LOCALIZATION OF PHOSPHATASE ACTIVITIES IN COLONIC GOBLET AND ABSORPTIVE CELLS

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INTRODUCTION

The goblet cell synthesizes protein-mucopolysaccharides, some of which are sulfated (7, 17). In a preceding paper, evidence that sulfation takes place in the Golgi lamellae was offered (8); the protein component is thought to be formed in the endoplasmic reticulum. Some of the endocellular membrane systems involved in the synthetic or the secretory process can be characterized by their hydrolytic phosphatase activities (4, 12). A generalized scheme, based on these properties, which attempts to explain the interrelation between endocellular membranes and their participation in secretion has been proposed (4, 12). In this Note, we compare the distribution of a group of phosphatases in the secretory and absorptive cells of the rat colonic mucosa, to provide evidence on some possible relations between the organelles involved in sulfation, secretion, and absorption.

MATERIALS AND METHODS

The colons from stock Sherman rats were removed under ether anesthesia and fixed for 18 hours in 4 per cent formaldehyde in 0.05 M cacodylate buffer containing 1 per cent $CaCl_2$ at pH 7.4 to 7.6, as previously described (16). Fixation was followed by several rinses in 0.05 M cacodylate buffer with 10 per cent sucrose. Occasionally, tissues were kept in buffered sucrose at 4°C for periods up to a month. Frozen sections were cut in a cryostat at 30 to 50 μ and were collected in cacodylate-buffered sucrose plus 0.05 M $MgCl_2$ at 4°C until they were transferred to the incubation mixtures.

The sections were incubated in modified Gomori lead mixtures to test for nucleoside diphosphatase

(13), acid phosphatase (2), and adenosinetriphosphatase (16) activities. Control sections were incubated in substrate-free media and in complete media containing the substrate plus an inhibitor, as follows: (*a*) inosine diphosphate,[1] or thiamine pyrophosphate,[2] plus 0.01 M uranylnitrate (13), (*b*) β-glycerophosphate[3] plus 10^{-3} M sodium fluoride, and adenosinetriphosphate (ATP)[1] plus 10^{-3} M *N*-ethyl maleimide (16). All incubations were followed by rinses in distilled water, then briefly (*ca* 20 seconds) in 0.005 M acetic acid, and two additional rinses in distilled water, at room temperature.

Tissues were postfixed in 1 per cent OsO_4 buffered with 0.1 M phosphate or veronal-acetate at pH 7.4 to 7.6. The tissues were embedded in Epon after dehydration through graded alcohols and propylene oxide. Sections were cut on a Huxley-Cambridge microtome and mounted on grids in the usual way. All tissues were routinely screened without staining, but for critical examination the sections were stained for 30 to 60 minutes in a saturated solution of uranyl acetate in 50 per cent alcohol.

RESULTS

I. *Nucleosidediphosphatase*

(a) INOSINE DIPHOSPHATE: The principal (or absorptive) and goblet cells show different distribution of reaction products after incubation with inosine diphosphate as a substrate. In the principal cells, the deposits are extensive and are found in the endoplasmic reticulum (particularly in the apical region of the cell), the outer nuclear membrane, and the lamellar components of the Golgi apparatus (Fig. 1). The mitochondria of the principal cells, often seen in close proximity to the endoplasmic reticulum, have no deposits, nor do the interdigitations of the lateral cell membranes (Figs. 1 and 2).

In the goblet cells the endoplasmic reticulum and the nuclear membranes are free of reaction product (Fig. 2); only a region of the lamellar component of the Golgi apparatus exhibits nucleosidediphosphatase activity.

(b) THIAMINE PYROPHOSPHATE: The deposits observed after incubation with thiamine pyrophosphate were restricted to the lamellar component of the Golgi apparatus in both the goblet (Fig. 3) and the principal cells.

II. *Acid Phosphatase*

In the principal cells, only the multivesicular bodies, which are usually found in the apical region (6, 21), were heavily laden with reaction product after incubation for acid phosphatase activity; the Golgi apparatus, mitochondria, and the microvilli were unreactive.

The less numerous multivesicular bodies of the goblet cells also exhibited various degrees of acid phosphatase activity (Fig. 4). The Golgi apparatus and the membranes of mucinogen granules of the goblet cells were devoid of significant deposits.

III. *Adenosinetriphosphatase*

In a previous paper (16) it was reported that both epithelial cell types of formaldehyde-fixed colonic mucosa exhibited extensive deposits of reaction product upon the inner mitochondrial membranes and the cristae mitochondriales when ATP was used as substrate. The lateral and basal cell membranes were also reactive and the interdigitations between adjacent epithelial cells were outlined in detail by the lead deposits (Fig. 5). The microvilli and the multivesicular bodies of both types of cells also exhibited intense activity with ATP as substrate.

