OBSERVATIONS ON THE MANTLE OF THE NORTHERN QUAHOG, MERCENARIA MERCENARIA L.[1]

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ABSTRACT

Preliminary histo- and cytochemical studies on the mantle of Mercenaria mercenaria L. revealed a fourth fold of the mantle edge which produces a copious supply of mucus and may have a mantle-cleansing function. Transverse sections of the mantle were treated with toluidine blue O and periodic acid-Schiff reagent. The G-Nadi reaction for cytochrome oxidase activity and the triphenyl tetrazolium chloride method for succinic dehydrogenase activity were carried out on whole fresh mantles. With toluidine blue O, the epithelium of the mantle edge stained considerably darker than the epithelium of other areas of the mantle. Schiff-positive material was localized primarily in the connective tissue and around the groove between the sensory and muscular folds. Cytochrome oxidase activity was most marked in the central area enclosed by the pallial line and in the outer edges of the mantle folds. Succinic dehydrogrenase activity was most marked in the central region of the mantle and in the groove between the sensory and muscular folds.

INTRODUCTION

The lamellibranch mantle edge generally consists of three folds which are usually described according to their position and function as an outer secretory fold, a middle sensory fold, and an inner muscular fold. In the northern quahog, Mercenaria mercenaria L., there is a fourth fold, bordered by a prominent ridge, along the inner surface of the mantle edge (Fig. 1).

We have been investigating the histo- and cytochemistry of the mantle edge and of the mantle in juxtaposition with the shell and ligament as part of a study upon ecological aspects of shell deposition. The purpose of this paper is to report some preliminary histological and histochemical observations made on the mantle of M. mercenaria. There is sufficient previous work (e.g. Rawitz, 1888, 1890; Moynier de Villepoix, 1892; Bevelander and Benzer, 1948; Kado, 1953, 1954;

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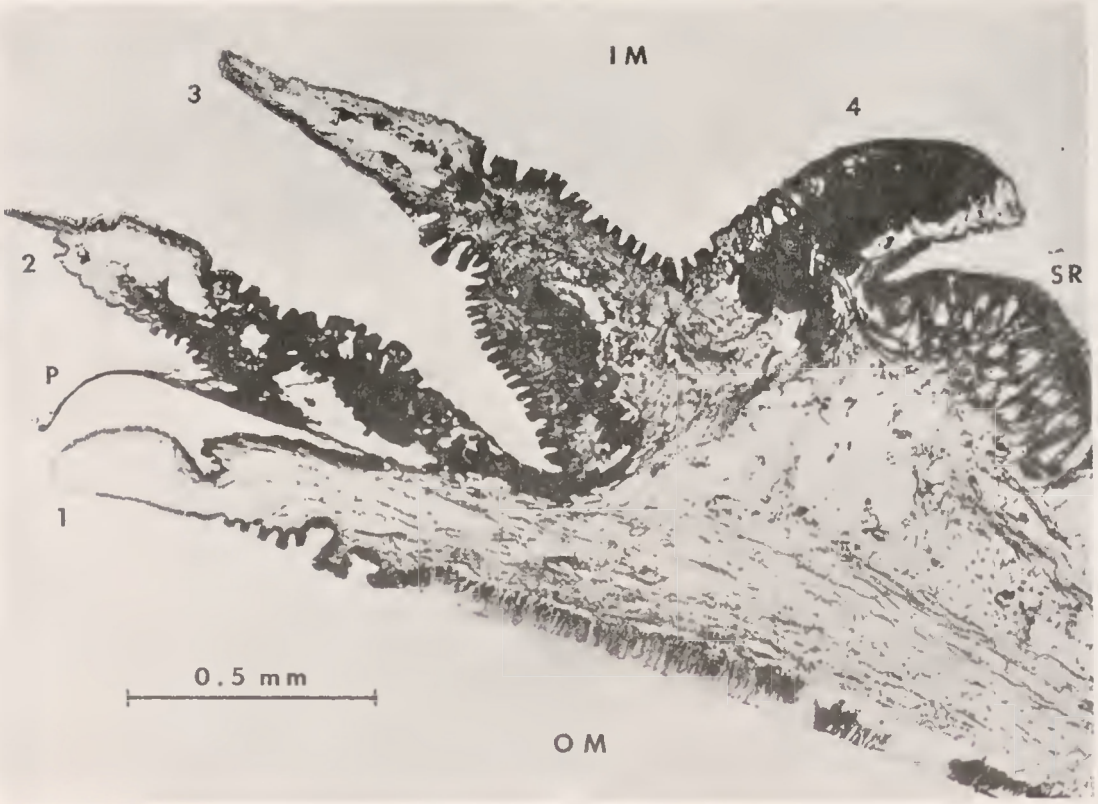

Fig. 1. Photomicrograph of a transverse section of the mantle edge from a specimen with a shell height of 4 cm. Section, stained with toluidine blue O, shows heaviest mucus concentration (darkest areas) in the second through fourth folds and in the secretory ridge.

