

PASSAGE OF FUCOSE-³H LABEL FROM THE GOLGI APPARATUS INTO DENSE AND MULTIVESICULAR BODIES IN THE DUODENAL COLUMNAR CELLS AND HEPATOCYTES OF THE RAT

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Fucose-³H is a stable sugar selectively taken up into glycoproteins (1, 2). Since fucose residues are located at the end of carbohydrate side chains (3), the uptake of fucose indicates completion of the synthesis of these side chains and presumably of the glycoprotein molecules themselves. When duodenal columnar cells were examined after fucose-³H injection into rats, the label was observed only in the Golgi apparatus at 2 min, but by 20 min after injection was also found at all cell surfaces (4). Presumably a glycoprotein completed in the Golgi apparatus was rapidly transferred to the outer surface of the plasma membrane to be added to the "cell coat." In the rest of the cell, some radioactivity was associated with vesicles, which may be the carriers of glycoprotein from Golgi apparatus to cell coat (4, 5). It was also noted recently that some radioactivity was located in dense and multivesicular bodies (6, 7). A similar finding has been reported in lymphocytes (8). The present investigation is a systematic examination of this phenomenon in duodenal columnar cells and hepatocytes.

MATERIALS AND METHODS

Young rats weighing 30–40 g and aged 2–3 wk were given a single intravenous injection of L-fucose-³H (5.0 mCi per animal; specific activity 4.3 Ci/mmole). Under these conditions, the fucose label decreases rapidly in blood and tissues (2), and, therefore, this procedure approximates pulse labeling. The animals were sacrificed 2, 5, 10, 20, and 35 min, 1, 4, and 30 hr later by glutaraldehyde perfusion through the left ventricle under ether anesthesia. The 2.5% aqueous solution of glutaraldehyde contained 37% of 0.05 M Sørensen's buffer, 0.1% sucrose, and either 0.25 or 1% fucose. Short segments of duodenum and small blocks of liver were removed from the animals and kept for 2 hr in the same fixative as used for perfusion. The tissues were then trimmed, washed in 0.15 M Sørensen's buffer, postfixed for 2 hr in 1% osmium tetroxide in 0.1 M Sørensen's buffer, dehydrated in acetone, and embedded in Epon. Thin (silver to gold) sections of the lower third of duodenal villi and of liver were radioautographed with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England) and,

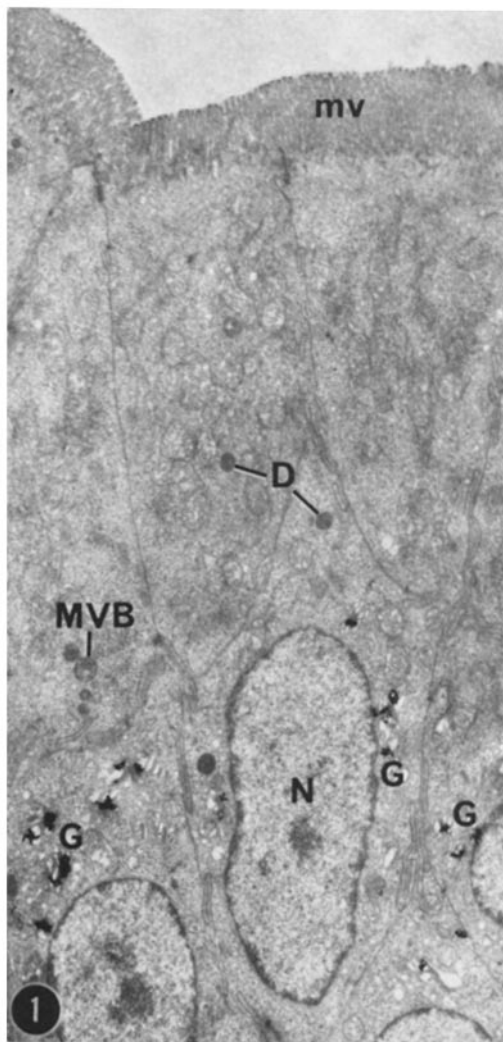


FIGURE 1 Electron microscope radioautograph of columnar cells from a duodenal villus at 2 min after intravenous injection of fucose-³H. Exposed for 14 wk. This radioautograph (and all others shown) was stained with uranium and lead. Nearly all of the silver grains present are over the Golgi stacks (G) while the other components of the cells are unlabeled. MVB, multivesicular body; D, dense body; N, nucleus; mv, apical microvilli. $\times 6500$.