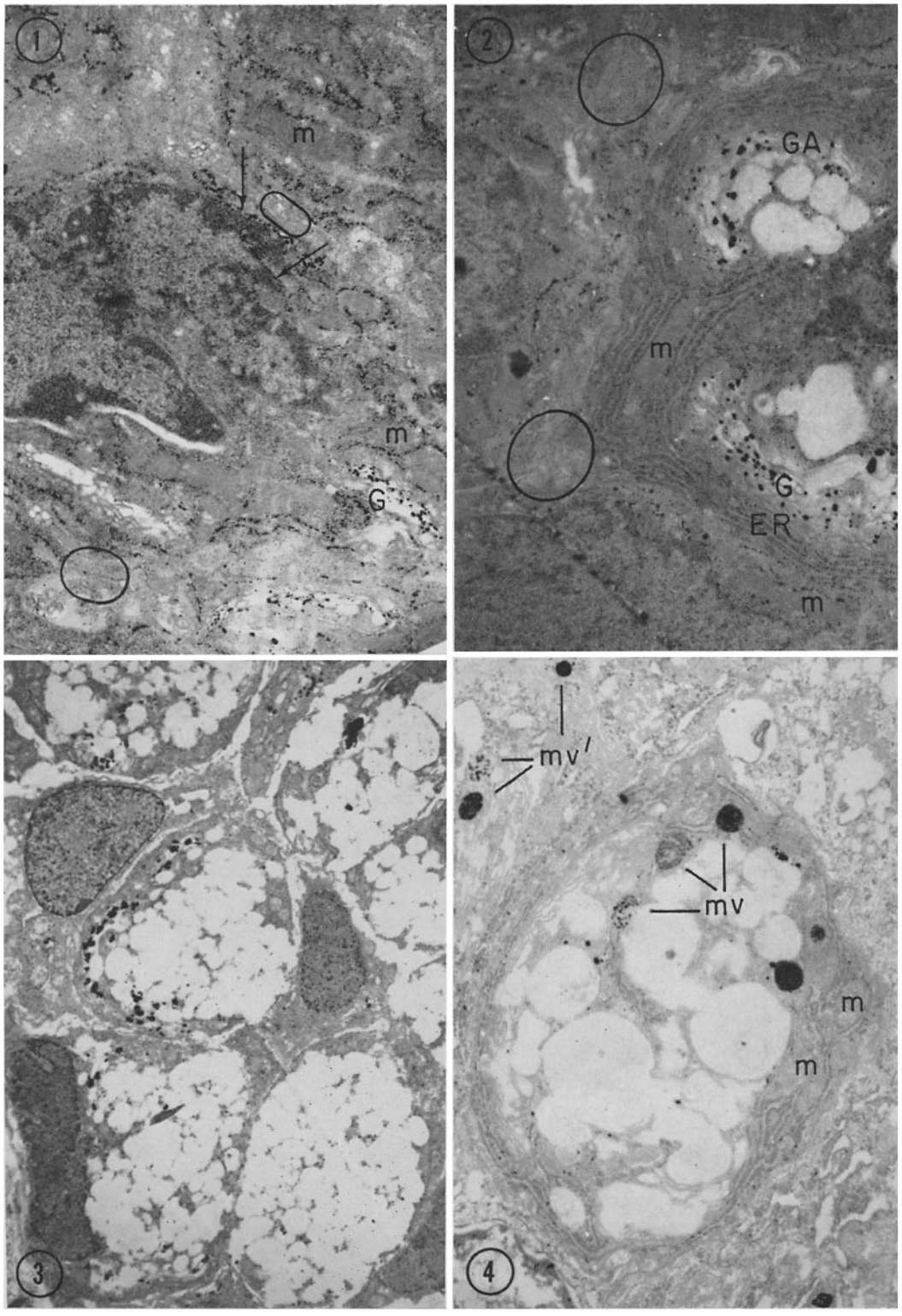
DISCUSSION

The dozen or so cell types (4, 11–14) in which nucleosidediphosphatase activity has been seen in the endoplasmic reticulum for the most part are not engaged in active protein formation. Since nucleoside diphosphatase activity is not present in the endoplasmic reticulum of many protein-synthesizing cells, this enzyme has been considered unrelated to the synthesis of the secretory protein by this organelle (12). On the other hand, the presence of intense enzyme activity between the luminal poles and the nuclei of polarized cells, such as those of the small intestine, kidney, yolk sac, placenta, stomach, and uterus, is believed to be suggestive of an involvement in cell transport (12). This relation is, indeed, nicely shown in the epithelial cells of the colon, where the endoplasmic reticulum of the absorptive cells has inosine diphosphatase activity, but no activity is evident in the elaborate endoplasmic reticulum of the protein-synthesizing goblet cells. Furthermore, the restriction of nucleoside diphosphatase activity to

[1] Obtained from Pabst Research Laboratories, Milwaukee, Wisconsin.

[2] Sigma Chemical Co., St. Louis, a gift from Dr. A. B. Novikoff.

