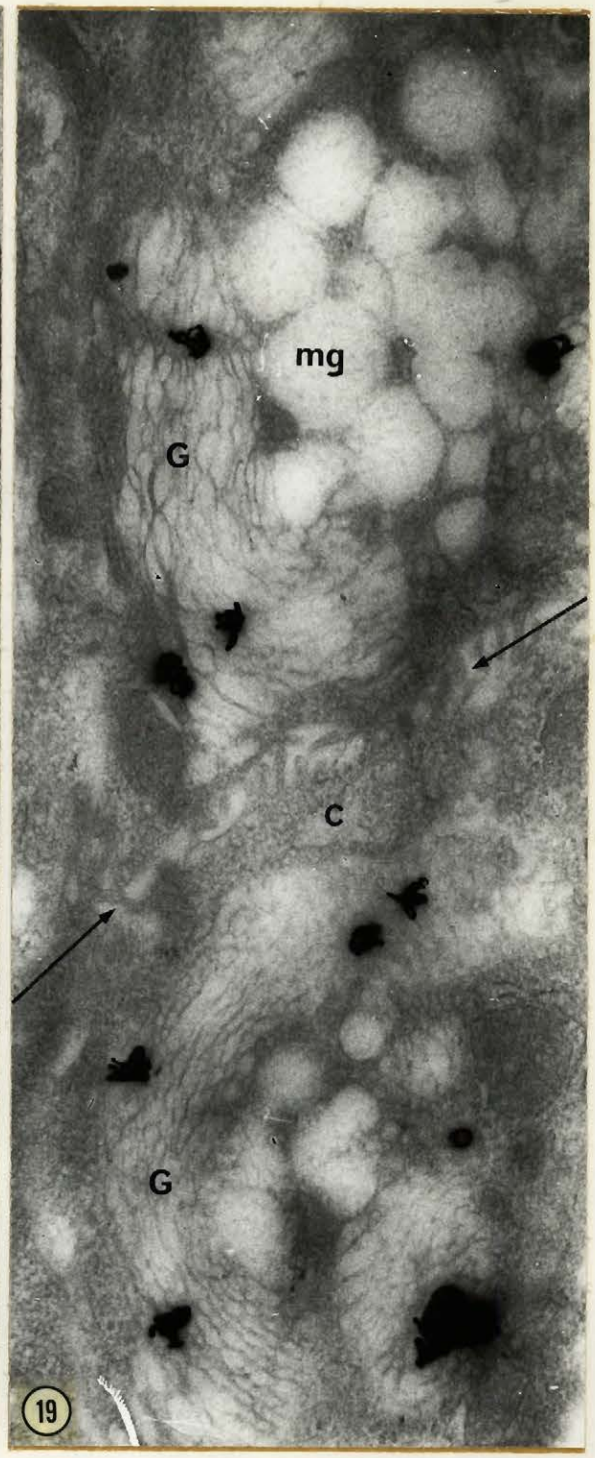

mg
G
C
G
19

Fig. 20 Radioautograph of a colonic goblet cell at the 20-minute interval, showing the supranuclear region. In the oddly-oriented Golgi complex, the arrow runs from flat peripheral saccule to dilated central saccule. (Gevaert 307 emulsion, exposed 2.5 months). 40000 X

Some silver grains are seen over Golgi saccules and others, over nearby mucigen granules (mg).

Fig. 21 Twenty minute interval. (The dark area at upper left is the grid.) (Gevaert 307 emulsion, exposed 2.5 months). 56000 X

A stack of Golgi saccules (G) is separated from the lateral cell membrane (LM) by rough-surfaced endoplasmic reticulum (rER). The label is again seen at all levels of the Golgi stack and in an adjacent mucigen granule (mg).


CC

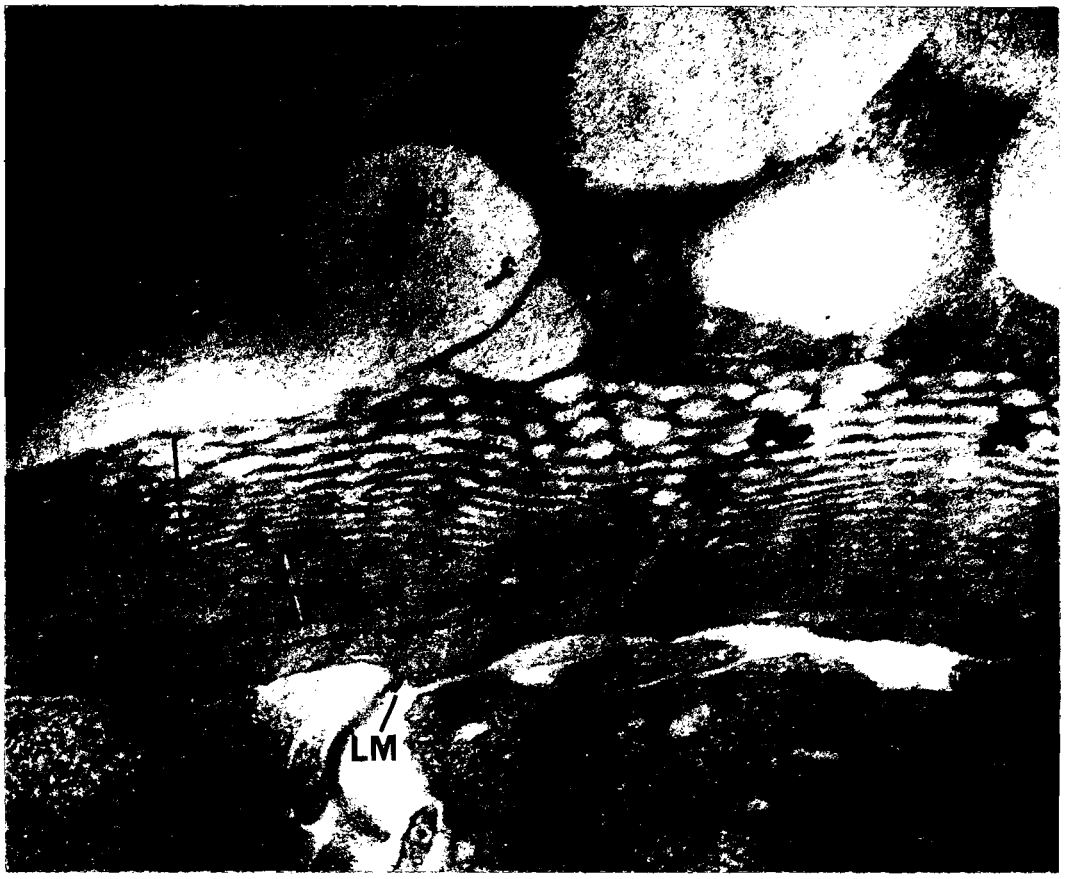

LM

Fig. 22 Radioautograph of a goblet cell in longitudinal section, with neighbouring columnar cells (cc) 40 minutes after injection of ^{3}H-glucose. (Gevaert 307 emulsion, exposed 2.5 months)

24000 X

The flattened saccules of the Golgi complex (G) are no longer detectably labeled. Instead, silver grains indicate the presence of label in the central mucigen granules (mg).

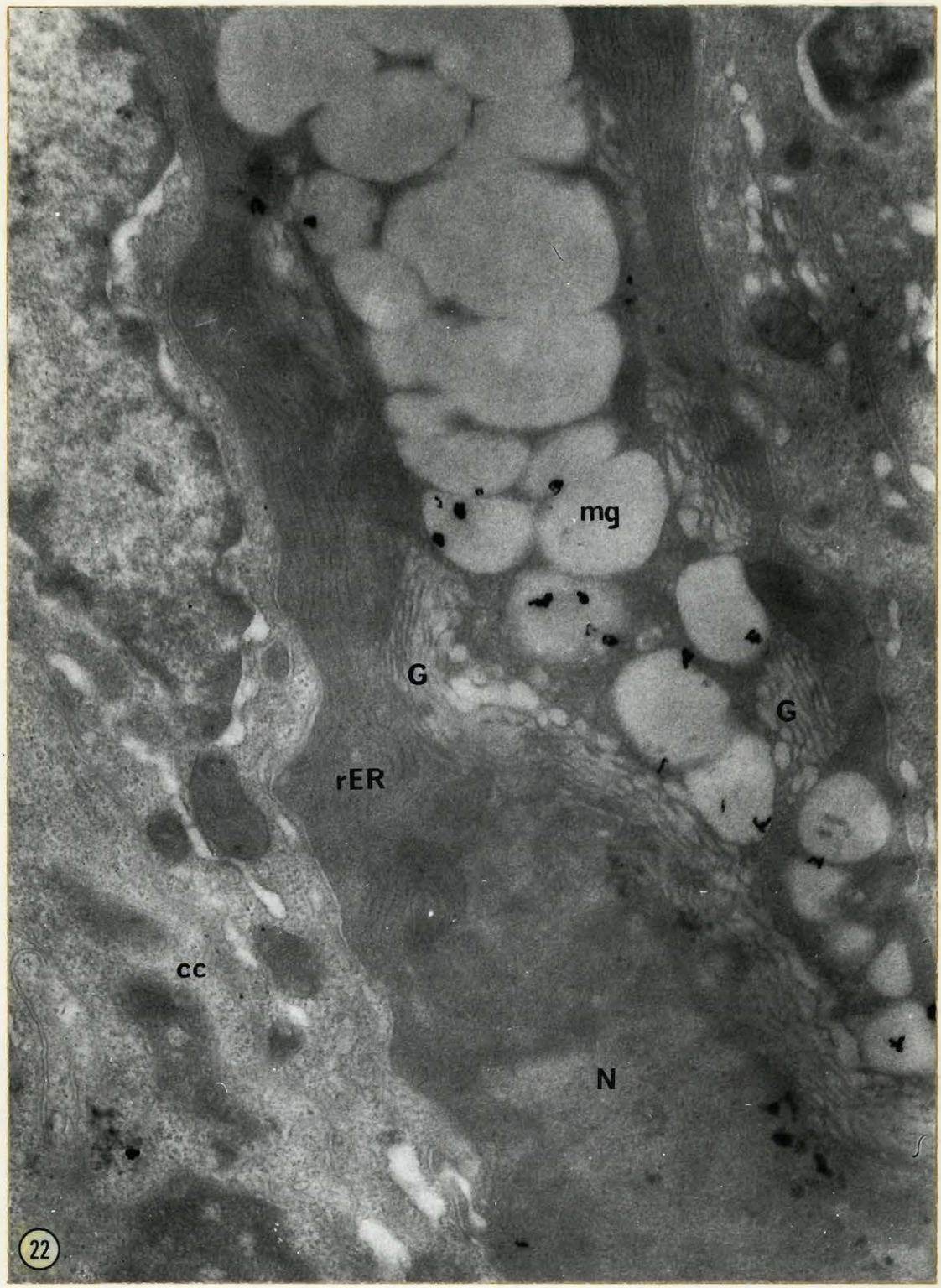

mg
G
G
rER
cc
N
22

Fig. 23 Longitudinal section of a goblet cell, 40 minutes after ^{3}H-glucose injection. Columnar cells (cc) are on both sides. (A deposit of gelatin from the emulsion shows at lower right). (Gevaert 307 emulsion, exposed 2.5 months). 24,000 X

The Golgi complex (G) is not labeled. Silver grains lie over the central mucigen granules (mg) but not over the apical mucigen granules. (rER, rough-surfaced endoplasmic reticulum; LM, lateral cell membrane).

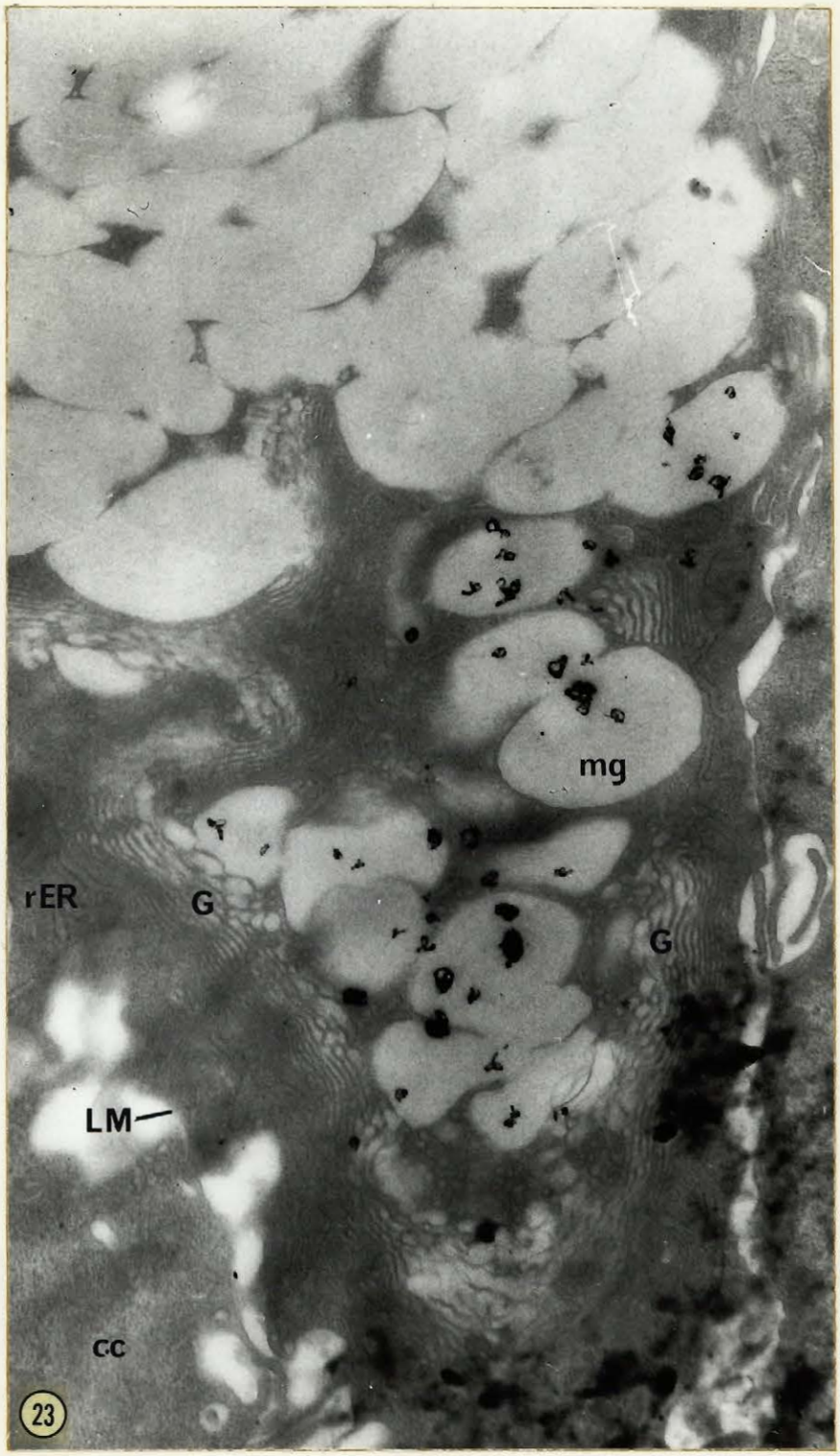

mg
rER
G
G
LM
cc
23

Fig. 24 Radioautograph of a goblet cell showing part of the supranuclear region, 1 hour after injection of ^{3}H-glucose. (Gevaert 307 emulsion, exposed 2.5 months) 24000 X

Although the Golgi complex (G) shows little or no label, nearby mucigen granules are heavily labeled. More distant mucigen granules (mg) are not labeled. (rER, rough-surfaced endoplasmic reticulum; LM, lateral cell membrane).

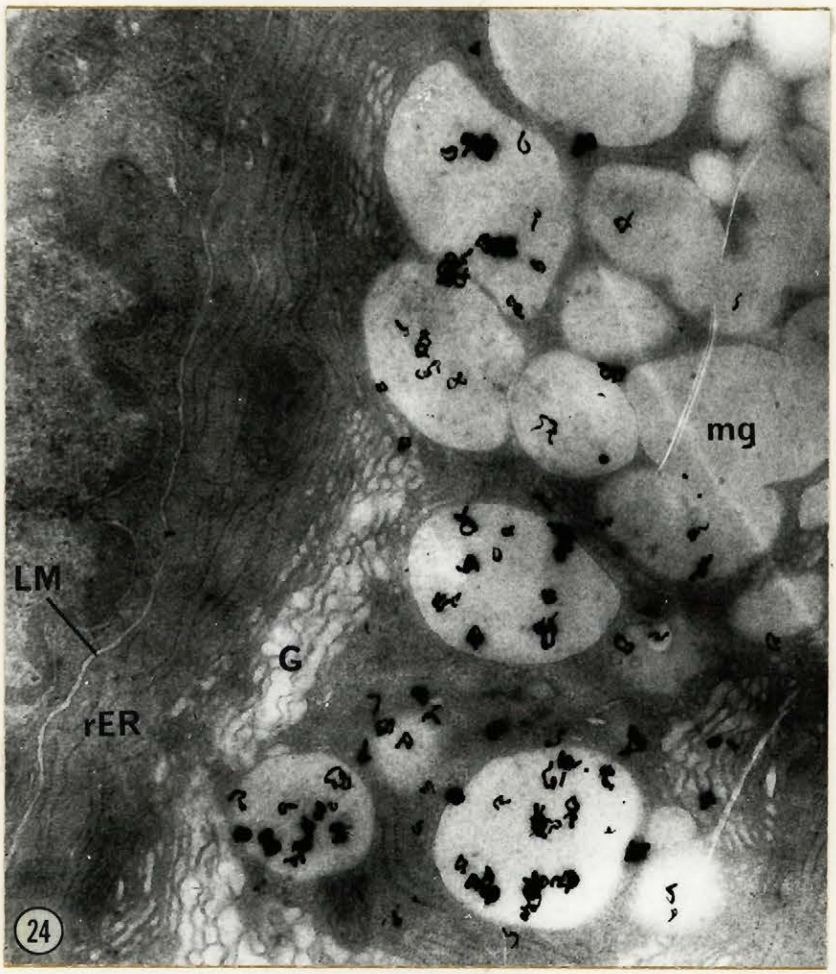
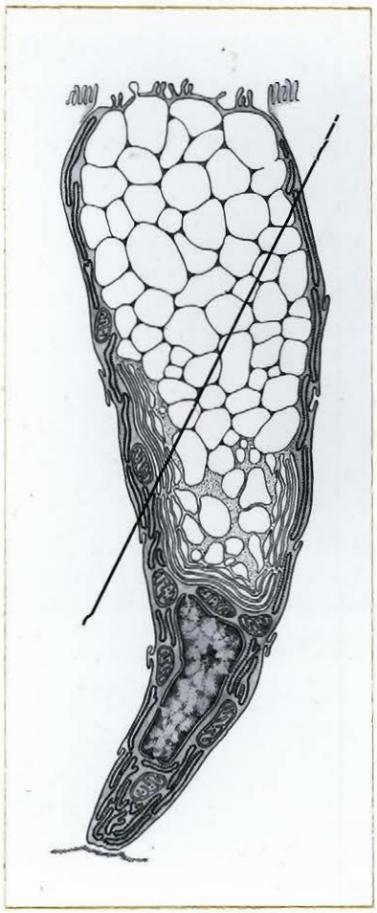

mg
LM
G
rER
24

Fig. 25 A colonic goblet cell sectioned obliquely through the brim of the cup-like Golgi complex (as in the diagram below). Radioautograph, one hour after injection of ^{3}H-glucose. (Gevaert 307 emulsion, exposed 2.5 months) 30000 X



The three stacks of Golgi saccules (G) present in the section are not labeled, but 4 nearby mucigen granules (mg) contain radioactive material. The granules' membranes are intact (arrowheads). A few silver grains are scattered over other organelles (sc).

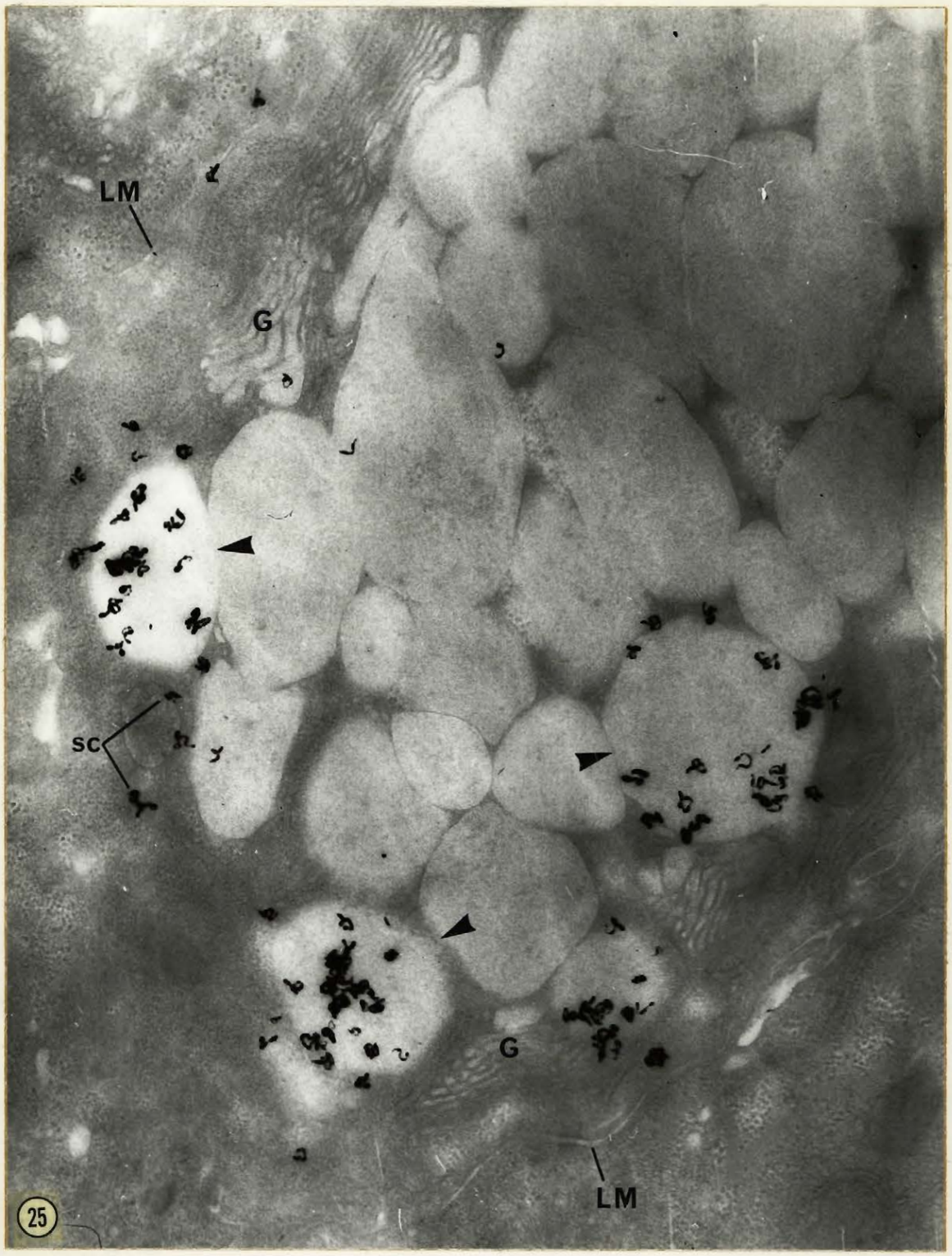

LM
G
SC
G
LM
25

Fig. 26 One and a half hours after ^{3}H-glucose injection. (Exposed 2.5 months, Gevaert 307 emulsion). 14,000 X

Discretely labeled mucigen granules are located mainly in the supranuclear region. The Golgi stacks (G) are little or not labeled. A few silver grains are scattered over the cells (sc).

