COMPARATIVE DISTRIBUTION OF CARBOHYDRATES AND LIPID DROPLETS IN THE GOLGI APPARATUS OF INTESTINAL ABSORPTIVE CELLS

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INTRODUCTION

Complex carbohydrates have been demonstrated cytochemically in the Golgi apparatus of the rat intestinal absorptive cell by specific stains for acid mucopolysaccharides (4) and for glycoproteins (15). A staining gradient in the cisternae was observed in these studies, increasing towards one face of the Golgi apparatus, generally referred to as the mature face. In addition, the early accumulation of labeled carbohydrates from radioactive precursors has suggested an involvement of the Golgi apparatus in the synthesis of this product for transport to other positions (2, 6, 9, 14). In the absorptive cell, much of this carbohydrate is subsequently transported to and incorporated into the surface coat, especially that covering the microvilli.

Lipid droplets are common in absorptive cells of animals on a normal diet. In the Golgi apparatus, these droplets are present in varying numbers, depending on the absorptive state of the cell, and they show a preferential accumulation in vacuolar dilations of the cisternae closest to the plasma membrane (7, 11). There is some evidence to sug- gest that these droplets represent chylomicrons or lipoproteins formed during lipid absorption and destined for release from the cell along the lateral boundaries (5, 7, 10).

A simultaneous occurrence of carbohydrates and lipid droplets in the Golgi apparatus and their respective distributions have not been demonstrated previously. A bifunctional condition is presently described in which the lipid droplets and carbohydrates are processed primarily on opposing faces of the Golgi apparatus.

MATERIALS AND METHODS

The upper jejunum of Wistar rats, fed ad lib., was obtained under Nembutal anesthesia (3 mg/100 g body weight). Tissues were fixed in 1% phosphate-buffered OsO_4, pH 7.2 (13), or in 5% glutaraldehyde in 0.2 M sodium cacodylate, pH 7.2. Tissues fixed in the latter were extracted with alcohol to remove lipids (8), and postfixed in OsO_4. All tissues were dehydrated in a graded series of ethanols and embedded in Epon (12).

Two rats received about 1000 R X-irradiation to the surface of the abdomen at 250 kv, 15 ma, 0.5 mm Cu and 1.0 mm Al added filtration, and an

exposure of 82.6 R/min at 50 cm (4). Portions of the jejunum were removed 20 min later and processed as above.

Routine sections were double stained with uranyl acetate and lead citrate. Complex carbohydrates were localized according to the procedure of Thiéry (17). The periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-Ag) technique was used as follows: tissue sections were floated on 1% periodic acid, 30 min; five rinses of distilled water, 10 min each; 0.2% thiocarbohydrazide (TCH) in 20% acetic acid, 48 hr; 10% acetic acid, 15 min; 5% and 2% acetic acid, 3 min each; three rinses of distilled water, 20 min each; 1% silver proteinate, 30 min in the dark; three rinses of distilled water, 10 min each. Sections were transferred to grids and examined without additional staining. Control sections were similarly processed, but without oxidation in periodic acid.

RESULTS

In routine sections, the Golgi apparatus of the absorptive cell generally consists of stacks of several cisternae primarily oriented parallel to the long axis of the cell (Fig. 1). One side of the stack, usually closest to the plasma membrane, characteristically shows vacuolar dilations in the cisternae. In this study, this side is referred to as the *vacuolar face*. Vacuolar enlargements are also common at the periphery of the stack. The opposite face frequently shows the presence of tubular and vesicular profiles which may extend some distance from the cisternae. The relative number of profiles visible of each type on this face varies. For simplicity, this face will be called the *tubular face*. The tubules are narrow and of relatively constant diameter compared to nearby smooth endoplasmic reticulum. In some places they appear as tubular extensions of cisternal plates of the Golgi apparatus.

