FINE STRUCTURAL LOCALIZATION OF LYSOSOMAL ENZYMES IN THE ABSORBING CELLS OF THE DUODENAL MUCOSA OF THE MOUSE

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Some enzymatic activities of lysosomes have been localized, with histochemical techniques applied to light microscopy, as fine granular deposits in the absorbing cells of the intestinal mucosa (1, 19). Barka (2), with the electron microscope, showed, in the cells of the duodenal villus of mouse, that acid phosphatase activity was confined to the dense bodies, the multivesicular bodies, and some Golgi vesicles. We obtained identical results in the stem cells of the duodenal crypt (10). Two other enzymatic activities related to the lysosomes can be located by electron microscopy, i.e. those of arylsulfatase and E-600[1]-resistant esterase, considered by some authors as probably cathepsin C (6). Moreover, alkaline phosphatase activity has been demonstrated in various types of dense bodies and multivesicular bodies of the absorbing cells of the duodenum of the mouse (12). Owing to the heterogeneity of these bodies, we have studied the fine structural localization of three lysosomal enzymes in this tissue, in order to identify more accurately these alkaline phosphatase-positive dense bodies as lysosomes.

[1] Diethyl-*p*-nitrophenyl phosphate.

MATERIAL AND METHODS

BALB/c+ adult male mice, fasted for 18 hr, were sacrificed by ether anesthesia. The duodenum was fixed in 5% cacodylate-buffered glutaraldehyde (filtered before use through Dowex No. 2) for 2 hr at 4°C. After a rinse of 2 hr in the buffer, the small blocks were quenched in precooled isopentane and sectioned at 30 μ with a freezing microtome. The sections were incubated in the following media.

For acid phosphatase activity, Gomori's medium as modified by Barka (2) was employed at pH 5 for 15 min at 37°C. For arylsulfatase activity, a medium derived from media described by Hopsu et al. (7) and by Goldfischer (55) was employed:

Acetate buffer pH 5.2	0.1 M	10 ml
P-Nitrocatechol sulfate	0.06 M	5 ml
Barium chloride	0.2 M	5 ml

7.5% sucrose was added to the medium.

The incubation was carried out at 37°C for 60 min at pH 5.2.

For E-600-resistant esterase activity, a method derived from Crevier and Bélanger (4) and modified by others (3, 13, 15, 20) was used. The sections were first incubated in E-600 10^{-5} M in 0.2 M acetate buffer at

pH 6 for 60 min at 37°C. Then, without rinsing, they were transferred to the following medium at pH 5.4 for 90 min at 0°C (ice bath):

Cacodylate buffer	0.05 M	pH 5.2	23	ml
$MgCl_2$	1 M		0.5	ml
Thiolacetic acid	0.24 M		1	ml
Lead nitrate	0.1 M		0.2	ml

7.5% sucrose was added to the medium.

For alkaline phosphatase activity, the sections were incubated for 5 min, at 4°C, in the medium described in preceding papers (11, 12).

An acid rinse was always omitted after the incubations. The postfixation was made in 2% buffered OsO_4 for 30 min at 4°C. The embedding was carried out in Epon according to Luft (14). For each experiment, control sections were incubated in substrate-free media

The thin sections were examined, unstained or after uranyl acetate–lead nitrate staining, in a Philips EM 200.

RESULTS

Acid Phosphatase Activity (Figs. 1–2)

The localization of acid phosphatase activity confirms the findings of Barka (2). The deposits of lead phosphate are mainly situated in the dense bodies which are especially abundant in the apical part of the duodenal absorbing cells. The precipitation is lacking in some of the dense bodies but is, however, present in them in another plane of sectioning. Several vesicles of smooth endoplasmic reticulum and multivesicular bodies covered with the precipitate are encountered in the supranu-