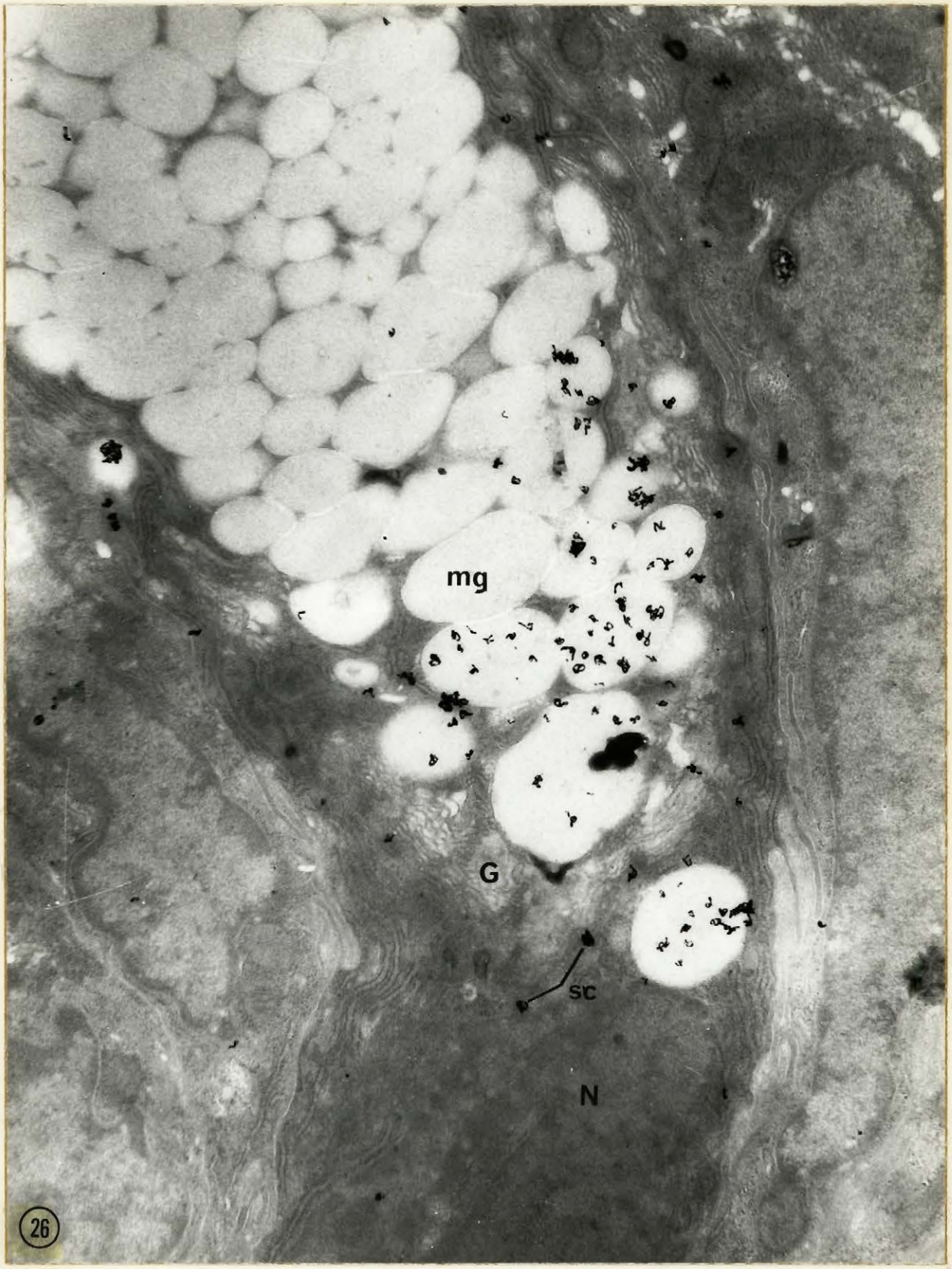

mg
G
sc
N
26

Fig. 27 Four hours after ^{3}H-glucose injection. (Gevaert 307 emulsion, exposed 1 month). 16,500 X

The heavily-labeled granules are commonly found near the apical cell membrane with a "trail" of lighter label below. A layer of cytoplasm containing rough-surfaced endoplasmic reticulum (rER) extends along the lateral cell membrane (LM) up to the apical membrane.

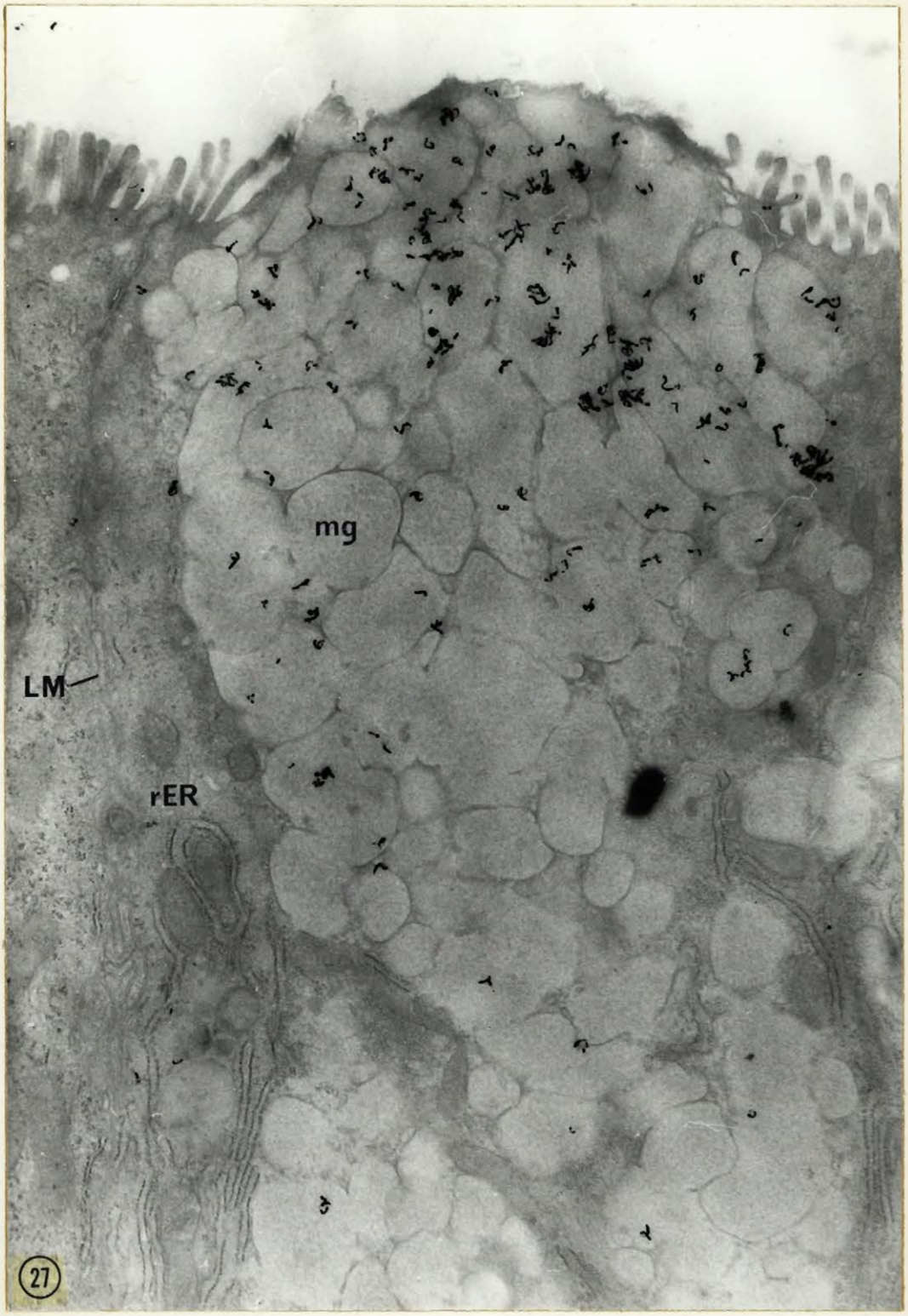

mg
LM
rER
27

Fig. 28 The apical region of another surface goblet cell, 4 hours after injection of ^{3}H-glucose. (Gevaert 307 emulsion, exposed 1 month).

27,500 X

The "front" of labeled granules reflects the U-shape of the Golgi complex where the granules arose, 4 hours earlier. Laterally, labeled mucigen granules have reached the apical surface (arrows) and one is being released (see Fig. 30).

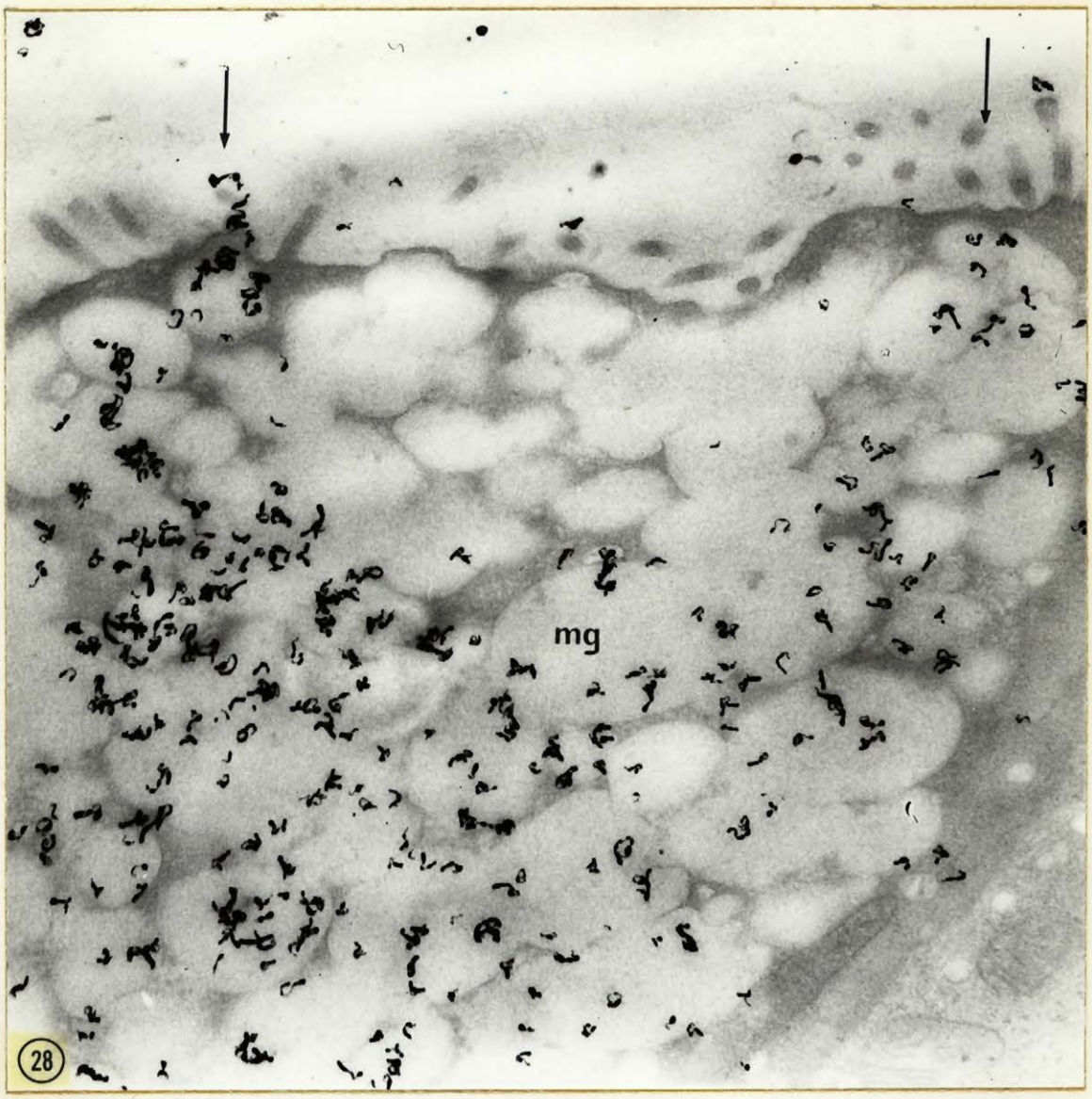

mg
28

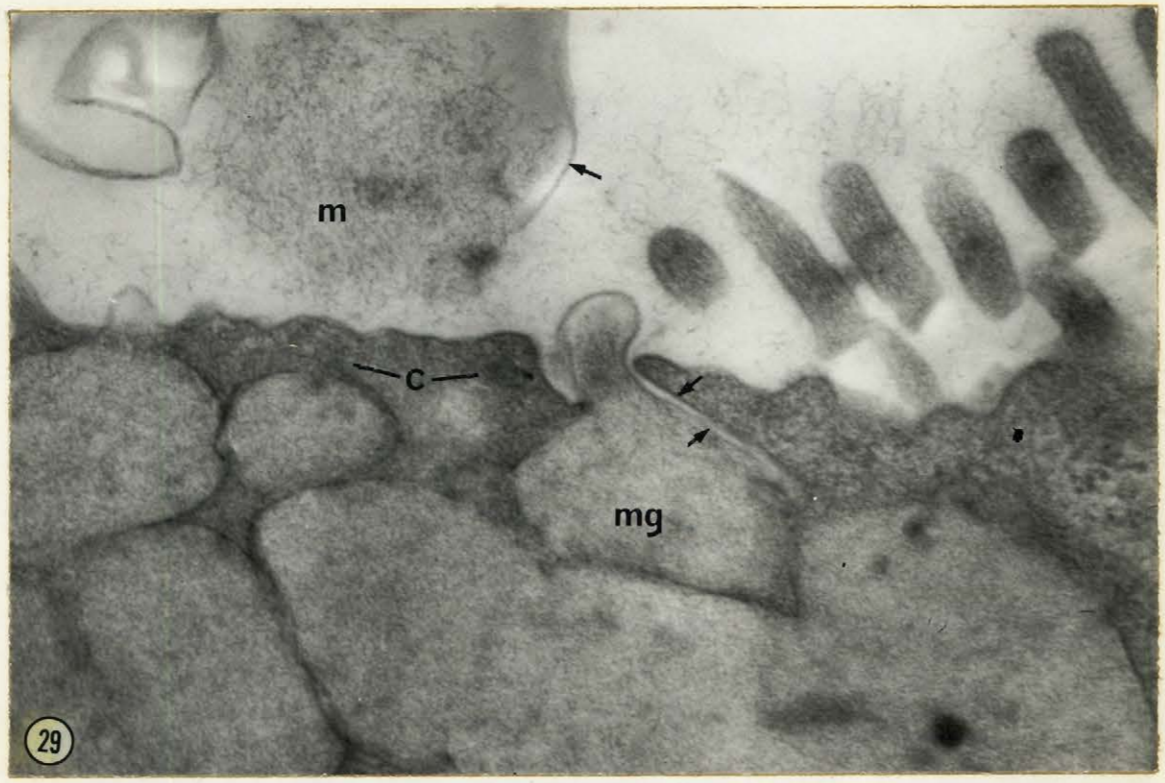
Fig. 29 The apical surface of a goblet cell at high magnification.

65,000 X

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 above down, the unit membranes which limit part of the free, broken granule, the granule in passage, and the cytoplasmic cleft.

Fig. 30 Radioautograph (shown at lower magnification in Fig. 28) four hours after ^{3}H-glucose injection; the area seen here is comparable to that of Fig. 29. (Gevaert 307 emulsion, exposed 1 month). 65,000 X

A radioactive mucigen granule is being extruded. Thus, 4 hours after the labeling of its contents in the Golgi region, this intact granule has completed its migration through the cell.



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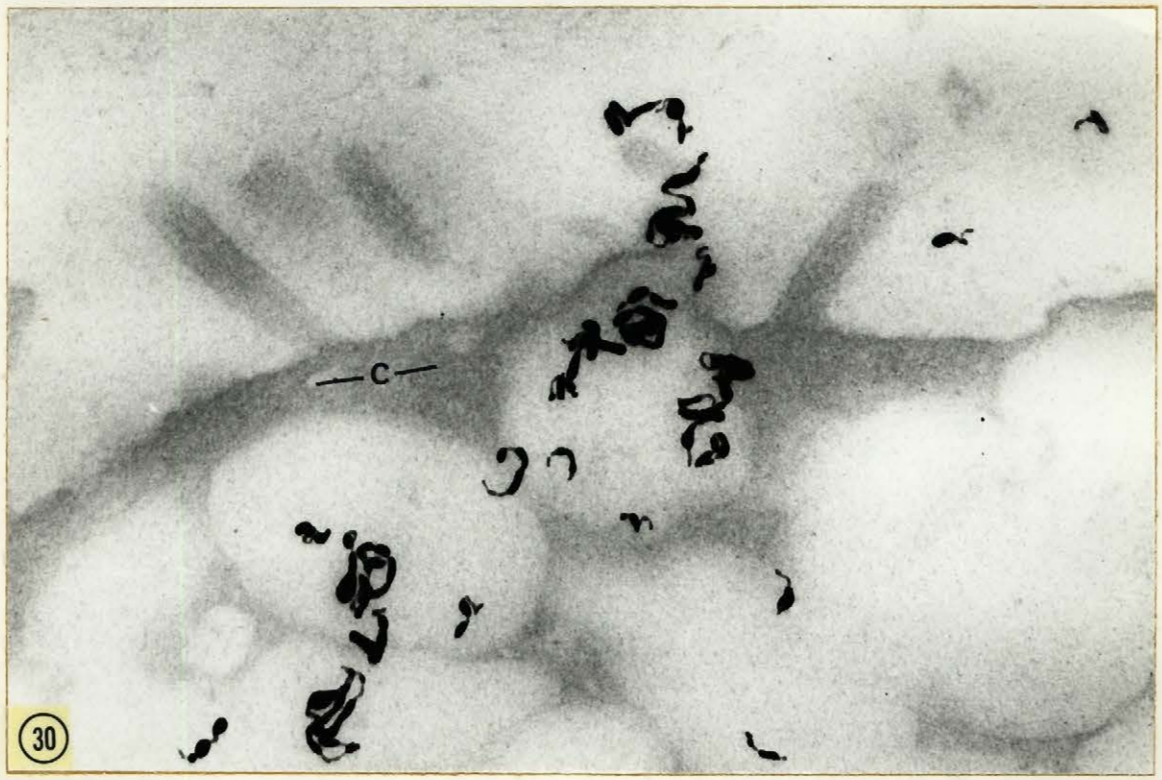
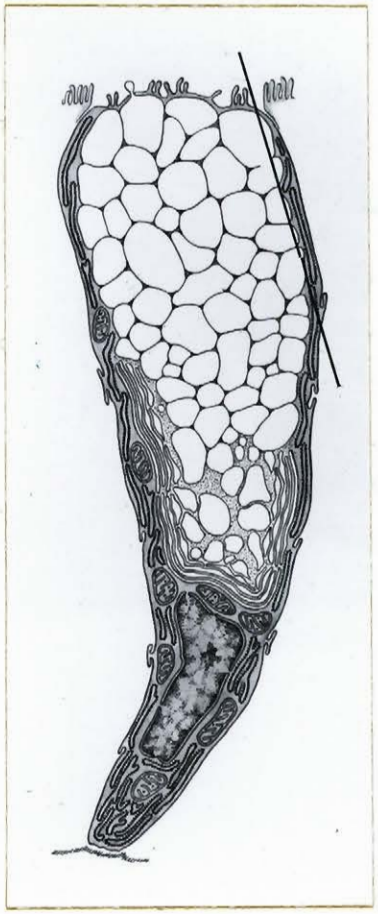


30

Fig. 31 Radioautograph of a surface goblet cell, 4 hours after injection of ^{3}H-glucose, sectioned as in the diagram below. (Gevaert 307 emulsion, exposed 1 month). 33,750 X



At the edge of the mass of apical mucigen granules, two membrane-bound granules are being released (arrows).

