

## Ultrastructural and Histochemical Studies on the Mucous Granules in the Tube Feet of the Starfish *Echinaster brasiliensis*

Former electron microscopic observations on thin section of the starfish *Asterina stellifera* tube feet have revealed the presence of 3 morphologically different secretory gland cells in the epithelial layers. These glands were also distinguished by their histochemical contents<sup>1,2</sup>.

One of the gland cells (called type A) is characterized by containing granules that present a highly organized structure<sup>3</sup>. These granules are delimited by a very distinct membrane and have circular or oval profiles, an indication that in 3 dimensions they have an ellipsoidal shape, with the major diameter averaging about 1.0  $\mu$ m. They present 2 distinct regions, an external one characterized by its low opacity to the electron beam and of variable thickness, and a second central part showing a regularly organized electron dense structure. The latter region could be interpreted as a bundle of hexagonal rods, organized in a regular array, which appear in cross sections as hexagons and in longitudinal sections as parallel bars.

Electron microscopic studies on the tube feet of the starfish *Echinaster brasiliensis* revealed the presence of a different structure displayed by the granule contents of the type A secreting gland cells; as in *Asterina stellifera* these cells are located in the same position, directly above the walking surface of the tube foot. A morphological and histochemical study followed to ascertain the details of this structure, as well as the nature of its mucous contents.

**Material and methods.** Specimens of *Echinaster*<sup>4</sup> were fixed with their tube feet in a relaxed state, accomplished by anesthetizing them in a 4% aqueous solution of magnesium chloride. The fixatives used were Bouin's (24 h) and Zenker's (16 h) solutions. The paraffin embedded sections were cut at 7 and 10  $\mu$ m. Several histochemical techniques were employed to ascertain the chemical nature of the granule contents: a) the periodic acid-Schiff (PAS) technique as described by McMANUS<sup>5</sup>, blocked by acetylation (McMANUS and CASON<sup>6</sup>) and controlled by salivary amylase<sup>7</sup>; b) the Alcianblue technique at pH 2.5 for acid mucopolysaccharides after LISON<sup>7</sup>; c) the Alcian-blue technique at pH 0.5 for sulfopolysaccharides; d) the colloidal iron as described by MÜLLER<sup>8</sup>; e) the colloidal iron plus PAS<sup>7</sup>, blocked by methylation and followed by saponification<sup>9</sup>; f) meta-

<sup>1</sup> H. SOUZA SANTOS, *Experientia* 22, 812 (1966).

<sup>2</sup> H. SOUZA SANTOS and W. SILVA SASSO, *Acta anat.* 69, 41 (1968).

<sup>3</sup> H. SOUZA SANTOS, *J. Ultrastruct. Res.* 16, 259 (1966).

<sup>4</sup> J. MÜLLER and F. H. TROSCHER, *System der Asteriden*. Braunschweig. 12 lams., Figs. 44-45, 1842, p. 154.

<sup>5</sup> J. F. A. McMANUS, *Nature*, Lond. 158, 202 (1946).

<sup>6</sup> J. F. A. McMANUS and J. E. CASON, *J. exp. Med.* 91, 651 (1950).

<sup>7</sup> L. LISON, *Histochimie et Cytochimie animales; principes et méthodes*. 3rd edn. (Gauthier-Villars, Paris 1960).

<sup>8</sup> G. MÜLLER, *Acta Histochem.* 2, 68 (1955).

<sup>9</sup> S. S. SPICER and R. D. LILLIE, *J. Histochem. Cytochem.* 7, 123 (1959).