TABLE I
Labeling of Dense Bodies and Vesicle-Containing Bodies in Columnar Cells of Duodenal Villi at Various Time Intervals after an Injection of Fucose-³H

Time after fucose- ³ H injection	Uniform dense bodies		Bodies with one to three vesicles		Bodies with four or more vesicles (MVB)	
	No.	% labeled	No.	% labeled	No.	% labeled
2 min	99	2.2	4	0	16	0
	77	1.3	44	2.2	41	0
5 min	88	6.8	37	8.1	58	1.7
10 min	137	22.0	50	16.0	50	6.0
20 min	28	35.8	38	50.0	50	26.6
35 min	51	38.2	80	35.0	110	22.8
1 hr	168	25.0	94	69.1	86	62.8
	64	56.3	132	55.4	87	41.4
4 hr	214	57.0	205	64.0	98	61.2
	46	8.7	40	27.5	66	31.8

after exposure, poststained with uranyl acetate followed by lead citrate. Counts of labeled and unlabeled dense and multivesicular bodies were made from radioautographs. A body was counted as labeled only if a silver grain lay over it or appeared to touch it.

RESULTS

In the *columnar cells of duodenal villi*, most dense bodies show a uniformly dark matrix. The multivesicular bodies display vesicles in a matrix as dark as in dense bodies, but a few show a light matrix. For the assessment of labeling, the bodies were arbitrarily classified into three groups: (a) dense bodies whose contents were uniformly dark; (b) bodies which showed one to three vesicles in section; and (c) bodies showing four or more vesicles. Only these last ones were called "multivesicular bodies." At 2 min after fucose-³H injection, silver grains were found over the Golgi apparatus only (Fig. 1). By 5 min after injection, however, a significant number of grains were found over dense bodies and bodies with one to three vesicles (Table I). Over two-thirds of the labeled bodies were close to the Golgi apparatus at this early time. Between 10 and 35 min after injection, labeling increased rapidly in all three types of bodies, although more slowly in multivesicular

bodies than in the others. Labeling was heavy over the three types of bodies at 1 hr (Fig. 2) and 4 hr, when over 50% of all three types were labeled (Fig. 3, Table I). By 30 hr after injection, the percentage of dense bodies labeled had decreased to 8.7%, but about 30% of the other two types remained labeled (Table I). It may be pointed out that even at their peak of labeling (4 hr after injection), these bodies accounted for only 4.5% of the total grain count since the absolute number of silver grains over other structures such as the cell membranes was much greater (Fig. 3).

In the *liver cells* of our animals dense bodies were numerous but multivesicular bodies were few. At 2 min after fucose-³H injection, none of these bodies was labeled, while over 80% of the total grain count overlay the Golgi apparatus (Fig. 4). The radioactivity of this organelle decreased to 60% of the cell total at 35 min and 19% at 4 hr, while dense bodies acquired as much as 10% of the label by 35 min and 23% by 4 hr. The percentage of dense bodies showing label was 24% at 35 min and 42% at 4 hr (Fig. 5).

DISCUSSION

Bodies similar to the ones observed in the present study have been shown to contain acid phospho-