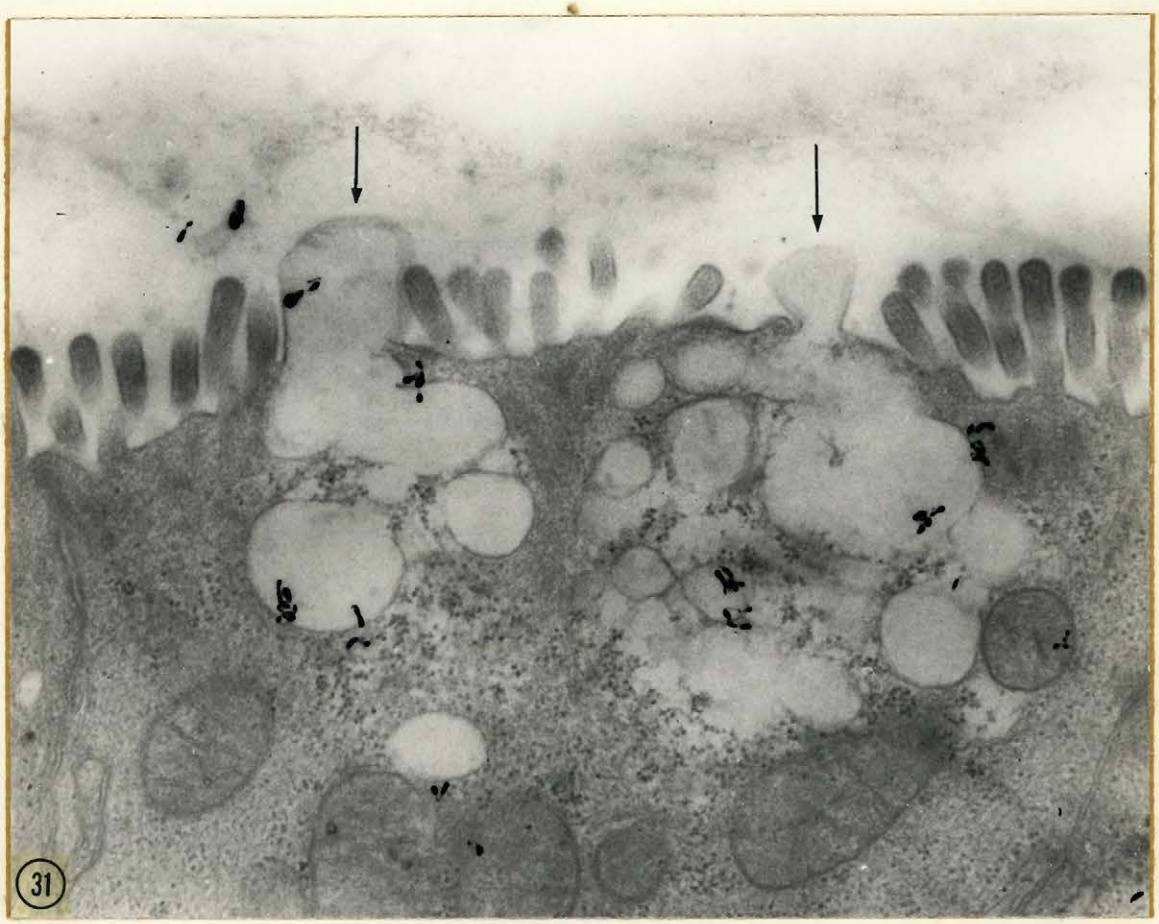
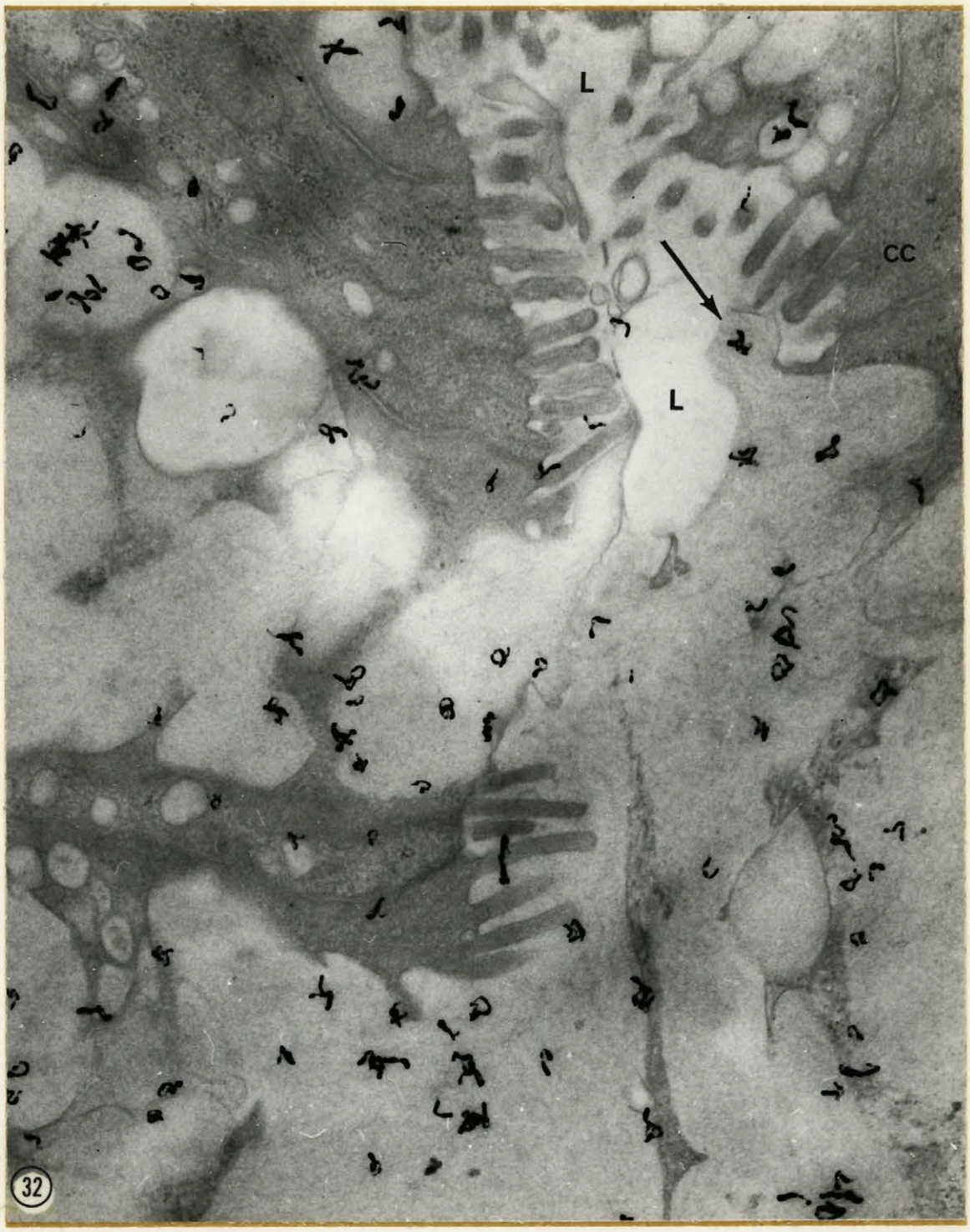

31

Fig. 32 Radioautograph showing the apical regions of goblet and columnar cells (cc) at the base of a crypt, 4 hours after injection of ^{3}H-glucose. (Gevaert 307 emulsion, exposed 1 month) 35,000 X

The cells are arranged around the lumen (L). Radioactive material is present in membrane-bound mucigen granules (upper left) and in masses of mucigen, where granule membranes seem to have broken (lower right). A labeled mucigen granule (arrow) is being released into the lumen.


L
CC
L
32

Figs. 33-36 Adjacent segments of colon from a single 10-gram rat, 3 hours after injection of ^{3}H-glucose. The effects of two fixatives are compared. 600 X

Fig. 33 Carnoy fixation, PA-Schiff and hematoxylin stain.

Several tall goblet cells (gob) are present in the surface epithelium. Although PA-Schiff stained mucigen fills the region above the nucleus (N), the apical region is empty. The apical mucigen seems to have been extruded into the lumen.

Fig. 34 Carnoy fixation, colloidal iron-Feulgen stain followed by radioautography. (Exposed 6 weeks).

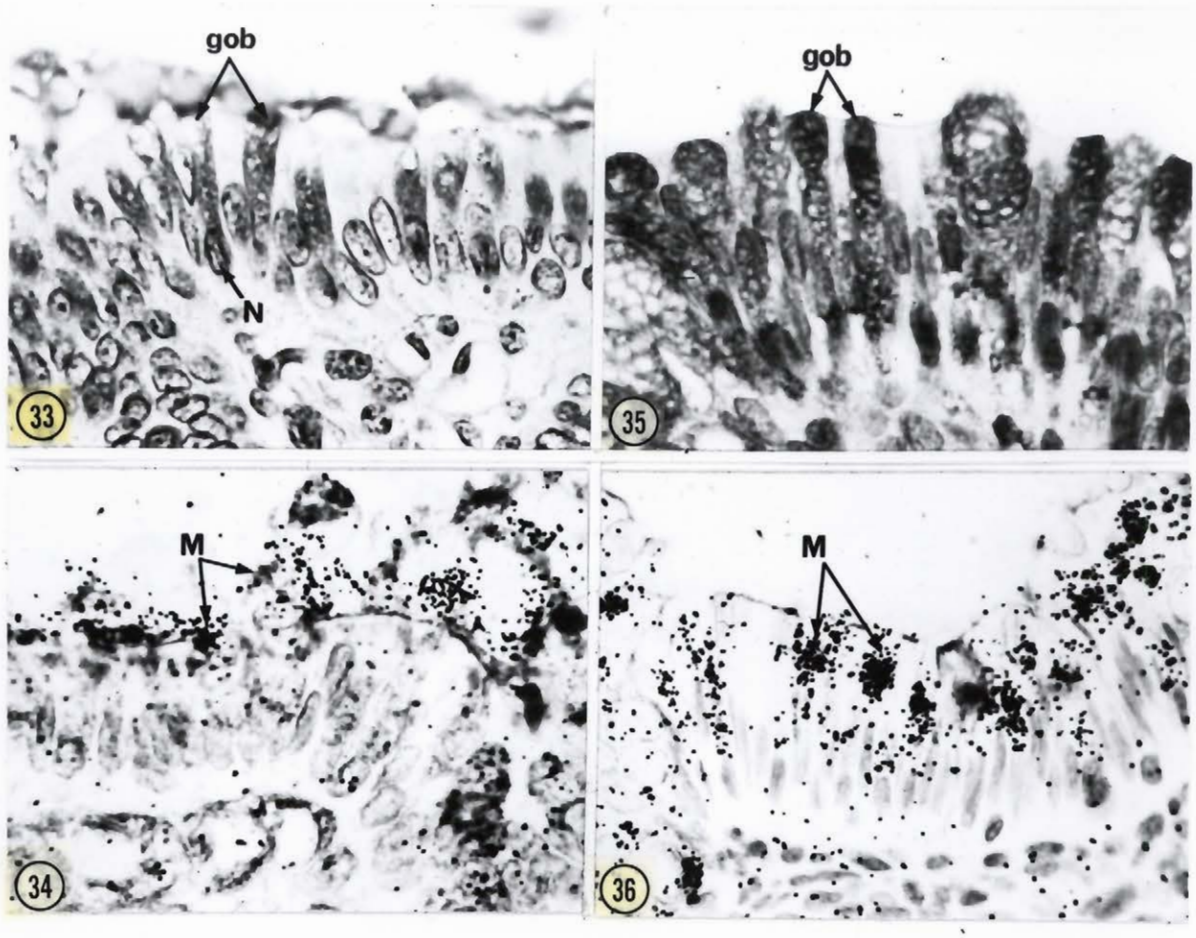
At 3 hours after its synthesis in the Golgi region, most or all of the labeled mucigen is in the lumen.

Fig. 35 Neutral buffered formalin fixation, PA-Schiff and hematoxylin stain.

In most goblet cells, the PA-Schiff stained mucigen is retained in the apical region; it has not been extruded into the lumen.

Fig. 36 Neutral buffered formalin fixation, colloidal iron-Feulgen stain followed by radioautography. (Exposed 6 weeks).

The labeled mucigen has not been secreted en masse within 3 hours after its synthesis. Rather, it will be gradually released, about an hour later.


gob
N
33
gob
35
M
34
M
36

Figs. 37-40 Adjacent sections of colon of 10-gram rat, 1.5 hours after injection of ^{3}H-glucose, subjected to various treatments before colloidal iron-Feulgen staining and radioautography. (Exposed 9 days) 600 X

Fig. 37 Control section, incubated in phosphate buffer alone.

The goblet cells on the surface (gob) and in the crypts (arrows) are distinguished by their colloidal iron-stained mucigen which is partially covered by dense clusters of silver grains.

Fig. 38 Control section, incubated in phosphate-buffered beta glucuronidase.

The staining of goblet cell mucigen is enhanced but the radioautographic reaction is unchanged, both on the surface (gob) and in the crypts (arrows).

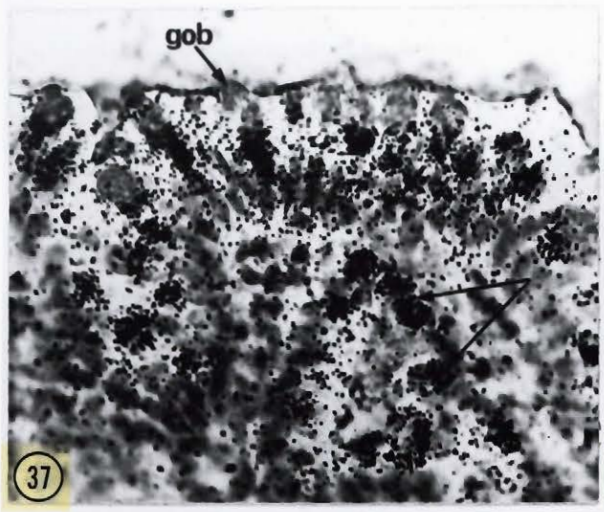
Fig. 39 Control section, oxidized in peracetic acid.

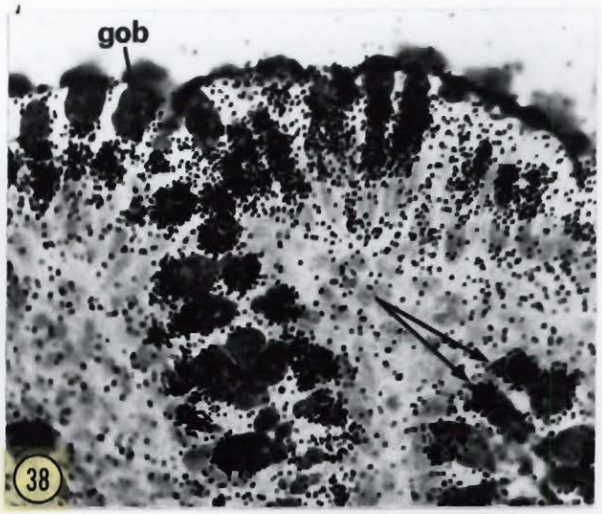
Stained mucigen is present in goblet cells, but is less abundant than in other controls (Figs. 37 and 38). The radioautographic reactions are still intense on the surface (gob) but are somewhat diminished in the crypts (arrows).

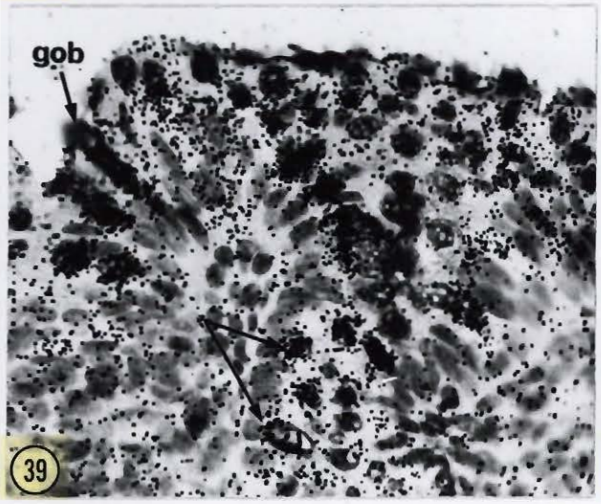
Fig. 40 Section oxidized in peracetic acid and subsequently incubated in phosphate-buffered beta glucuronidase.

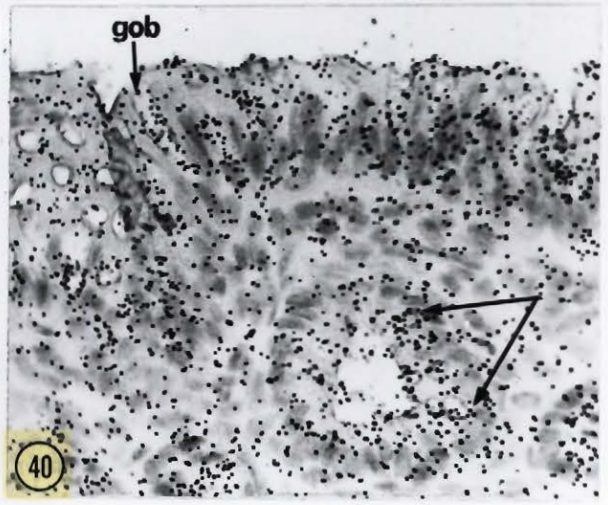
The goblet cells (gob, arrows) have lost their stainable mucigen and most of their radioactive material.

In contrast, the label scattered throughout epithelial and connective tissue cells has not been extracted.


gob
37


gob
38


gob
39

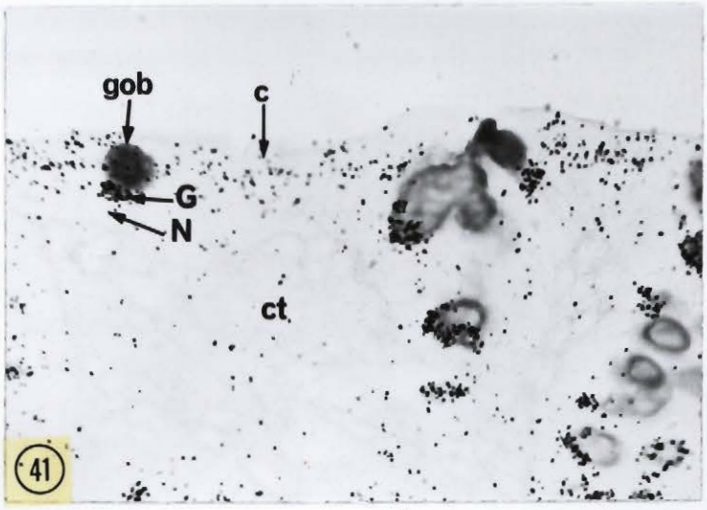

gob
40

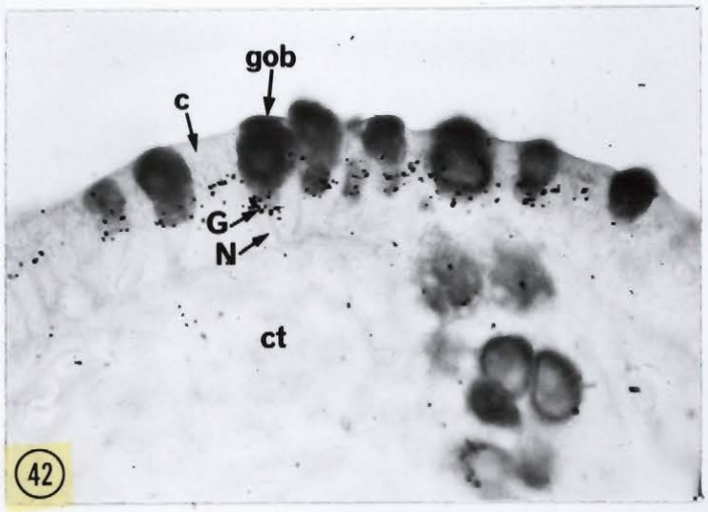
Figs. 41-43 Colon from 20-gram rats, 10 minutes after similar injections of ^{3}H-glucose (exp. 9) or ^{3}H-galactose (exp. 10). After extraction of glycogen with amylase, the PA-Schiff technique strongly stains the mucigen of goblet cells (gob) but leaves nuclei (N), columnar cells (C) and the underlying connective tissue largely unstained. (Exposed 2 months).

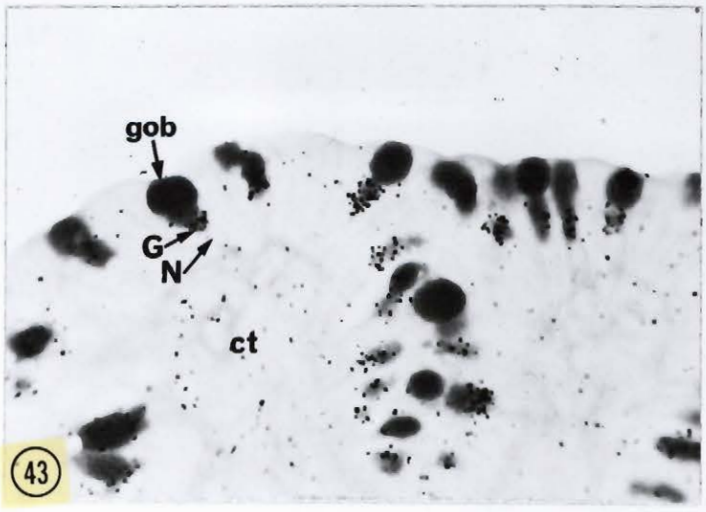
Fig. 41 Ten minutes after ^{3}H-glucose injection, radioactive material is present in the Golgi region (G) of goblet cells. In columnar cells, it is difficult to distinguish a localized Golgi reaction because of the scattered label throughout the cytoplasm.

Fig. 42 Ten minutes after ^{3}H-galactose injection, radioactive material is detected in the Golgi region (G) of goblet cells, with small amounts in the Golgi region of columnar cells. The rest of the cytoplasm of both cell types is free of label.

Fig. 43 Thirty minutes after ^{3}H-galactose injection, the radioautographic reaction over the Golgi region (G) of goblet cells is enhanced: it is now as intense as that seen at 10 minutes after ^{3}H-glucose (Fig. 41). Still, the rest of the cytoplasm of all epithelial cells is free of label.


gob
c
G
N
ct
41


gob
c
G
N
ct
42


gob
G
N
ct
43

Figs. 44-50 Radioautographs of 20-gram rat colon after injection of ^{3}H-galactose or ^{14}C-glucosamine. (Half-micron Epon sections, Ilford L-4 emulsion, toluidine blue stain).

Fig. 44 A single dark goblet cell in the surface epithelium, 10 minutes after ^{3}H-galactose injection. (Exposed 7 months). 1300 X

A cluster of silver grains indicates radioactivity in the Golgi region (G), between nucleus (N) and mucigen (M). The columnar cells are scarcely labeled.

Fig. 45 A surface goblet cell, 30 minutes after ^{3}H-galactose injection. (Exposed 7 months) 1300 X

An enhanced Golgi reaction (G) covers the supranuclear region of the goblet cell. Some labeled material is scattered outside the Golgi region, particularly in the basal cytoplasm of goblet and columnar cells. Since such label was completely removed from comparable paraffin sections by alpha amylase (see Fig. 43) this material is identified as labeled glycogen (gly).

Fig. 46 Three surface goblet cells, 30 minutes after injection of ^{3}H-galactose. (Exposed 7 months) 1300 X

Again, the Golgi region (G) of goblet cells is heavily labeled, while small amounts of labeled glycogen are detected in the basal cytoplasm.

Fig. 47 An oblique section through the upper part of a crypt, 10 minutes after ^{14}C-glucosamine injection. (Exposed 3 months). 1300 X

(Continued)

A goblet cell is obliquely sectioned, so that its mucigen appears not to reach the lumen (L). Above and lateral to the nucleus (N) radioactive material is detected. EM radioautography identified this as the Golgi region (G).

Fig. 48 A surface goblet cell, 30 minutes after injection of ^{3}H-glucosamine. (Exposed 4 months). 1300 X

The localization of radioactivity in the supranuclear Golgi region (G) resembles that seen after ^{3}H-galactose injection (Fig. 45).

Fig. 49 Base of a crypt, 30 minutes after injection of ^{3}H-galactose. (Exposed 2 months). 1200 X

The cells are oriented around the lumen (L). In the goblet cells, a radioautographic reaction appears over the Golgi region (G).

Fig. 50 Base of a crypt, 30 minutes after injection of ^{14}C-glucosamine. (Exposed 3 weeks). 1200 X

Radioactive material is present in the Golgi region (G) of goblet cells. Although the position of the Golgi complex in crypt goblet cells is variable (Figs. 9 and 10), EM radioautography confirmed that this label was in or near the Golgi complex.