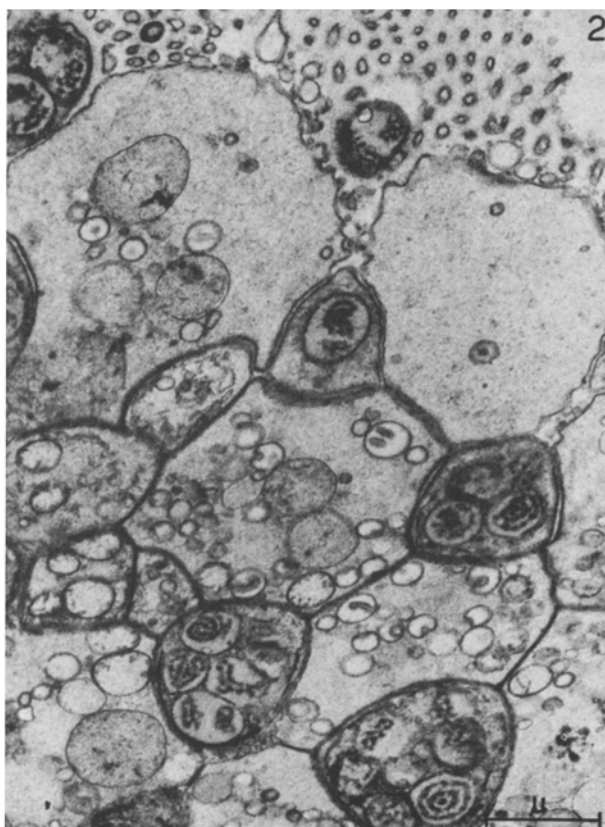
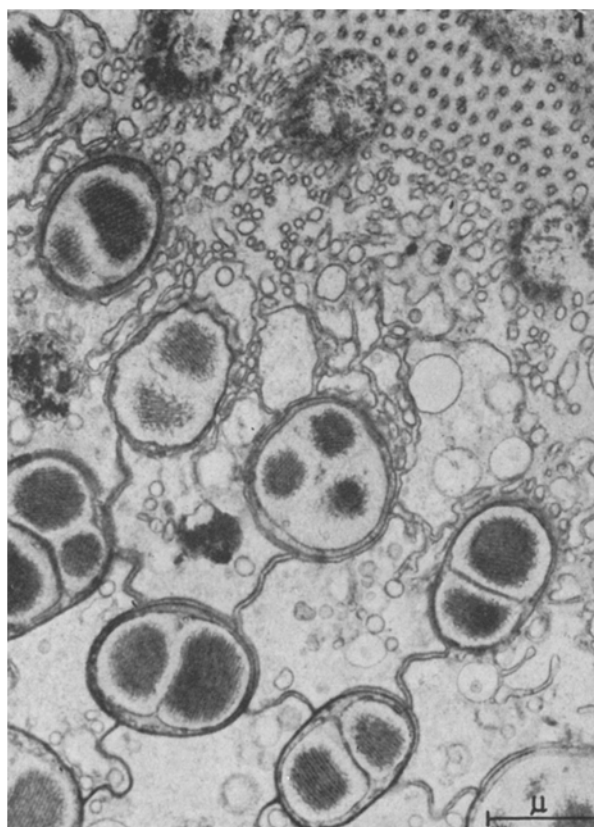


Fig. 1 and 2. Comparison of these figures shows that the same location is occupied by the mucous gland cells in the tube feet of both asteroids: 1. *Asterina stellifera*; 2. *Echinaster brasiliensis*.  $\times 15,000$ .

chromatic studies by treating the sections with 0.01% toluidine blue at pH 3.5, 4.2 and 5.4.

Electron microscopic specimens were fixed in 1.5% potassium permanganate in filtered sea water for about 1 h at 5°C. After dehydration in propylene oxide, the specimens were embedded in a catalyzed epoxy resin mixture, Epon 812, by a method similar to LUFT's<sup>10</sup>. Thin sections were cut on a Porter-Blum microtome equipped with glass knives. The sections were mounted on uncoated metal grids, doubly stained for enhancing electron contrast, first in uranyl acetate<sup>11</sup> and later in lead citrate<sup>12</sup> aqueous solutions, and examined and photographed in a Siemens Elmiskop I electron microscope, operating at 80 kV.

**Results.** Comparison of Figure 1 (*Asterina*) and Figure 2 (*Echinaster*) demonstrates that the mucous gland cells occupy the same position in the tube feet of both asteroids. The contents of the mucous granules present in the

tube feet of *Echinaster*, although also well organized, have a different arrangement compared with those displayed by *Asterina*<sup>3</sup> (Figure 3a). These contents appear as concentric circles (Figure 3b and c) or spirals (Figure 3d) in the micrographs. The components are rods (probably also having an hexagonal cross section as in *Asterina*) arranged in concentric circles about an axis. In cross sections each bundle of rods appears as circles (3c), and in oblique cuts, as ellipsis (3b) or spirals (3d) depending on the inclination of the cut. The major diameter (averaging 0.4  $\mu$ m) of the entire granule structure is about half the size of those found in *Asterina* (0.8  $\mu$ m).