[3] Mann Research Laboratories, New York, a gift from Dr. H. W. Deane.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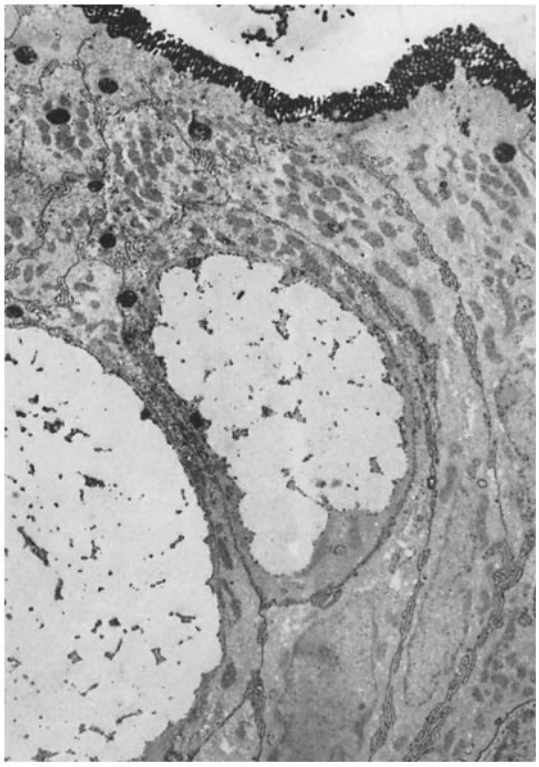

FIGURE 5 Rat colonic mucosa fixed as in Fig. 1; incubated for 40 minutes in ATP-containing medium. The brush border and the multivesicular bodies are covered with heavy deposits. The cell membranes are accentuated by the reaction product. Most of the mitochondria exhibit deposits upon the cristae. Uranyl stained. × 4,200.

the lamellar components of the Golgi apparatus in such protein-secreting cells as cartilage (15) and goblet cells is also correlated with that function most frequently attributed to the Golgi apparatus, namely, the segregation of substances produced elsewhere in the cell (5, 17).

ATPase activity in cell membranes is thought to be related to the transport mechanisms at the

FIGURE 1 Rat colonic mucosa fixed overnight in buffered formaldehyde at 4°C and incubated for 15 minutes in presence of inosine diphosphate. The deposits cover the endoplasmic reticulum of the absorptive cells and the nuclear membrane (arrows) and are present on lamellae of the Golgi apparatus (*G*). The mitochondria (*m*), often encircled by endoplasmic reticulum, are free of deposits. The cell membrane (circled areas) is also unreactive. Uranyl stained. × 10,000.

FIGURE 2 Same as Fig. 1. The endoplasmic reticulum (*ER*) of the goblet cell is free of reaction product. The lamellar components of the Golgi apparatus contained the only extensive deposits found in this type of cell (*G*). The endoplasmic reticulum of two absorptive cells is covered with deposits. Uranyl stained. × 11,000.

FIGURE 3 Goblets from rat colonic mucosa, fixed as in Fig. 1, after 40 minutes' incubation with thiamine pyrophosphate. The deposits were restricted to the lamellar components of the Golgi apparatus of goblet and principal cells. Uranyl stained. × 3,500.

FIGURE 4 Cross-section of a goblet cell, incubated 30 minutes for acid phosphatase activity after fixation as in Fig. 1. The deposits are limited to the multivesicular bodies in the cytoplasm of the goblet (*mv*) and principal cells (*mv'*). The contrast observable in the endoplasmic reticulum resulted from the uranyl stain. × 10,500.

surfaces of many different kinds of cells (10, 12, 16). The widespread occurrence of this enzyme suggests that it may have a general, rather than a specific, function in cellular transport.

The function, origin, and significance of the multivesicular bodies have been variously interpreted (9, 18–20). It has been suggested that these bodies are self-reproducing units which in maturity release small vesicles of constant size (20). It has also been suggested that these bodies may arise from the Golgi apparatus (19), and/or that they represent a type of lysosome or micropinocytotic vacuole (9). The multivesicular bodies of the colonic epithelial cells, like those of the small intestine (1), have been found to contain acid phosphatase. The presence of acid phosphatase activity fulfills at least the cytochemical criterion for classifying the multivesicular bodies as lysosomes (11). Whether or not they contain the other hydrolases usually contained in lysosomes (3) is not known.

It has been alleged that the early secretory granules of the goblet cells of the intestine, *i.e.* those which seem to have recently separated from the Golgi apparatus, have apparent acid phosphatase activity, while the mature secretory granules do not have such activity (11). We have observed acid phosphatase activity, using the naphthol phosphate–pararosaniline method (2), in the supranuclear region of goblet cells at all levels of the crypt. However, no activity has been detected with the lead method in relation to either the early secretory granules or the Golgi apparatus. This activity has been confined entirely to the multivesicular bodies which are seen in the cytoplasm around the periphery of the base and midportion of the goblet proper (Fig. 4). These bodies have not been observed to participate in the sulfation of the secretory product (8), nor do they appear to take part in any other aspect of secretion. It would seem therefore, that acid phosphatase is not directly involved in the elaboration of mucinogen granules in the colonic goblet cells.

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