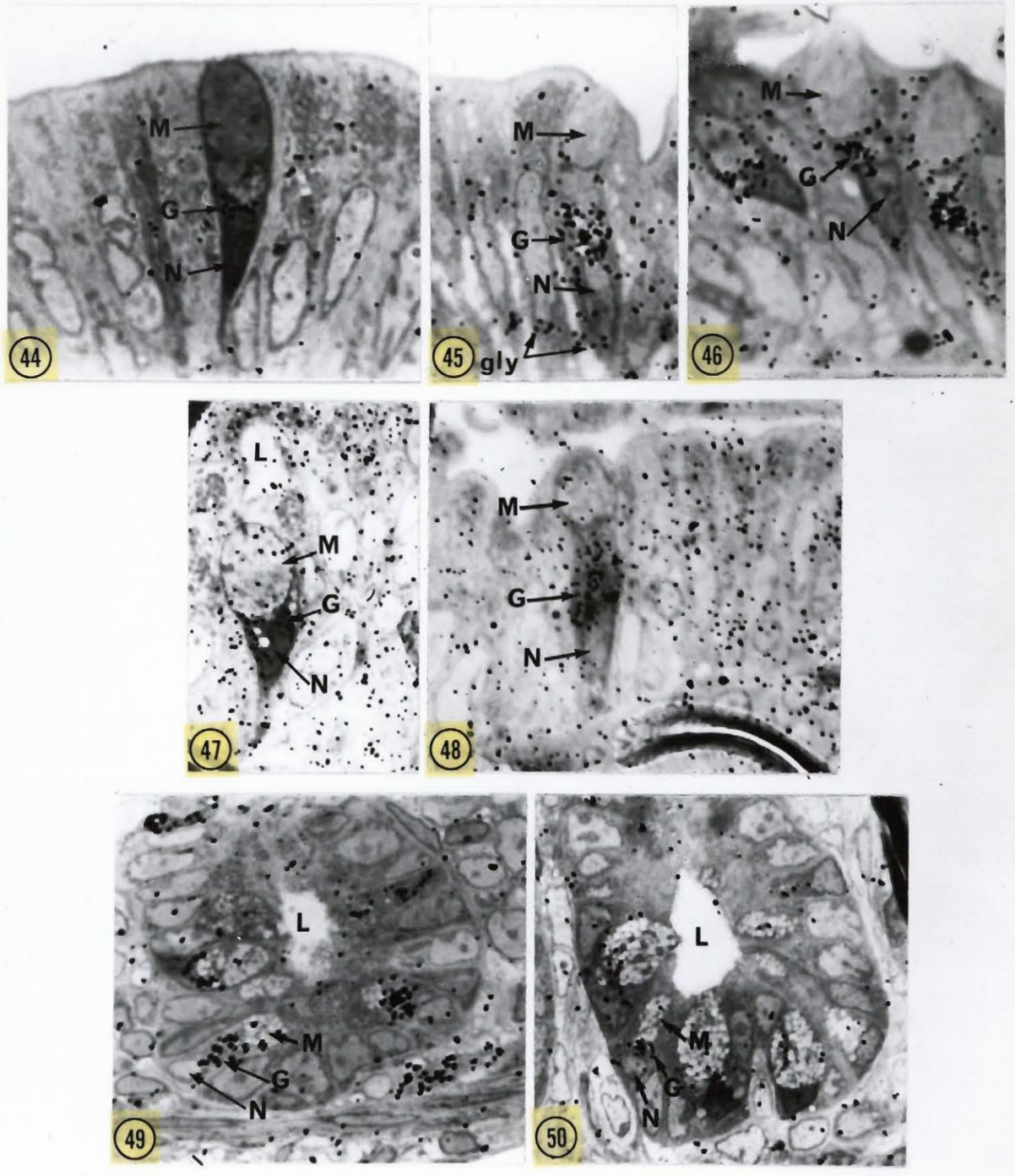

M
G
N
44
M
G
N
gly
45
M
G
N
46
L
M
G
N
47
M
G
N
48
L
M
G
N
49
L
M
G
N
50

Fig. 51 The epithelial cells of colon of 20-gram rat, 10 minutes after injection of ^{14}C-glucosamine. (Ilford L-4 emulsion, exposed 3 months). 35,000 X

A goblet cell (left) and columnar cell (right) are separated by lateral cell membranes (LM). Portions of their nuclei are visible (N,N^1). In the goblet cell, silver grains overlie the flattened Golgi saccules, while rough-surfaced endoplasmic reticulum (rER) and mucigen granules (mg) are not labeled.

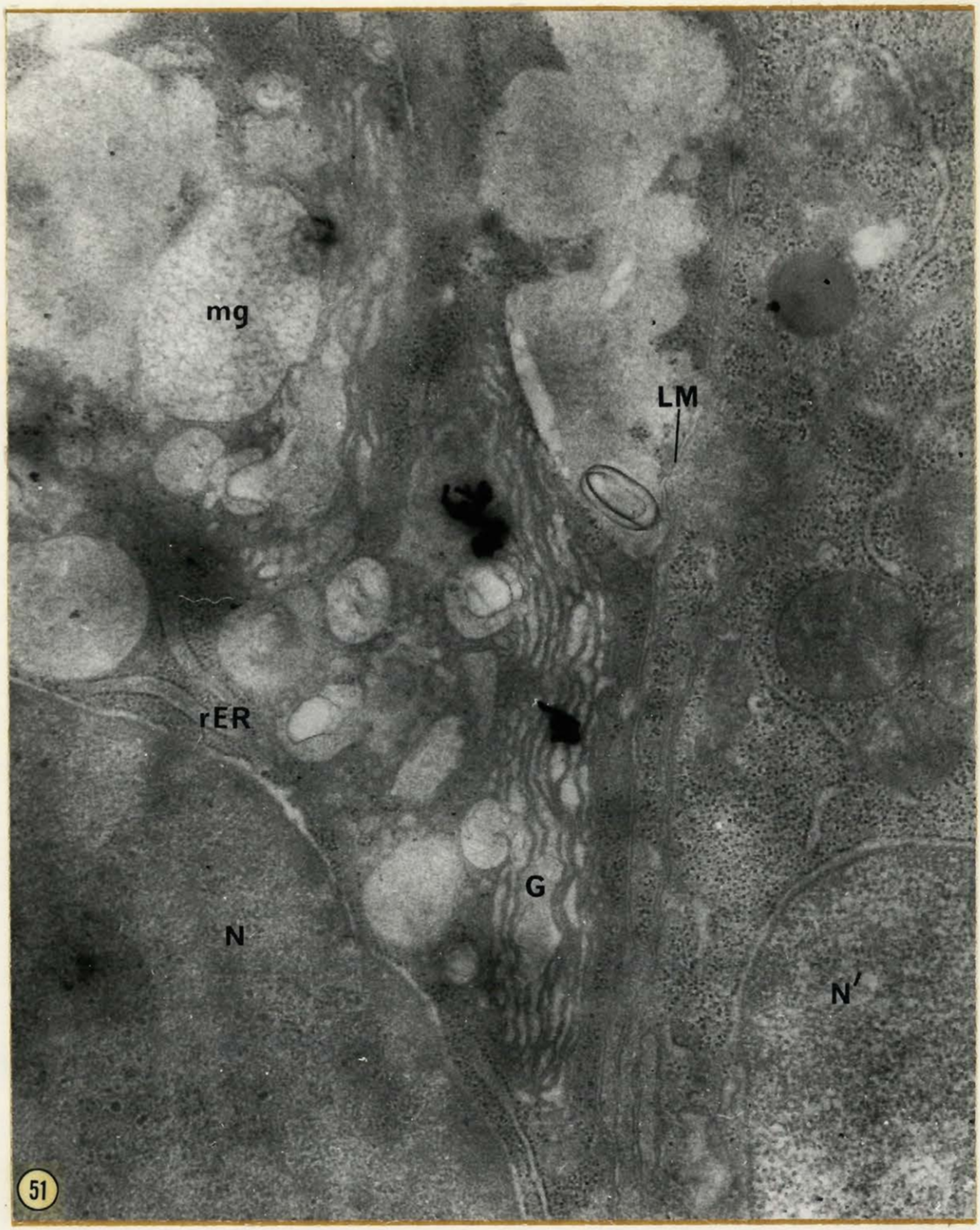
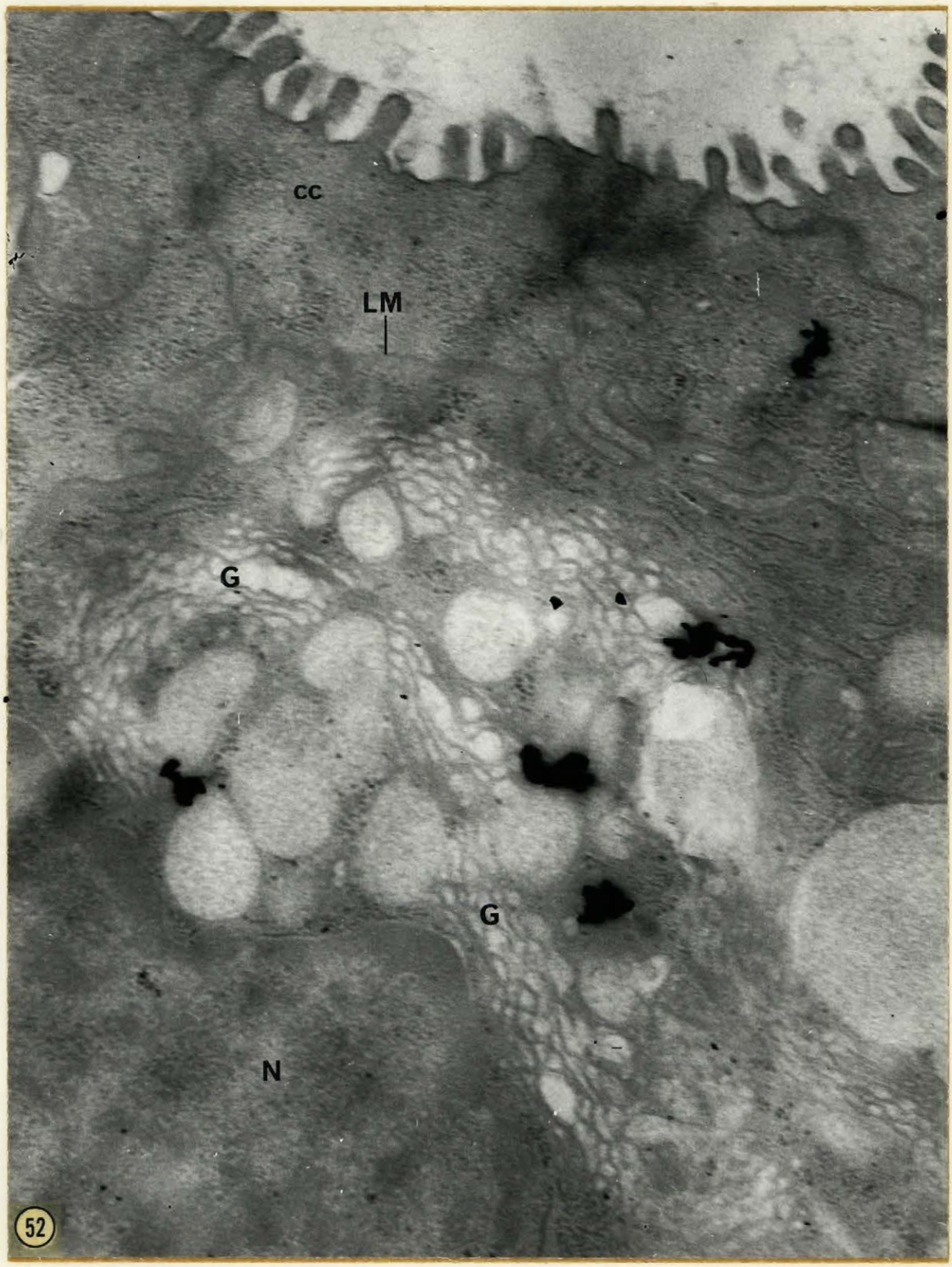

mg
LM
rER
G
N
N′
51

Fig. 52 Obliquely-sectioned epithelial cells of colon of 20-gram rat, 30 minutes after injection of ^{14}C-glucosamine. (Ilford L-4 emulsion, exposed 3 months) 35,000 X

Present in the section are the nucleus (N) and supranuclear region of a goblet cell, and the apical regions of 3 columnar cells (cc). In the goblet cell, silver grains lie on or near Golgi saccules (G) or mucigen granules (mg).


CC
LM
G
G
N
52

Figs. 53-57 Radioautographs of colonic surface epithelium after injection of ^{35}S-sulfate or ^{3}H-leucine. (Half-micron methacrylate sections, toluidine blue stain). 1200 X

Fig. 53 Colonic surface epithelium of 100-gram rat, 15 minutes after injection of ^{35}S-sulfate. (Exposed 3 months).

Radioactive material is localized in the Golgi region (G) between basal nucleus (N) and apical mucigen (M).

Fig. 54 Same as above, one hour after injection of ^{35}S-Sulfate. (Exposed 3 months).

Now, the label is distributed over the supranuclear region.

Fig. 55 Same as above, 3 hours after injection of ^{35}-Sulfate. (Exposed 3 months).

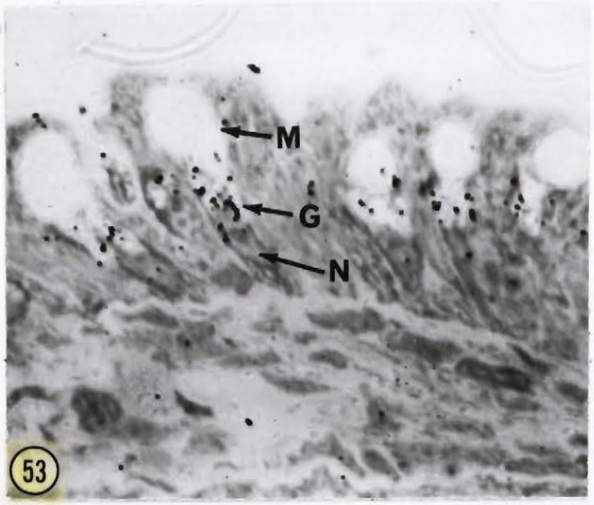
A variable region of the apical mucigen (M) is labeled.

Fig. 56 Two goblet cells in the colonic epithelium of 50-gram rat, 6 minutes after intravenous injection of ^{3}H-leucine. (Exposed 1 month).

The radioautographic reaction over the basal and perinuclear cytoplasm of goblet cells corresponds to the distribution of rough-surfaced endoplasmic reticulum (rER, seen in Figs. 3 and 4). In columnar cells (C) label is scattered throughout the cytoplasm.

Fig. 57 A single goblet cell in the colonic epithelium of 50-gram rat, 20 minutes after injection of ^{3}H-leucine. (Exposed 1 month).

The reaction again appears over the basal and perinuclear cytoplasm of the goblet cell, while the cytoplasm of columnar cells (C) is generally labeled.



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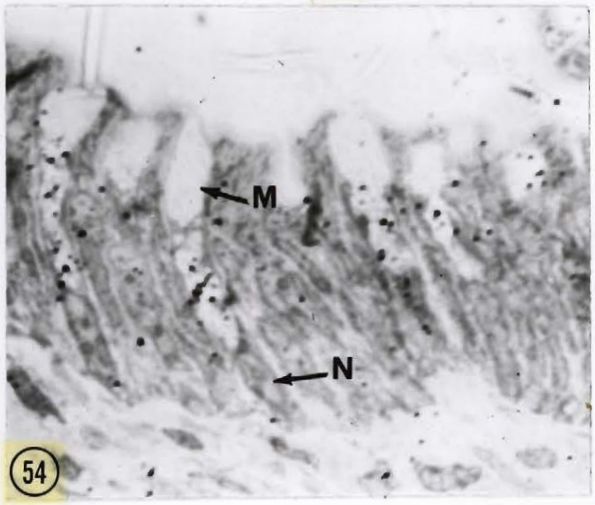

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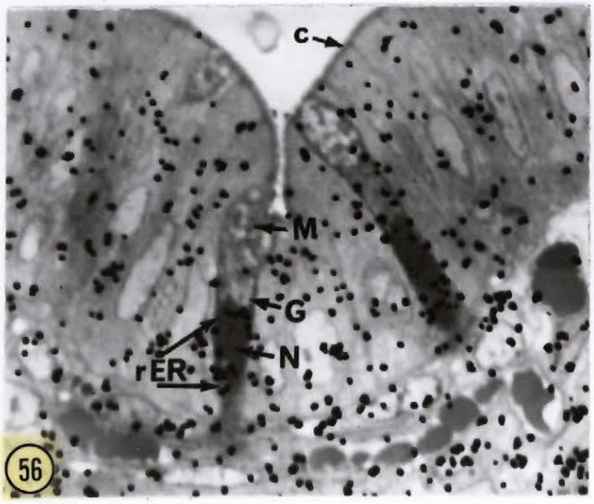

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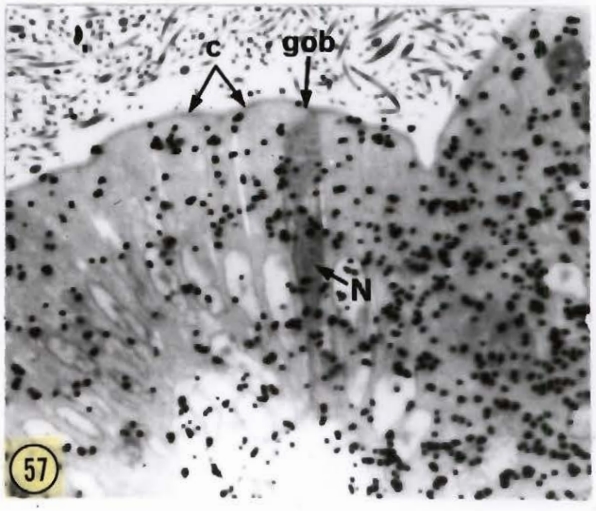


57

Figs. 58-62 Small intestine of rats, showing the surface epithelium stained with the PA-Schiff technique (for detection of glycoproteins). 1200 X

Fig. 58 Duodenum of 20-gram rat.

Above the unstained nucleus(N, left) of goblet cells (gob) is a column of stained mucigen granules and the Golgi region (G, left) extending up to the heavily-stained apical mucigen (M). Above the unstained nuclei (N, right) of columnar cells (C) the Golgi region (G, right) is stained by PA-Schiff. In addition, the lateral and basal cell surfaces are lightly stained while the apical surfaces are intensely stained.

Fig. 59 Jejeunum of 100-gram rat, 15 minutes after intraperitoneal injection of ^{3}H-l-glucose. Nuclei (N) are counterstained with hematoxylin. (Exposed 4.5 months).

After a low dose of labeled glucose, radioactive material is detected only in the Golgi region (G) of goblet cells.

Fig. 60 Duodenum of 100-gram rat, 5 minutes after local injection of ^{3}H-6-glucose. (Exposed 5.5 weeks).

Above the unstained nuclei (N), the Golgi region of goblet cells (G, right) is heavily labeled, while the Golgi region of columnar cells (G, left) shows little or no radioactivity. A light scatter of label is seen in the columnar cells.

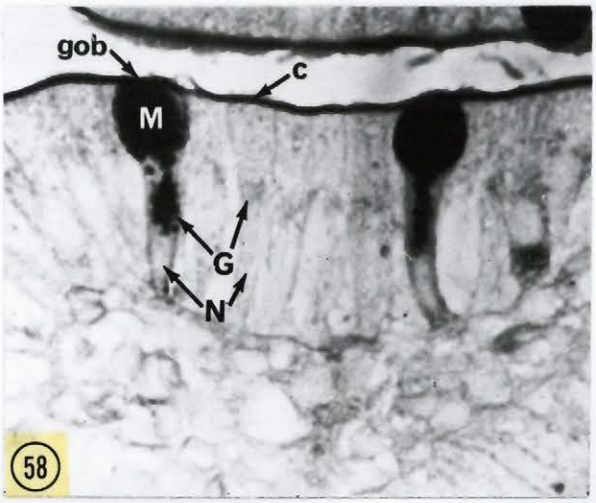
Fig. 61 Duodenum of 110-gram rat, 15 minutes after intraperitoneal injection of ^{3}H-6-glucose. (Exposed 7 weeks).

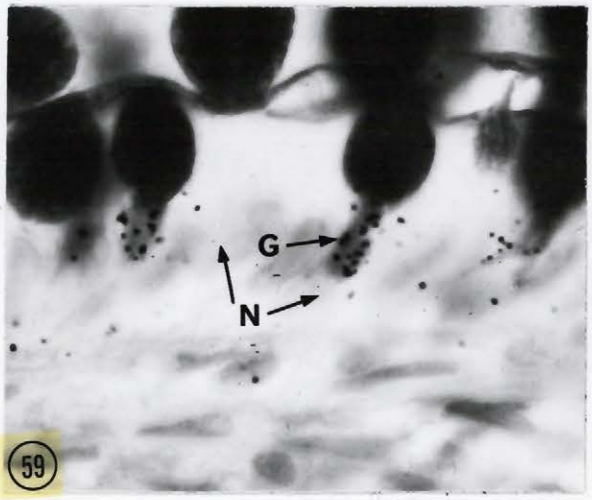
Again, label from ^{3}H-glucose appears in the Golgi region of goblet cells (G) while the columnar cells are scarcely labeled.

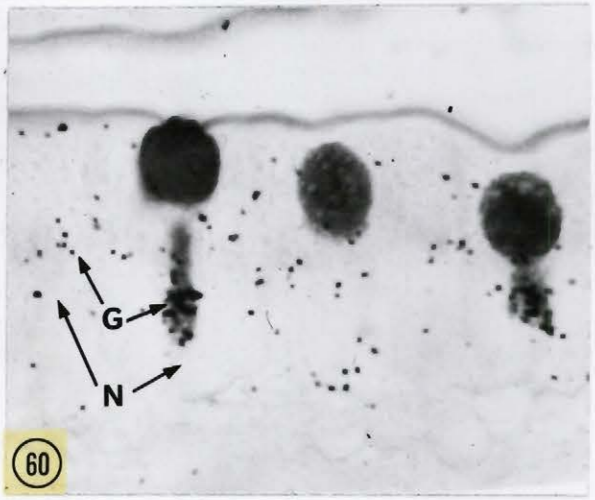
(Continued)

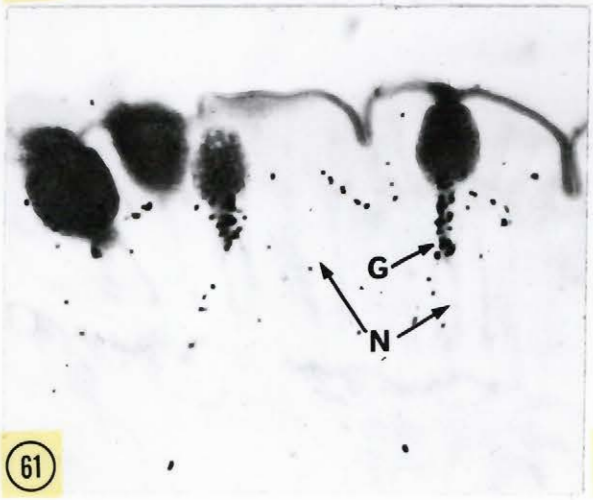
Fig. 62 Duodenum of 20-gram rat, 10 minutes after intraperitoneal injection of ^{3}H-l-galactose. (Exposed 2 months).