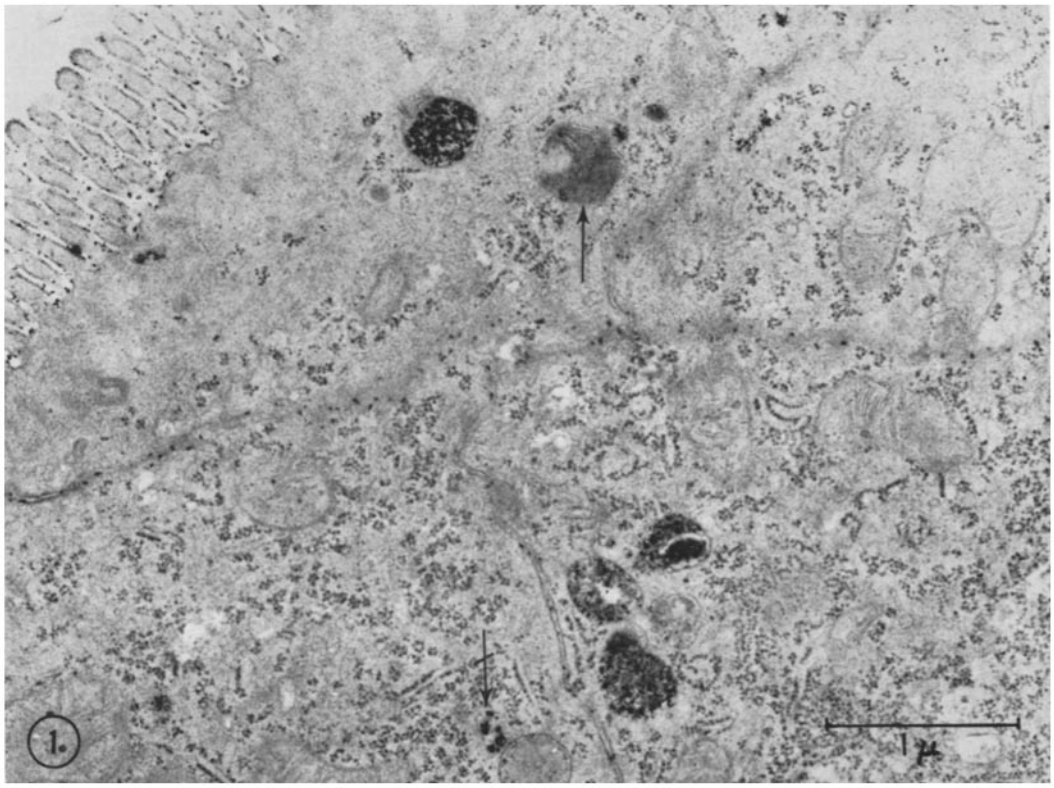

FIGURE 1 Acid phosphatase activity. Apical part of four absorbing cells. Four dense bodies present a precipitate of lead phosphate. At the upper arrow, a dense body which lacks the deposits. At the barred arrow, a profile of smooth endoplasmic reticulum with reaction product. Small nonspecific deposits are present on the cell membranes. × 26,000.