When present, lipid droplets occupy dilations in the cisternae along the vacuolar face and at the periphery of the stack (Figs. 1, 2). After PA-TCH-Ag staining, these droplets contain a reaction product (Fig. 2). The reaction product is seen as small, distinct, electron-opaque grains of silver. Other contents of the Golgi cisternae in absorptive cells from the normal animal usually show only a slight staining reaction which is not above the background level (see controls below). In contrast, considerable staining routinely occurs in tubular and vesicular profiles on the opposing tubular face (Figs. 2, 3). After X-irradiation, the Golgi

apparatus exhibits a more intense and more consistent staining gradient across the cisternal stack (Fig. 4). Here, two or three cisternae on the vacuolar face remain unstained, while those toward the tubular face show an increased staining of the luminal contents. Staining of the lipid droplets is the only exception to the staining gradient observed. Tubules and many vesicles on the tubular face stain intensely. Other unstained vesicles appear in clusters near the Golgi cisternae or intermingled with stained profiles (Figs. 2, 4). Their significance is not established in this study. Staining of profiles of rough and smooth endoplasmic reticulum in the vicinity of the Golgi apparatus likewise was not detected at any time (Figs. 3, 4).

Lipid-extracted tissues, stained by the PA-TCH-Ag technique, are not as well preserved morphologically; however, organelles can be identified (Fig. 5). Staining is restricted to cisternae, vesicles, and tubules along the tubular face. Neither lipid droplets nor reaction product are seen in the dilations of the vacuolar face. Controls in which periodic acid was omitted show only a slight staining in the lumina of Golgi cisternae and associated tubules and vesicles (Fig. 6). Lipid droplets, however, continue to stain.

DISCUSSION

In studies on the localization of complex carbohydrates in duodenal absorptive cells with silver methenamine (15), or in colonic epithelial cells with colloidal iron (18), the Golgi apparatus was shown to display a staining gradient in which the contents of cisternae on one face showed little staining, while cisternae on the opposing face stained intensely. Vesicles present along the stained face typically showed the presence of reaction product. Similar results were obtained with the PA-TCH-Ag procedure in the present study. However, as with the use of colloidal thorium (3, 4), a staining reaction within the Golgi cisternae was demonstrated less consistently than in the adjacent tubules and vesicles in normal animals. In agreement with the study by Berlin (4), X-irradiation of the abdomen results in an increased staining of the Golgi cisternae at the tubular face. An increased involvement of the organelle in the production and release of complex carbohydrates from this face is suggested.
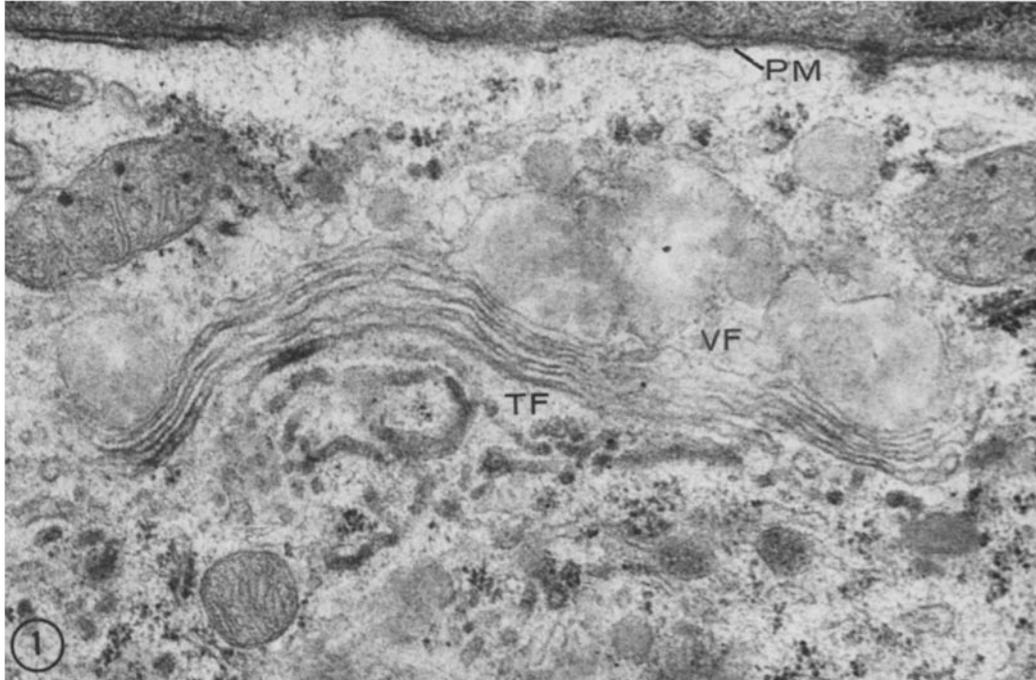
FIGURE 1 Portion of the Golgi apparatus of an intestinal absorptive cell from a normal animal. The section is stained with uranyl acetate and lead citrate. A stack of five flattened cisternae is present. The face closest to the lateral plasma membrane (PM) shows a number of vacuolar dilations which contain accumulations of lipid droplets of moderate density (vacuolar face, VF). Dark-staining tubular and vesicular profiles are present along the opposite, tubular face (TF). $\times$ 42,000.
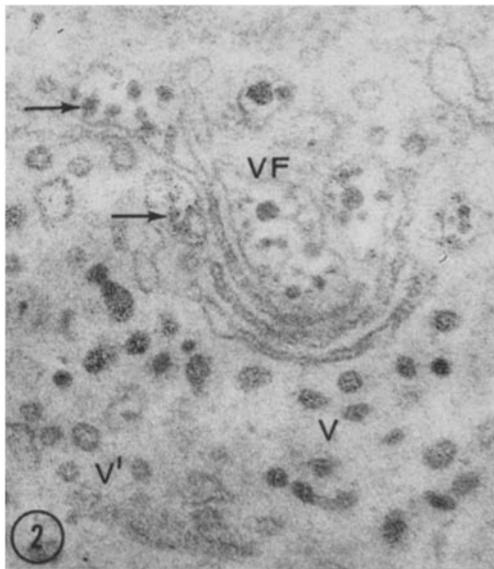
FIGURE 2 Golgi apparatus from a normal animal. PA-TCH-Ag staining. Lipid droplets are present in large dilations of the cisternae on the vacuolar face (VF). These droplets are stained by small grains of silver (arrows). Vesicles (V) on the opposite face show considerable staining. Other vesicles (V') and the Golgi cisternae are not noticeably stained. $\times$ 42,000.