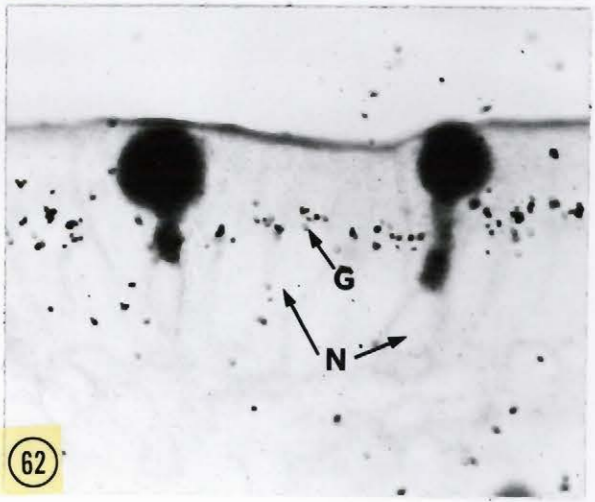
In contrast with ^{3}H-glucose label, the ^{3}H-galactose label is not taken up in goblet cells. Instead, considerable label is localized in the Golgi region of columnar cells (G), with little or no scatter in the rest of the cytoplasm.


gob
c
M
G
N
58


G
N
59


G
N
60


G
N
61


G
N
62

Figs. 63-68 Duodenal villi of 10-gram rats sacrificed at various time intervals after injection of ^{3}H-glucose. (Amylase-digested, PA-Schiff stain, exposed 10 days). 600 X

Fig. 63 The four goblet cells are readily identified by their mucigen which is stained by PA-Schiff. At 5 minutes after injection, small clusters of silver grains overlie the Golgi region (G) above the unstained nucleus (N).

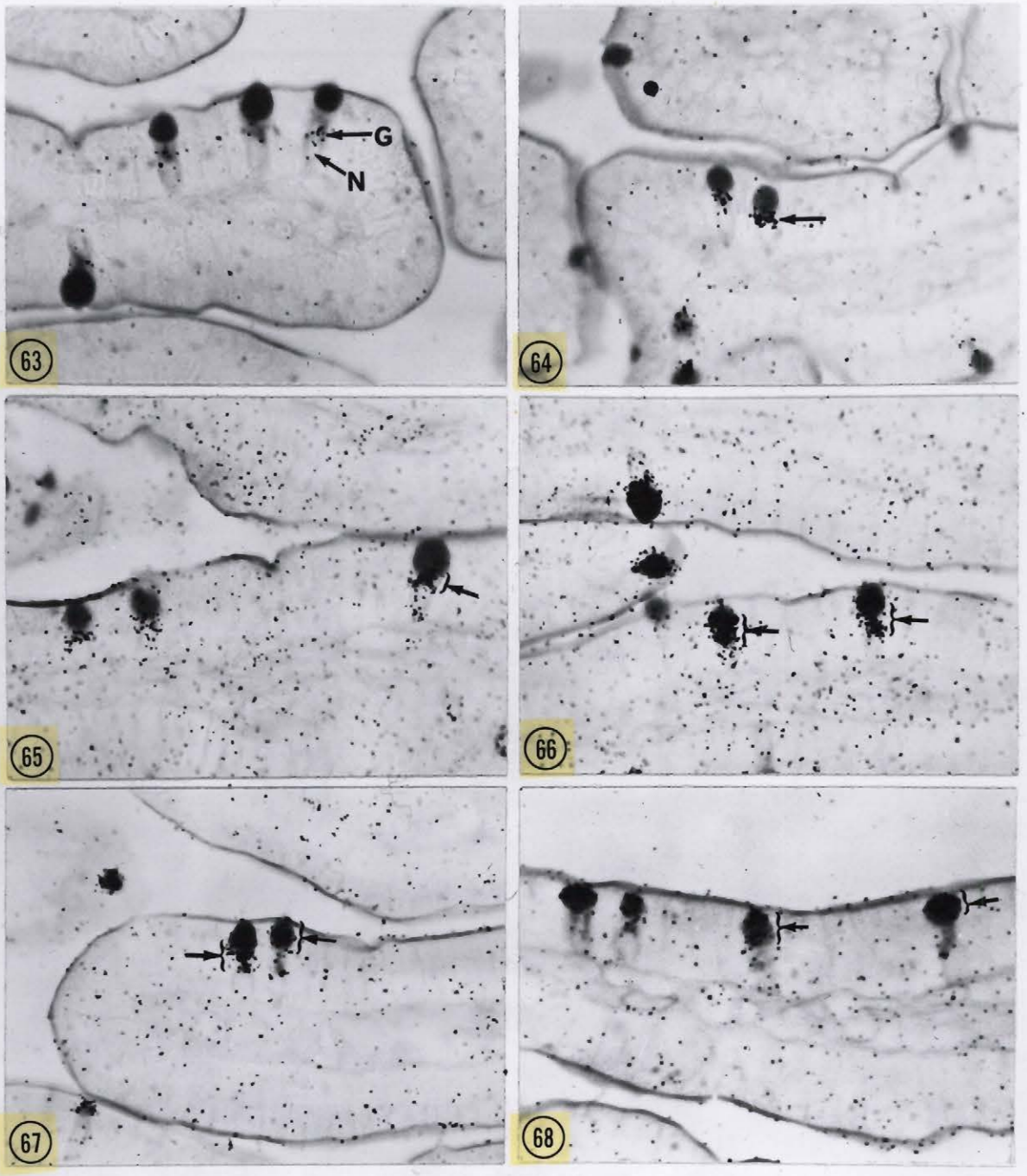
Fig. 64 At 20 minutes, the supranuclear Golgi reaction (G) is more intense.

Fig. 65 At 40 minutes, labeled material is detected both in the supranuclear region and at the lower edge of the mucigen. In addition, scattered label appears in the basal cytoplasm of goblet cells and throughout the columnar cells.

Fig. 66 By 1 hour, labeled material has spread farther into the apical mucigen.

Fig. 67 At 1 1/2 hours, the labeled material has migrated farther in some goblet cells than in others (brackets).

Fig. 68 By 4 hours after injection, no label is detected in the supranuclear region of most goblet cells. The apical mucigen is heavily labeled. The scattered label persists.


G
N
63
64
65
66
67
68

Figs. 69-72 Adjacent sections of duodenum from a 10-gram rat, 1 hour after injection of ^{3}H-glucose, subjected to various treatments before colloidal iron-Feulgen staining and radioautography. (Exposed 9 days) 600 X

Fig. 69 Control section, incubated in phosphate buffer alone.

The upper third of the picture is occupied by a villus, the middle third by crypts and the lower part by Brunner's glands. On the villus, the goblet cells (gob) show colloidal iron-stained mucigen, Feulgen-stained nuclei, and an intense radioautographic reaction. The mucous cells of Brunner's glands (B gl) are so heavily labeled that only the lumen is visible (small white space above tip of arrow).

Fig. 70 Control section, incubated in phosphate-buffered beta glucuronidase.

Above, a goblet cell is heavily stained and intensely labeled. Below, Brunner's glands are blackened by the radioautographic reaction.

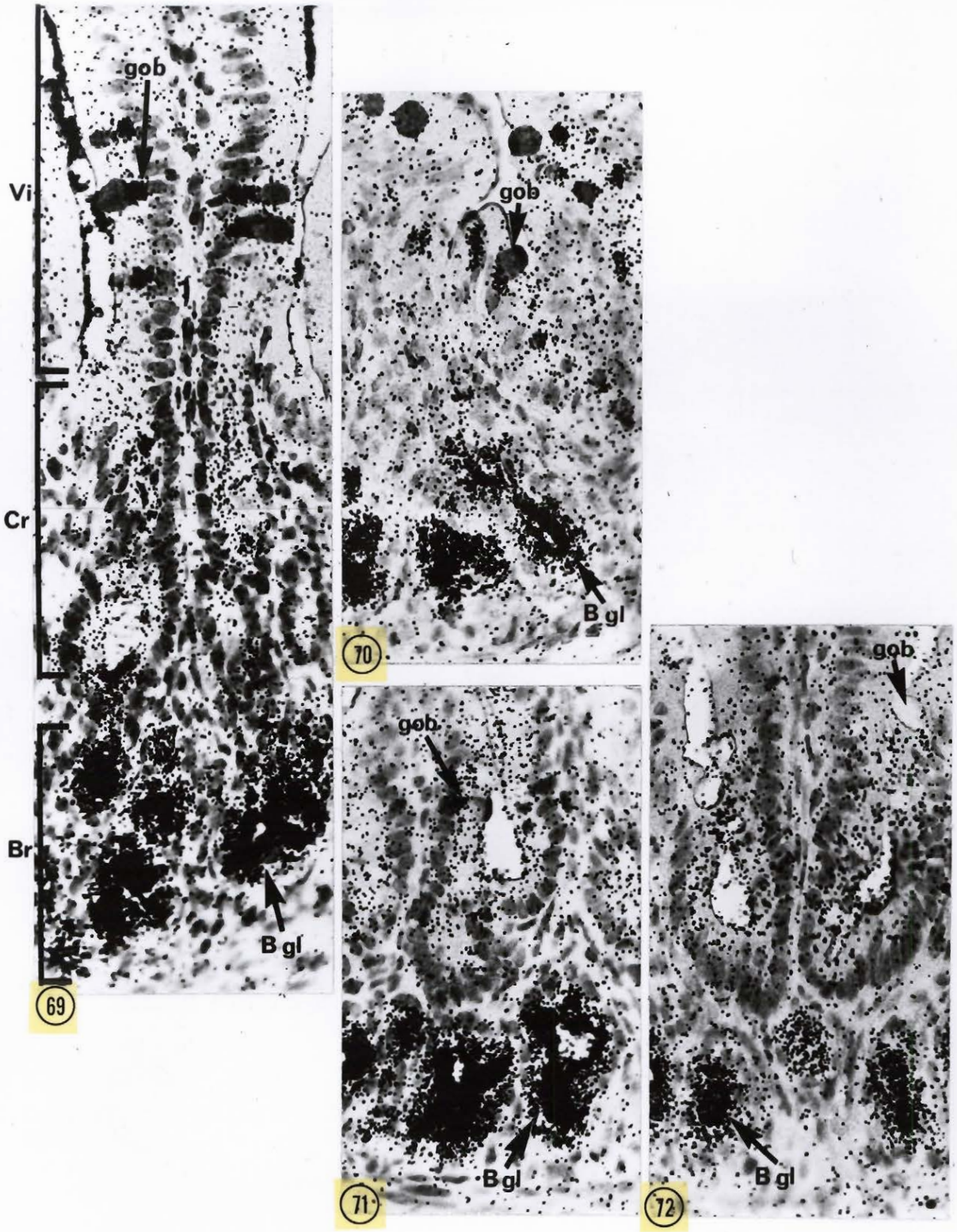
Fig. 71 Control section, oxidized in peracetic acid.

The colloidal iron staining of goblet cells is somewhat decreased, but radioactivity is still present both here and in Brunner's gland below.

Fig. 72 Section oxidized in peracetic acid and subsequently incubated in phosphate-buffered beta glucuronidase.

The apex of goblet cells (gob) appears empty. In the supranuclear region, the radioautographic reaction is considerably diminished.

Note that the scattered reaction over the epithelium and connective tissue was not affected by these treatments.


gob
Vi
Cr
Br
B gl
69
gob
B gl
70
gob
B gl
71
gob
B gl
72

Figs. 73-75 Surface epithelium of stomach of 20-gram rat. 1200 X

Fig. 73 Hematoxylin-eosin stain.

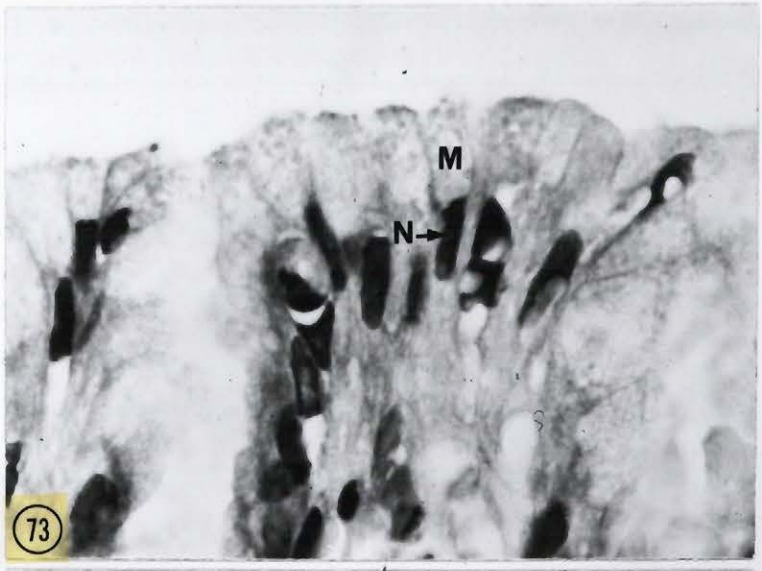
Above the dark nucleus (N) the cell apex is filled with light, unstained mucigen (M).

Fig. 74 Radioautograph, 10 minutes after injection of ^{3}H-glucose. (PA-Schiff stain, exposed 2 months).

Above the unstained nucleus (N), the black silver grains indicate the presence of radioactivity in or near the small stained mucigen granules of the Golgi region (G). The heavily-stained mucigen in the cell apex is not labeled.

Fig. 75 Radioautograph, 10 minutes after injection of ^{3}H-galactose. (PA-Schiff stain, exposed 2 months).

The small mucigen granules in the supranuclear region are now easily seen, for no radioautographic reaction is present. (Compare with ^{3}H-glucose uptake, above).



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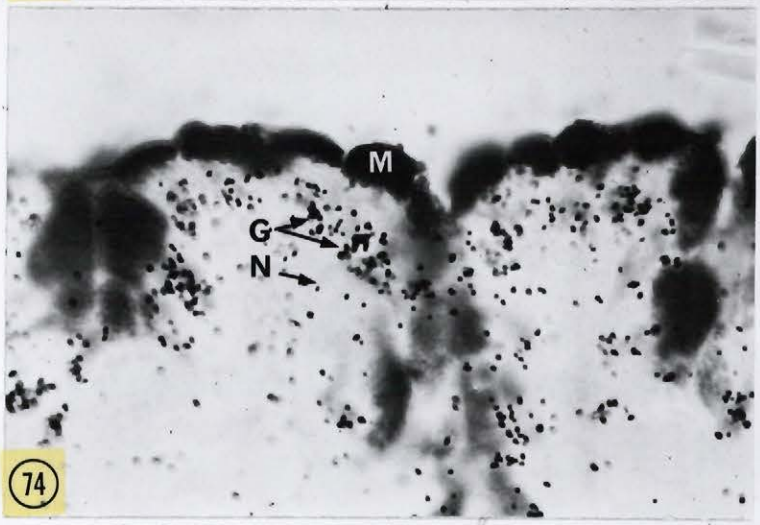

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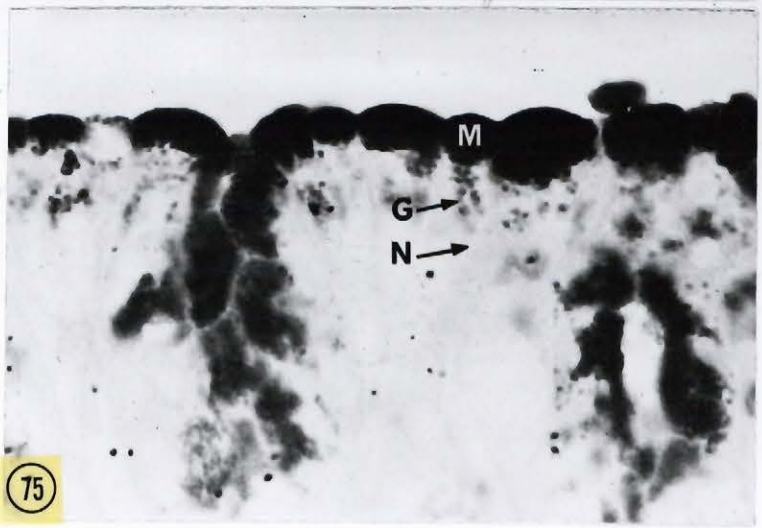

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Figs. 76-79 Adjacent sections of stomach from a 10-gram rat, 1 hour after injection of ^{3}H-glucose, subjected to various treatments before colloidal iron-Feulgen staining and radioautography. (Exposed 9 days) 600 X

Fig. 76 Control section, incubated in phosphate buffer alone.

Mucous cells cover the surface and the pits, forming an infolded row. The dense clumps of silver grains in their supranuclear regions coalesce to form a band between nuclei and mucigen.

Fig. 77 Control section, incubated in phosphate-buffered beta glucuronidase.

The apical mucigen and the free mucus in the lumen are more deeply stained. In the cells, the radioactivity is undiminished.

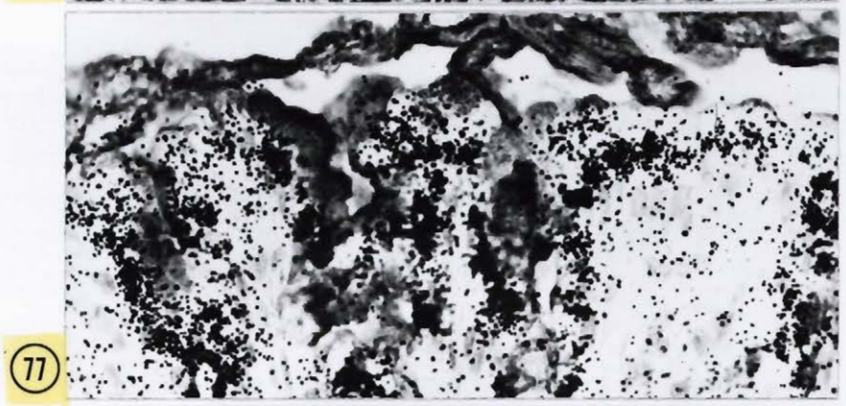
Fig. 78 Control section, oxidized in peracetic acid.

The staining of nuclei and apical mucigen is enhanced, but the radioactivity in the cells is somewhat diminished.

Fig. 79 Section oxidized in peracetic acid and subsequently incubated in phosphate-buffered beta glucuronidase.

Nuclei are still strongly stained, but the staining of apical mucigen is abolished and the radioautographic reactions are considerably diminished. When compared to Fig. 76, it is evident that most of the localized radioactivity has been extracted, while the scattered radioactivity has not been affected.

76



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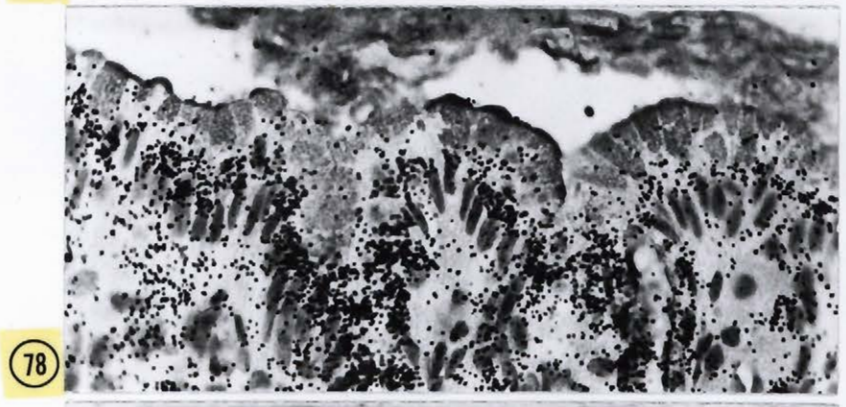


