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Ultrastructural changes in the lysosomal-vacuolar system in digestive cells of *Mytilus edulis* as a response to increased salinity

R. K. Pipe and M. N. Moore

Natural Environment Research Council, Institute for Marine Environmental Research; Prospect Place, The Hoe, Plymouth PL1 3DH, Devon, England

Abstract

The effects of increasing salinity on the ultrastructural morphology of the lysosomal-vacuolar system in digestive cells of the common mussel *Mytilus edulis* were investigated in order to relate structural changes to previous biochemical and cytochemical observations. After 3 h of increased salinity, from 21 to 35‰, the digestive cells showed an increase in numbers of heterolysosomes. There was some evidence of digestive cell breakdown, the contents forming membrane-bound bodies and being released into the tubule lumen. After 12 h of increased salinity, heterolysosomes were prevalent in the digestive cells. There was increased evidence for digestive-cell breakdown, many of the tubule lumina being packed with membrane-bound bodies. It is concluded that increasing salinity from 21 to 35‰ stimulates the lysosomal-vacuolar system, as a result of autophagocytosis or apoptosis; this is consistent with the hypothesis that intracellular, lysosomally-mediated, catabolism of proteins is a source for free amino acids during the adaptation of mussels to increased salinity.

Introduction

The common mussel *Mytilus edulis* is an osmoconformer when exposed to varying external salinities, i.e., it does not have the ability to alter extracellular osmoconcentration. It is, however, able to achieve some cell-volume regulation by control of the intracellular amino acid concentration (Bishop, 1976). The increase in free amino acids required when salinity is increased may be brought about by sequestering of intracellular components, with subsequent degradation by lysosomes during autophagocytosis (Moore *et al.*, 1980; Bayne *et al.*, 1981).

The digestive gland of bivalve molluscs consists of blind-ending tubules with an epithelial lining of digestive, basophilic and immature cells (Sumner, 1966 a, b; McQuiston, 1969 a, b; Owen, 1970, 1972; Pal, 1971, 1972; Platt, 1971). The principal role of the digestive cells is to digest material supplied to the lumen of the tubules and, for this purpose, they have a highly developed lysosomal-vacuolar system (Owen, 1972).

It has been shown, in related studies on the digestive gland of *Mytilus edulis*, that experimental raising of salinity increases the activity of the lysosomal enzymes aminopeptidase-I and hexosaminidase, while lowering salinity decreases the activity. The cytochemically-determined lysosomal stability and levels of lysosomal and cytosolic free-amino-acid concentrations were also found to be responsive to salinity changes (Koehn *et al.*, 1980; Moore *et al.*, 1980; Bayne *et al.*, 1981).

The objectives of the present study were to investigate changes in the ultrastructural morphology of the digestive cells in response to increased salinity, and to relate such structural changes to previous biochemical and cytochemical observations (Koehn *et al.*, 1980; Moore *et al.*, 1980; Bayne *et al.*, 1981), supporting the hypothesis that intracellular lysosomal proteolysis provides free amino acids for effecting isosmotic cell-volume regulation with increasing salinity.

Materials and methods

Mytilus edulis were collected in October from Shinnecock, a lagoon site connecting to the open ocean on the south shore of Long Island on the east coast of North America. The mussels were acclimated in the laboratory for 3 to 4 wk in artificial sea water at 21 or 35‰ S, and were fed regularly with *Isochrysis galbana* at a level greater than maintenance ration. Mussels were transferred directly from the lower to the higher salinity and sampled after 3 and 12 h. Digestive glands were dissected-out, and small pieces of tissue (approximately 1 mm^3) were fixed in 2.5% glutaraldehyde in phosphate buffer (+2.5% NaCl) at

pH 7.4 for 2 h at 4 °C. The samples were rinsed overnight in phosphate-buffered sucrose and post-fixed in 1% osmium tetroxide in phosphate buffer at pH 7.4 for 1 h. They were then dehydrated through an ascending ethanol series into propylene oxide, before infiltration overnight in several changes of Taab resin. Polymerisation of the blocks was carried out at 60 °C for 48 h. Silver-to-gold sections (70 to 90 nm) were cut on a Reichert ultramicrotome and stained with aqueous uranyl acetate and lead citrate. The sections were examined in either a Philips 200 or 300 electron microscope with an accelerating voltage of 60 or 80 kV.