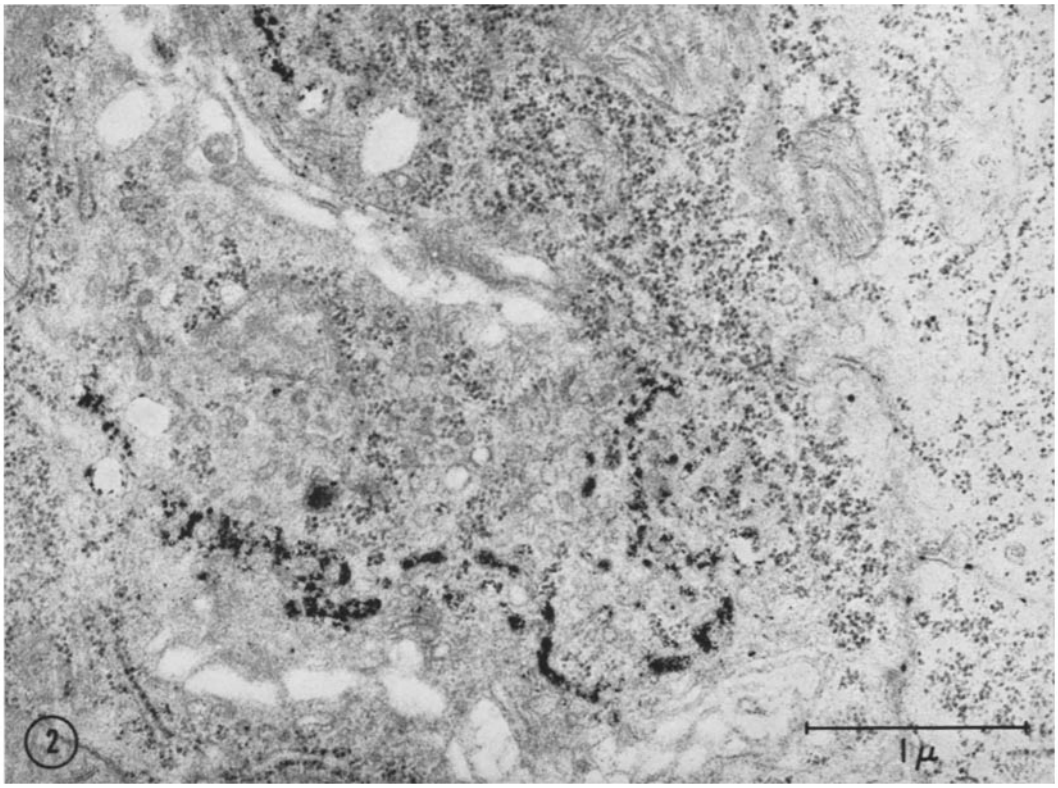

FIGURE 2 Acid phosphatase activity. Vesicles and small saccules of a Golgi apparatus and profiles of smooth endoplasmic reticulum are filled with lead phosphate. × 29,500.

clear zone. Vesicles and only the first row of saccules along the mature side of the Golgi apparatus are filled with the reaction product.

Arylsulfatase Activity (Figs. 3–4)

The dense bodies show a heavy precipitate of barium sulfate which is generally localized on one side of the bodies. However, reactive bodies are found to be less abundant than after the acid phosphatase reaction, even if serial sections are made. Multivesicular bodies are seldom regularly stained but frequently contain one or two vesicles with the specific precipitate. Very few Golgi vesicles show the deposits.

E-600–Resistant Esterase Activity (Figs. 5–6)

The precipitate of lead sulfide is clearly localized on specific structures, particularly on nearly all the dense bodies. Multivesicular bodies show faint deposits of reaction product. Infrequently the vesicles of the Golgi apparatus may be stained. Occasionally, in the Golgi region, a cisterna of the smooth endoplasmic reticulum is in continuity with a positive vacuole containing dense inner structures (Fig. 5).

Alkaline Phosphatase Activity (Fig. 7)

The precipitate of lead phosphate is localized on the microvilli, the smooth reticulum profiles, the multivesicular bodies, the Golgi cisternae, and on nearly all the dense bodies (5). The latter have the same morphological aspect as those bodies that are positive for the three preceding lysosomal enzymes.

DISCUSSION

Very few biochemical studies have been devoted to the intestinal lysosomes (8, 9). Until recently, only Gomori's technique for acid phosphatase was applied to electron microscopy and it was assumed that the numerous dense bodies observed in the mucosal cells of the intestine were lysosomes

because of their acid phosphatase activity. This presumption now seems accurately proved with the fine structural localization of two other lysosomal enzymes in morphologically identical bodies.

If the presence of these enzymes is observed in the dense bodies, their localization in the Golgi zone is unequal. Whereas acid phosphatase activity is frequently found in the Golgi vesicles, E-600–resistant esterase is seldom seen and arylsulfatase is hardly ever present. These findings in the duodenum are not in total agreement with the first assumption made by Novikoff et al. (16) as to the similarity of the Golgi vesicles of the liver and the primary lysosomes. In our experiments, Golgi vesicles seem to contain mainly acid phosphatase. On the other hand, sections (Fig. 5) show a direct continuity between an already elaborated dense body and a smooth reticulum profile. This corresponds to the second assumption of Novikoff et al. (17) as to the origin of lysosomes from a dilation of the endoplasmic reticulum. It may be that some hydrolases pass through the Golgi apparatus and that others do not. However, a particularly strong enzymatic inhibition due to the fixative or a chemically inactive condition of the enzymes may impede visualization of the Golgi reactive sites.