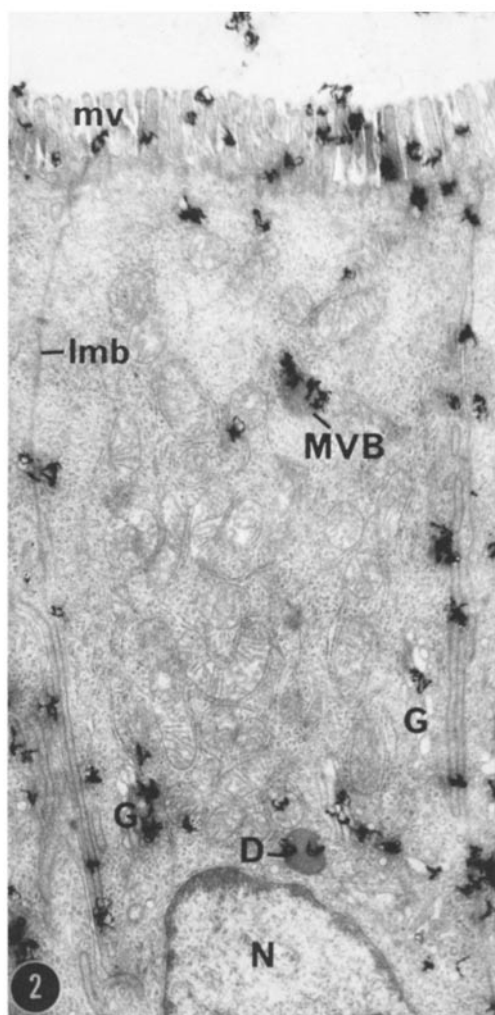


FIGURE 2 EM radioautograph of a duodenal columnar cell 1 hr after fucose-³H injection. Exposed for 7 wk. Silver grains are still numerous over the Golgi stacks (G), but now many silver grains are also seen over the lateral cell membranes (*lmb*) and over the apical microvilli (*mv*). In addition, the one dense body (*D*) and one multivesicular body (*MVB*) in this figure are both labeled. *N*, nucleus. $\times 9900$.

tase in both duodenal columnar cells (9) and liver (10). It is therefore believed that these bodies are lysosomes. In accord with the reported staining of lysosomes by the periodic acid-Schiff technique (11), we have found that the bodies under study stain with the periodic acid-silver methenamine technique and with phosphotungstic acid at low pH. The glycoproteins revealed by these methods

may be the lysosomal hydrolases themselves (12, 13).

At early times after fucose-³H injection, most of the label is restricted to the Golgi apparatus in duodenal columnar cells (Fig. 1) and hepatocytes (Fig. 4), indicating that the completion of carbohydrate side chains of glycoproteins occurs there. Later migration of the label reveals that some of the newly completed glycoproteins appear within lysosomes (Figs. 2-3, 5). These results are in accord with those of Cohn et al. (14) who showed the passage of leucine-³H label from rough endoplasmic reticulum through the Golgi apparatus to lysosomes in mononuclear phagocytes. In the present study, the labeling of some lysosomes of duodenal columnar cells at 10 min after injection (Table I), i.e. slightly before significant labeling is observed at the cell surface, indicates that lysosomes receive their newly completed glycoprotein directly from the Golgi apparatus rather than indirectly from the cell surface.

It has been suggested that multivesicular bodies evolve into dense bodies (9, 15, 16). The present results indicate, however, that dense bodies do not receive newly synthesized glycoprotein by this mechanism, since they were labeled earlier and lost label sooner than multivesicular bodies (Table I), a fact indicating that labeled glycoprotein in multivesicular bodies could come from dense bodies, or from the transformation of dense bodies into multivesicular bodies. The fact that the bodies with one to three vesicles in this study exhibit a labeling pattern intermediate between that of dense and that of the multivesicular bodies could suggest that these bodies are a transition stage in the evolution of one type of body to the other. The possibility remains, however, that the three types of bodies may receive their label independently from the Golgi apparatus, but at varying rates.

The mechanism of transfer of labeled glycoprotein from the Golgi apparatus to lysosomes in these cells is not yet clarified. However, several possibilities which are not mutually exclusive may be considered. One possible mechanism could be the direct transformation of Golgi saccules into lysosomes, as reported in thyroid follicular cells (17), kidney adenoma cells (18), intestinal crypt columnar cells (19), and promyelocytes (20, 21).

A second possibility is the transport of labeled glycoprotein from Golgi apparatus to lysosomal bodies via small Golgi vesicles (14, 15, 22, 23), as indicated by observations suggesting fusion of