Results

Digestive-cell structure

The fine structure of the digestive cells in *Mytilus edulis* is very similar to that in other bivalve molluscs (Sumner, 1966 a, b; McQuiston, 1969 a, b; Owen, 1970, 1972; Pal, 1971, 1972), and has been described in detail by Platt (1971) and Owen (1972). Basically, the cells are columnar to cuboidal in form, with well-developed apical microvilli and a large basally-situated nucleus. The most characteristic feature of the cells is the various types of membrane-bound micro- and macro-vesicles.

Owen (1970) described two types of microvesicle and four types of macrovesicle in digestive cells of *Cardium edule;* in *Mytilus edulis,* Platt (1971) distinguished between two types of microvesicle and three types of macrovesicle. It was recognised by Owen and Platt, however, that the types of macrovesicle they described most probably represent stages in the heterophagic cycle rather than discrete bodies. Thompson *et al.* (1974) only differentiated between macrovesicles and microvesicles in *M. edulis,* and considered the macrovesicles to be heterolysosomes and equivalent to Owen's Types 1 and 2 macrovesicles; the microvesicles were considered to be equivalent to Owen's Type 4 macrovesicle, which these authors referred to as residual bodies.

In the present study we will distinguish between three types of macrovesicle as described by Platt (1971), and follow the terminology of Owen (1972) to describe them, although it is realised that some may be autophagic rather than heterophagic bodies.

Type 1 macrovesicles are confined to the sub-apical region of the cell. They are often irregular in shape and usually appear empty or with only small amounts of electron-dense material distributed round the periphery. These macrovesicles are considered by Platt (1971) and Owen (1972) to be heterophagosomes and will be referred to as such.

Type 2 macrovesicles usually occur in the mid-region of the cell and are by far the largest of the macrovesicles. They range in appearance from being empty to being completely full. The contents, when present, are moderately electron-dense and homogeneous in appearance, with occasional, small, highly electron-dense granules typically around the periphery of the vesicles. Platt (1971) and Owen (1972) consider these macrovesicles to be heterolysosomes.

Type 4 macrovesicles are typically located in the basal region, although they may be found throughout the cell. They are bound by two membranes with an irregular interspace, and have very electron-dense contents. These macrovesicles are considered by Platt (1971) and Owen (1972) to be residual bodies.

Responses to increased salinity

Representative digestive cells from mussels acclimated to 35‰ S are shown in Fig. 1: *1*. These cells showed the typical structure as described above and by Platt (1971) and Owen (1972). All three types of macrovesicle were present in the cells. There appeared to be relatively few heterophagosomes, but large numbers of residual bodies and heterolysosomes, ranging from completely full to almost empty.

Digestive cells typical of individuals acclimated to 21‰ S are shown in Fig. 1: *2*. The cells from these mussels had a condensed appearance, with darkly staining cytoplasm and an apparent reduction in cell size, with relatively few macrovesicles. In particular, heterolysosomes appeared to be comparatively scarce. Large numbers of lipid droplets were found associated with some of the digestive cells.

After 3 h of increased salinity, from 21 to 35‰, the digestive cells showed an increase in numbers of heterolysosomes which tended to be extremely large, with variable amounts of contents (Fig. 2: *3*). There was also some evidence for digestive-cell breakdown, the contents forming membrane-bound bodies and being released into the tubule lumen (Fig. 2: *4*).

