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ULTRASTRUCTURAL LOCALIZATION OF ACID PHOSPHATASE ACTIVITY IN THE KIDNEY SAC NEPHROCYTES OF HELIX ASPERSA

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Ultracytochemical analysis of acid phosphatase in the nephrocytes of *Helix aspersa* renal sac was carried out using beta-glycerophosphate as the substrate of reaction. The reaction product was localized on dictyosomes, lysosomes, apical and lateral plasma membranes and, occasionally, in dense digestive bodies. In the latter, the lack of reaction or the weakness of it is discussed. Reaction product was affected by sodium fluoride, except in apical and the most lateral membranes. This fact could be interpreted as the result of phosphatase activity at these sites being different from that of lysosomal acid phosphatase.

The lytic capacity of a cell has frequently been reported to be directly related to the acid phosphatase activity in its lysosomes (1, 3, 10, 12, 13, 19–21).

It has likewise been widely demonstrated that such enzymatic activity is not an exclusive property of lysosomes. On the contrary, non-lysosomal acid phosphatase does occur (5, 10, 12, 13, 17, 18). The general view is that high activity exists in cells carrying out degradative processes, as well as in low differentiated cells and tissues (12, 13, 20).

An excretory spherule or concretion, which represents the final product of nitrogen metabolism, is elaborated as the result of the action of enzymatic organelles in *Helix aspersa* renal sac (15, 16). Since the acid phosphatase plays an important role in the catabolic processes, the activity of this enzyme in the *Helix aspersa* nephrocytes has been studied in the present report.

MATERIAL AND METHODS

For this work, the renal sac was divided into three consecutive zones: a) posterior, from the region adjacent to the hepatopancreas as far as the renopericardial canal, b) medial, bordering on the heart and c) anterior or the portion in which primary ureter begins.

50 μm thick slices, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer

at pH 7.2 for 35 min, were incubated at 37°C for 35–40 min in a medium described by Lewis and Kwinght (8). The medium contained 0.01 M beta-glycerophosphate in 0.05 M acetate buffer (pH 5) containing 0.004 M lead nitrate. After washing in cacodylate buffer, they were post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 hr at 4°C. They were then dehydrated and embedded in Spurr's resin.

Thin sections were either examined without staining or stained for 30 min with uranyl acetate.

Cytochemical controls: the three renal zones were incubated a) in medium from which the substrate had been removed, and b) in medium containing 10 mM sodium fluoride.

RESULTS

According to Lewis and Kwight (8), by using beta-glycerophosphate as a substrate, acid phosphatase has been demonstrated in *Helix aspersa* renal sac. This organ was divided into three zones (anterior, medial and posterior) and a compara-

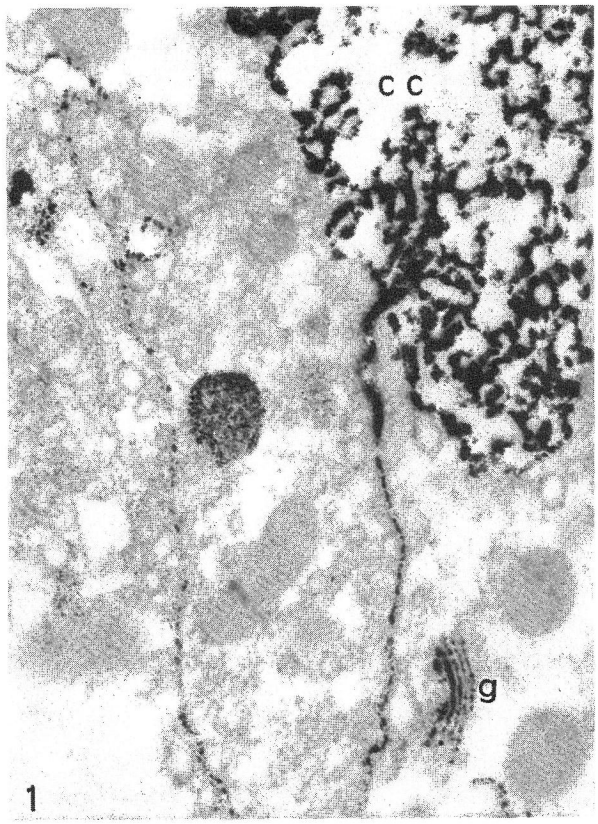

FIG. 1. Acid phosphatase activity in *H. aspersa* nephrocytes. Note the reaction at the apical and lateral plasma membranes. cc: coelomic cavity or renal sac lumen; g: Golgi apparatus. ×10,000

