RADIOAUTOGRAPHIC COMPARISON OF THE UPTAKE OF GALACTOSE-H^3 AND GLUCOSE-H^3 IN THE GOLGI REGION OF VARIOUS CELLS SECRETING GLYCOPROTEINS OR MUCOPOLYSACCHARIDES

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ABSTRACT

The radioautographic distribution of the label of galactose-H^3 was compared with that of glucose-H^3 in a series of secretory cells of the rat. Whereas the glucose label appeared in all mucous cells, the galactose label was incorporated only into certain mucous cells. Whenever either label was incorporated, however, it was located first in the Golgi region and later in the secretion product, mucus. Several lines of evidence, including extraction of glucose label with peracetic acid—beta glucuronidase, indicated that the material synthesized in the Golgi region was glycoprotein in nature. In chondrocytes, both the galactose and the glucose label appeared first in the Golgi region and later in cartilage matrix; extraction of glucose label with hyaluronidase indicated that much of it consisted of mucopolysaccharide. In all secretory cells, the extraction of glycogen by amylase had no effect on Golgi radioactivity. Such extraction did not eliminate the scattered cytoplasmic label also seen after glucose-H^3 injection, but completely eliminated that seen after galactose-H^3. Consequently, the galactose-H^3 label in the Golgi region stood out more clearly, and was detected in many cells: pancreas, liver, epididymis, and intestinal columnar cells. In the latter, label later appeared in the surface coat. Thus, radioautography after injection of galactose-H^3, as after glucose-H^3, indicates that synthesis of complex carbohydrates takes place in the Golgi region of many secretory cells.

INTRODUCTION

A radioautographic reaction appears over the Golgi region of many secretory cells after injection of labeled glucose (37). In the goblet cells of rat colon examined with the electron microscope, the reaction has been pinpointed to the Golgi saccules (35). The evidence strongly suggests that the glucose label taken up into the saccules is used for the synthesis of the carbohydrate moiety of mucus glycoprotein.

However, not all glucose is incorporated into complex carbohydrates. Some 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 with that of labeled glucose.

Furthermore, in the hope of throwing light on the nature of the substances labeled by glucose-H^3 and galactose-H^3, we examined the effects of three enzymes, hyaluronidase, alpha amylase, and

TABLE I

Summary of Radioautographic Experiments

Radioactive materials	Specific activity	Injected dose per gram body weight ‖	Animal weight	Time elapsing between injection and sacifice	Sections
	mc/mmole	*μc*	*g*		
Main experiment					
glucose-6-H^3*	1300	20	20	10, 30 min	paraffin, 4 μ
galactose-1-H^3‡	83	20	20	10, 30 min	paraffin, 4 μ Epon, ½ μ
Subsidiary experiments					
glucose-6-H^3	1300	4	10	5, 15, 30, 45 min 1, 2, 3 hr	paraffin, 3 μ
"	1300	100	10	5, 20, 40 min 1, 1½, 4 hr	paraffin, 4 μ Epon, ½ μ
"	250	60	110	15 min	paraffin, 4 μ Epon, ½ μ
Na-sulfate-S^{35}§	carrier-free	2	100	5, 15 min 1, 3 hr	paraffin, 4 μ Epon, ½ μ
"	carrier-free	2	10	10, 30 min 1, 3 hr	paraffin, 4 μ
4,5-leucine-H^3‡	5450	350	50–55	2, 6, 10, 20, 30 min	Epon, ½ μ

The exposure times varied for each experiment; they will be indicated in the legends of the figures.

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‡ New England Nuclear Corporation, Boston, Massachusetts.

§ Charles E. Frosst & Co., Montreal, Canada.

‖ All injections were given intraperitoneally, except for the first of the subsidiary experiments, in which the material was injected subcutaneously.

beta glucuronidase, on the radioautographic reactions.

MATERIALS AND METHODS

The radioautographic experiments were performed on Sprague-Dawley and Fischer rats, as summarized in Table I. To compare the uptake of glucose and galactose, similar doses of the two labeled sugars were injected into 20-g rats under similar conditions (Table I, main experiment). In further experiments using glucose-H^3, the migration and release of secretory material was followed with time. Finally, the uptake of sulfate and leucine was compared to that of the monosaccharides (Table I, subsidiary experiments).

HISTOLOGICAL METHODS: The organs were collected under ether anesthesia and fixed in neutral buffered formalin pH 7.0 (36) at 4°C for 24 to 48 hr. Paraffin sections were cut at 3 or 4 μ, and were stained with hematoxylin (H) and eosin (E), periodic acid(PA)–Schiff (36) without counterstain, or colloidal iron (33). Counterstaining of nuclear DNA with the Feulgen reaction seemed to enhance the definition of the colloidal iron stain, and to prevent the partial desensitization of the emulsion produced when colloidal iron alone was used to prestain radioautographs.

Certain tissues were processed for sectioning at ½ μ. Thus, after injection of labeled glucose or galactose, colon and liver were fixed in isotonic phosphate-buffered 2.5% glutaraldehyde (4°C, 1 to 2 hr), rinsed in buffer, postfixed in isotonic buffered 1% osmium tetroxide (48), and embedded in Epon. In experiments with labeled sulfate and leucine, the colon was fixed in glutaraldehyde or osmium tetroxide (50) and embedded in methacrylate.

RADIOAUTOGRAPHIC METHODS: The *paraffin sections*, either unstained or prestained as above, were coated by dipping into liquid Kodak NTB2 emulsion (24). The ½ μ *Epon sections* were coated with Ilford L-4 emulsion (glucose and galactose experiments) or with Kodak NTB2 emulsion (sulfate and leucine experiments), and were poststained with toluidine blue (35).