Legend: 1, first, outer, secretory fold; 2, second, middle, sensory fold; 3, third, inner, muscular fold; 4, fourth, elaborating fold; IM, inner surface of mantle; OM, outer surface of mantle; P, periostracum; and SR, secretory ridge.

Rosso, 1954; Genesi, 1955; Beedham, 1958) to indicate that the histology and histochemistry of the bivalve mantle epithelium varies according to location and, presumably, function. For example, Beedham (1958), working with the mantle of Anodonta cygnea, suggested that the variation in histological appearance in different regions of the mantle is due in part to the relative amounts of conchiolin produced, and the secretory rate of the cells.

Although M. mercenaria is common along the east coast of North America, there is virtually nothing known of the mantle histology or histochemistry, except for work by Bevelander and Benzer (1948). The techniques referred to in our paper were selected to provide a background of information on the synthetic areas and the energy sources for synthesis and secretions in the mantle of M. mercenaria, so that more specific work can be carried on in the future. The reported results are by no means definitive.

MATERIALS AND METHODS

Transverse sections of the mantle were stained either with toluidine blue O or periodic acid-Schiff reagent. Fresh mantle tissue was treated with the G-Nadi reagent for cytochrome oxidase activity or with 2-3-5 triphenyl tetrazolium chloride for succinic dehydrogenase activity. All material to be sectioned was first embedded in paraffin. Stained sections were dehydrated through a series of ethanols, cleared in xylene, and mounted in Permount.

The following methods are referred to in this paper:

a. Toluidine blue O. Mantle tissue was fixed in Carnoy's fixative and stored in 70 per cent ethanol until used. Sections were hydrated and stained for five minutes in a 0.1 per cent aqueous solution of toluidine blue O. This stain was used by Ronkin (1952) in studies on mucus formation and secretion in the large marine snail, Busycon canaliculatum.

b. Periodic acid-Schiff reaction. This reaction (after Hotchkiss, 1948; cited by Pearse, 1954) is employed for the localization of various substances.

Sections fixed in 10 per cent neutral formalin and embedded in paraffin were hydrated and treated with periodic acid solution for 5 minutes, rinsed in 70 per cent ethanol, immersed in a reducing bath for 1 minute, again washed in 70 per cent ethanol and then stained in

Schiff's solution for 20 minutes. They were then washed in running water for 10 minutes, stained in Delafield's hematoxylin for 3 minutes, differentiated in 1 per cent acid alcohol and washed in running water for 30 more minutes. They were then counterstained with Orange G for 10 seconds and rewashed in running water for 30 seconds, dehydrated in ethanol and cleared in xylene. Permount was used as the mounting medium.

c. G-Nadi reaction. This method employed for cytochrome oxidase activity was after Moog (1943; cited by Pearse, 1954). A whole fresh mantle was incubated in "Nadi reagent" at 37 C. Two control sections were washed in sodium azide and phenylurethane solutions respectively. One section was incubated in "Nadi reagent"/ phenylurethane for 5 minutes and the other section in "Nadi reagent"/ sodium azide for the same time. All sections were washed in 0.7 per cent NaCl and mounted in 5 per cent potassium acetate. Permanent sections were not made.

d. Triphenyl tetrazolium chloride method. This method (after Straus et al; cited by Pearse, 1954) is used to localize succinic dehydrogenase activity. Fresh whole mantle was washed in phosphate buffer at pH 7.2, and then incubated in TTC at room temperature for 30 minutes. After incubation the section was again washed in the buffer solution and fixed in neutral formalin for 2 hours.

OBSERVATIONS

Histology of Mantle Epithelium

The shell-depositing, outer epithelium of the mantle consists largely of columnar epithelial cells with a small central nucleus. Most of these cells measure about 7 microns wide by 41 high; the nucleus has a diameter of 6 microns. In the region of the mantle edge, however, these cells are 4.5 microns wide by 50 high. Near the pallial line there are a large number of mucous gland cells but these decrease in number toward the umbo of the shell. The inner epithelium of the mantle, which lines the mantle cavity, is made up of cuboidal cells (11 microns wide by 15 high) with a relatively large central nucleus (diameter, 7.5 microns). The tissues in most of the mantle, that area between the pallial line and the umbo region, consist chiefly of connective and vascular tissue between the two epithelial layers. Distal to the pallial line the mantle thickens. This distal portion of the mantle, the pallial border, includes additional tissue. Muscular elements of the border, mucous cells and other secretory cells are

conspicuous. Epithelial cells of the mantle edge stain darker with toluidine blue O than do those cells proximal to the pallial line.