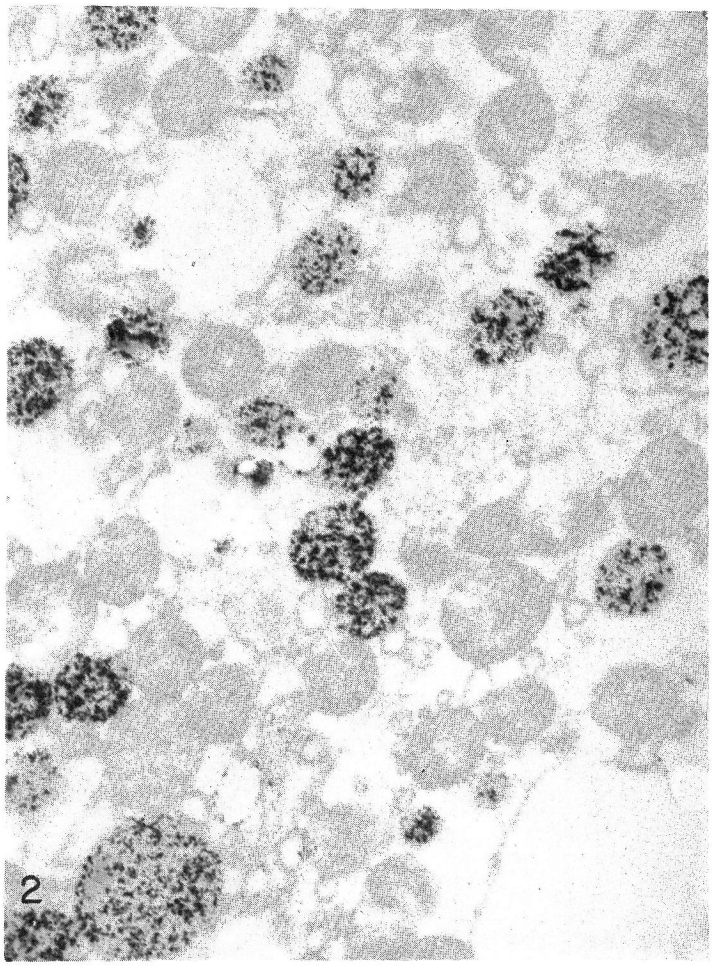

FIG. 2. Acid phosphatase reaction in nephrocytes lysosomes. ×8,000

tive study was carried out.

Nephrocyte lysosomes, of several sizes and distributed over the whole cytoplasm, show a variable degree of staining (Figs. 1, 2). Besides, in the course of our study, slight differences were observed to exist according to the renal zone examined. Both the medial and anterior zones was proved to be more intensely stained.

Although in most cases the acid phosphatase reaction in dense digestive bodies was absent, restricted specific reactivity were occasionally detected (Fig. 3).

In the small dictyosomes, the cisternae of the trans side were intensely stained where those of the cis side were stained less strongly (Fig. 1).

The apical and lateral cell surfaces are other areas of enzyme reactivity. At the above mentioned apical plasmalemma the precipitate discretely surrounds the microvilli, the base showing a higher concentration. As regards the lateral plasma membrane, in most cases, the reaction was shown to be discontinuous (Fig. 1).

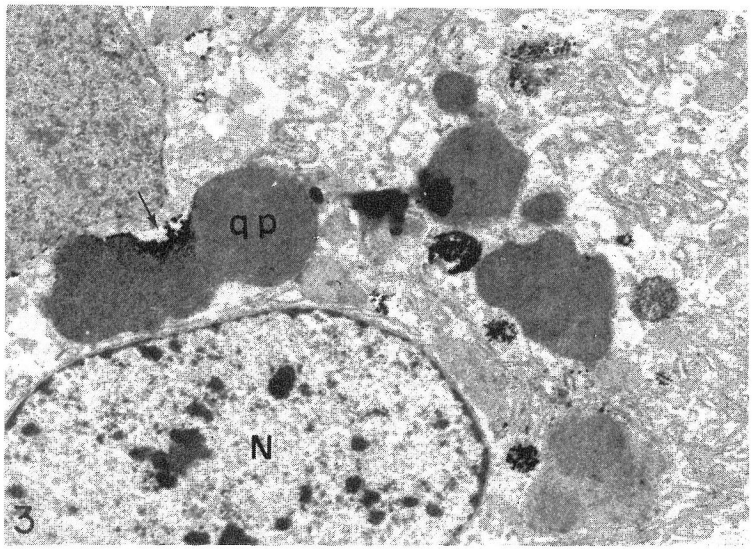

FIG. 3. Positive activity is observed in lysosomes, Golgi apparatus (g) and at located sites in dense digestive bodies (arrow). db: digestive body; N: nucleus. ×3,000