ENZYMATIC DIGESTIONS: In the galactose-H^3 experiment, small blocks of glutaraldehyde-fixed liver were immersed in a phosphate-buffered 1% solution of alpha amylase (Worthington Biochemical

Corp., Freehold, New Jersey, hog pancreas, twice crystallized, activity 712 U/mg) for 2 hr at 37°C, before OsO_4 postfixation and Epon embedding. Controls were incubated in buffer without enzyme (5). The other digestions were performed on deparaffinized 4 μ sections prior to staining and radioautography. In all glucose-H^3 and galactose-H^3 experiments, a few sections were treated with salivary *alpha amylase* for removal of glycogen, as described in the preceding article (35). Other sections were treated with testicular *hyaluronidase* or with *beta glucuronidase* (35).

After control and enzyme-treated sections had been radioautographed, the percentage of radioactive material extracted by the three enzymes was measured by comparing the counts of silver grains over enzyme-treated sections with those over controls. These counts were done in some of the tissues known to produce secretory glycoprotein and/or mucopolysaccharide. The results were expressed as per cent reduction in the counts of silver grains per unit area (tracheal cartilage matrix, pulp of incisor tooth) or per cell (chondrocyte).

RESULTS

Mucous Cells

GOBLET CELLS OF COLON: When the goblet cells of colon were examined after injection of *galactose-H^3*, a strong radioautographic reaction was seen over the Golgi region at 10 min (Fig. 1) and 30 min (Figs. 2 and 3). Relatively few silver grains were scattered outside this region (Figs. 1 to 3), even after long exposure; and all this scatter was removed by amylase digestion. In contrast, after *glucose-H^3*, the intense Golgi reaction was accompanied by a general scatter of silver grains over the rest of the cell (Figs. 5 to 7 in the preceding article, reference 35) and most of this scatter was *not* removed by amylase digestion.

Radioautographs of intestinal epithelium 5 and 15 min after *sulfate-S^{35}* injection also showed a Golgi-localized reaction in goblet cells (Fig. 4) as reported by previous authors (18, 26).

In sharp contrast, radioautographs of colon at 6 to 30 min after *leucine-H^3* injection showed silver grains widely distributed over the epithelium. The reaction over goblet cells was particularly heavy in the basal and perinuclear cytoplasm. Label was not localized in the Golgi region (Fig. 5).

OTHER MUCOUS CELLS: The rough estimates of reaction intensities given in Table II indicate that the Golgi region of all mucous cells took up substantial quantities of glucose-H^3. With galactose-H^3, on the other hand, some mucous cells were heavily labeled in the Golgi region (cells of sublingual and Brunner's glands, and goblet cells of colon), others were labeled only weakly (surface mucous cells of stomach), and still others, not at all (goblet cells of duodenum and other parts of small intestine, tracheal mucous glands). Similarly, sulfate-S^{35} was incorporated into only some mucous cells, as reported by other workers (18).

At later times after injection, the radioautographic reactions of mucus-secreting cells appeared over mucigen. The labeled mucigen was found to migrate to the cell apex and later to be excreted. The approximate migration speed (Table III) was estimated from radioautographs obtained at numerous intervals between 5 min and 4 hr after injection of glucose-H^3 in 10-g rats (Table I, subsidiary experiments). With a wide range of variation, the labeled material began to leave the cells as mucus 3 to 4 hr after injection, except in the case of Brunner's glands where labeled material appeared outside the cells at 1 to 2 hr.

Other Epithelial Cells

COLUMNAR CELLS OF DUODENUM: The duodenal villi of young rats are covered by uniform columnar cells with the occasional goblet cell. The Golgi complex of columnar cells is in a supranuclear position (Fig. 6) and is stained by colloidal iron–Prussian blue (Fig. 7) and by the periodic acid–Schiff technique (27, 41). In addition, both methods stain the striated border (Figs. 7 and 8).

It had previously been reported that, soon after administration of glucose-H^3 in very high doses, or after local application, a weak but distinct reaction appeared over the Golgi region of columnar cells, in addition to the intense reaction over the Golgi region of goblet cells (38). Furthermore, such high doses produced a light scatter of label throughout the cytoplasm of columnar cells (as in the colon, Figs. 5 to 7 in reference 35). With lower systemic doses of glucose-H^3 (Table I, main experiment), columnar cells were not significantly labeled even though goblet cells still showed a Golgi reaction (Fig. 9).

When a similar low dose of galactose-H^3 was administered under the same conditions (Table I, main experiment), a strikingly different radioautographic reaction was produced. The goblet

cells incorporated no detectable label. As for the columnar cells, at 10 min after injection they showed considerable uptake of label in the Golgi region, with little or no scatter in the rest of the cell (Figs. 8 and 10), while at 30 min after injection, most of the galactose label was located in the apical cytoplasm and along the apical cell surface (Fig. 11).

Pancreatic acinar cells showed a supranuclear reaction 10 min after injection of galactose-H^3 (Figs. 12 and 13). In *liver parenchymal cells*, the galactose label (after removal of glycogen) was located in the cytoplasm close to bile canalicules (Fig. 14). In the epithelial cells of *epididymis* and of *kidney proximal convoluted tubule*, galactose-H^3–labeled material was present in the supranuclear region at 10 min after injection. By 30 min, it had spread to the apical cytoplasm and accumulated at the surface.