78

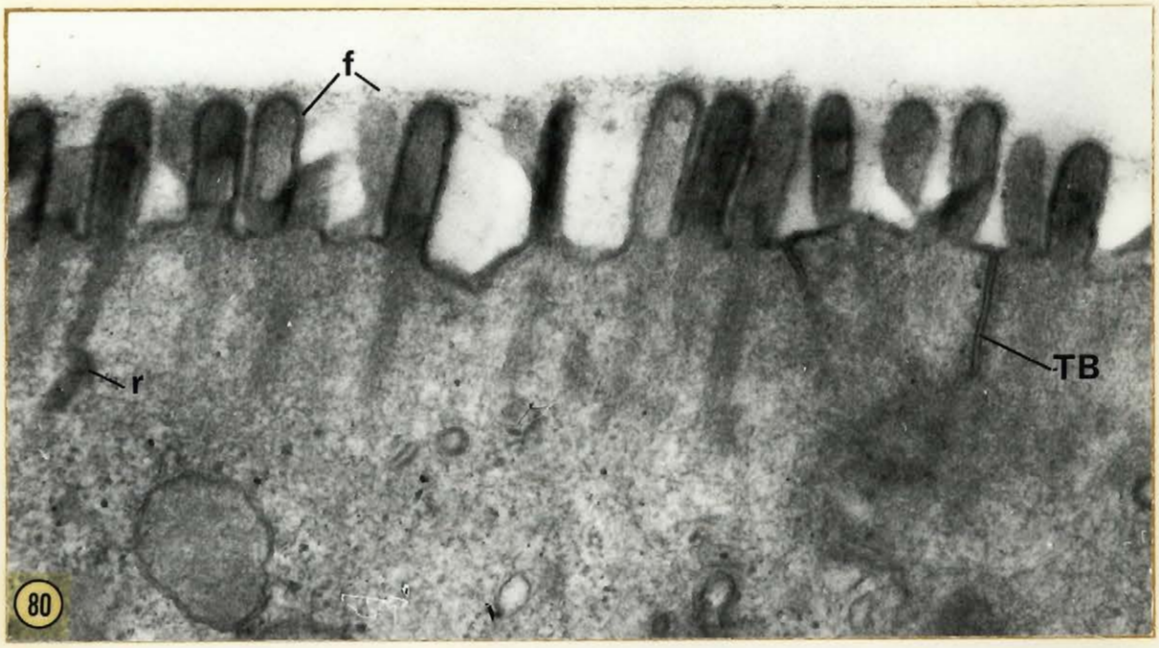
79

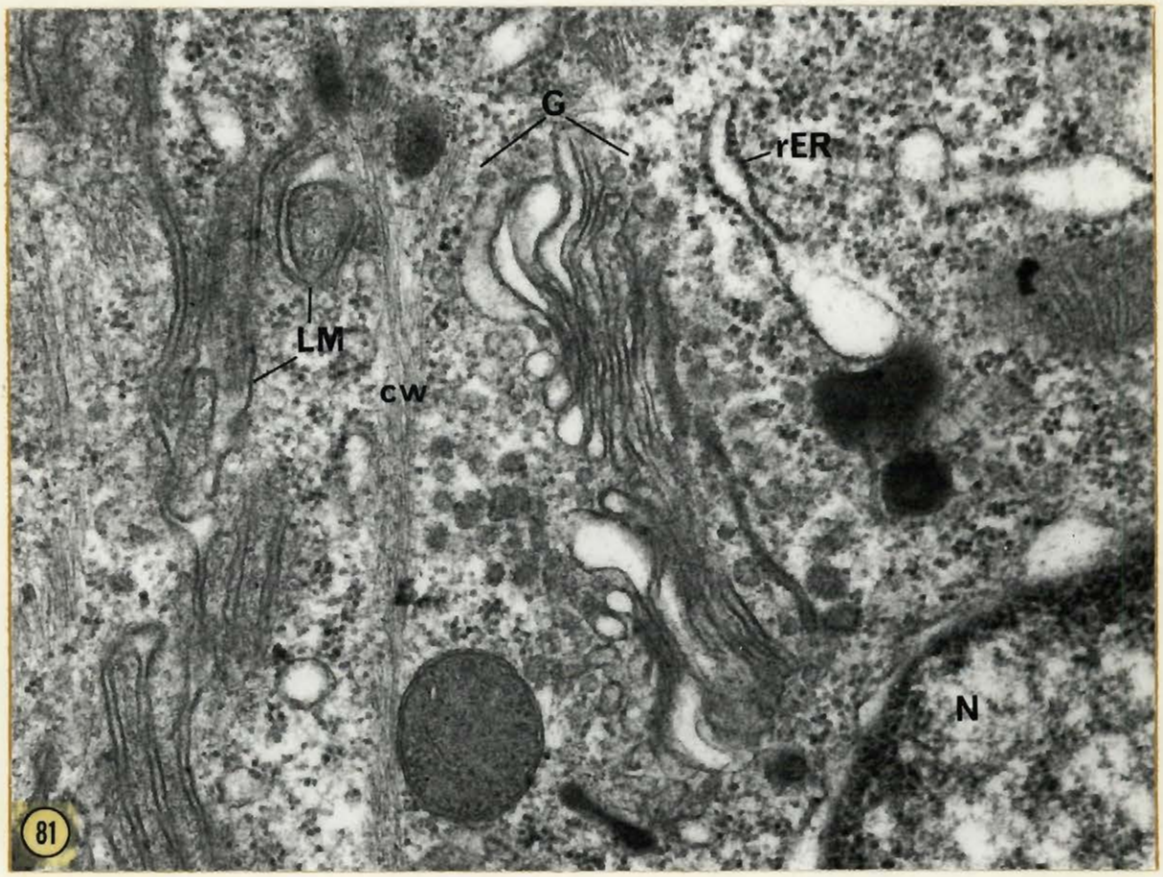
Fig. 80 The lumenal border of the surface epithelium of 20-gram rat colon, showing the apical regions of 3 columnar cells, tightly joined at terminal bars (TB). 52000 X

Uniform microvilli with rootlets (r) are covered by the apical cell membrane. On the outer surface of the apical membrane, a short dense layer of fuzzy material is seen (f, left). At the tips of microvilli, the fuzz merges with a continuous filamentous layer (f, right).

Fig. 81 The supranuclear region of a surface columnar cell of colon of 20-gram rat (area shown in Text Fig. B) 70000 X

Between the interdigitating lateral membrane (LM) and the nucleus (N), a stack of Golgi saccules is vertically oriented. (cw, cell web; rER, rough-surfaced endoplasmic reticulum; m, mitochondrion).


f
r
TB
80


G
rER
LM
cw
N
81

Figs. 82-86 Radioautographs of the surface epithelium of colon of 100-gram rats after administration of labeled precursors. The epithelium consists mainly of columnar cells.

Fig. 82 Five minutes after local injection of ^{3}H-l-glucose. (The dark spots at right are goblet cells which are out of focus). (PA-Schiff - hematoxylin stain, exposed 4.5 months). 1300 X

Above the row of faintly-stained nuclei (N), radioactivity has been incorporated in the Golgi region (G) of columnar cells. (SB, striated border).

Fig. 83 Five minutes after local injection of ^{3}H-6-glucose. (Colloidal iron-Feulgen stain, exposed 5.5 weeks). 1300 X

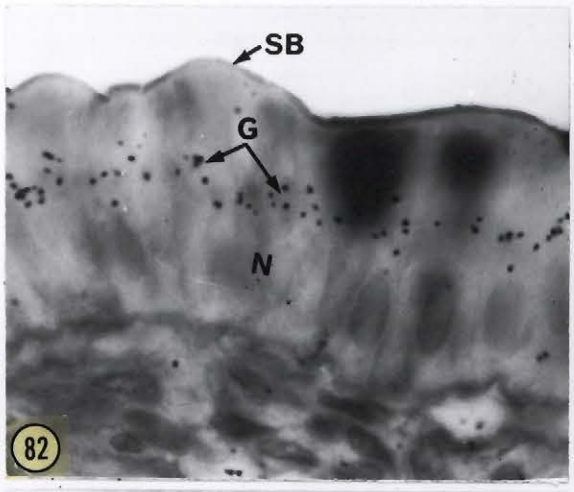
The stained Golgi complex (G) which appears as grey lamellae above the nucleus (N), is heavily labeled. A light scatter of label is seen over the rest of the cytoplasm. The striated border (SB) is intensely stained by colloidal iron.

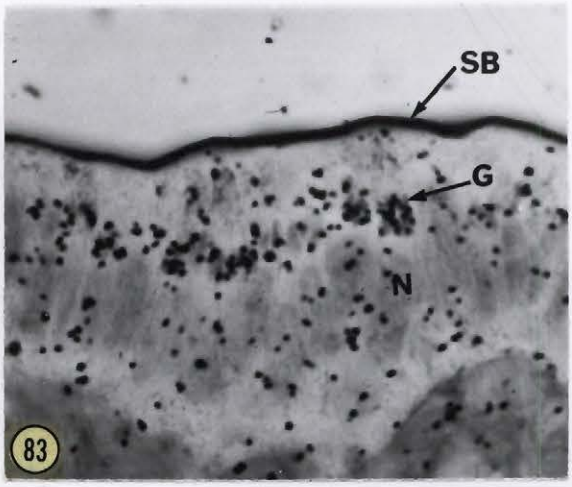
Figs. 84-85 Segments of colon exposed *in vitro* to ^{3}H-6-glucose. (Amylase digested, H & E stain, exposed 3 weeks). 600 X

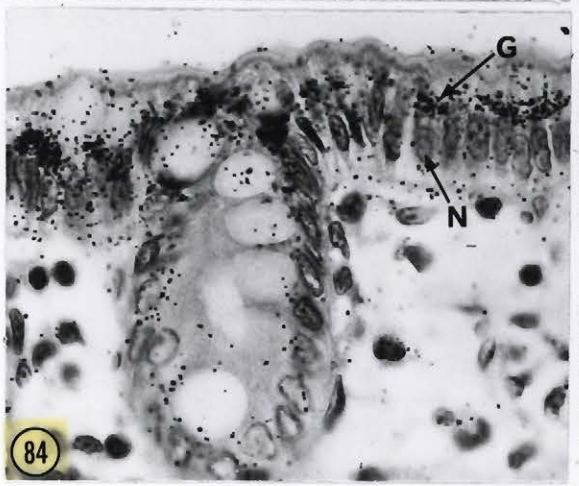
Within 5 minutes (Fig. 84), considerable amounts of glucose label have been incorporated in the Golgi region (G) of columnar cells. After 15 minutes' incubation (Fig. 85), the Golgi region is intensely labeled. (N, nuclei).

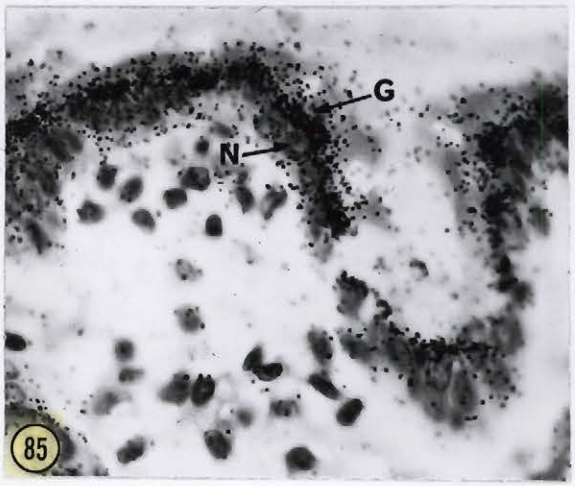
Fig. 86 Five minutes after local injection of ^{3}H-leucine. (Colloidal iron-Feulgen stain, exposed 11 days). 1300 X

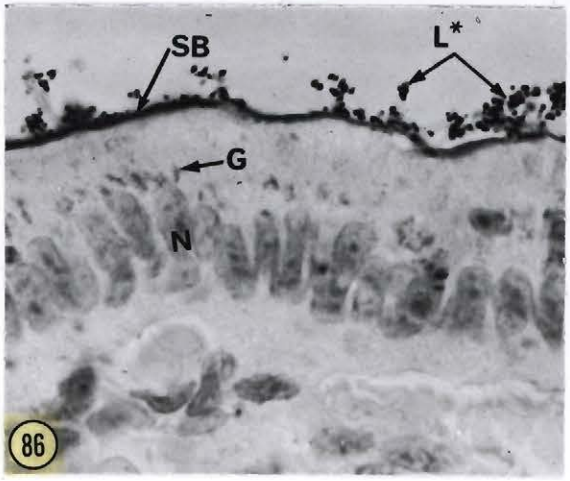
The labeled precursor (L*) remains in the lumen above the colloidal iron-stained striated border (SB). In the unlabeled columnar cells, the Golgi complex (G) is stained blue (here, grey).


SB
G
N
82


SB
G
N
83


G
N
84


G
N
85


SB
L*
G
N
86

Figs. 87-92 Cross or oblique sections through duodenal villi of 20-gram rats showing the connective tissue core covered by a simple columnar epithelium. 660 X

Fig. 87 Maillet technique, to demonstrate the position of the Golgi complex in columnar cells.

The osmium-stained Golgi region (G) lies just above the nucleus (N). In goblet cells (gob), the Golgi region is not stained by this technique.

Fig. 88 Colloidal iron technique (for detection of acidic carbohydrates).

The columnar cells show blue (here, grey) stained material corresponding to the Golgi complex (G) above the unstained nucleus (N). The strongly-stained material on the striated border (SB) is presumably the surface coat on the microvilli, (shown in Fig. 80). The mucigen of goblet cells (gob) is also stained.

Fig. 89 Radioautograph, 10 minutes after ^{3}H-galactose injection, stained by the periodic acid-Schiff technique (for detection of glycoproteins). (Kodak NTB-2 emulsion, exposed for 2 months).

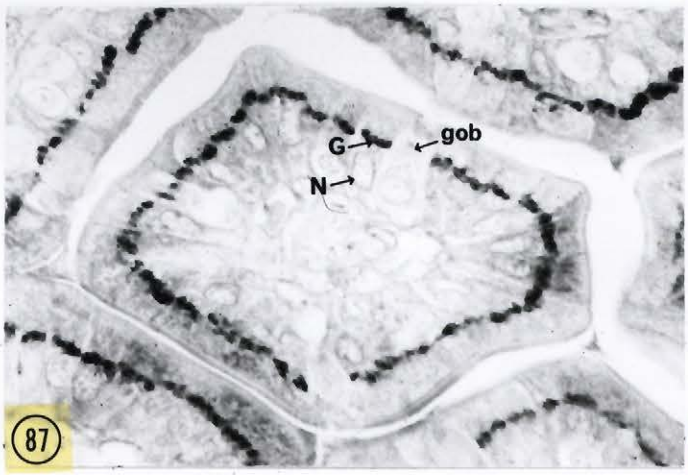
Stained are the basement membrane (BM), striated border (SB) and lateral surfaces of columnar cells. There is also a faint staining of the Golgi region (G) of these cells. The Golgi region is covered with silver grains, indicating uptake of the galactose label. In contrast, the heavily-stained goblet cell (gob) shows none. This figure may be compared with figure 90.

Fig. 90 Colloidal iron-Feulgen stained radioautograph, 10 minutes after injection of ^{3}H-glucose (in a dose comparable to that of ^{3}H-galactose, Fig. 89).

The columnar cells (c) have incorporated no detectable glucose label while the goblet cells (gob) are heavily labeled in the Golgi region (G).

Figs 91 and 92 Colloidal iron-Feulgen stained radioautographs (Kodak NTB-2 emulsion, exposed 2 months).

Ten minutes after ^{3}H-galactose injection (Fig. 91), the goblet cell (gob) is unlabeled, while columnar cells show Golgi-localized radioactivity (G). By 30 minutes (Fig. 92), a small amount of label is still present in the Golgi region of columnar cells, but most of it is in the apical cytoplasm and on the striated border (SB).



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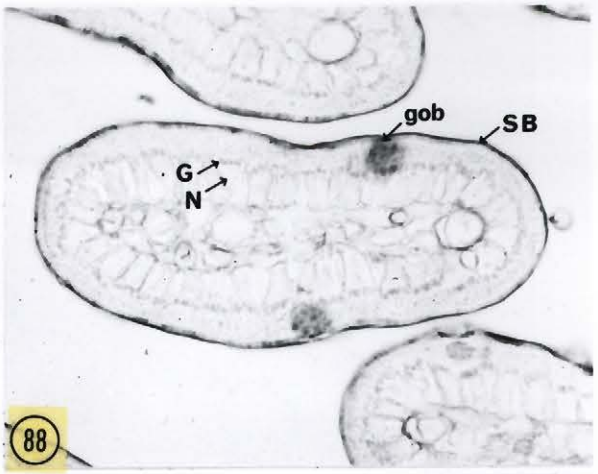

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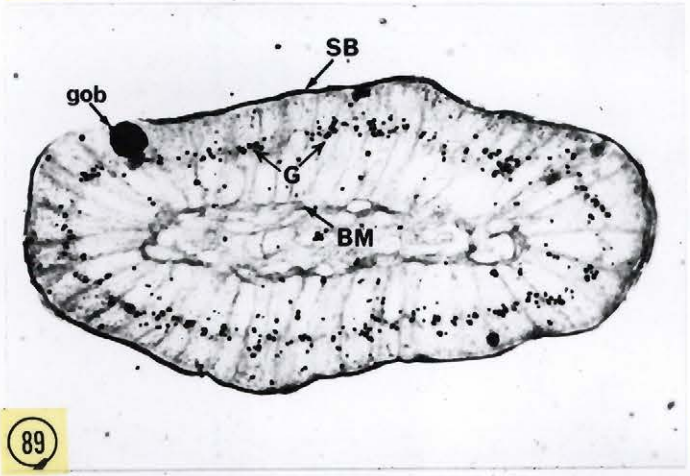

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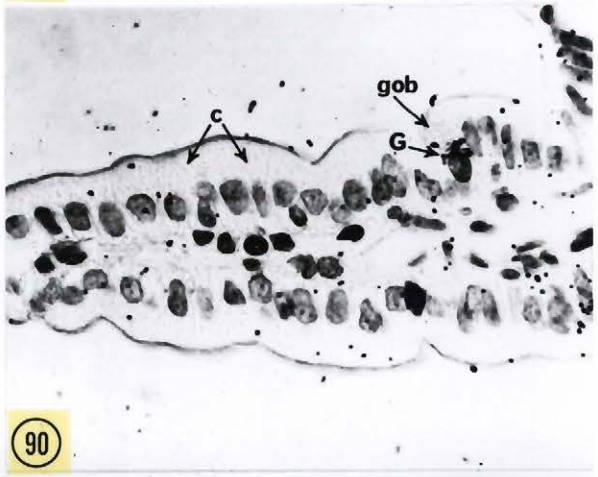

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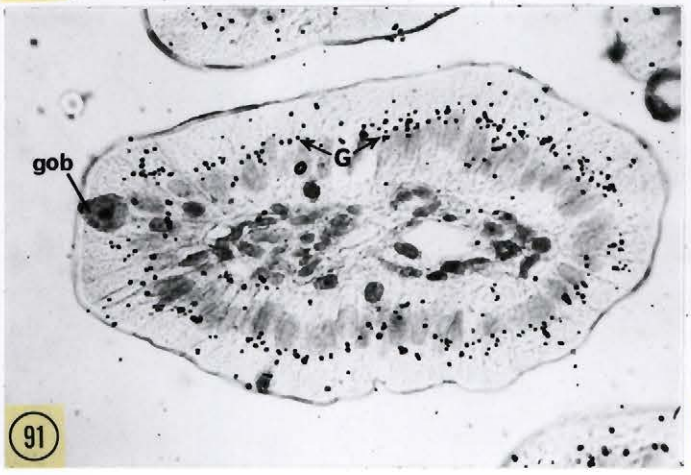

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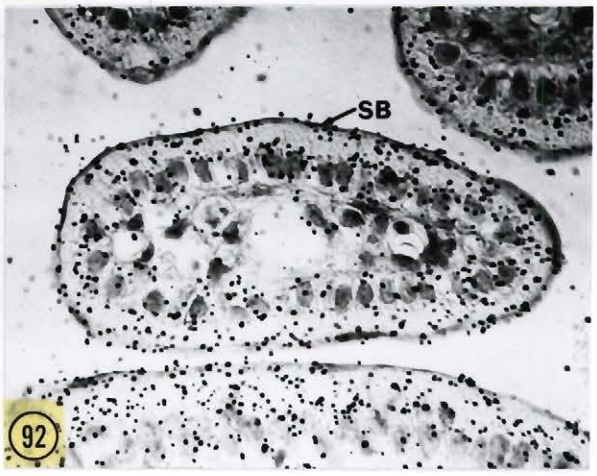


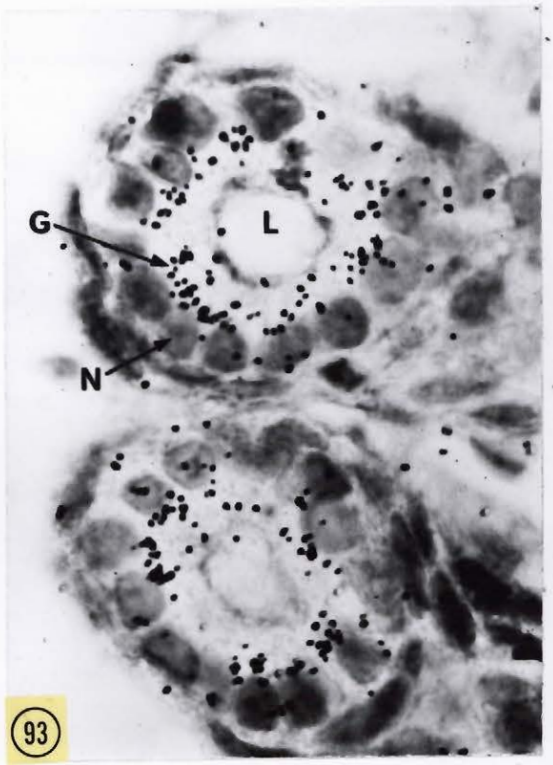
92

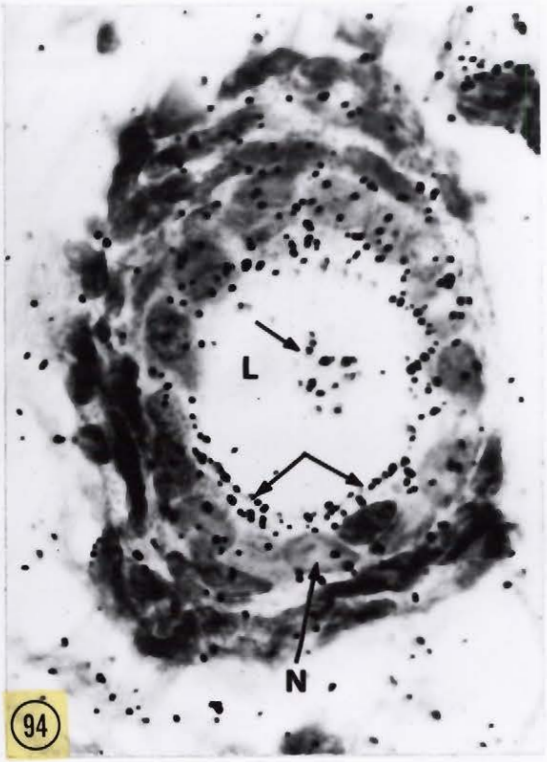
Figs. 93 and 94 Radioautographs of epididymal tubules of 20-gram rats, after injection of ^{3}H-galactose. (Colloidal iron-Feulgen stain, exposed 5 months). 1200 X

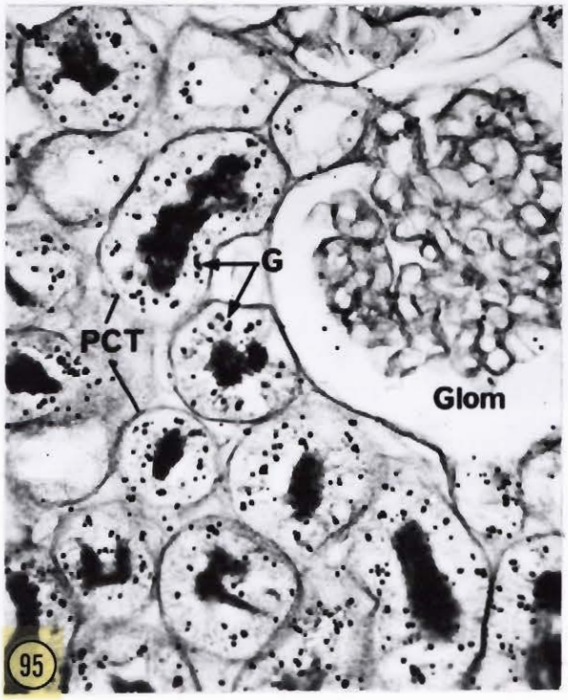
The nuclei (N) of epithelial cells form a ring around the wide lumens (L). At 10 minutes after injection (Fig. 93) radioactive material is located in the supranuclear Golgi region (G). The apical cell borders are stained by colloidal iron, but are not labeled. By 30 minutes after injection, (Fig. 94) most label is associated with the apical borders and with material in the lumen.