The arylsulfatase activity visualized is probably of type B (18). A pH of 5.2 is, indeed, more specific for this enzyme. However, it can not be proved that arylsulfatase A, with an optimum pH at 4.2

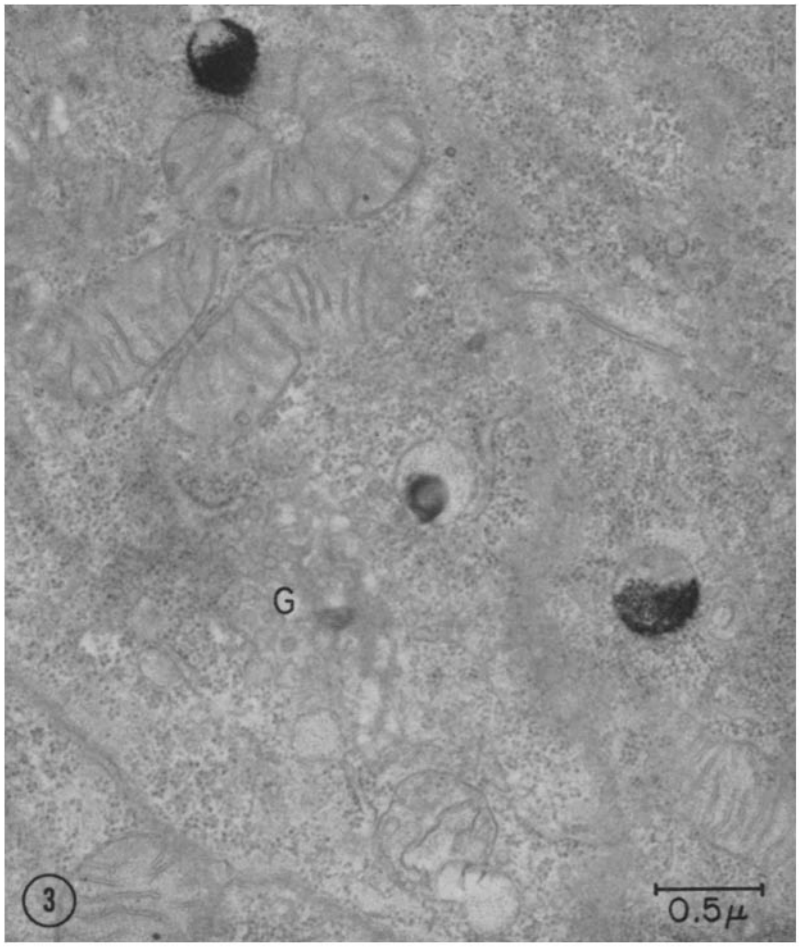

FIGURE 3 Arylsulfatase activity. Two dense bodies present the precipitate of barium sulfate. The Golgi apparatus (*G*) is unreactive. × 29,500.