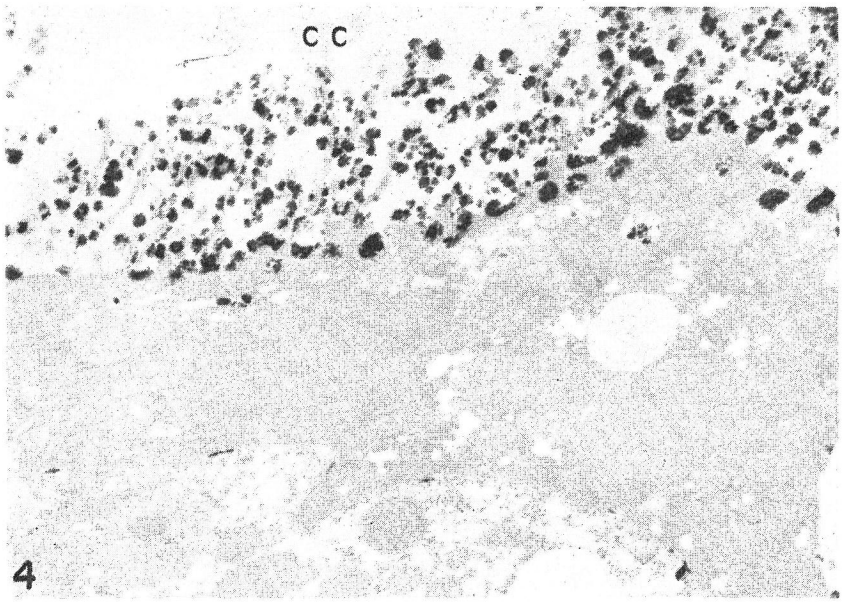

FIG. 4. Maintenance of reaction in the apical plasma membrane after addition of 10 mM NaF. cc: coelomic cavity. ×8,000

Over the three renal zones considered the cell surface staining is not uniform. Nevertheless, such differences are too small to be considered distinctive of each zone.

As well as nephrocytes, other cell types present in the excretory system give positive reaction in lysosomes, Golgi apparatus and, occasionally, on lateral plasma membranes. In no case was the apical plasmalemma site of deposition.

In order to compare sites of enzymatically produced precipitates with those produced non-enzymatically, substrate was deleted from the medium. Under these conditions, no precipitate was observed in the renal regions studied.

After addition of 10 mM sodium fluoride to the medium, the apical surface was slightly affected. However, lateral plasma membranes was unreactive in some cells but in others showed a reaction. On the other hand, no dictyosomes appeared with reaction product and lysosomal precipitate was severely affected (Fig. 4).

Despite this, in the medial zone this lytic organelle retained some ability to hydrolyze the substrate. This latter result could be due to an inhibitory effect of sodium fluoride and acid phosphatase less than 100% (4).

DISCUSSION

Although the acid phosphatase activity is a common way to identify lysosomes, such activity cannot be ruled out in other cell components. On the other hand, the number of isoenzymes involved is not yet well established (10). Nevertheless, by using the specific substrate on different tissues the characterization of some of them has been possible (10, 18).

Since beta-glycerophosphatase and nitrophenylphosphatase are non-specific substrates and can thus be hydrolyzed by almost all acid phosphatase isoenzymes, they have been widely employed in both biochemical and cytochemical demonstration (10). As compared with nitrophenylphosphate, however, beta-glycerophosphate has proved to be a more suitable substrate for the characterization of acid phosphatase in most tissues (10, 11).

As has previously been described in other cell types (9, 12, 13, 20), we have clearly detected a positive staining of lysosomes in our material. Remarkable differences were not observed among the three renal zones considered.

As might be expected (2, 6), positive reaction was not restricted to lysosomes. Dyctiosomes have shown enzymatic activity, especially in the cisternae of the trans side of Golgi apparatus, such as in other cell types (6).

In dense digestive bodies, acid phosphatase activity was absent or restricted to located sites. On this basis, as has previously been reported in other invertebrates (13), it could be postulated that hydrolases other than acid phosphatase are involved in the digestive metabolism of *Helix aspersa* nephrocytes. Nevertheless, other explanations are possible: a) inadequate chemical composition of the matrix could be responsible for the lack of precipitation of lead phosphate with the cytochemical medium employed in this study. This possibility could be inferred from the studies carried by De Jong (4), b) instead of this, as has been proposed by Bainton and Farquhar (1), the matrix could be too compact to allow the enzyme accessibility.

The microvillar border and the most lateral plasmalemma of many nephrocytes are clearly reactive to the cytochemical method employed. This result agrees with

that previously described in *Candida albiians* (20), some invertebrates (13) and in newborn rat intestine (12). The role of acid phosphatase in plasma membrane has not yet been well established although its function in extracellular degradative processes has been suggested (13, 20). On this basis, it can be proposed that activity in the plasmalemma of *Helix aspersa* nephrocytes could be the consequence of a process of recuperation of substances that need a previous extracellular digestion. In the coelomic cavity, these substances could have been eliminated with the excretory spherule (16), while in lateral spaces such molecules could be in the fluid filtered between the cells (7, 14).

An explanation of the maintenance of enzymatic activity in lateral and apical plasmalemma in the presence of sodium fluoride could be that the lytic enzymes of these membranes are different from lysosomal acid phosphatase (17, 18). Another possibility, suggested by the unspecificity of beta-glycerophosphate, could be that the above-mentioned reaction is due to phosphatases involved in the active transport of ions.

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