The mucous granule contents in the *Echinaster* tube feet showed a intense positive reaction to PAS; exhibited a positive metachromasia only with toluidine blue at pH 5.4; were colloidal iron-positive and lightly alcian-blue positive. With colloidal iron + PAS they showed intense positive reaction to PAS. With the usual histochemical controls it was observed that after acetylation blocking, the PAS positivity was no longer obtained. The specificity of the colloidal iron reactions were investigated by methylation and the previous colloidal iron positive material was found negative. This is an indication that the granules contents is a mucopolysaccharide. The positivity to colloidal iron after methylation plus saponification, the positivity of the metachromasia at pH 5.4 and the negativity of the alcianblue at pH 0.5 exclude the possibility of a sulfated polysaccharide.

**Discussion.** The *Echinaster* mucous granules contents contain an association of neutral and acid mucopolysaccharides with a neutral predominance, whereas in *Asterina*<sup>2</sup> these granules present the same association but with an acid predominance. This difference may be responsible for the distinct morphological organization.

It was suggested<sup>3</sup> that the secretory product of the type A mucous gland cell in *Asterina* acts as a sealing lubricant that helps in the locomotion process. The loosely packed arrangement displayed by the mucous granules of *Echinaster*, when compared with the closely packed arrangement of *Asterina*, could reflect the latter's need for a larger amount of mucus at the same walking surface of the tube feet.

**Resumen.** Es estudiada la ultraestructura y la histoquímica de gránulos de secreción de células glandulares mucosas del pié ambulacrario de la estrella de mar *Echinaster brasiliensis*. Estos gránulos secretores tienen una organización distinta comparada con aquellos encontrados en la estrella del mar *Asterina stellifera* anteriormente estudiada. Así son organizados en forma de círculos concéntricos en torno de un eixo.

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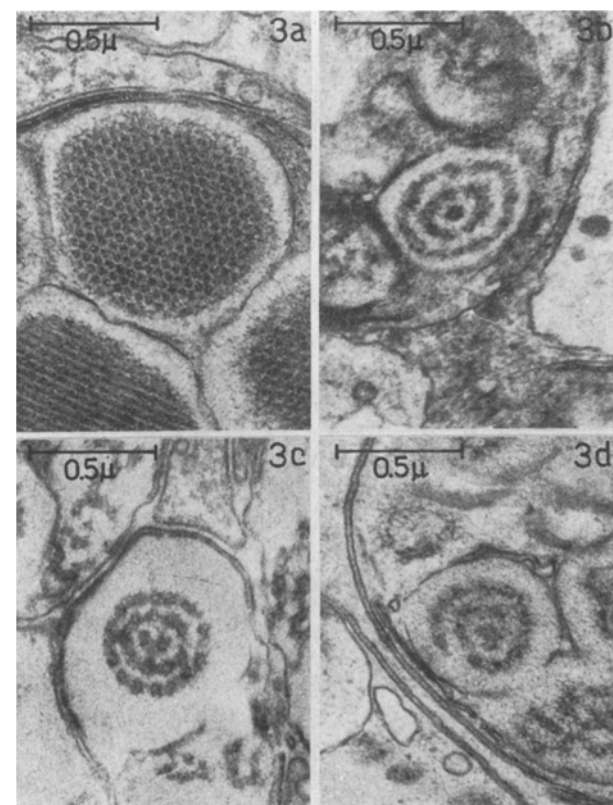


Fig. 3. a) Electron micrograph presenting type A mucous granules found in the tube feet of *Asterina*. The highly organized structure is demonstrated, the rods appearing both in transverse and longitudinal section. b and c) Mucous granules found in the tube feet of *Echinaster*. The contents of these mucous granules appear in the micrographs as concentric circles and ellipsis. d) The mucous granules contents appear as spirals in the micrograph, probably due to the length of the rods when cut obliquely.  $\times 35,000$ .

<sup>10</sup> J. H. LUFT, J. biophys. biochem. Cytol. 9, 409 (1962).

<sup>11</sup> M. L. WATSON, J. biophys. biochem. Cytol. 4, 475 (1958).

<sup>12</sup> E. S. REYNOLDS, J. exp. Med. 91, 651 (1950).

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