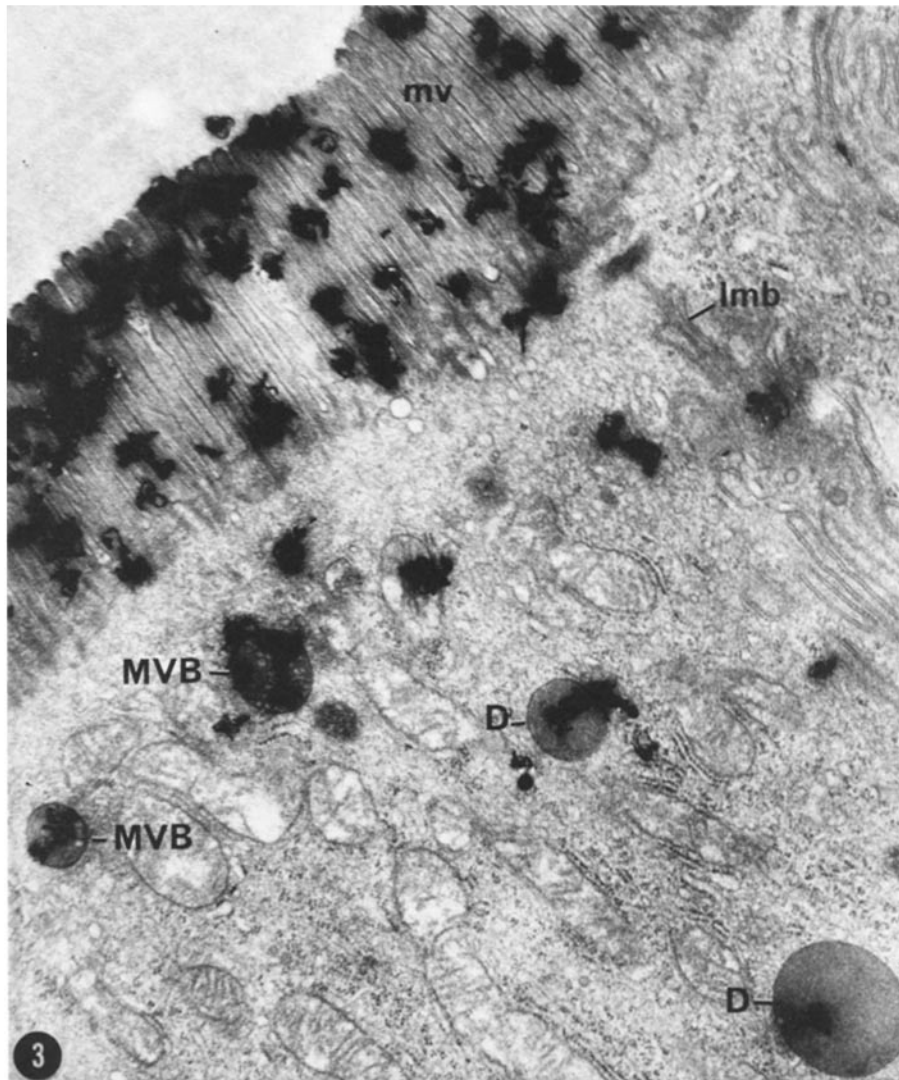


FIGURE 3 EM radioautograph of the apical region of a duodenal columnar cell 4 hr after fucose-³H injection. Exposed for 7 wk. Silver grains are seen over the two dense bodies (D) and the two multi-vesicular bodies (MVB) present in this cell. In addition, reaction is present over the microvillous border (mv) and along the lateral cell membrane (lmb). $\times 19,500$.

Golgi vesicles with these bodies (14, 15, 24) as well as by the presence of acid phosphatase in all these structures (16, 22).

A third possibility is derived from the finding of Golgi-related tubules in negatively stained Golgi fractions (25) or in thick sections stained by glycoprotein techniques (26). The occasional presence of tails on lysosomes, which was observed here and elsewhere (9, 22, 27), suggests that, at least in the early stage of their formation, these bodies may

be connected to Golgi tubules. It is, therefore, possible that labeled material is transported from Golgi apparatus to lysosomes via connecting tubules.