After 12 h of increased salinity, from 21 to 35‰, heterolysosomes, predominantly of an empty appearance, were prevalent in the digestive cells (Fig. 3: *5*). There appeared to be fusion of some heterolysosomes to form "giant" lysosomes, which again characteristically had an empty appearance. Some of these large, secondary lysosomes appeared to contain cytoplasmic organelles, indicating that they may be autophagic vacuoles (Fig. 3: *5*). Digestive-cell breakdown into membrane-bound bodies appeared more frequently in the 12 h increased-salinity individuals, and many of the tubule lumina were packed with these membrane-bound bodies (Fig. 3: *6*).

Discussion

The results show that in *Mytilus edulis* the digestive-cell morphology, in particular the lysosomal-vacuolar system, is affected by alterations in salinity, as evidenced by an increase in the numbers of large heterolysosomes. The changes are apparent after 3 h of increased salinity but more pronounced after 12 h.

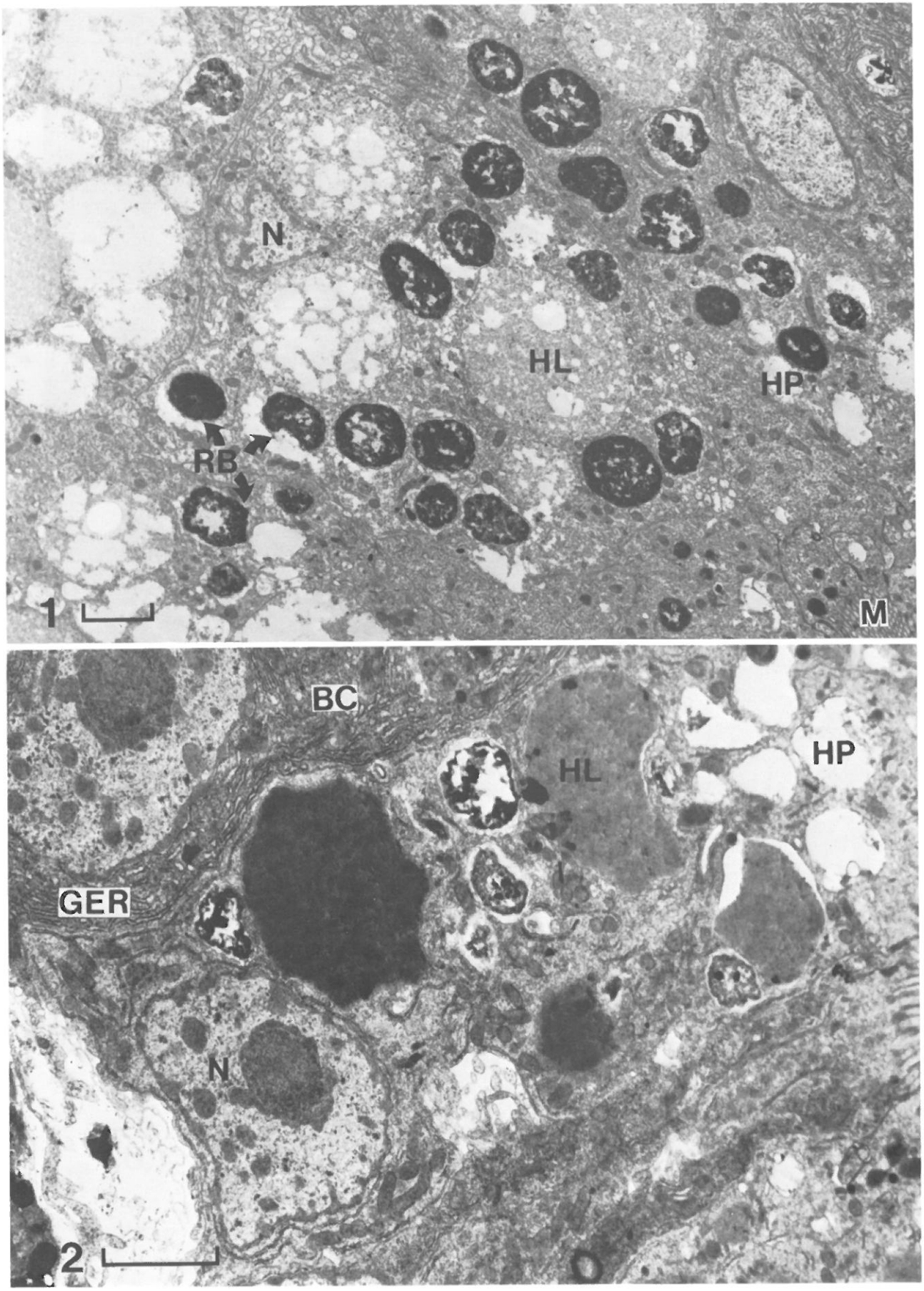


Fig. 1. *Mytilus edulis.* Transmission electron micrographs (TEMs) of sections through digestive glands of mussels acclimated to (*1*) 35‰ S and (*2*) 21‰ S. BC: basophilic cell; GER: granular endoplasmic reticulum; HP: heterophagosome; HL: heterolysosome; RB: residual body; N: nucleus; M: microvilli. (Scale bars = 2 μm)

