Demonstration of Acid Phosphatase Activity in the Normal Rat Jejunum by Means of Electron Microscopy^{1), 2)}

by

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Introduction

The electron microscopic demonstration of acid phosphatase (ACP) activity was first tried by Sheldon et al. (1955) who applied Gomori's method on the osmium-fixed mouse jejunum. Their method is, however, unnecessarily intricate and yet the preservation of fine structural details of epithelial cells is not successfully good.

Holt (1959) recommended the use of cold formol-calcium fixation for the preservation of both ACP activity and morphological details in the light microscopic histochemistry, and Novikoff (1961) tried to apply the formol-calcium to the electron microscopic demonstration of ACP activity in hepatic lysosomes.

In the present investigation an attempt was made to demonstrate the ACP activity in the normal rat jejunum in the electron microscope by our own method.

Material and Methods

Material used in the present study was the jejunum of normal Wistar strain rats weighing 100-150 g. Rats were killed by a blow on the neck without anesthesia. Immediately after the sacrifice, the abdominal wall was incised and before the excision of tissue cold formol-calcium containing 8% sucrose (FCS), pH adjusted

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2) A part of this work has been published as a letter to the editor in the *Journal of Histochemistry and Cytochemistry* (Ogawa et al., 1962a).

to 7.4 with 1 N NaOH before use, was poured into the abdominal cavity. Small pieces of jejunum were then excised and fixed in cold FCS.

For the light microscopic demonstration of ACP small pieces of jejunum were fixed for 30-60 minutes in cold FCS, washed thoroughly in cold distilled water, mounted on wooden blocks and frozen by the method described previously (Ogawa and Shinonaga, 1961; Ogawa et al., 1962b) for the preparation of cryostat sections. Cryostat sections placed on the coverslips No. 1 were incubated in the modified Gomori ACP medium containing 8% sucrose and 1 mM lead acetate (Ogawa et al., 1962b) for 30-45 minutes at 37°C, washed in distilled water, treated with diluted (approximately 1%) yellow ammonium sulfide solution for 2-5 minutes, washed in distilled water and mounted in glycerine jelly on slide glasses.

For the demonstration of ACP in the electron microscope small pieces of jejunum fixed for about 5 minutes in cold FCS were taken out, diced by the razor blade on the paraffin bed and fixed again in cold FCS for 1-3 hours. During the dicing on the paraffin bed, tissues were kept in cold FCS in order not to be dried up. Diced specimens were then washed briefly in the Gomori's ACP medium described previously instead of water, incubated in the Gomori medium for 30-45 minutes at 37°C, rinsed briefly in cold Caulfield solution (Caulfield, 1957), post-fixed in cold Caulfield solution for 1 hour in vacuo, washed briefly in distilled water, dehydrated in gradual ethanols, and embedded in prepolymerized methacrylate (1:2.3 mixture of methyl and butyl containing 24 g. per 100 ml. benzoyl peroxide). After polymerization for 24-36 hours at 45°C, sections were cut with either glass or diamond knives, mounted on collodion-coated copper grids, examined with a HITACHI HU-11 electron microscope and photographed at original magnifications of 3,800 to 14,500.

In some cases unfixed as well as cold Hanks-formol-sucrose-fixed cryostat sections were used for the light microscopic demonstration of ACP. Hanks-formol-sucrose solution consists of 90 ml. Hanks' balanced salt solution (Hanks and Wallace, 1949), 10 ml. original formalin and 8 g. sucrose.

Substrate-free media were used for controls.

Results and Discussion

A. Light microscopic observation

In addition to the positive reaction in the striated border, the ACP activity was diffusely positive in the cytoplasmic area corresponding to the Golgi zone of jejunal epithelial cells in unfixed cryostat sections (Fig. 1), whereas in FCS-fixed sections, ACP was positive not only in the striated border, but also in granules presumed to be lysosomes (de Duve, 1959; Novikoff, 1961) lying in the cytoplasmic area between the striated border and the nuclear zone of the absorptive cells (Figs. 1-7). It was likely that in the cases of unfixed frozen sections the membranes lining lysosomes were ruptured or altered by the treatment of freezing and thawing (de Duve, 1959), resulting in the release of ACP and subsequent diffuse cytoplasmic stain. Lysosomes were observed even in the cytoplasmic area immediately beneath the striated border, suggesting the possibility that they correspond to "pinosomes" named by Novikoff (1960) on the basis of the finding obtained in *Chaos* and *Amoeba proteus*. In some cases lysosomes (pinosomes) were concentrated in the Golgi zone. The size of lysosomes in absorptive cells in FCS-fixed preparations varied from 0.2μ to 4μ with an average of 0.5μ in their greatest diameter. Lysosomes situated in and around the Golgi zone were larger than those found in the cytoplasm immediately beneath the striated border, suggesting the possible coalescence of lysosomes. The coalescence of lysosomes has not infrequently been observed in cultured cells (unpublished data).