Grains of reaction product are observed in the lipid droplets following the PA-TCH-Ag staining procedure. It is known that the use of osmium tetroxide in fixation can interfere with the specificity of certain stains for carbohydrates (15). As used in the present study, silver can be bridged to tissue-bound osmium through TCH forming granular deposits (16). The use of osmium was necessary, however, to preserve the droplet lipids in relation to Golgi structures (8, 9). The reaction observed in lipid droplets is considered to be non-specific, as it is retained in the absence of periodic acid pretreatment. On the other hand, staining of the structures along the tubular face of the Golgi
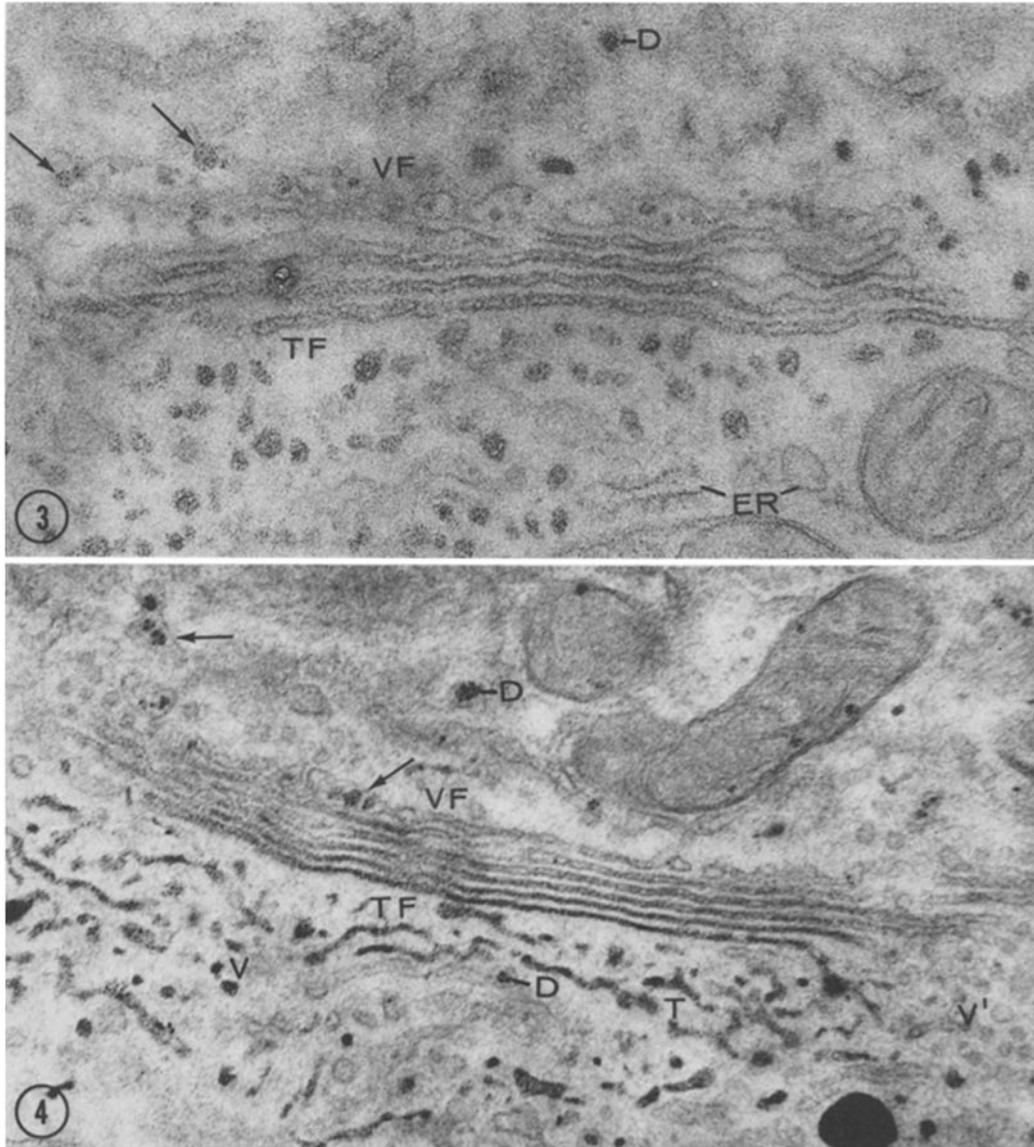
FIGURE 3 Golgi apparatus from a normal animal. PA-TCH-Ag staining. The contents of numerous vesicular profiles on the tubular face (*TF*) are stained. The unstructured contents of the Golgi cisternae are not stained, although a faint staining of the membranes is visible and similar to that in control preparations (Fig. 6). Lipid droplets are present within cisternal dilations along the vacuolar face (*VF*) and show a reaction to the stain (arrows). A droplet (*D*) within the endoplasmic reticulum (*ER*) also shows typical staining. The unstructured contents in the surrounding *ER* does not stain. × 45,500.

