SYNTHESIS OF COMPLEX CARBOHYDRATES IN THE GOLGI REGION, AS SHOWN BY RADIOAUTOGRAPHY AFTER INJECTION OF LABELED GLUCOSE

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Biochemical work (1, 2) has shown that free glucose is incorporated into mucopolysaccharides and glycoproteins in the course of their synthesis. (The word glycoprotein is used to refer to protein-associated carbohydrates, reference 3.) Little is known, however, about the sites of these syntheses. Kumamoto (4) observed that, after injection of radioactive glucose, many cells, in particular those secreting mucus, became radioactive, presumably due to uptake of glucose into the complex carbohydrates of mucus. However, her work gave no indication of the intracellular site of this synthesis.

Attention was drawn to the Golgi zone because it is often stained with the periodic acid–Schiff technique (5) (believed to be specific for glycoproteins, reference 6) and with the colloidal iron technique (7) (believed to stain acid mucopolysaccharides, reference 8). Further evidence was provided by the radioautographic finding that sulfate-S^{35} was taken up into the Golgi zone of chondrocytes and of certain mucus-secreting cells (9, 10), indicating that this zone was the site of sulfation of complex carbohydrates. Was the Golgi zone also the site of synthesis of these carbohydrates? In the hope of clarifying the problem, labeled glucose was injected into rats; and radioautographs were prepared of a variety of cells, including those which are active in the elaboration of complex carbohydrates.

METHODS

Systemic injections of 50 μc of D-glucose-6-H^3 (Radiochemical Centre, Amersham, England; 1.3 mc/mmole) in 0.05 ml saline were given to 4-day-old Sprague-Dawley rats by the subcutaneous route. The animals were sacrificed by exsanguination under ether, 5, 15, 30, 45 minutes, 1, 2 or 3 hours later. Tissues were fixed in neutral formalin; 3-μ sections were stained with the periodic acid–Schiff or modified Hale's colloidal iron method and were radioautographed by the coating technique (11), while other samples were prepared by Maillet's technique for detection of the Golgi zone (12). Another group of similar animals was injected intraperitoneally with 20 μc sodium sulfate-S^{35} (Charles E. Frosst & Co., Montreal, carrier-free) in 0.1 ml saline and sacrificed 10, 30 minutes, 1, and 3 hours

after injection. Parallel experiments were also done in 100-gm rats using a single injection of either tracer. In addition, one 100-gm male rat received four intraperitoneal injections of 250 μc glucose-6-H^3 each at 5 minute intervals, and was sacrificed 5 minutes after the last injection. Tissues were processed as described above.

Local injections of D-glucose-6-H^3 were made into the intestinal lumen of duodenum (50 μc) and colon (50 μc) of 100-gm male rats. Five minutes later, segments of intestine were removed from the sites of injection and processed as after systemic injection.

RESULTS

The Golgi zone was identified in most cells as a supra- or paranuclear region staining with the PA-Schiff (Fig. 1), colloidal iron, and Maillet techniques (Figs. 6 and 8). By 5 to 15 minutes after *systemic injection* of glucose-H^3, a radioautographic reaction was seen over the Golgi region in the goblet cells of intestine (Fig. 2) and in a series of other cells, some of which are listed in Table I. After sulfate-S^{35} injection, a Golgi reaction was

FIGURE 1 Duodenal epithelium from 100-gm rat (PA-Schiff technique; photographed with green filter). × 1300.

Columnar chief cells and one goblet cell may be seen. In chief cells, the stain outlines the basement membrane (*bm*), the side membranes (*sm*), the striated border (*sb*), and the Golgi zone, in which vertical lamellae may be seen (*cG*). In the goblet cell, the stain shows the goblet proper (*gob*) and the supranuclear cytoplasm in which the Golgi zone is located (*gG*).

FIGURE 2 Radioautograph of duodenal epithelium from 100-gm rat, 5 minutes after last of 4 systemic injections of glucose-H^3 (stained with PA-Schiff, exposed 48 days). × 1300.

An intense reaction covers the Golgi region of the goblet cells (*gG*). (The scattered grains predominate over the faintly stained Golgi material of chief cells.)

FIGURE 3 Radioautograph of colonic epithelium from 100-gm rat, 5 minutes after intraluminal injection of glucose-H^3 (stained with colloidal iron, counterstained with Feulgen reaction, and exposed 11 days). × 1300.

Only columnar chief cells are seen, with nuclei (*N*) and striated border (*Sb*). The Golgi material, stained by colloidal iron and appearing faintly gray, is seen in the supranuclear region (arrows), and is often overlaid by silver grains.

FIGURES 4 and 5 Same as Fig. 3, but exposed for longer periods, that is, for 21 days (Fig. 4) and 39 days (Fig. 5). × 1300.

A reaction stronger than that in Fig. 3 is localized over the vertical lamellae of the Golgi zone, which are seen as light gray streaks (arrows) under the black silver grains.

FIGURE 6 An acinus of the sublingual gland from 4-day-old rat, stained by the Maillet technique (12) to demonstrate osmiophilic elements of the Golgi apparatus. × 1100.