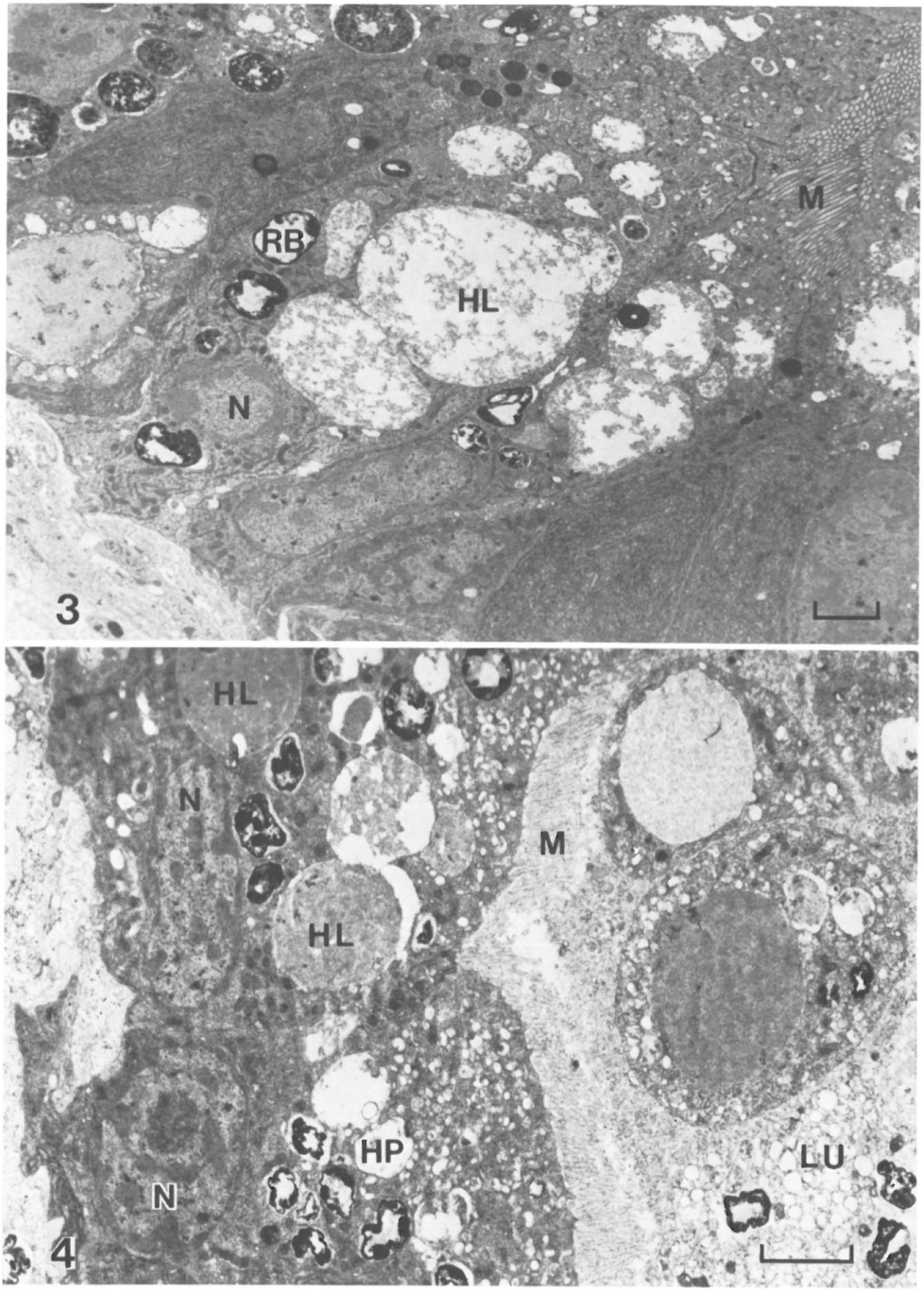