SUMMARY

When young rats were given a single intravenous injection of fucose-³H, and radioautographs of duodenal villus columnar cells and hepatocytes were examined at various time intervals later, the

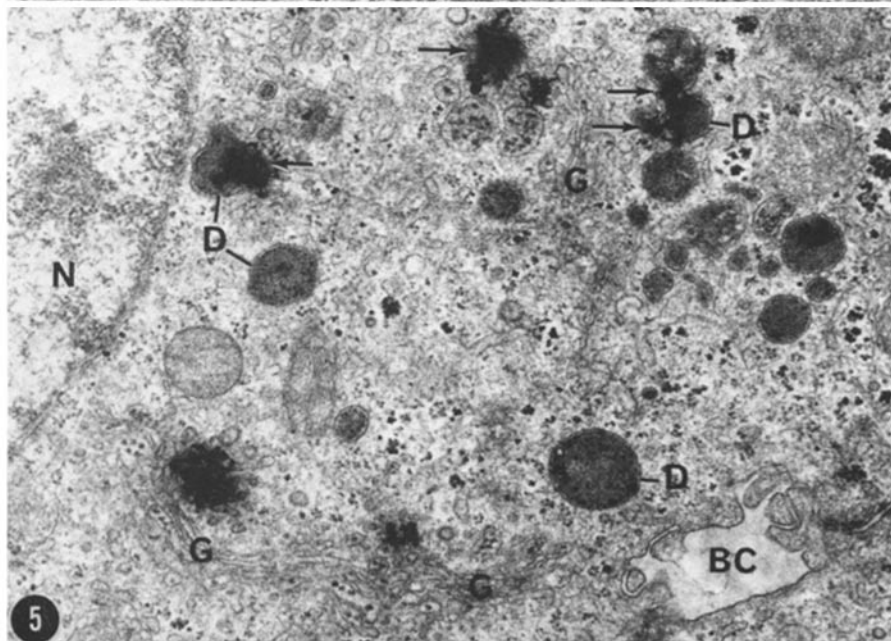
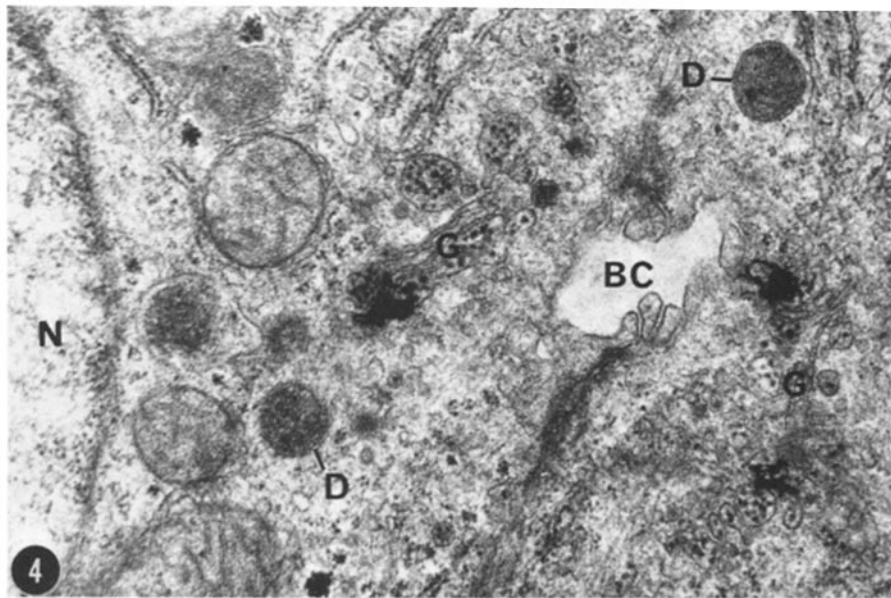


FIGURE 4 EM radioautograph of the Golgi region of hepatocytes 2 min after fucose-³H injection. Exposed for 7 months. All three silver grains overlie the saccules of the Golgi apparatus (*G*). The dense bodies present (*D*) are unlabeled. *N*, nucleus; *BC*, bile canaliculus. $\times 27,000$.

FIGURE 5 EM radioautograph of the Golgi region of hepatocytes 4 hr after fucose-³H injection. Exposed for 3 months. Some silver grains remain over the Golgi stacks (*G*) where they appear to be associated with the maturing face. In addition, four silver grains (arrows) are localized over dense bodies (*D*). *N*, nucleus; *BC*, bile canaliculus. $\times 26,000$.

label was found to be at first localized to the Golgi apparatus, but later appeared in lysosomes. These results indicate that the Golgi apparatus is the site of completion of synthesis for lysosomal glycoproteins.

This work was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada.

Received for publication 11 May 1971, and in revised form 16 July 1971.

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