There are two types of mucous cells. One is cuboidal, occurring in the cuboidal epithelium and in the connective tissue beneath both cuboidal and columnar epithelium. The other type of cell is narrow and flask-shaped with a long apical region terminating in a small bulb-shaped tip. This mucous cell type is found most often in the columnar epithelium.

The periodic acid-Schiff reaction shows concentrations of Schiff-positive material around the groove between the sensory and muscular folds and in the pallial muscle fibers. The loose connective tissue also shows appreciable amounts of Schiff-positive material.

The epithelium of the outer surface of the mantle along the distal border forms a very irregular, almost corrugated surface in histological sections. The proximal portion of the outer surface of the secretory fold is also "corrugated," while the distal outer surface appears smooth.

The epithelial cells of the secretory fold are columnar and contain both basal and apical granules. At the tip of the fold, the nuclei of the epithelial cells are basal. Cells of the inner surface of the secretory fold, forming the periostracal groove with the cells of the outer surface of the sensory fold, are narrow, about 4.5 microns wide and 20 to 30 high, with basal nuclei up to 4 microns in diameter.

Epithelial cells of the outer surface of the sensory fold are small and cuboidal, while the cells of the inner surface tend to be more columnar, with a basal nucleus. Heavy concentrations of mucous cells underlie the epithelium of the sensory fold.

Both inner and outer surfaces of the muscular fold are comprised of cuboidal epithelium. Large numbers of mucous cells, which discharge mucus to the outer surface through many narrow channels, occur in the basal portion of the epithelium. Underlying the inner-surface epithelium there is another relatively heavy concentration of mucous cells which are distinct from the basally occurring concentrations.

The fourth fold (Fig. 1), possibly unique to Mercenaria mercenaria L. and related species, is smaller and more blunt than the other folds. Its distal edge is oriented opposite to the direction of the other three folds and overlaps a prominent ridge, SR, bordering it. The fourth fold contains a large amount of granular mucus.

Enzyme Activity

Cytochrome oxidase activity was demonstrated most markedly in the central area enclosed by the pallial line and in the outer edges of the mantle folds. It was relatively sparse or absent elsewhere in the folds.

Succinic dehydrogenase activity, as demonstrated by a red coloration, was also most marked within the area of the mantle enclosed by the pallial line. The groove between the sensory and muscular folds was also high in succinic dehydrogenase activity.

DISCUSSION

Generally, the lamellibranch mantle edge is considered to be divided into three folds (Beedham,1958; Morton, 1958). The presence of a fourth fold in the mantle of Mercenaria mercenaria L. is, therefore, significant and is reported here.

According to the late Dr. Thurlow C. Nelson (personal communication), the quahog, when exposed to air, is able to void mud and other debris without the use of the siphons. The mud is always mixed with large amounts of mucus. Dr. Nelson suggested that the fourth fold might function in this process.

The staining techniques (toluidine blue O and PAS) have revealed a heavy concentration of mucus in the fourth fold and in the ridge which is covered by the fold in fresh material. Since it has been observed in opened specimens that these portions of the mantle edge copiously secrete mucus and that debris collecting in their vicinity becomes coated with mucus, this mucus apparently aids in consolidating debris that collects around the periphery of the mantle. It is assumed that the mucus-coated debris is voided by ciliary and muscular action of the mantle edge.

The significance of the localization of cytochrome oxidase and succinic dehydrogenase activity is not clear at this time, except that these enzymes are associated with the energy-producing Krebs cycle (see Wilbur, 1960, for a review of energy relations in bivalve mantles). The observations reported here are only preliminary and much more work with the enzyme systems of M. mercenaria has to be done. The ultimate objective is to determine seasonal activity as well as sites of activity. When this has been done, perhaps a clearer picture of the role of the mantle in such functions as shell secretion can be obtained.

CONCLUSIONS

1. There is a fourth fold of the mantle edge of M. mercenaria. It is possible that this fold aids in voiding debris through copious secretion of mucus.

2. The histology of the epithelium in different regions of the mantle and two types of mucous gland cells, a cuboidal type and a flask-shaped cell, are described.

3. Transverse sections of mantle treated with toluidine blue O showed differences in the staining reactions to this stain in different regions of the mantle. Large mucus deposits were localized in the fourth fold and mucous gland cells were evident in the epithelium.

4. Schiff-positive material was found to be localized primarily in the connective tissue and pallial muscle fibers, and around the groove between the sensory and muscular folds.

5. The G-Nadi reaction for cytochrome oxidase and the TTC method for succinic dehydrogenase activity were performed on fresh mantle. Cytochrome oxidase activity was found to be most marked in the region from near the hinge to the pallial line and in the distal portion of the pallial border folds. Succinic dehydrogenase activity was most clearly demonstrated in the area behind the pallial line and at the juncture of the middle and inner mantle folds.

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