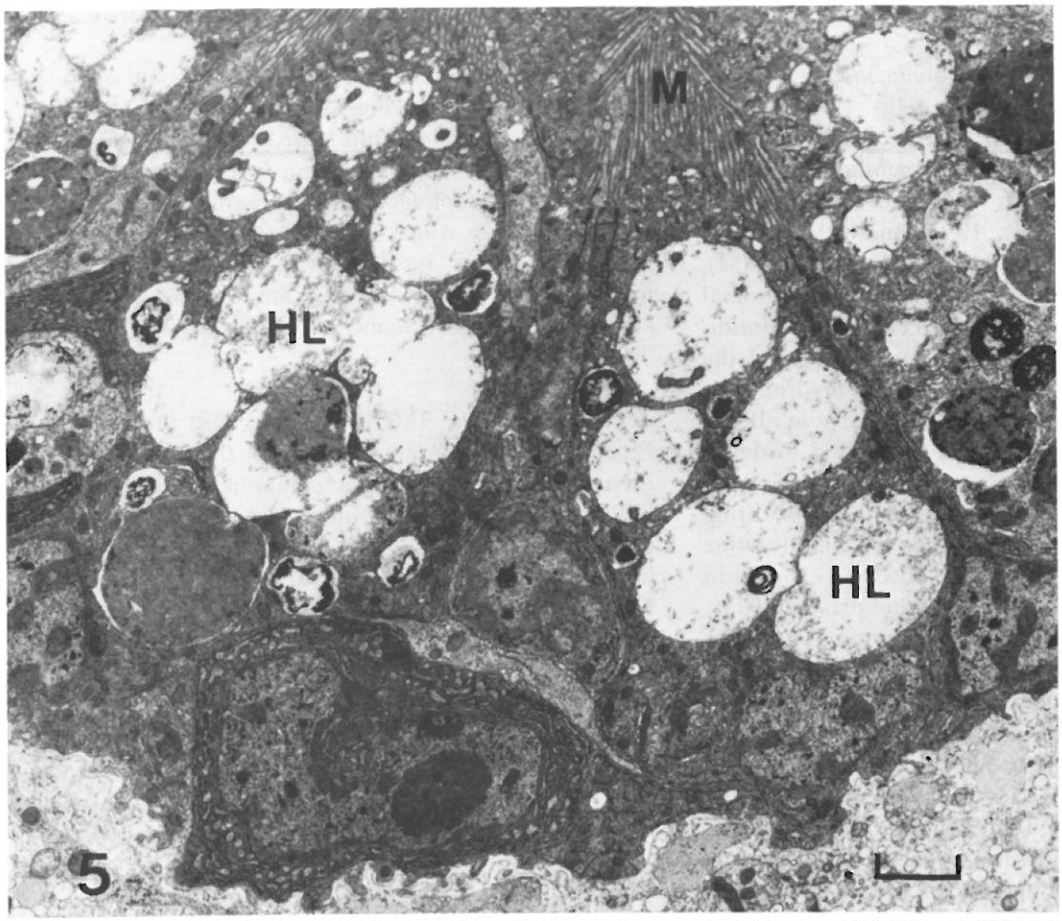