Chondrocytes

Chondrocytes of tracheal and epiphyseal cartilages showed a reaction in a paranuclear position which was assumed to correspond to the Golgi region (Table II). The material labeled with glucose-H^3 or galactose-H^3 made its appearance in the adjacent matrix at 30 to 40 min after injection (Table III).

Enzymatic Digestions

AMYLASE-LABILE RADIOACTIVITY: The radioactivity removed by alpha amylase treatment (presumed to be in glycogen) was assessed after injection of either precursor. It was thus shown that the radioactive material in the Golgi region was not glycogen. After galactose-H^3, all the scattered cytoplasmic label (found only in liver, intestinal epithelium, and muscle) consisted of glycogen. After glucose-H^3, however, much of the scattered label was not glycogen.

HYALURONIDASE-LABILE RADIOACTIVITY: Hyaluronidase had no effect on the radioactivity of epithelia: for instance, control sections of duodenal columnar epithelium showed an average of 5.0 gr per unit area, and hyaluronidase-treated sections, 4.9 gr per unit area. However, treatment of tooth pulp, cartilage, aortic wall, and other types of connective tissues extracted radioactive

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FIGURES 1 to 5 Radioautographs of rat colon in $\frac{1}{2}$ μ Epon sections, stained with toluidine blue.

FIGURE 1 A single goblet cell in the surface epithelium, 10 min after galactose-H^3 injection in a 20 g rat (Ilford L-4 emulsion, 7 month exposure). In the goblet cell, a cluster of silver grains overlies the Golgi region (*G*) between nucleus (*N*) and mucigen (*M*). The columnar cells are not significantly labeled. × 1200.

FIGURE 2 Four surface goblet cells, 30 min after galactose-H^3 injection in a 20 g rat (Ilford L-4 emulsion, 7 month exposure). The Golgi region (*G*) of the goblet cells is heavily labeled. (A few grains are seen over the goblet cell base and columnar cell cytoplasm. Since such label was completely removed from comparable paraffin sections by alpha amylase, these grains are attributed to labeled glycogen.) × 1200.

FIGURE 3 Base of a crypt in the colon of a 20 g rat, 30 min after injection of galactose-H^3. (Ilford L-4 emulsion, 2 month exposure). The cells are oriented around the lumen (*L*). In the goblet cells, a radioautographic reaction appears over the Golgi region (*G*). × 1100.

FIGURE 4 Surface epithelium of 100 g rat colon, 15 min after injection of sulfate-S^{35} (Kodak NTB2 emulsion, 3 month exposure). Radioactivity is located in the Golgi region (*G*). × 1200.

FIGURE 5 Surface epithelium of 50 g rat colon, 6 min after intravenous injection of leucine-H^3 (Kodak NTB2 emulsion, 1 month exposure). In goblet cells, the reaction around the nucleus (*N*) and in the basal region corresponds to the distribution of the rough-surfaced endoplasmic reticulum (*rER* shown in detail in Fig. 1 of reference 35). The supranuclear Golgi region (*G*) shows no localized label. In columnar cells, label is scattered throughout the cytoplasm. × 1100.

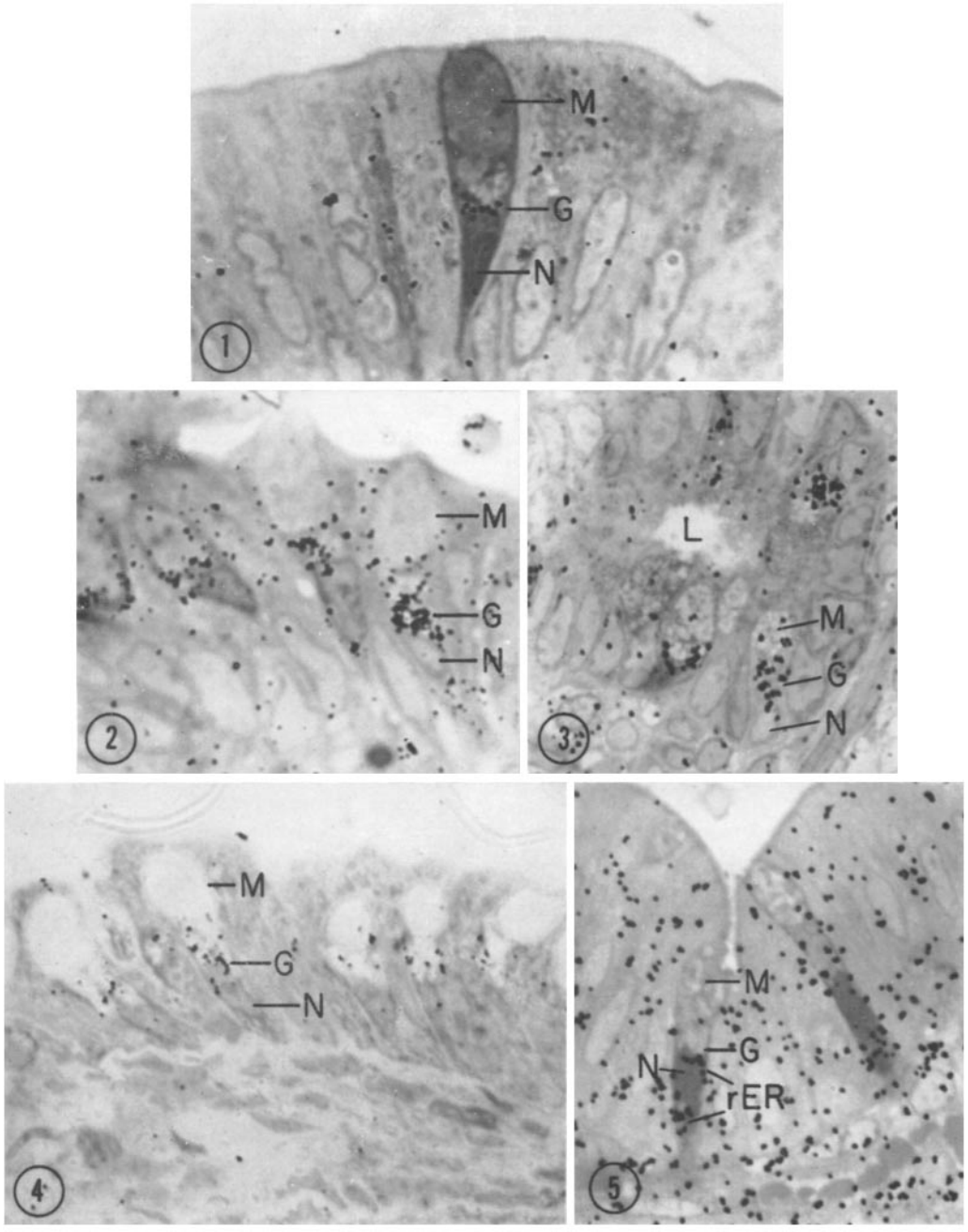
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TABLE II