FIGURE 4 Golgi apparatus from an irradiated animal showing a gradient in PA-TCH-Ag staining. Reactive lipid droplets (arrows) are present on the vacuolar face (*VF*) in dilations of otherwise unstained cisternae. Staining of the cisternae increases toward the tubular face (*TF*) where the staining intensity is similar to that in adjacent tubular (*T*) and vesicular profiles (*V*). Other vesicles (*V'*) and the ER are not stained. Lipid droplets (*D*) in the ER, however, do stain. × 37,000.
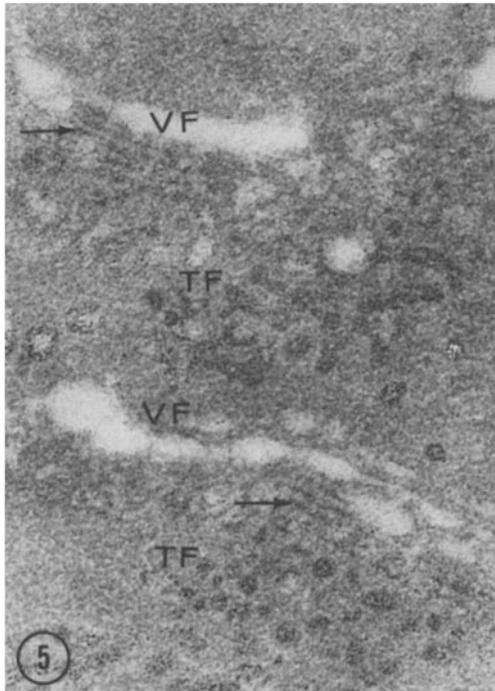
FIGURE 5 Golgi apparatus from an irradiated animal. PA-TCH-Ag staining of glutaraldehyde-fixed, lipid-extracted specimen. Two units are seen, each with a vacuolar (*VF*) and a tubular face (*TF*). Cisternae (arrows), tubules, and vesicles on the latter face are stained. Lipid droplets are lacking and the vacuolar face is not stained. × 42,000.