Under the high magnification, ACP was in many cases positive in the form of the ringlet in lysosomes (Figs. 5-7), suggesting that ACP was positive in membranes delineating lysosomes.

The ACP positive membranous structures were also seen in goblet cells (Fig. 8), which will be described in detail in the electron microscopic observation.

Paneth cells and blood cells in the lamina propria contained ACP-rich granules (lysosomes) (Figs. 2, 3, 9, 10). Lysosomes were occasionally found massively in the cytoplasm of Paneth cells (Fig. 9).

Fine structural details were not so well preserved in Hanks-formol-sucrose-fixed cells as in FCS-fixed cells, and therefore FCS is recommended as the fixative of choice for the demonstration of

ACP at the cellular level in the light microscope, although Holt & Hicks (1961) prefers lately the formol-phosphate-sucrose fixative for electron microscopy.

B. Electron microscopic observation

Before going into details of results of the electron microscopic observation, it should be mentioned here that the lowering of lead ion concentration in the incubating medium was necessary in order to avoid the substrate-independent non-enzymatic lead deposition in the plasma membranes of microvilli. The final concentration of 1 mM of lead ion in the incubating medium has been found satisfactory to reveal no metallophilia of the plasma membranes of microvilli incubated in the substrate-free medium for controls (Ogawa et al., 1962b) (Fig. 11).

The positive ACP activity in the striated border under the light microscope was confined to the plasma membranes of microvilli under the electron microscope (Figs. 12-14). The ACP activity was positive in the terminal bars (Fig. 15), the membranes corresponding to the interdigitating plications of the lateral surfaces of the adjacent cells (Fig. 13), and lysosomes (Figs. 15-17) in the absorptive cells. Membranes composing caveolae (Fig. 14) and pinocytotic vesicles (Fig. 16) also appeared to be positive for ACP. These findings seem to support the concept that lysosomes originate from the plasma membranes of microvilli in connection with pinocytosis and the subsequent local differentiation of smooth membranes. In relation to this problem, a recent report of Straus (1962) is of interest. He suggested that the small phagosomes in the renal tubule cells serve in the transport or exchange of materials between the blood and special regions of epithelial cells, and the large phagosomes represent a protective reaction of the cells against the invasion of foreign materials. The relation of ACP to phagosomes has also been found in *Tetrahymena* (Seaman, 1961).

It was of considerable interest to find the positive ACP activity in the smooth membrane system of the Golgi complex in the goblet cell (Figs. 18-23). The ACP activity was positive not only in the smooth membranes composing packed flattened cisternae but also in the agranular membranes surrounding Golgi vesicles which are considered to collect the secretory material (Palay, 1958). As the droplets of mucus coalesce and fine membranes limiting them disappear during the maturation of droplets in goblet cells, the ACP

activity is also lost. The positive ACP reaction in components of the Golgi complex of goblet cells may be explained in terms of a probable involvement of ACP in the mechanism of mucus formation. The relation of lysosomes to the Golgi lamellae has also been discussed in the previous publication (Ogawa et al., 1962c).

Mitochondria did not reveal any ACP activity.

As was in the case of the light microscopic observation, ACP-rich lysosomes were observed in the cytoplasm of Paneth cells (Figs. 24-26) and in blood cells of unknown type (mast cell or leukocyte?) in the lamina propria (Fig. 27) in the electron microscope. In the case of lysosomes (corresponding perhaps to so-called secretory granules) in Paneth cells, the ACP activity was frequently demonstrated in membranes surrounding lysosomes (Figs. 24, 25). This finding speaks for the concept that electron dense materials observed inside may be produced from the surrounding ACP positive membranes. Occasionally the lead deposition is noted in the nuclei, which has been known non-enzymatic (Ogawa et al., 1962b, c).