Incorporation of Complex Carbohydrate Precursors in the Golgi Region of 10- to 20-g Rats

Tissue and cell	Radioautographic reaction in Golgi region 5 to 15 min after injection of		
	Glucose-H^3	Galactose-H^3	Sulfate-S^{35}
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 g rat (Table I, subsidiary experiment).

material (presumed to be hyaluronic acid and/or chondroitin sulfuric acids A and C, reference 36). Assessment of the amounts removed from cartilage (Table IV) revealed that, at 5 min after injection, the labeled material present in the chondrocyte cytoplasm was not hyaluronidase-labile; at 20 min, 10% of it was labile; and, at later times, when labeled material had appeared in the matrix, half of it was extracted from both cells and matrix. Similarly, in pulp of developing incisor tooth, the enzyme failed to remove the label at early time intervals, but removed 74% of it at 4 hr (Table IV). Thus, labeled material in both cartilage and tooth pulp was at first resistant to the action of hyaluronidase, but later became largely labile.

RADIOACTIVITY EXTRACTED BY THE SEQUENCE PERACETIC ACID–BETA GLUCURONIDASE: It is known that successive treatment by peracetic acid and beta glucuronidase removes PA-Schiff–stained material from various mucous cells (10). In our sections, this treatment extracted colloidal iron–stained material from cells lining the gastric mucosa and from the goblet cells of colon and duodenum, whereas peracetic acid or beta glucuronidase alone was not effective. Similarly, the radioactivity present in these cells after glucose-H^3 injection was considerably decreased only by treatment with the complete acid-enzyme sequence (Figs. 20 and 21 in preceding article, reference 35). In contrast, the sequence removed no radioactivity from connective tissues (cartilage and pulp of tooth).

DISCUSSION

Mucous Cells

Goblet Cells of Colon

Radioautographs of the goblet cells of colon obtained soon after injection of glucose-H^3 showed a heavy reaction localized over the Golgi complex and a light, scattered reaction distributed over the basal and lateral cytoplasm (Figs. 5 to 7 in preceding article, reference 35). The Golgi reaction was attributed to the synthesis of the carbohydrate moiety of the glycoprotein of mucus, but no explanation was given for the scatter in the rest of the goblet cell. Was this scatter due to the synthesis of complex carbohydrates outside the Golgi region?

Let us first consider the metabolic pathways which the glucose-H^3 could have followed (Fig. 15). A portion of the body glucose is transformed, without breakdown of the 6-carbon chain, into nucleotide sugars which are used for the synthesis of complex carbohydrates: glycogen, glycolipids, glycoproteins, and mucopolysaccharides. Another portion is metabolized via glycolytic pathways and the Krebs cycle to give rise to amino acids, acetate, and other small molecules which in turn may be used in the synthesis of nucleic acids, proteins, lipids, etc. Since radioautography usually detects large, nonlipid molecules rendered

TABLE III

Migration of Labeled Secretory Material

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

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

insoluble by fixation, the reactions observed after glucose-H^3 injection could a priori be due either to complex carbohydrates (other than glycolipids) or to nucleic acids or proteins built up from labeled metabolites of glucose.

First, if the label scattered in the cytoplasm of goblet cells after glucose-H^3 injection were due to uptake of intact glucose into glycogen, it should be removed 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. Secondly, if the cytoplasmic scatter remaining after alpha amylase treatment were due to labeled nucleic acid, a similar scatter should be observed soon after injection of an RNA-DNA precursor such as cytidine-H^3; however, after such injection, the early reactions of goblet cells were restricted to the nucleus (Fig. 20 in reference 1). Thirdly, if the scatter were caused by labeled protein, a similar distribution of label should be seen soon after injection of a protein precursor such as leucine-H^3. When this was injected, the Golgi region of goblet cells was unlabeled, but the basal and lateral cytoplasm showed a reaction (Fig. 5) which was not unlike the scatter observed after glucose-H^3 (Figs. 5 to 7 in preceding article, reference 35).

However, before concluding that the scattered amylase-resistant label consisted of protein arising from glucose breakdown and not of glycoprotein or mucopolysaccharide, we thought it would be useful to examine the fate of a monosaccharide which is not broken down as readily as glucose.