Fig. 2. *Mytilus edulis.* TEMs of sections through digestive glands of mussels exposed to increased salinity (from 21 to 35‰ S) for 3 h. Increased numbers of heterolysosomes are evident (*3*), and there is evidence of digestive-cell breakdown, the contents forming membrane-bound bodies and being released into the tubule lumen (*4:* LU). Abbreviations as in legend to Fig. 1. (Scale bars = 2 μm)



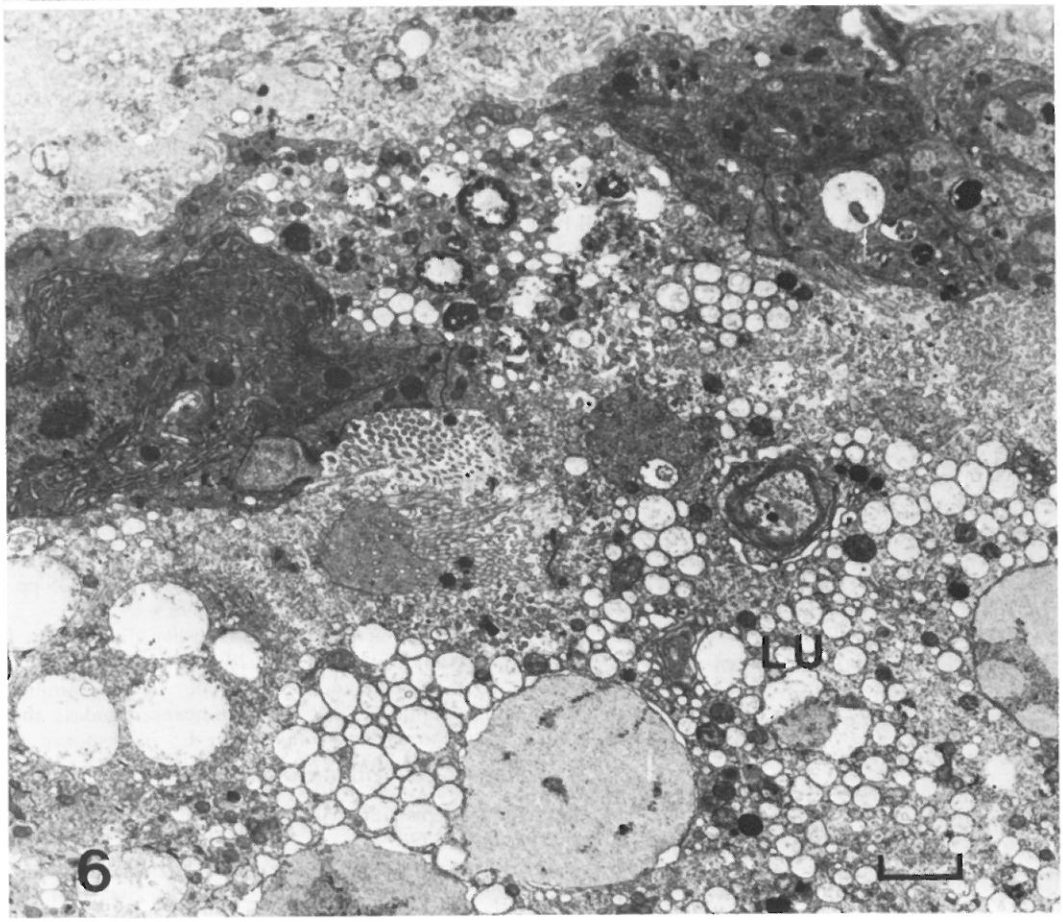


Fig. 3. *Mytilus edulis.* TEMs of sections through digestive glands of mussels exposed to increased salinity (from 21 to 35‰ S) for 12 h. Some large, secondary lysosomes appear to contain cytoplasmic organelles, indicating that they may be autophagic vacuoles (*5*). Many tubule lumina are packed with membrane-bound bodies (*6*). Abbreviations as in legend to Fig. 1. (Scale bars = 2 μm)

Lysosomes are known to be involved in the turnover and catabolism of intracellular proteins (Segal, 1975; Mortimore and Schworer, 1977; Amenta and Brocher, 1980) and to have relatively high concentrations of free amino acids in comparison to the cytosol (Ward and Mortimore, 1978; Koehn *et al.*, 1980). In related studies (Koehn *et al.*, 1980; Moore *et al.*, 1980; Bayne *et al.*, 1981), increasing salinity elevated the activity of the lysosomal enzymes aminopeptidase-1 and N-acetyl-β-hexosaminidase. The integrity of the lysosomal membrane and the concentration of lysosomal and cytosolic ninhydrin-positive substances were also found to be responsive to altered salinities. These findings were all put forward as evidence supporting the hypothesis that lysosomal hydrolysis provides a source of free amino acids in digestive cells during exposure to increased salinities, in order to facilitate some degree of cell-volume regulation. The observed changes in ultrastructural morphology found in the present study, showing an obvious stimulation of the lysosomal-vacuolar system, also appear to be in agreement with this hypothesis. In particular, the presence of giant lysosomes in some digestive cells after 12 h at increased salinity is consistent with the concurrent evidence of reduced lysosomal membrane stability (Bayne *et al.*, 1981); this is in turn indicative of increased membrane fluidity, which would facilitate fusion of these organelles, leading to the formation of abnormally enlarged lysosomes (Poste and Allison, 1973; Szego, 1975).