Some of lysosomes in blood cells were vacuolated, which may be artificial (Fig. 27).

Results obtained in the present investigation indicate that ACP is very closely related to the membrane system of the endoplasmic reticulum (ER), in particular to the smooth ER. Lysosomes may be considered a specially differentiated organelle originating from the smooth ER. It should not be forgotten, however, that, although ACP is undoubtedly one of the lysosomal enzymes and has duly been considered a marker of lysosomes (de Duve, 1959; Novikoff, 1961), it is by no way exclusively localized in lysosomes and related structures.

Summary

Using normal Wistar strain rat jejunum, electron microscopic demonstration of acid phosphatase (ACP) activity was performed. By and large results obtained in the electron histochemical observation were in good agreement with those observed in the light histochemical study.

In the electron microscope the ACP activity was positive in the plasma membranes of microvilli, the terminal bars, membranes corresponding to the interdigitating plications and lysosomes (pinosomes) in absorptive cells. The secretory granules in Paneth cells

were also positive for ACP. The ACP activity was positive in the agranular membranes of the Golgi complex and membranes surrounding Golgi vesicles in goblet cells. Lysosomes were also demonstrated in blood cells of unknown type in the lamina propria.

The findings obtained in the present investigation indicate the close relationship of ACP to the membrane system of the endoplasmic reticulum (ER), particularly to the smooth ER. It is tentatively assumed that lysosomes are a specially differentiated organelle originating from the smooth ER.

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Explanation of figures

Figs. 1-10. Light micrographs. Demonstration of acid phosphatase (ACP) activity by modified Gomori's method. Rat jejunum.

Fig. 1. Unfixed cryostat section. Note the diffuse activity in the striated border (arrows) and underlying cytoplasmic area corresponding to the Golgi zone. $\times 670$.

Figs. 2-10. Cold formol-calcium-fixed sections.

Figs. 2-4. The ACP activity was positive in the striated border and lysosomes in absorptive cells. Blood cells of unknown type in the lamina propria (LP) also contain ACP-rich granules. Fig. 2, $\times 570$. Fig. 3, $\times 510$. Fig. 4, $\times 740$.

Figs. 5-7. Higher magnification of lysosomes (pinosomes) in absorptive cells. G represents the ghost of the goblet cell. Lysosomes localized deeper in the cytoplasm (Figs. 6 & 7) are larger than those lying beneath the striated border (Fig. 6). $\times 2,800$.

Fig. 8. A goblet cell. ACP positive striations (arrow) are noted. $\times 1,700$.

Figs. 9 & 10. Paneth cells. Secretory granules in Paneth cells are positive for ACP, indicating that secretory granules belong to a class of lysosomes. Fig. 9, $\times 2,800$. Fig. 10, $\times 2,500$.

Figs. 11-27. Electron micrographs. Demonstration of ACP activity.

Figs. 11-17. Absorptive cells.

Fig. 11. Section incubated in the substrate-free medium. G represents the goblet cell. No lead deposition is observed.

Figs. 12-14. The ACP activity is positive in the plasma membranes of microvilli and membranes corresponding to the interdigitating plications of the lateral surfaces of the adjacent cells.

Fig. 15. Terminal bars and lysosomes are positive for ACP.

Figs. 16 & 17. ACP positive lysosomes are noted. Membranes composing pinocytotic vesicles appear to be positive for ACP (arrow).

Figs. 18-23. Goblet cells. Compact agranular membranes and membranes surrounding Golgi vesicles are positive for ACP. Mitochondria (M) are free of activity. Figure 22 denotes a cross section of goblet cells.

Figs. 21 & 23. The droplets of mucus in goblet cells are not surrounded by intact membranes any more. The apical plasma membranes have burst and mucus

is oozing out into the lumen.

Figs. 24-26. Paneth cell. Paneth cells contain lysosomes. ACP is in many cases positive in membranes delineating lysosomes. Fig. 25 is a higher magnification of a portion of Fig. 24. N indicates the nucleus.

Fig. 27. A blood cell of unknown type. Note ACP-rich lysosomes. Some of lysosomes are vacuolated. N represents the nucleus.

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