Biochemical evidence obtained so far indicates that galactose administered in vivo is not subject to glycolysis; rather, the galactose that enters metabolism is first converted to UDP-galactose (Fig. 15, and reference 19). Then, at least in liver, some UDP-galactose is converted to UDP-glucose (13), a nucleotide sugar which does not enter directly into glycolytic pathways, but may be used for glycogen synthesis (29). However, most of the UDP-galactose is used for the synthesis of glycoproteins and glycolipids (19, 32, 43). Since most lipids are removed from tissues by the organic solvents of histological processing, labeled galactose may be considered, at least in radioautographic studies of amylase-treated sections, as a precursor of glycoproteins.

Within 10 min after injection of galactose-H^3, label was incorporated into the Golgi region of colonic goblet cells, with a slight scatter at the base of the cells (Fig. 1). After amylase treatment the Golgi reaction was not reduced (and therefore was due to labeled glycoproteins), but the scatter was removed, and therefore consisted of glycogen.

Hence, with the doses of galactose-H^3 employed here, no synthesis of complex carbohydrate (other than glycogen) was detected outside the Golgi region.

In this light, it seemed unlikely that the substances synthesized from *glucose* outside the Golgi region were glycoproteins and mucopolysaccharides. Since as pointed out above, the distribution of the amylase-resistant scatter resembled that of the newly synthesized protein observed after leucine-H^3 injection, it is reasonable to attribute the scatter to labeled amino acids formed from catabolized glucose-H^3 and taken up into proteins. Briefly, in colonic goblet cells, only the Golgi region showed signs of synthesis of complex carbohydrates (other than glycogen).

Other Mucous Cells

Mucous secretions contain up to 75% carbohydrate (46). After glucose-H^3 injection, all those cell types which produce mucous secretions showed, besides a light cytoplasmic scatter, a heavy reaction over the Golgi complex at first, and over their mucus later (Tables II and III). This fact may appear paradoxical since most mucous secretions do not contain glucosyl residues (15, 22, 28, 46). However, it is known that glucose may give rise to all the monosaccharide components of complex carbohydrates (Fig. 15, and references 8, 45). Through these conversions, the glucose label may find its way into newly formed mucus.

The labeled glucose and galactose employed differed in specific activities (Table I) and in over-all metabolism (6, 7, 13, 21), so that incorporation of the two precursors in a given cell could be compared only roughly. Nevertheless, in examining their radioautographic distributions in various tissues (Table II), clear differences emerged. In contrast to the uptake of glucose label by mucous cells of all types, the *galactose* label was taken up only by certain types of mucous cells. Thus, it appeared in the goblet cells

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FIGURES 6 to 11 Cross- or oblique paraffin sections through duodenal villi of 20-g rats, showing the connective tissue core covered by a simple columnar epithelium. × 660.

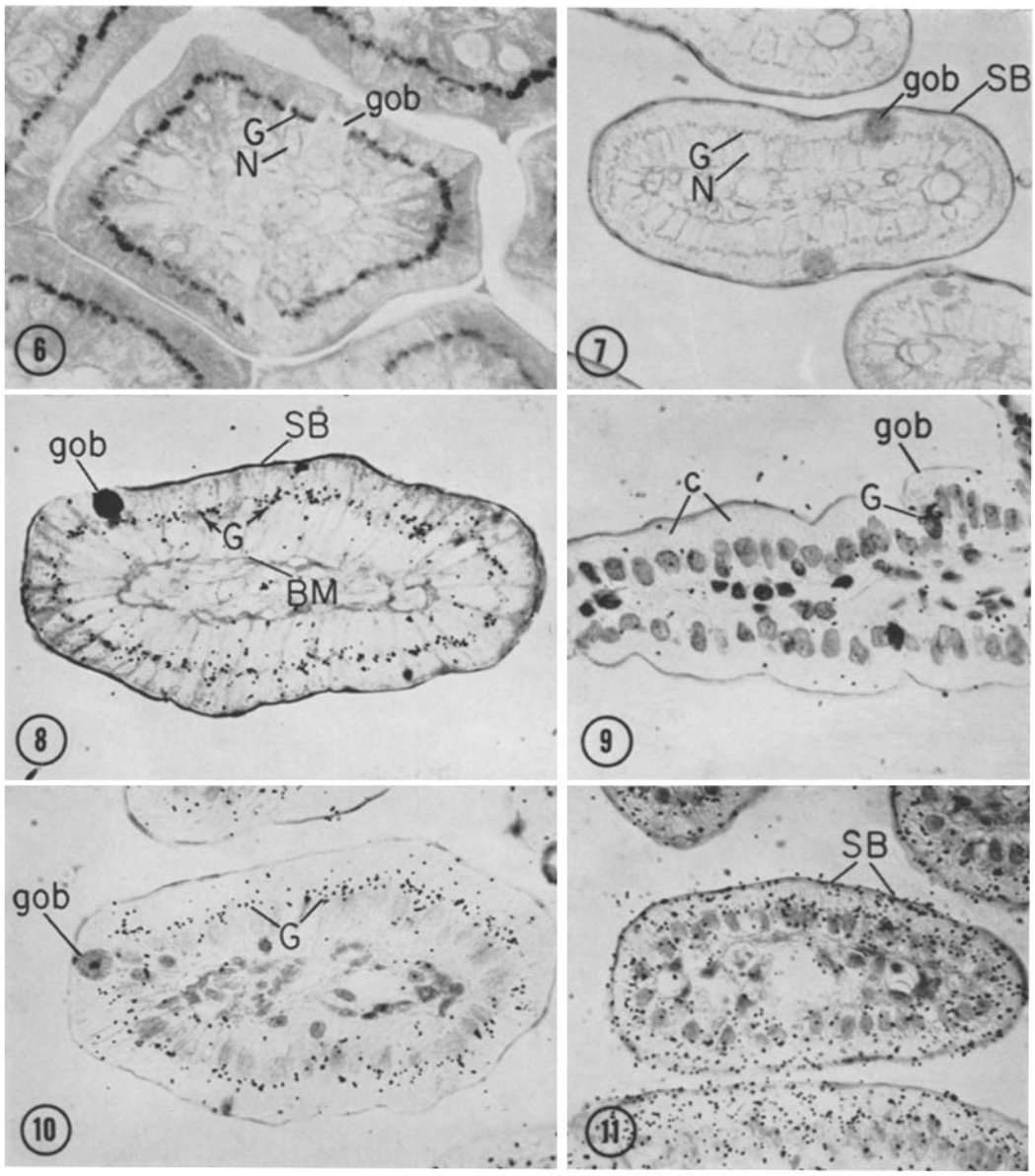
FIGURE 6 Maillet technique (30) to demonstrate the position of the Golgi complex in columnar cells. The osmium-stained Golgi region (*G*) lies just above the nucleus (*N*). In goblet cells (*gob*), the Golgi region is not stained by this technique.