The results indicate two possible mechanisms whereby proteins may be catabolised lysosomally in order to alter cellular free amino acid concentration. The process could be one of autophagocytosis, which is the intracellular sequestration of cytoplasmic components and subsequent degradation by lysosomes (reviews by: Ericsson, 1969; Glaumann *et al.*, 1981). Alternatively, it could be a process of apoptosis which is controlled cell-deletion and involves the degeneration of cells into membrane-bound apoptotic bodies and subsequent phagocytosis by surrounding cells (reviews by: Bowen, 1981; Wyllie, 1981). Cytoplasmic organelles were clearly seen in the large Type 2 macrovesicles in digestive cells of mussels subjected to raised salinity for 12 h, indicating that they may be autophagic vacuoles. However, breakdown of digestive cells into membrane-bound bodies with subsequent extrusion into the lumen of the tubules and phagocytosis by surrounding digestive cells was also evident in mussels subjected to increased salinity. Clearly, it is not possible, on the basis of morphology, to differentiate between autophagic vacuoles and phagocytosed-apoptotic bodies, particularly in digestive cells where the principal role is heterophagic digestion. It was for this reason that Ericsson (1969) introduced the term cytosegresome to cover both types of body. However, this term does not seem to have been universally accepted, as the terms autophagic and heterophagic vacuole still predominate in the literature. Our results provide very strong evidence for the occurrence of apoptosis but this does not necessarily rule out concurrent autophagocytosis, since both mechanisms could operate as a means of lysosomal protein catabolism within the cells.

In conclusion, the results appear to reveal a stimulation of the lysosomal-vacuolar system with increasing salinity, as a result of autophagocytosis or apoptosis. This supports previous findings which are consistent with the hypothesis that intracellular, lysosomally-mediated, catabolism of proteins is a source for free amino acids during the adaptation of mussels to increased salinity.

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