Figs. 95 and 96 Radioautographs of kidney cortex of 20-gram rats, after injection of ^{3}H-galactose. (Amylase digested, PA-Schiff stain, exposed 2 months). 600 X

Most of the field is occupied by glomeruli (glom) and proximal convoluted tubules (PCT). Radioactivity is present mainly in the proximal convoluted tubules. At 10 minutes (Fig. 95) it is seen as clusters of silver grains in the supranuclear cytoplasm, perhaps corresponding to the Golgi region (G). By 30 minutes (Fig. 96) much of the radioactivity has spread into the PA-Schiff-positive brush border (BB).


G
L
N
93


L
N
94


G
PCT
Glom
95

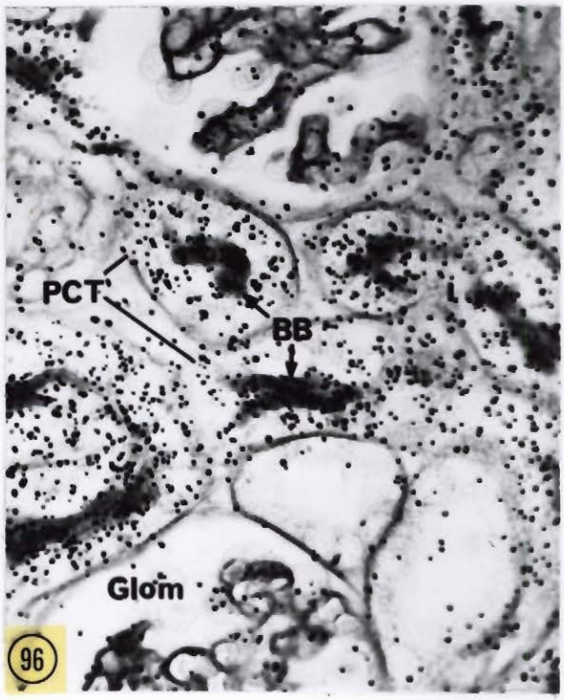

PCT
BB
Glom
96

Fig. 97 Pancreatic acini from a 20-gram rat, stained with hematoxylin-eosin. 1200 X

The peripheral nuclei (N) are embedded in basophilic ergastoplasm. Central to the nuclei, pale cytoplasm containing secretion granules extends to the lumen (L) where a centroacinar cell (CA) is seen.

Fig. 98 Pancreatic acini from a 10-gram rat, prepared by the Maillet technique to demonstrate the Golgi region. 1100 X

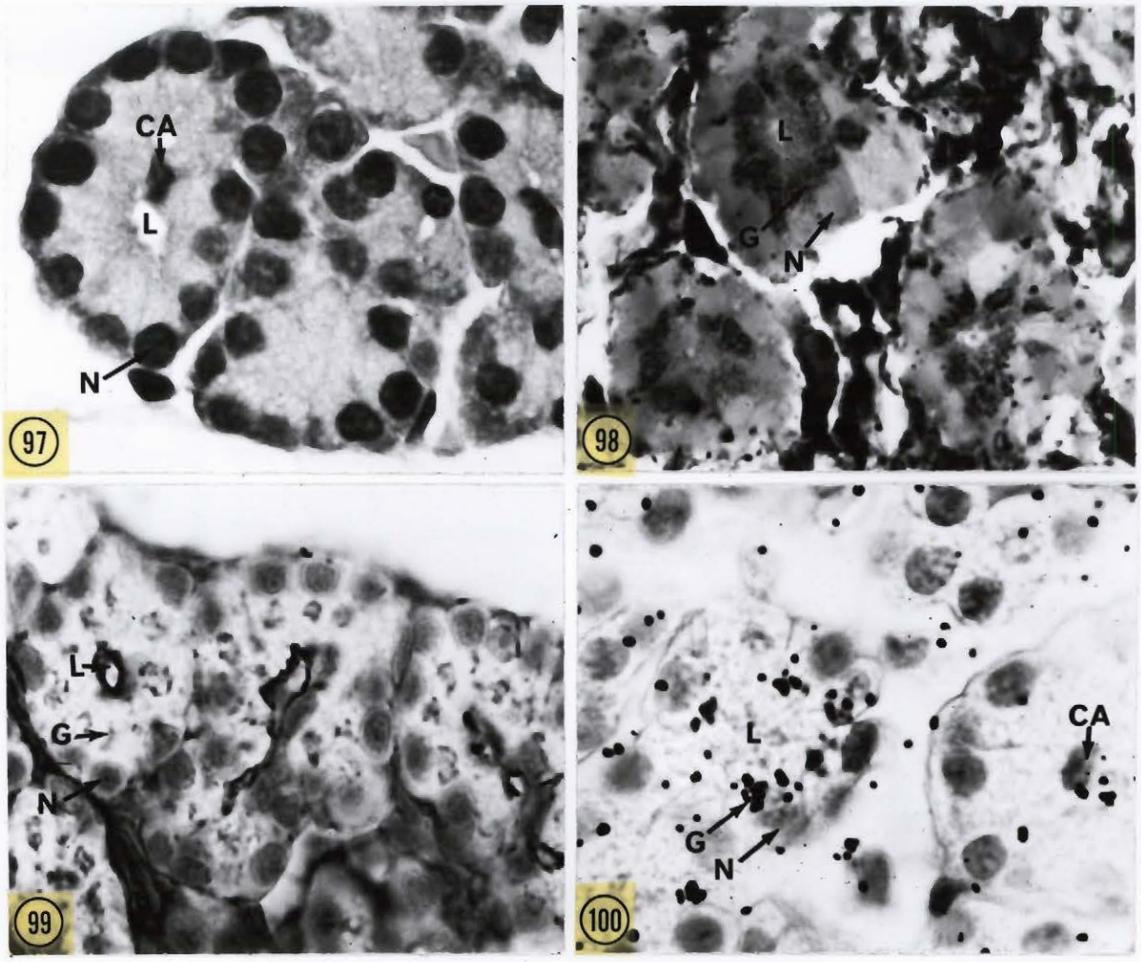
Three acini are present, each with a small central lumen (L). Above the unstained nuclei (N) osmium has been deposited in the Golgi region (G).

Fig. 99 Pancreatic acini from a 10-gram rat, stained with colloidal iron. Nuclei (N) are stained by the Feulgen technique. 1100 X

The border of the lumen (L) and the supranuclear Golgi region (G) are colloidal iron-positive, indicating the presence of acidic carbohydrates.

Fig.100 Radioautograph of a pancreatic acinus from a 20-gram rat, 10 minutes after injection of ^{3}H-galactose. (Colloidal iron-Feulgen stain, Kodak NTB-2 emulsion, exposed 5 months). 1200 X

The nuclei (N) of acinar cells are oriented around the central lumen (L). Small clumps of grains are seen in the supranuclear cytoplasm, a localization corresponding to the Golgi region (G). In the acinus at right, only the centroacinar cell (CA) is labeled.

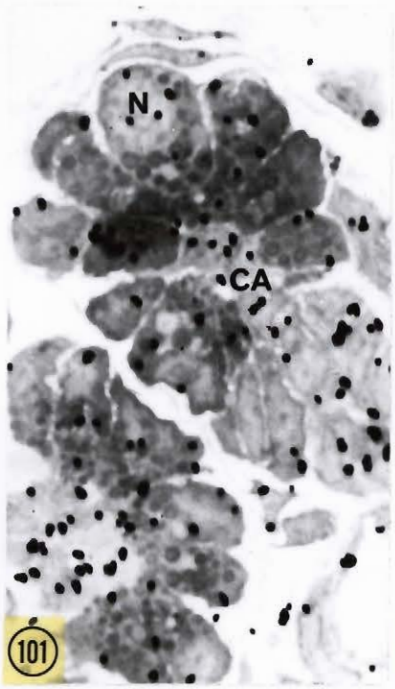

CA
L
N
97
L
G
N
98
L
G
N
99
CA
L
G
N
100

Figs. 101-103 Radioautographs of acini in pancreas of 10-gram rats, at various time intervals after injection of ^{3}H-glucose. (Half-micron Epon sections, toluidine blue stain, Kodak NTB-2 emulsion, exposed 8 weeks) 1200 X

At 5 minutes after injection (Fig. 101) most of the label is in centroacinar cells (CA) while acinar cells are lightly labeled. At 20 minutes (Fig. 102) radioactive material is detected in acinar cells, both in the supranuclear Golgi region (G) and in the basal cytoplasm. Little label is seen in the centroacinar cell (CA). By 40 minutes (Fig. 103) the basal and supranuclear cytoplasm of acinar cells is heavily labeled.

Figs. 104-105 Radioautographs of acini in pancreas of 20-gram rat, after injection of ^{14}C-glucosamine. (Half-micron Epon sections, toluidine blue stain, Ilford L-4 emulsion, exposed 14 and 8 weeks, respectively). 1200 X

At both time intervals, little or no label is detected in the cytoplasm of acinar cells. Near the lumen (L), centroacinar cells (CA) are labeled, lightly at 10 minutes (Fig. 104) and heavily at 30 minutes (Fig. 105).



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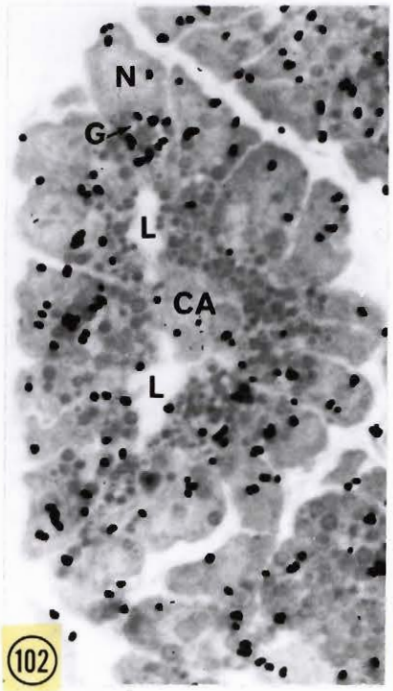

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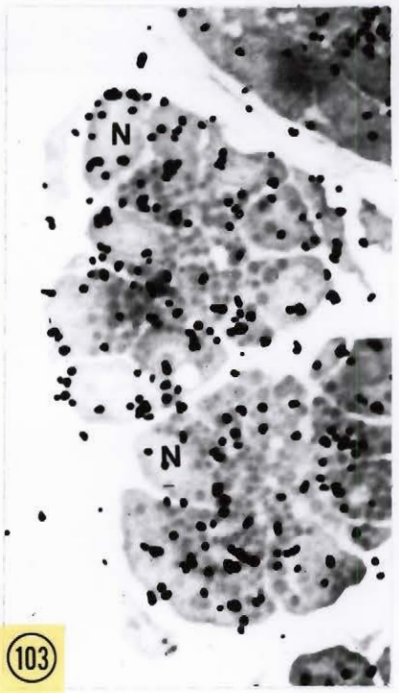

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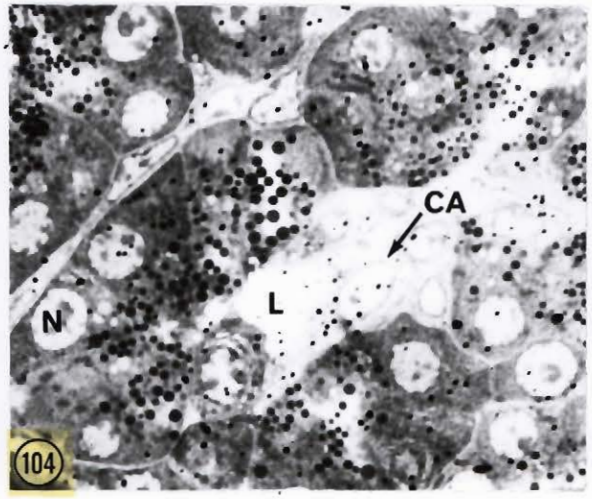

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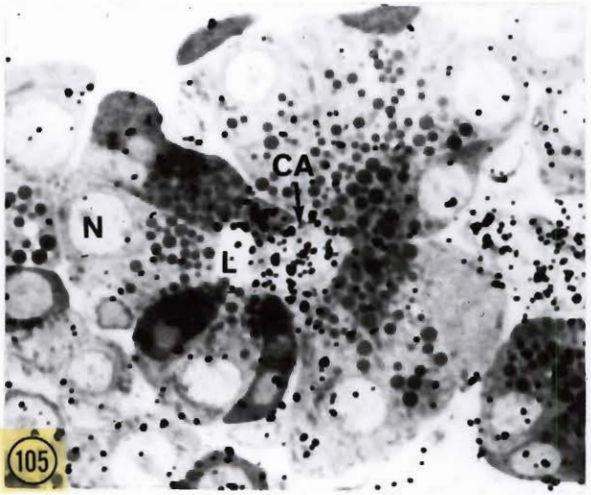


105

Figs. 106-109 Radioautographs of liver of 20-gram rat, 10 minutes after injection of ^{3}H-glucose (exp. 9) or ^{3}H-galactose (exp. 10) stained with the PA-Schiff technique for detection of glycogen.

600 X

Fig. 106 ^{3}H-glucose, control. The glycogen is stained an intense magenta (here, black) so that the light radioautographic reaction is not visible. (Exposed 5 weeks).

Fig. 107 ^{3}H-glucose, amylase-digested. Removal of glycogen caused little or no decrease in the radioautographic reaction; ^{3}H-glucose label is scattered throughout the cytoplasm of liver cells. (Exposed 5 weeks).

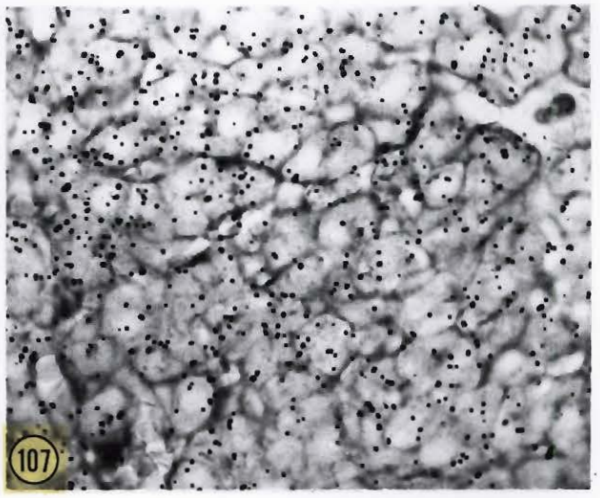
Fig. 108 ^{3}H-galactose, control. The intensely-stained glycogen masks a heavy radioautographic reaction. (Exposed 2 months).

Fig. 109 ^{3}H-galactose, amylase-digested. Much of the radioactive material was extracted by amylase, and was thus identified as glycogen. That which remains appears as dense clumps of silver grains, seen at higher magnification in Fig. 110. (Exposed 2 months).

Fig. 110 Radioautograph of liver parenchymal cells, 10 minutes after injection of ^{3}H-galactose. Glycogen has been extracted with amylase. (Colloidal iron-Feulgen stain, exposed 5 months) 1100 X

Clumps of silver grains are localized in the juxtanuclear cytoplasm of liver cells, and near the liver cell interfaces, but not near sinusoids (S).

106



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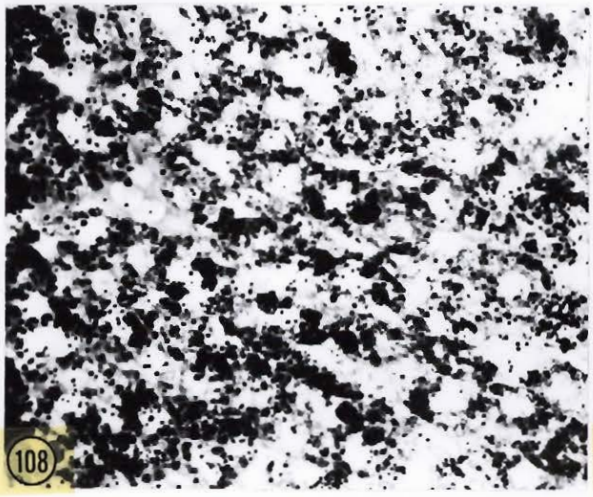

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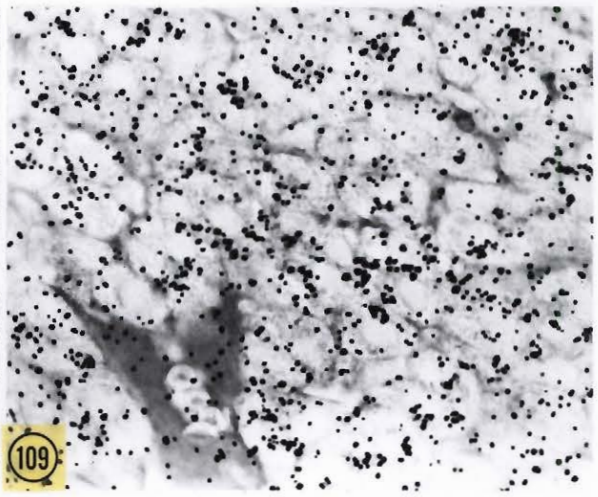

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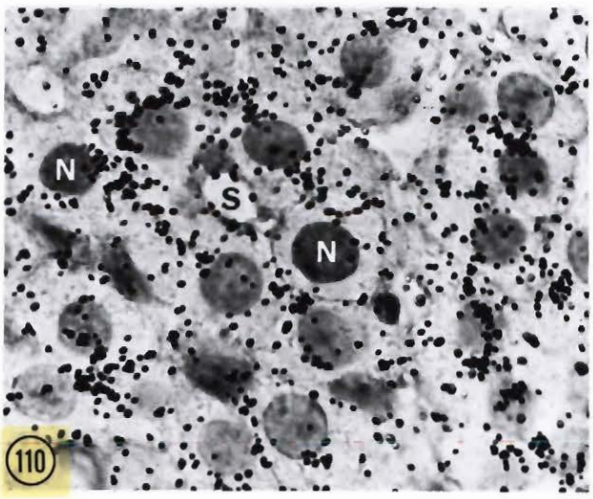


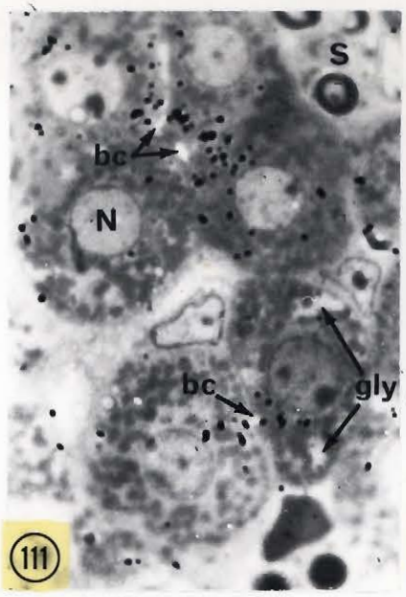
110

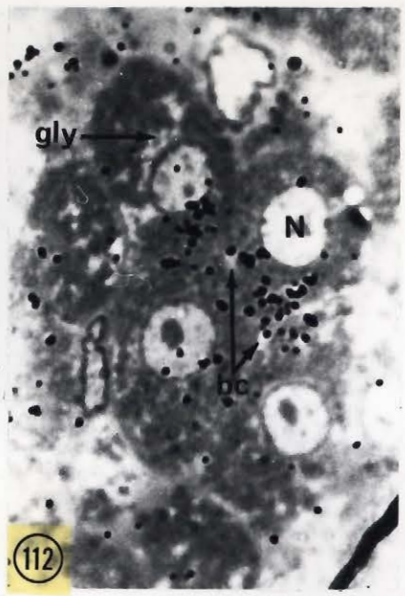
Figs. 111 and 112 Radioautographs of liver parenchymal cells, 10 minutes after injection of ^{3}H-galactose. Glycogen has been removed by digestion with alpha-amylase. (S, sinusoid) (Half-micron Epon sections stained with toluidine blue; Ilford L-4 emulsion, exposed 3 months). 1200 X

In the cytoplasm around the central nuclei (N), are dark mitochondria and pale glycogen areas (gly), here emptied by amylase digestion. Between adjacent cells, bile canaliculi (bc) are visible. Radioactivity is localized in the peri-canalicular cytoplasm, a position also occupied by the Golgi complex.

Fig. 113 Radioautograph of liver parenchymal cells, 10 minutes after injection of ^{14}C-glucosamine. (Half-micron Epon section, toluidine blue stain, Ilford L-4 emulsion, exposed 8 weeks). 1200 X

Although some silver grains are seen over the nuclei (N) and cytoplasm of parenchymal cells, many of them appear near bile canaliculi (bc).


S
bc
N
bc
gly
111


gly
N
bc
112

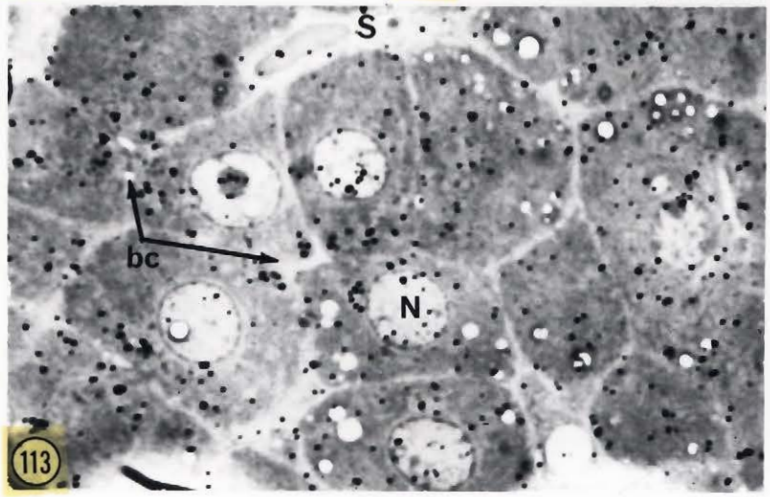

S
bc
N
113

Fig. 114 Electron micrograph of parenchymal cells in the liver of a 20-gram rat, showing a region near a bile canalicule (BC).

45,000 X

Portions of 2 cells are present, with their plasma membranes (PM) closely applied. A vacuole (V) lies near the nucleus (N) of one of the cells. In the cytoplasm adjacent to the bile canalicule, the Golgi complex is found (G). Although rough-surfaced endoplasmic reticulum (rER) mitochondria (m) and glycogen (gly) also lie in this region, they are equally distributed in other parts of the cell.