FIGURE 7 Colloidal iron–Prussian blue (for detection of acidic carbohydrates). The columnar cells show blue (here, light 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 a surface coat on the microvilli. The mucigen of goblet cells (*gob*) is also stained.

FIGURE 8 Radioautograph, 10 min after galactose-H^3 injection, stained by the periodic acid–Schiff technique (for detection of glycoproteins). (Kodak NTB2 emulsion, 2 month exposure). 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 Fig. 9.

FIGURE 9 Colloidal iron–Feulgen-stained radioautograph, 10 min after injection of glucose-H^3 (in a dose comparable to that of galactose-H^3 in Fig. 8). The columnar cells (*c*) have incorporated no detectable glucose label while the goblet cells (*gob*) are heavily labeled in the Golgi region (*G*).

FIGURES 10 and 11 Colloidal iron–Feulgen-stained radioautographs (Kodak NTB2 emulsion, 2 month exposure). The goblet cell (*gob*) is unlabeled 10 min after galactose-H^3 injection (Fig. 10), while columnar cells show Golgi-localized radioactivity (*G*). By 30 min (Fig. 11), 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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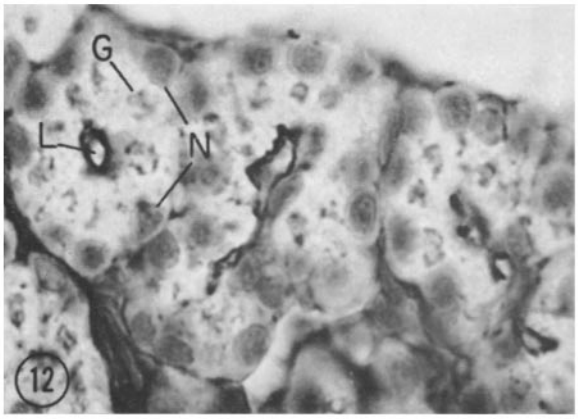

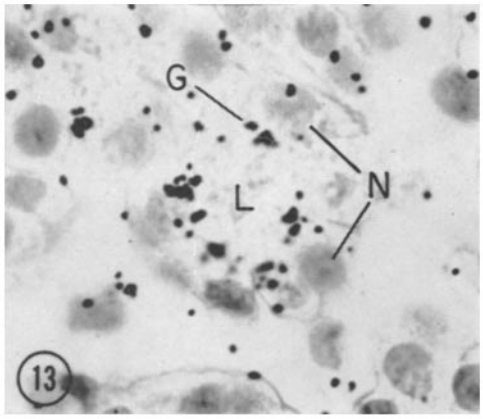

FIGURE 12 Pancreatic acini from a 10 g rat, stained with colloidal iron. Nuclei (*N*) are stained by the Feulgen technique. The border of the lumen (*L*) and the supranuclear Golgi region (*G*) are colloidal iron–positive, indicating the presence of acidic carbohydrates. × 1100.

FIGURE 13 Radioautograph of a pancreatic acinus from a 20 g rat, 10 min after injection of galactose-H^3. (Colloidal iron–Feulgen stain, Kodak NTB2 emulsion, 5 month exposure). 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*). × 1200.

of colon (Figs. 1 to 3), but not in those of duodenum (Fig. 10). Since in most cells galactose is not converted to other monosaccharides (13), it might be thought that only those taking up the label should have a mucus rich in galactosyl residues. However, some galactosyl residues have been found in most types of mucus (15, 22, 28, 46). It may be that those mucous cells which showed no uptake of galactose-H^3 label do not have the enzymatic equipment to make use of free galactose, and instead obtain their galactosyl residues by conversion of glucose.

In any case, whenever galactose-H^3 was taken up by 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, as in colonic goblet cells, they were due to galactosyl-H^3 residues taken up into the carbohydrate moiety of mucus glycoprotein.