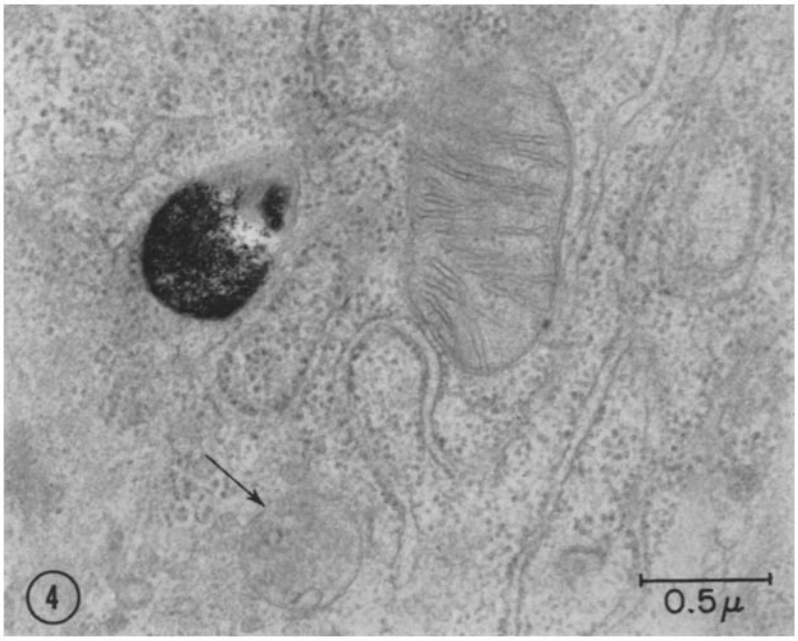

FIGURE 4 Arylsulfatase activity. A dense body shows the deposits of barium sulfate localized mainly on one side of the structure. At the arrow, a nonreactive multivesicular body. × 35,500.

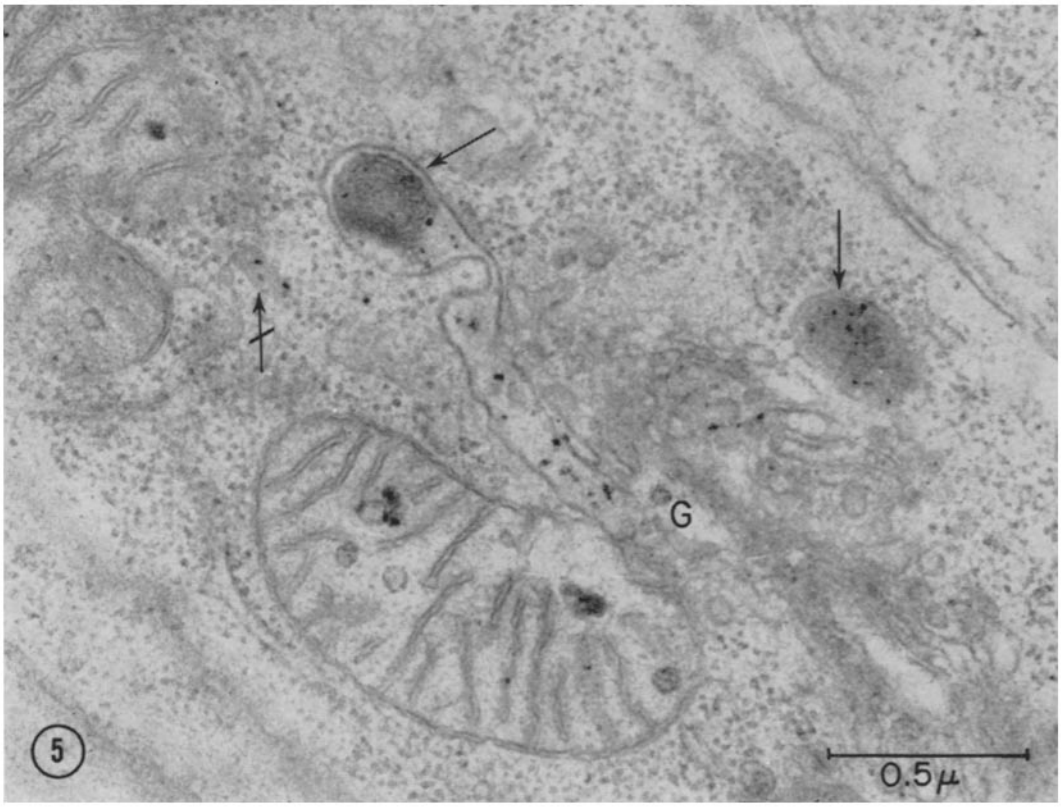

FIGURE 5 E-600-resistant esterase activity. A Golgi (*G*) zone with two reactive dense bodies (arrows). One of the dense bodies is in continuity with a dilated cisterna of smooth reticulum in which some deposits of lead sulfide are seen. At the barred arrow, tangential section of a dense elongated profile which contains fine deposits. × 53,500.