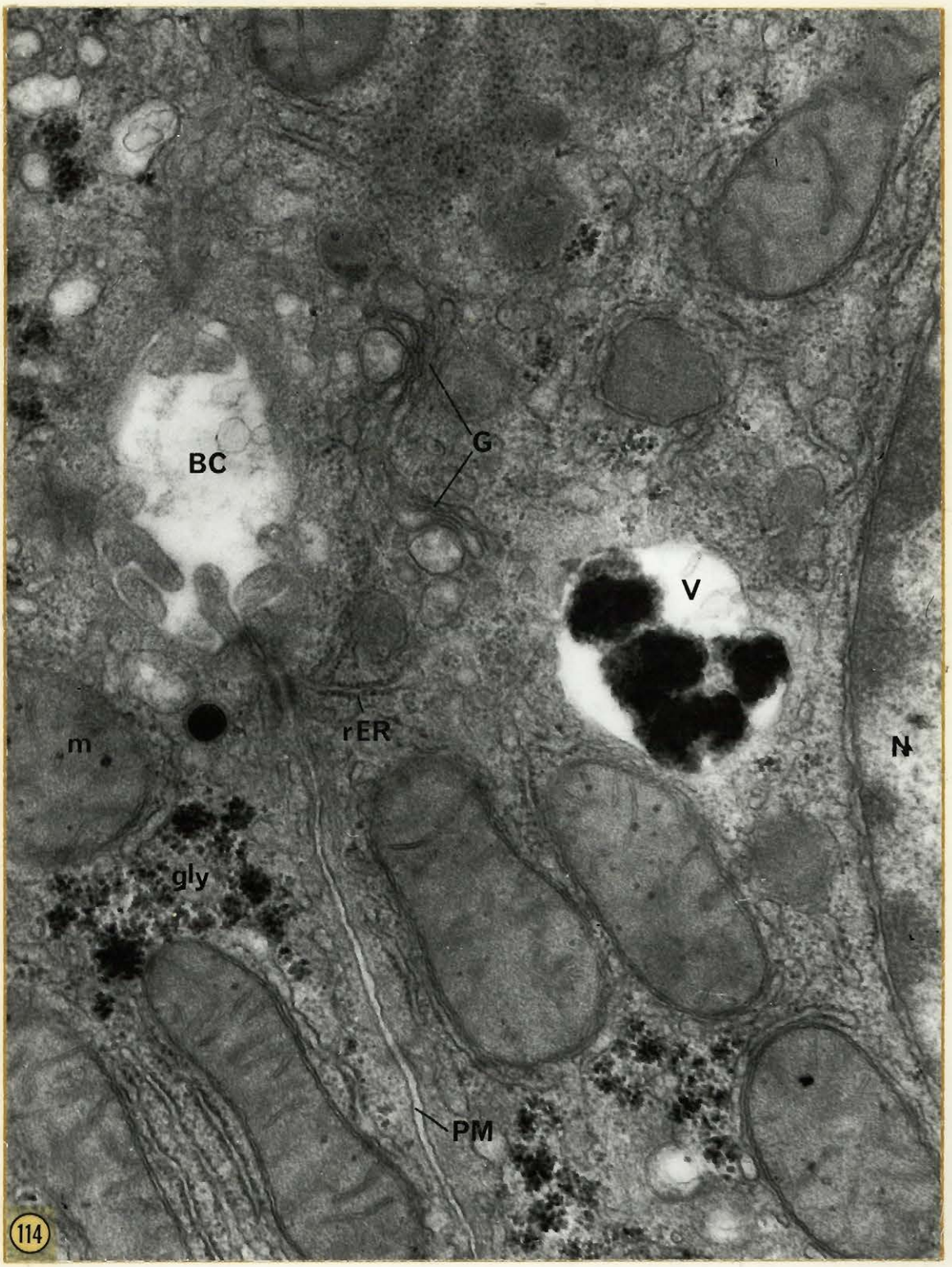

BC
G
V
m
rER
N
gly
PM
114

Fig. 115 Tracheal cartilage of 10-gram rat, prepared by the Maillet technique to demonstrate the Golgi region of chondrocytes.

1100 X

The Golgi region (G) is generally located to one side of the nucleus (N).

Fig. 116 Radioautograph of tracheal cartilage of 10-gram rat, 10 minutes after injection of ^{35}S-sulfate. (Colloidal iron-Feulgen stain, exposed 19 days) 1100 X

In the chondrocytes, radioactive material is present in the small juxtanuclear area where the Golgi region (G) is located.

Fig. 117 Radioautograph of epiphyseal cartilage of 10-gram rat, 5 minutes after injection of ^{3}H-glucose. (Colloidal iron-Feulgen stain, exposed 40 days) 1200 X

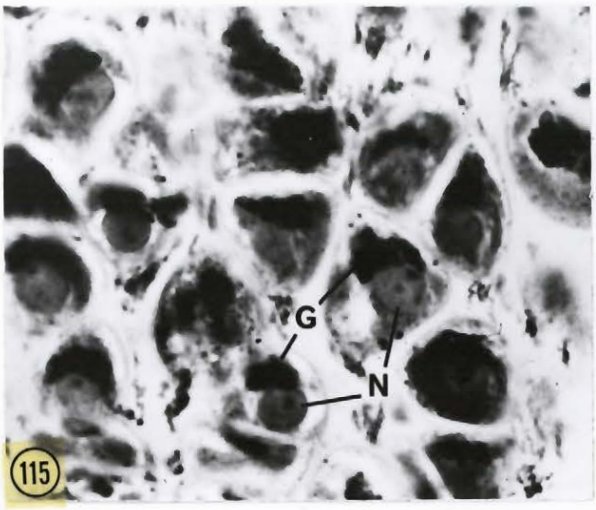
In most cells, the nuclei are not clearly seen. In the cell at left, radioactivity is mainly localized to one side of the nucleus (N) in the Golgi region (G).

Fig. 118 Radioautograph of epiphyseal cartilage of 20-gram rat, 10 minutes after injection of ^{3}H-galactose. (Colloidal iron-Feulgen stain, exposed 2 months) 1200 X

In the flattened chondrocytes of the zone of proliferation, small areas of radioactive cytoplasm are again seen next to the nuclei (N).

Fig. 119 Radioautograph of epiphyseal cartilage of 20-gram rat, 10 minutes after injection of ^{14}C-glucosamine. (Colloidal iron-Feulgen stain, exposed 6 days) 1200 X

Within a row of chondrocytes in the zone of cell proliferation, one cell in metaphase (M) has incorporated little label. The others show small juxtanuclear accumulations of radioactivity.



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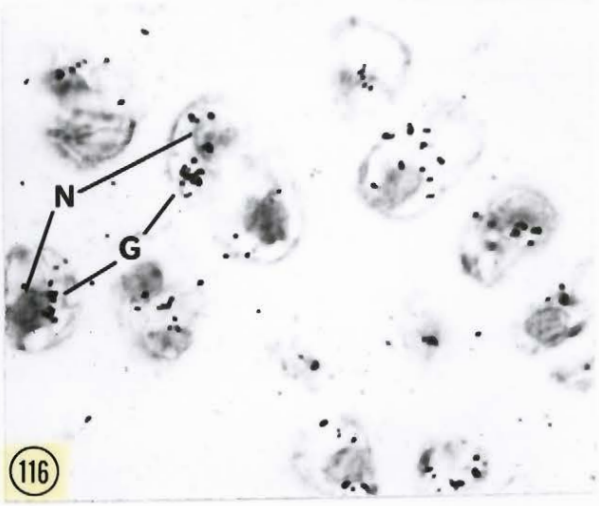

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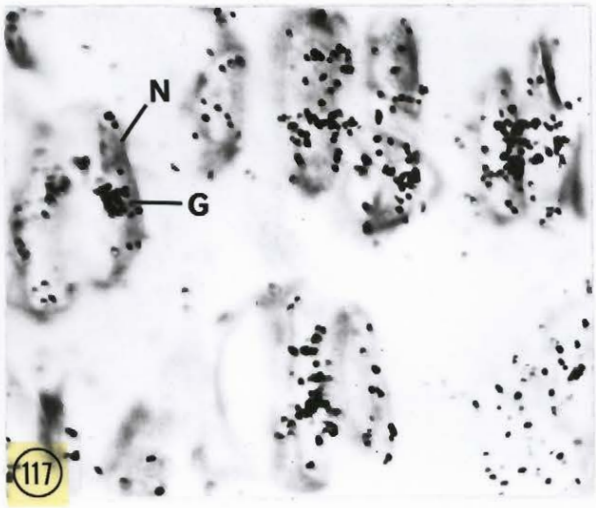

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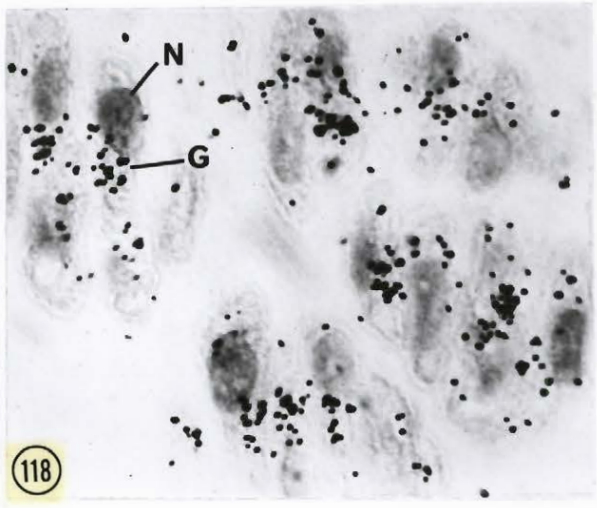

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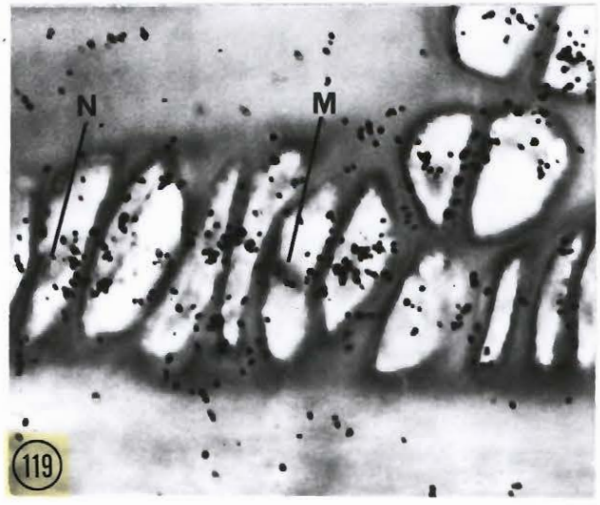


119

Figs. 120 and 121 Radioautographs of tracheal cartilage from 10-gram rat, 20 minutes after injection of ^{3}H-glucose. (PA-Schiff stain, exposed 10 days). 600 X

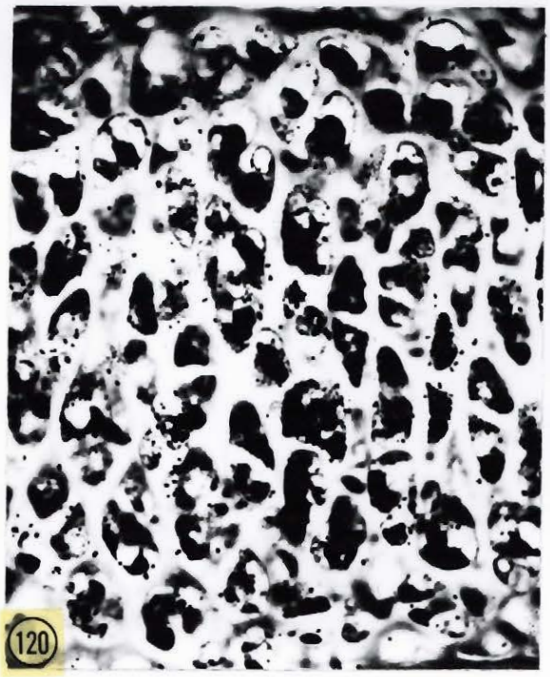
Fig. 120 Control. The intensely-stained glycogen in the cytoplasm of chondrocytes appears black in the photograph, so that the radioautographic reactions over the chondrocytes are not visible.

Fig. 121 Amylase-digested. After extraction of glycogen, the staining of chondrocytes is largely lost, but the radioautographic reaction, now visible, is not diminished.

Fig. 122 and 123 Radioautographs of epiphyseal cartilage from 100-gram rat, 15 minutes after injection of ^{3}H-glucose. (PA-Schiff stain, exposed 5 weeks) 600 X

Fig. 122 Control. A radioautographic reaction over the cytoplasm of chondrocytes is almost completely masked by abundant, darkly-stained glycogen.

Fig. 123 Amylase-digested. Extraction of glycogen leaves the cytoplasm of chondrocytes only weakly stained. None of the radioactive material in the cytoplasm has been removed.



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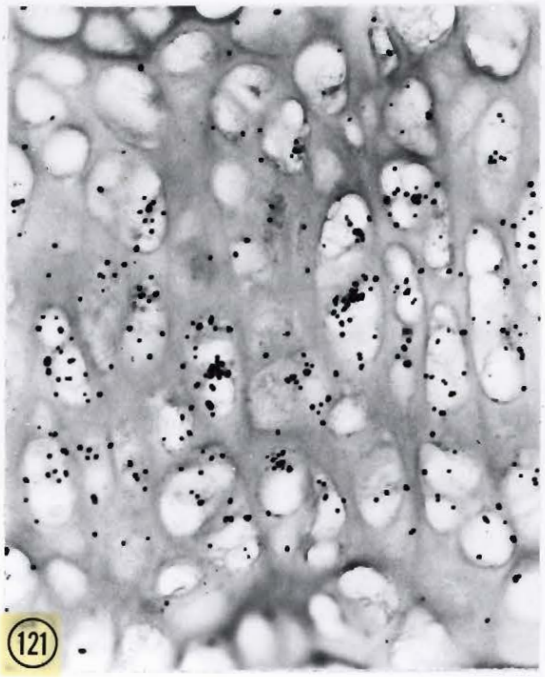

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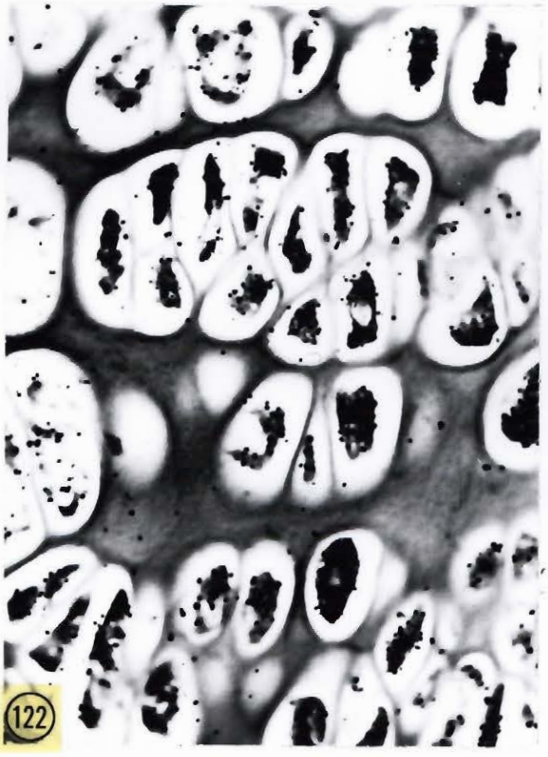

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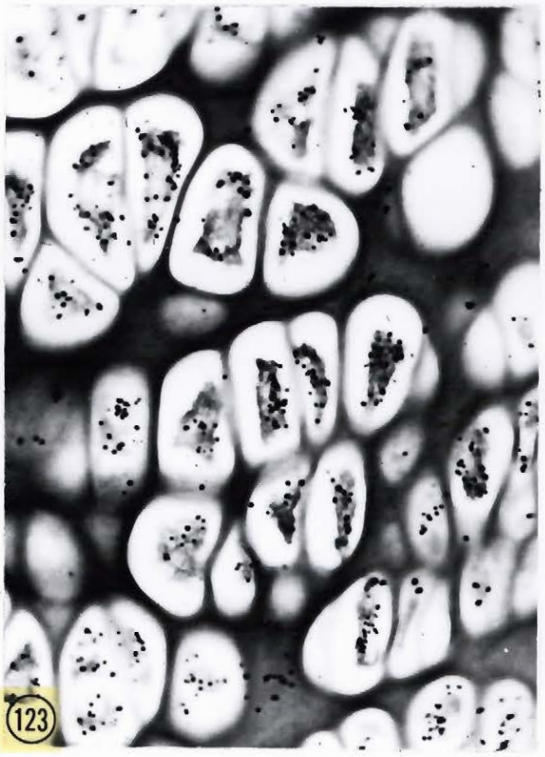


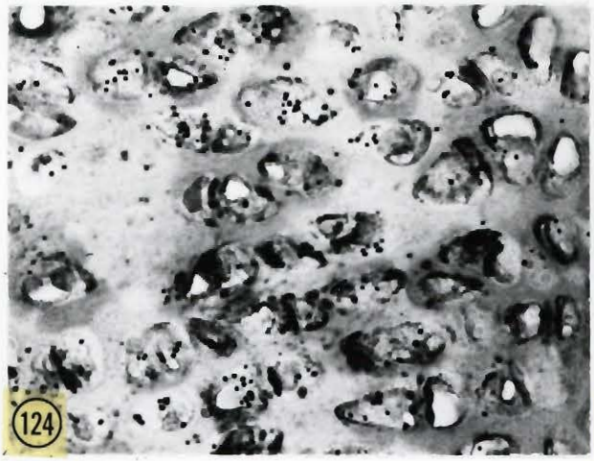
123

Figs. 124-129 Radioautographs of tracheal cartilage from 10-gram rats, at various time intervals after injection of ^{3}H-glucose. The control sections on the left were incubated in phosphate buffer alone. The corresponding adjacent sections on the right were incubated in phosphate-buffered hyaluronidase, to extract the acid mucopolysaccharide, chondroitin sulfate. (Colloidal iron-Feulgen stain, exposed 9 days) 600 X

Figs. 124 and 125 Twenty minute interval. The loss of colloidal iron stained material from the cytoplasm of chondrocytes would suggest that acidic carbohydrates were extracted by treatment with the enzyme. The radioautographic reaction, however, has been reduced by only 10%.

Figs. 126 and 127 Forty minute interval. Hyaluronidase treatment has extracted approximately half of the radioactive material from both cells and matrix.

Figs. 128 and 129 One and one half hour interval. Again, about half of the radioactive material in cells and matrix is hyaluronidase-labile.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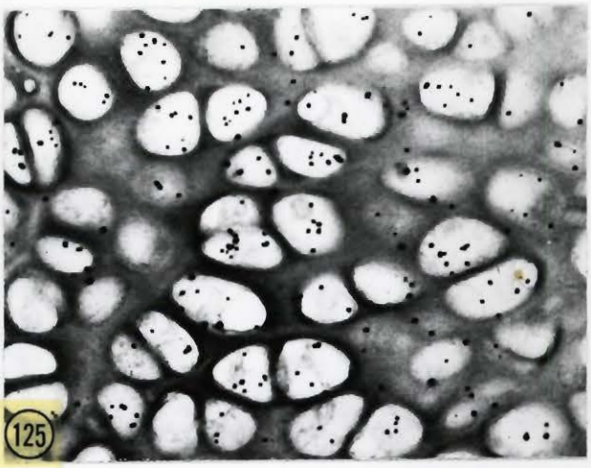
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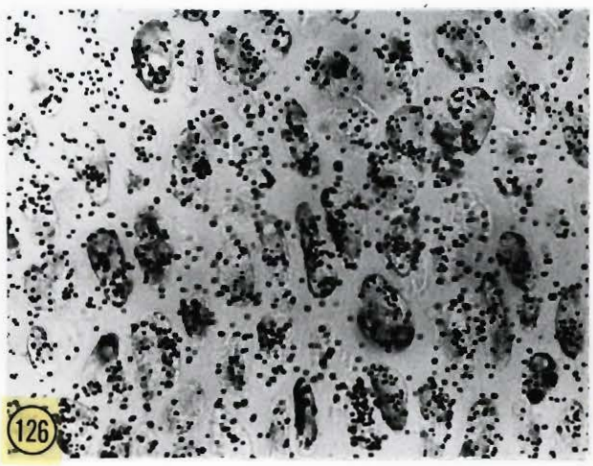
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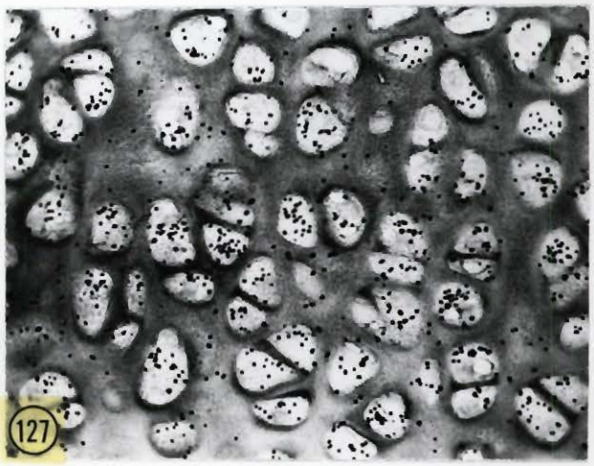
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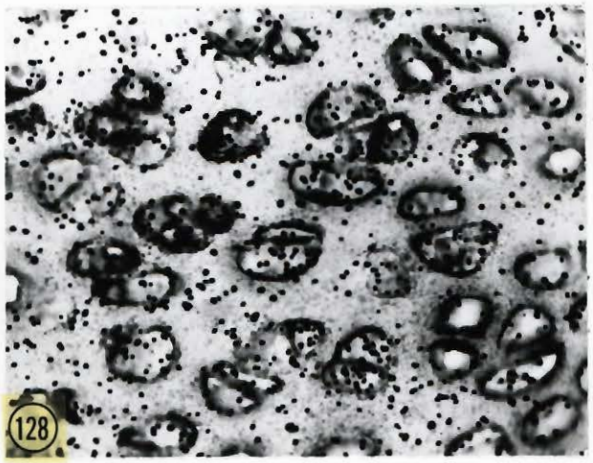
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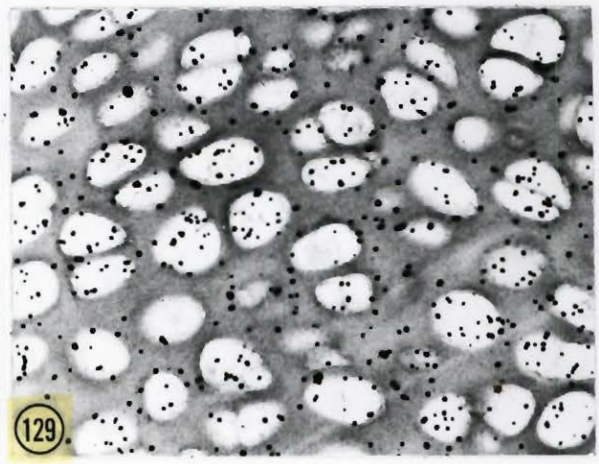
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