FIGURE 6 Golgi apparatus from an irradiated animal. Staining was done without previous oxidation in periodic acid. Lipid droplets on the vacuolar face (*VF*) continue to be reactive. Staining of structures on the tubular face (*TF*) is faint or lacking. A faint staining remains in dense bodies (*B*) and over membranes in general. Plasma membrane, *PM*. × 42,000.
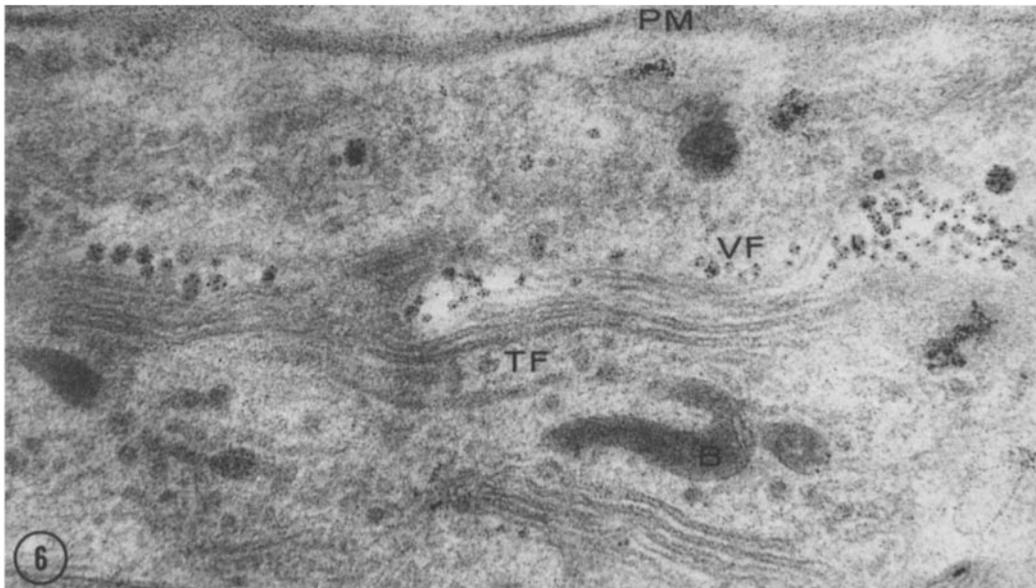
apparatus occurs only faintly or not at all without the periodic acid. Conversely, when lipids are extracted from tissues before staining with PA-TCH-Ag, reaction product is limited to the tubular face. Staining in this location, therefore, does not appear to be related to the presence of lipids, but to be dependent on the presence of complex carbohydrates. In light of other studies (4, 6, 15),

these carbohydrates most likely represent glycoproteins which may carry acid groups.

The evidence presented in this study therefore shows lipid droplets to preferentially accumulate in Golgi vacuoles along the face opposite to that of glycoprotein concentration. Furthermore, an increase in the glycoprotein content resulting from X-irradiation does not alter the observed polarity.

The simultaneous demonstration of these substances on opposing faces of the Golgi apparatus illustrates the bifunctional nature of this organelle in the intestinal absorptive cell. Bifunctional conditions have also been observed in rabbit polymorphonuclear leukocytes in time sequence during development (1) and in the amoeba (19). In the latter, glycoproteins and phosphatases also are present simultaneously on opposing faces of the Golgi apparatus. A spatial separation of functional compartments in the Golgi apparatus therefore appears possible in cells of varying specialization. A simple unidirectional movement of cisternae and their contents through the organelle from forming face to maturing face in such cells seems unlikely. Likewise, in absorptive cells, it appears that lipid droplets and glycoproteins are primarily emitted within secretory vesicles from different regions of the Golgi apparatus. The mechanism for the movement of the different products through the Golgi apparatus, however, requires additional study.

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