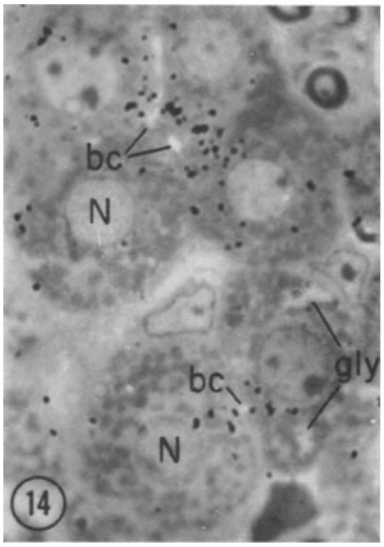

FIGURE 14 Radioautograph of liver parenchymal cells, 10 min after injection of galactose-H^3. Glycogen has been removed by digestion with alpha amylase. (½ μ Epon section stained with toluidine blue; Ilford L-4 emulsion, 3 month exposure.) In the cytoplasm around the central nuclei (*N*) are dark mitochondria and pale glycogen areas (*gly*), here emptied by amylase digestion. Between adjacent cells, bile canaliculi (*bc*) are visible. Radioactivity is localized in the pericanalicular cytoplasm, a position known to be occupied by the Golgi complex. × 1200.

Other Epithelial Cells

PANCREATIC ACINAR CELLS

Many serous secretions contain glycoproteins which are relatively poor in carbohydrate (28, 31). For instance, the secretion of pancreatic acinar cells includes at least one glycoprotein, the enzyme ribonuclease, which contains only 2%

carbohydrate (39). A histochemical study of these cells revealed the presence of acidic carbohydrates in the Golgi region as well as at the apical surface (Fig. 12). Radioautographs at 10 min after galactose-H^3 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. 13). (After glucose-H^3 injection, there was a heavy scatter of label, so that it was not even clear whether there was Golgi uptake.)

TABLE IV

Per Cent Reduction in Count of Silver Grains after Digestion with Testicular Hyaluronidase

Time after injection of glucose-H^3	Chondrocyte cytoplasm	Cartilage matrix	Pulp of tooth
5 min	0	*	0
20 min	10	*	0
40 min	37	50	12
1 hr	47	57	32
1½ hr	44	58	38
4 hr	68	42	74

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

The galactose-H^3 results suggest that a small amount of complex carbohydrate is synthesized in the Golgi region of pancreatic acinar cells. Presumably, this carbohydrate is added there to proteins coming from their own site of synthesis, the ergastoplasm, as they are "packaged" into zymogen granules (47).

Liver Parenchymal Cells

The metabolic studies quoted above indicate that all the galactose that enters the liver cell is changed to UDP-galactose, which may be converted to UDP-glucose to provide glucosyl resi

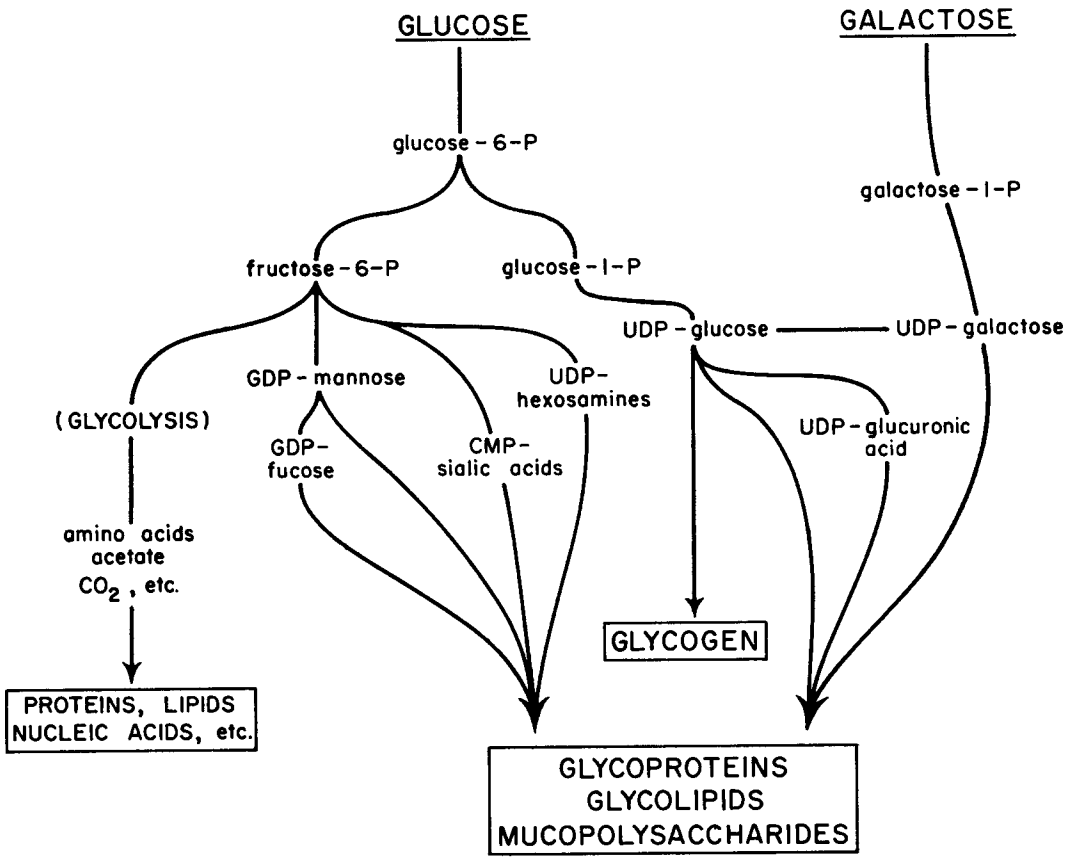

FIGURE 15 A simplified schematic representation of the metabolism of glucose and galactose. Some *glucose* is converted to various nucleotide sugars for the synthesis of complex carbohydrates; some, through glycolysis, gives rise to smaller molecules which enter other metabolic pathways. The fate of *galactose*, in contrast, is largely limited to the synthesis of complex carbohydrates.

