ELECTRON-MICROSCOPIC AUTORADIOGRAPHY OF THE EPITHELIUM OF THE MOUSE LARGE INTESTINE AFTER ADMINISTRATION OF GLUCOSE-H^3

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Incorporation of glucose-H^3 into goblet cells of the large intestine of adult mice was studied by electron-microscopic autoradiography. Glucose or its conversion product is initially incorporated into structures of the Golgi complex, and 15-30 min later it is found in the secretory granules. Glucose-H^3 is incorporated only into those goblet cells which are in the stage of synthesis of secretion.

The hypothesis of Hirch on the intracellular conveyor, and D. N. Nasonov's views regarding the role of the Golgi complex in secretion formation were confirmed by electron-microscopic autoradiography [6, 10]. It has now been shown that not only the "packing" of the final secretory product but also many important processes of carbohydrate synthesis take place in the Golgi complex: addition of the polysaccharide moiety of the glycoproteins secreted by the cell [1, 5].

Neutra and Leblond [11, 12] showed that glucose-H^3 is initially incorporated into lamellae of the Golgi complex in the goblet cells of the rabbit large intestine, from which it passes into the vacuoles and mucigen granules. The Golgi complex in the goblet cell is thus the site of glycoprotein synthesis. These workers concluded that the synthesis, migration, and liberation of glycoproteins continue uninterruptedly throughout the life of the cell. However, an electron-microscopic study of the goblet cells of the intestine in dogs and rats showed that as secretion accumulates in the goblet cell the number of membranous structures of the Golgi complex and of the granular endoplasmic reticulum decreases, and as the cell becomes overfilled with secretion they are displaced into the stem of the goblet. This suggested that processes of synthesis can hardly take place in the cell when distended with secretion [2-4, 9].

The object of the present investigation was to study synthesis of the secretion of the goblet cell by using incorporation of labeled glucose-H^3, detected by electron-microscopic autoradiography.

EXPERIMENTAL METHOD

To study synthesis of secretion by the goblet cells adult male albino mice weighing 20-22 g were used. After fasting for 24 h the animals were given an intraperitoneal injection of glucose-H^3 (specific activity 700 μCi/g) in a dose of 0.5 μCi/g body weight. The animals were sacrificed 5, 15, and 30 min and 1-2 h after the injection of glucose. Pieces of mucous membrane of the large intestine were fixed with glutaraldehyde and postfixed with osmic acid, and then embedded in Epon-812. Sections cut to a thickness of 800 Å were coated with type PR-2† emulsion by the "loop" method [8]. The emulsion, liquified at 40°C, was diluted with distilled water (1:1); a tanning agent (0.4% solution of chrome alum) was added at the rate of 2-3 ml to 100 ml emulsion, after which a plasticizer (a solution of ethyl alcohol with glycerol in the ratio of 1:1) was added at the rate of 2 ml per 100 ml emulsion.

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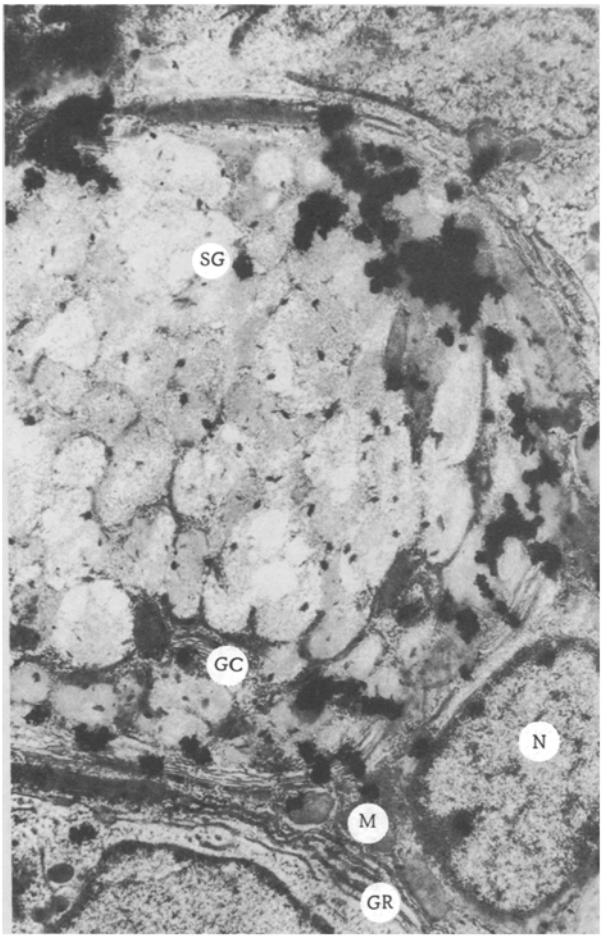