The Golgi material (*G*) occupies a region between the nucleus (*N*) and the mucus accumulation (*M*) in the apical part of the cell.

FIGURE 7 Radioautograph of a sublingual gland acinus from 4-day-old rat, 1 hour after systemic injection of glucose-H^3 (stained with colloidal iron, counterstained with Feulgen reaction, exposed 22 days). × 1100.

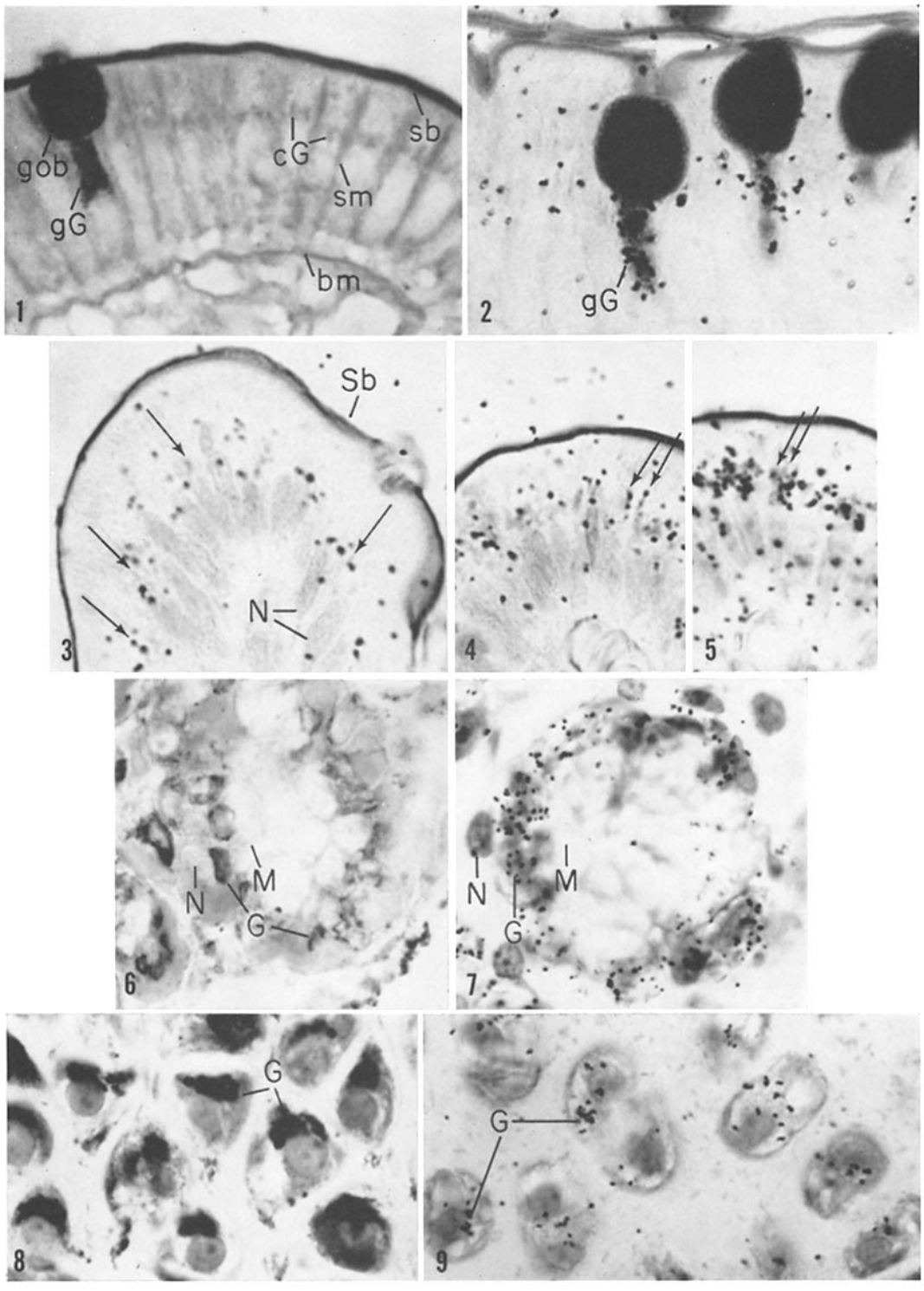
Radioactivity is located in the Golgi region (*G*), while the basal cytoplasm and the strands of apical mucus (*M*) are unreactive.

FIGURE 8 Tracheal cartilage from 4-day-old rat, stained with the Maillet technique (12) to demonstrate osmiophilic elements of the Golgi apparatus in chondrocytes. × 1100.

Note the juxtanuclear location of the black Golgi region (*G*).

FIGURE 9 Radioautograph of tracheal cartilage from 4-day-old rat, 10 minutes after systemic injection of sulfate-S^{35} (stained with colloidal iron, counterstained with Feulgen reaction, exposed 19 days). × 1100.

In the chondrocytes a small region next to the nucleus is radioactive, but the rest of the chondrocyte and the matrix show no significant radioactivity.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also observed, but only in some of the cells, as shown in Table I (Fig. 9).