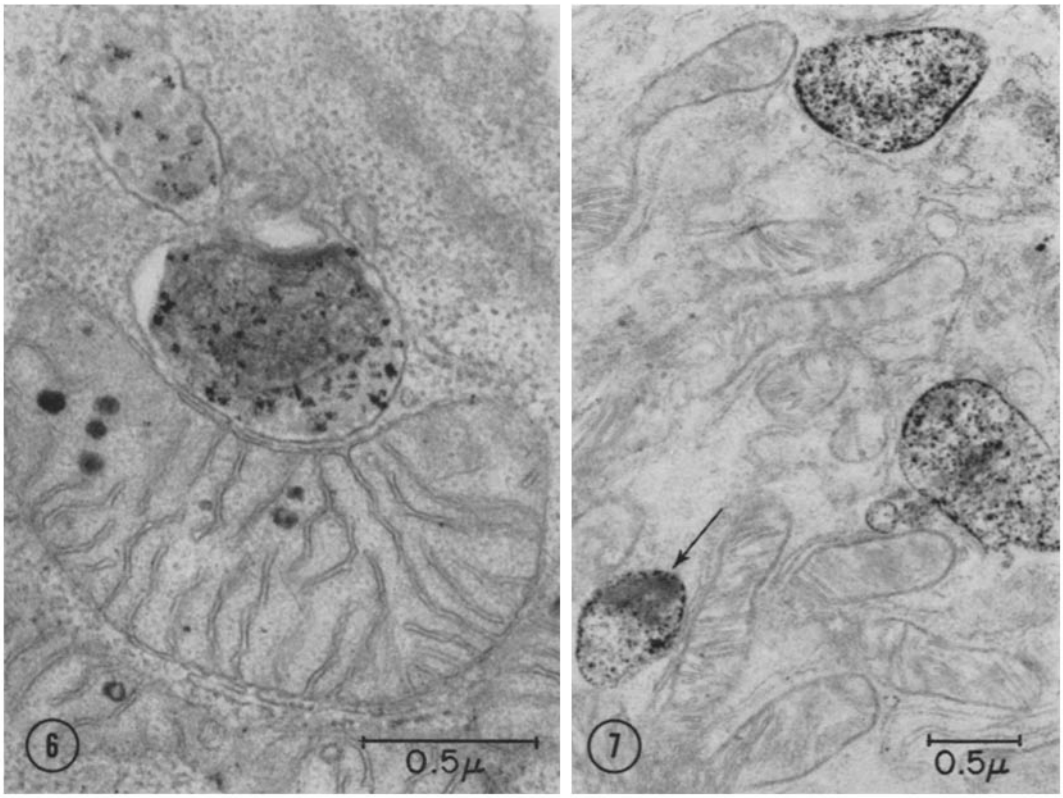

FIGURE 6 E-600-resistant esterase activity. A dense body and an elongated multivesicular body show the precipitate of lead sulfide. × 47,000.

FIGURE 7 Alkaline phosphatase activity. Three dense bodies show the precipitate of lead phosphate. The body marked with an arrow may be compared with the lysosome of Fig. 4. × 24,500.

is not involved. The enzymatic activity is not regularly located on the dense bodies, in agreement with previous observations of Goldfischer (5) and of Hopsu et al. (7). Since the tissues, the incubating media, and the coupling agent were different in our and these authors' experiments, it seems likely that arylsulfatase is not present in all the dense bodies. If this observation were confirmed in other tissues, one might assume that each lysosome does not contain all the hydrolases. Nevertheless, the cytochemical technique employed probably does not show low levels of activity. The frequent "one-side" deposit of barium sulfate is perhaps an artifact of postfixation of dehydration.

The presence of three acid hydrolase activities in bodies showing a morphologic pattern identical with that of bodies containing alkaline phosphatase activity brings a new argument for the presence of alkaline phosphatase in the lysosomes of the absorbing cells of the duodenum.

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