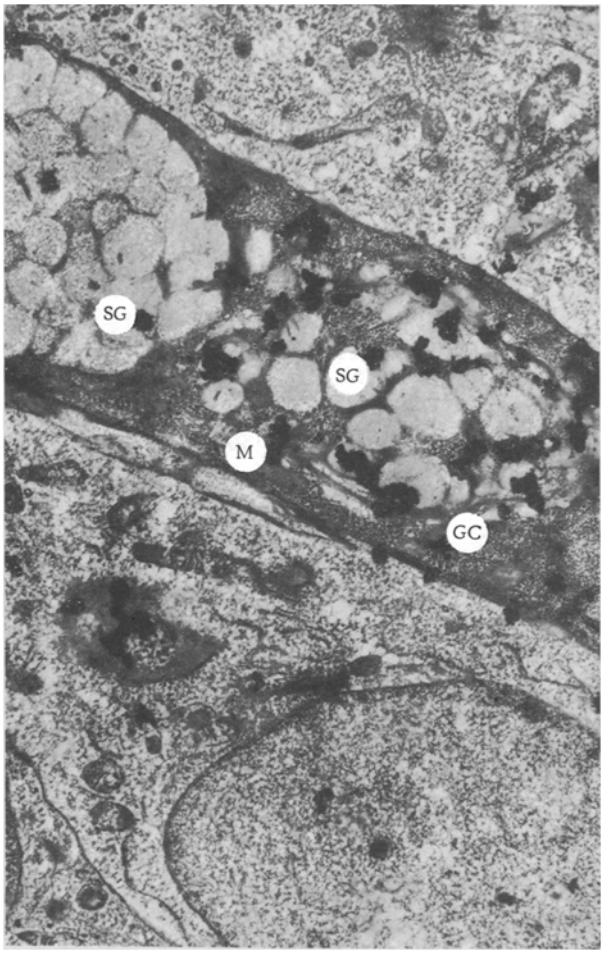

Fig. 1 Fig. 2

Fig. 1. Incorporation of glucose-H^3 or its conversion product into structures of the Golgi complex and secretory granules 15 min after injection of the label: N) nucleus; M) mitochondria; SG) secretory granules; GC) Golgi complex; GR) granular endoplasmic reticulum. 37,500×.

Fig. 2. Incorporation of glucose-H^3 or its conversion product into goblet cell 30 min after injection of label. Legend and magnification as in Fig. 1.

After the emulsion had been cooled on ice (1-2 min) Japanese copper grids with the sections, placed on glass slides, were coated with emulsion by means of a copper wire loop. Development was carried out after exposure for 45 days by the method of Perfilov et al. [7]. The sections were stained with uranyl acetate and lead citrate and examined in the IEM-7 electron microscope.

EXPERIMENTAL RESULTS

Electron microscopy of the goblet cells of the mouse intestine showed that their ultrastructure differs only a little from that in dogs and rats. The goblet cells of the mouse large intestine are at various stages of secretion formation. The principal distinguishing feature of the goblet cell at the height of secretion formation is the presence of a well-developed Golgi complex, which sits like a cup above the nucleus; laterally to the lamellae of the Golgi complex the outlines of the granular endoplasmic reticulum can be seen. In the goblet cell distended with secretion all the intracellular structures are displaced into the stem of the goblet where, because of the high electron density, they are difficult to distinguish.

Granules of reduced silver were found 5 min after injection of glucose-H^3 in the goblet cells in a stage of intensive secretion formation, above the lamellae of the Golgi complex, indicating that glucose or its conversion products are connected with the structures of the Golgi complex. In cells overfilled with secretion, no incorporation of the label could be seen. The largest number of silver grains above the structures of the

Golgi complex, both above its lamellae and its vacuoles, was observed 15 min after injection of the isotope, when single grains of silver also were observed above the mucigen granules lying close to the structures of the Golgi complex – the place where they are formed (Fig. 1).

Most of the label 30 min after injection of glucose-H^3 was located above the secretory granules in the immediate proximity of the structures above the mucigen granules, in the apical part of the secretion-distended cell (Fig. 2).

The label was uniformly distributed above the secretory granules 1-2 h after injection of glucose-H^3, but many tracks continued to be found above the structures of the Golgi complex.

The results thus indicate that glucose-H^3 or its conversion products, when incorporated into the structures of the Golgi complex, are gradually found in the synthesized secretory granules, and they leave the zone of the Golgi complex.

These results differ somewhat from those obtained by Neutra and Leblond, using electron-microscopic autoradiography of the large intestine of young rats (weighing 10 g), in which all the secretory granules were labeled 40 min after injection of glucose-H^3, and only solitary grains of silver were observed above the Golgi complex. The present results suggest that in adult mice the incorporation of glucose-H^3 into the Golgi complex takes place in the early stage of secretion formation or at the height of the secretory cycle. In the course of secretion the number of membranous structures of the Golgi complex is reduced, and this is accompanied by a decrease in the number of silver grains above its structures.

When the cells are distended with secretion and the membranous structures of the Golgi complex are displaced into the stem of the goblet, no incorporation of glucose-H^3 or of its conversion products takes place into them. This suggests that synthesis of the secretion by the goblet cell takes place in cycles. Synthetic processes are halted in the cell when overfilled with secretion and are resumed when the cell is emptied.

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