With the passage of time, the reactions observed with either label persisted in the Golgi region up to about 1 hour (Fig. 7). Later, reactions appeared in the secretory materials, which, in all cells cited except chondrocytes, consisted of mucus. In the case of chondrocytes, reactions eventually appeared over the cartilage matrix (13).

Five minutes after *local injection* of glucose-H^3 into the lumen of duodenum and colon, an intense radioautographic reaction was seen over a region of the goblet cells corresponding to the Golgi zone. In both tissues, a moderate reaction was also present over the Golgi zone of chief cells (Figs. 3 to 5).

DISCUSSION

That glucose is a precursor of glycoproteins and mucopolysaccharides was shown by a number of authors (1). Draper and Kent (2) incubated colonic mucosa in Krebs solution with glucose-C^{14}, and recovered a labeled glycoprotein fraction which showed a trace of C^{14} in the protein moiety, none in the nucleic acid contaminant, but a considerable amount in the monosaccharide residues, fucose (6.5 per cent of the radioactivity taken up from the medium), hexose (11.2 per cent), hexosamine (12.1 per cent), and sialic acid (6.1 per cent).

From this and other investigations (1), it is clear that the formation of glycoproteins and mucopolysaccharides occurs in two steps: (*a*) conversion of glucose into other monosaccharides, that is, aldoses, hexosamines, hexuronic acids, and sialic acids; and (*b*) linking of the monosaccharides for the final synthesis. When the tissues of animals sacrificed after glucose-H^3 injection are processed for radioautography, it may be assumed that the free glucose-H^3 and other labeled monosaccharides are washed out. Hence, our results give no information as to the site of conversion of glucose to other monosaccharides. On the other hand, at least after formalin fixation, glycoproteins and mucopolysaccharides are retained in sections (14), and, if they have taken up labeled monosaccharides, they can be detected by radioautography.

The earliest reactions should be located at the site of the linking of labeled glucose and monosaccharides into complex carbohydrates, that is, at the site of synthesis of these substances. Since 5- to 15-minute reactions (Table I; Figs. 2 to 5) were over the Golgi region, this region would appear to be the site of synthesis.

It remained to be seen whether the carbohydrates synthesized in the Golgi zone could be identified. That *glycoproteins* must have been involved was suggested not only by the work of Draper and Kent (2), but also by the abundance of glycoproteins in the secretions of most cells whose Golgi zone took up the glucose label, that is, in mucus (1), the secretion of the first ten cells listed in Table I, and in cartilage matrix (15), the secretion of the chondrocyte, the last cell in Table I. Hence, the carbohydrates synthesized in this zone must be, at least in part, glycoproteins. It is speculated that the elaboration of glycoproteins involves the following sequence of events: first the protein moiety would be synthesized in the ribosomes of the ergastoplasm (rough-surfaced endoplasmic reticulum) (16); later the protein moiety would

TABLE I

Intensity of Radioautographic Reaction in the Golgi Region of Cells Known to Be Elaborating Complex Carbohydrates, as Observed after Systemic Injection of D-*Glucose-6-*H^3 *or Sulfate-*S^{35} *into 4-Day-Old Rats*

Cell	Glucose-H^3 (15 min. after injection)	Sulfate-S^{35} (10 min. after injection)
Sublingual gland, mucous acinar cells	+++	−
Submaxillary gland, acinar cells	+	−
Stomach, surface mucous cells	+	+++
Mucous neck cells*	+	−
Duodenum, goblet cells	++	++
Brunner's gland cells*	+++	−
Ileum, goblet cells	++	++
Colon, goblet cells	+++	+++
Trachea, mucosal goblet cells	+	−
Submucosal mucous gland cells*	+++	+++
Chondrocytes	+	+++

* Reactions seen in 100-gm rats.

migrate to the Golgi zone (17–23); then, within this region, the monosaccharide residues would be linked not only to each other, but also to the protein moiety. The combination of protein and carbohydrate moieties might be the essential factor in the phenomenon described as the "packaging" of secretory material in the Golgi zone (24–28). Finally, the glycoprotein "package" would migrate toward the surface of the cells to be released as secretory material into the lumen (or into the matrix, in the case of chondrocytes). Such an interpretation would account for the fact that most secretory proteins are associated with carbohydrates (6, 29, 30).

Free glucose has also been shown to be a precursor of *acid mucopolysaccharides* (1). The uptake of sulfate-S^{35} in the Golgi region of some cells (Table I) (9, 10) indicated that complex carbohydrates were sulfated there; but, since most sulfated carbohydrates are acid mucopolysaccharides, then the sulfation of these substances must take place in the Golgi zone. Whether sulfation occurred before, during, or more probably after (31) polysaccharide synthesis was not clearly established by these results. In any case, the presence of mucopolysaccharides in the Golgi region as indicated by sulfation as well as by histochemical tests (7) would suggest that the observed uptake of the glucose label is, at least in part, due to synthesis of acid mucopolysaccharides.

In conclusion, it is suggested that an important role of the Golgi zone is to provide for the synthesis of glycoproteins and possibly acid mucopolysaccharides as well.

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