dues for glycogen synthesis (5, 29) or may directly supply galactosyl residues for the synthesis of plasma glycoproteins (43). Indeed, our galactose-H^3 injections produced considerable amounts of labeled glycogen throughout the cytoplasm of liver cells. After amylase extraction of this glycogen, the remaining label was located close to bile canalicules (Fig. 14), a region occupied by the Golgi complex (4). Since liver cells are known to synthesize and secrete plasma glycoproteins (25, 43), the Golgi complex may use galactose in the synthesis of the carbohydrate moiety of these glycoproteins.

Columnar Cells of Duodenum

Long considered to be mainly absorptive in function, the columnar cells have recently been shown to have secretory activity as well. It was reported that, 15 min after administration in vitro of glucose-H^3, sulfate-S^{35}, or acetate-H^3, the labels were in the cytoplasm of the columnar cells and that, by 1 hr, they appeared in the apical "fuzz" coating the surface of microvilli (17). Because the surface coat was stained by PA-Schiff, colloidal iron and Alcian blue (16, 41), we assume that it contains glycoproteins and acidic carbohydrate groups. Since the Golgi complex was also stained by these three techniques (references 3, 27, 44, 49; and Fig. 7) we wondered whether the carbohydrate component of the surface coat was formed in this organelle.

Indeed, a clear-cut uptake of label was observed in the Golgi region of columnar cells 5 to 10 min after administration of large doses of glucose-H^3 (38) or moderate doses of galactose-H^3 (Figs. 8 and 10). At later time intervals after *glucose-H^3*, heavy, scattered label appeared over the entire cytoplasm, so that the reactions could no longer be related to a specific part of the cell. But by 30 min after *galactose-H^3*, most of the label was clearly related to the apical cytoplasm and surface coat (Fig. 11). Thus, it appeared that the Golgi-synthesized material migrates through the apical cytoplasm to the surface. It followed that a component of the surface coat would be synthesized in the Golgi complex. This component is presumably a complex carbohydrate rich in galactosyl residues, in accord with Kalckar's recent proposal that galactose is an important constituent of the complex carbohydrates present at the cell surface (20).

Since all duodenal 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 coat. Yet the thickness of the coat seems to be constant; therefore, this addition must be balanced by a continual loss of coat material into the lumen. Such renewal may ensure rapid replacement of this protective layer in case of damage (17).

Epithelia of Kidney and Epididymis

In these cells, as in duodenal columnar cells, galactose label was seen at 10 min in the Golgi region and at 30 min in the apical cytoplasm and on the apical surface. These observations imply secretory activity in both these cell types. In proximal convoluted tubule cells, the galactose-labeled substance may have been secreted onto the apical surface to renew the PA-Schiff and colloidal iron–reactive surface coat which covers the microvilli of the brush border, or it may have been released into the lumen as the urinary glycoproteins known to be produced by these cells (23). Similarly, in the epididymis, the galactose-labeled material may serve to renew the surface coat and/or be released into the lumen.

Chondrocytes

Cartilage matrix contains collagen and the sulfated acid mucopolysaccharide, chondroitin sulfate, both of which are believed to be synthesized in chondrocytes (12, 40). In addition, glycoprotein is present in the matrix (2, 11, 14).

With the electron microscope, chondroitin sulfate has been identified in the Golgi complex and secretion granules of chondrocytes as hyaluronidase-labile material stained by colloidal thorium (42). Electron microscope radioautography, after injection of sulfate-S^{35}, revealed that the sulfation of this mucopolysaccharide occurs in association with the Golgi saccules (12) and vacuoles (9). Did the Golgi complex carry out the full synthesis of chondroitin sulfate?

Since we have observed an early uptake of labeled glucose and galactose in the Golgi region of chondrocytes, it seems that the synthesis of complex carbohydrates occurs in this location. By 30 to 40 min after injection, the material labeled with either precursor began to appear in the matrix, indicating that the Golgi-synthesized complex carbohydrates were released by the cells to become components of the matrix.

Treatment by the enzyme hyaluronidase, which is known to break down chondroitin sulfate (36),

removed all the metachromasia and about half (42 to 68%) of the radioactivity present at 1 hr and later after glucose-H^3 injection (Table IV). It is therefore likely that a fair proportion of the radioactivity was in a nonmetachromatic component of the cartilage matrix, perhaps the glycoprotein known to be present there (2, 11, 14).

At the early times after injection, the labeled material was particularly resistant to hyaluronidase (Table IV). Did it consist mainly of glycoprotein or perhaps of a hyaluronidase-resistant form of chondroitin sulfate? Or was the nascent chondroitin sulfate lost in the course of fixation so that only the glycoprotein remained?

In *conclusion* then, whereas the complex carbohydrate *glycogen*, which is not destined for secretion and is not protein-associated, is synthesized in the cytoplasm outside the Golgi complex, synthesis of *glycoproteins* and/or *mucopolysaccharides* occurs in the Golgi region of a variety of cell types. The substances synthesized in this region are all destined for secretion, but as widely differing materials: mucus, enzymes, extraneous cell coat, and cartilage matrix. These materials have a common denominator, for they consist of carbohydrate linked to protein. Thus, secretory proteins are not only "packaged" in the Golgi region—they are linked to carbohydrate there as well.

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