

# The fine structure of *Strongylocentrotus purpuratus* testes<sup>1</sup>

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**Summary.** The fine structure of *Strongylocentrotus purpuratus* testes has been examined. No obviously important differences appear to exist between the description reported here and the published fine structure of testes obtained from other sources. The most useful fixative was found to be a mixture of glutaraldehyde-paraformaldehyde.

Much information has been reported on the structural organization of sea urchin gonads at the light microscopy level<sup>2-4</sup> and some work has been done on the fine structure of sea urchin eggs<sup>5</sup>. In spite of the data accumulated by cytologists and biochemists on mature sea urchin gametes no one, to our knowledge, has yet investigated the fine structure of sea urchin testes. The purpose of this com-

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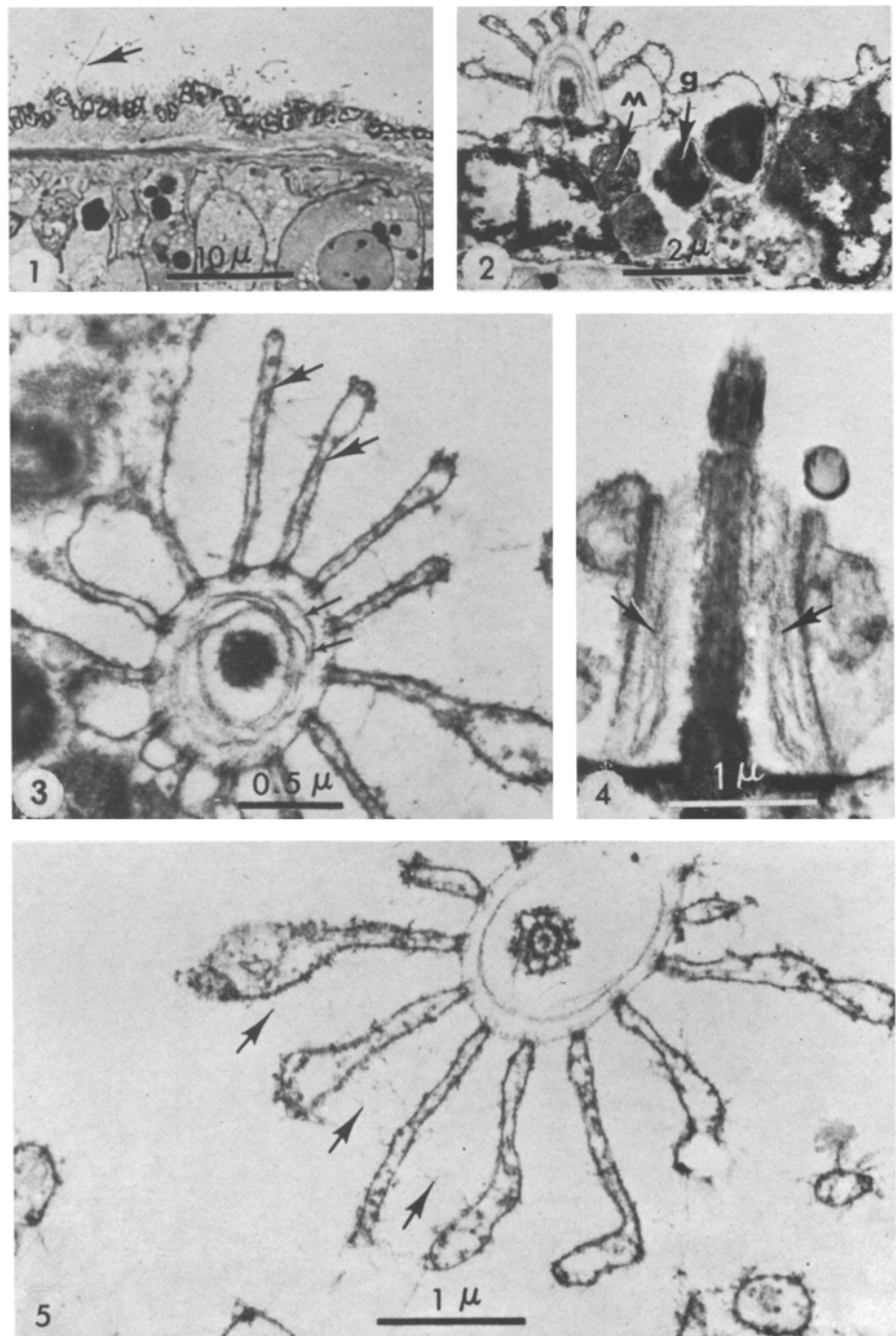


Fig. 1. Upper section of an acinus. Photomicrograph showing the ciliated epithelial layer. Cilium at arrow.  $\times 1800$ .

Fig. 2. Electron micrograph of ciliated epithelial layer. Mitochondrion (m); granule (g) filled with dark inclusions.  $\times 8700$ .

Fig. 3. Lower portion of a cilium in tangential section showing plasma membrane projections (large arrows) and a cylindrical sheath around the cilium (fine arrows).  $\times 30,000$ .

Fig. 4. Lower portion of a cilium in longitudinal section showing the cylindrical sheath (arrows) that surrounds the cilium outside the cell surface.  $\times 21,000$ .

Fig. 5. Base of a cilium in cross section showing the plasma membrane projection. Arrows indicate the fine filaments.  $\times 21,000$ .

munication is to report what appears to us to be a good preservation technique for the ultrastructural observation of the testes obtained from the sea urchin *Strongylocentrotus purpuratus*.

**Methodology.** The sea urchins were obtained from the Pacific Biomarine Supply Company, Venice, California. The animals were routinely kept in our laboratory aquaria at 15°C in natural filtered sea water. The animals were kept in the aquaria for the shortest time possible, and appeared to be in good condition. Small portions of the external part of a testis, including 2 or 3 acini, were excised under a low power microscope and placed in a petri dish containing one of the following sea water-prepared mixtures (pH 7.4): 4% for/5% glu; 6.4% glu/2% ac; 2.25% for/2% ac; 2% for /2.5% glu; 3.2% glu/1% ac; 1.1% for /1% ac; glu/parafor (50%). The ab-

brevisions used are the following: glutaraldehyde (glu); formaldehyde (for); acrolein (ac) and paraformaldehyde (parafor). 5 min after fixation at 23°C the material was placed in a 1% sea water-O<sub>2</sub>-O<sub>4</sub> solution at 4°C for 1 h. The specimens were dehydrated and embedded in Epon 812 after propylene oxide infiltration. The sections obtained were double-stained with uranyl acetate and lead citrate. The preparations were examined with a JEM 7A electron microscope.

**Results and discussion.** Figure 1 is a section through an acinus (this figure represents the only optical micrograph reported here, all the other figures are electron micrographs) and reveals a single layer of ciliated epithelial cells at the periphery which have very conspicuous nuclei and prominent cilia that possess a stalk measuring 6 µm long and 250 nm in diameter. These cells contain in their

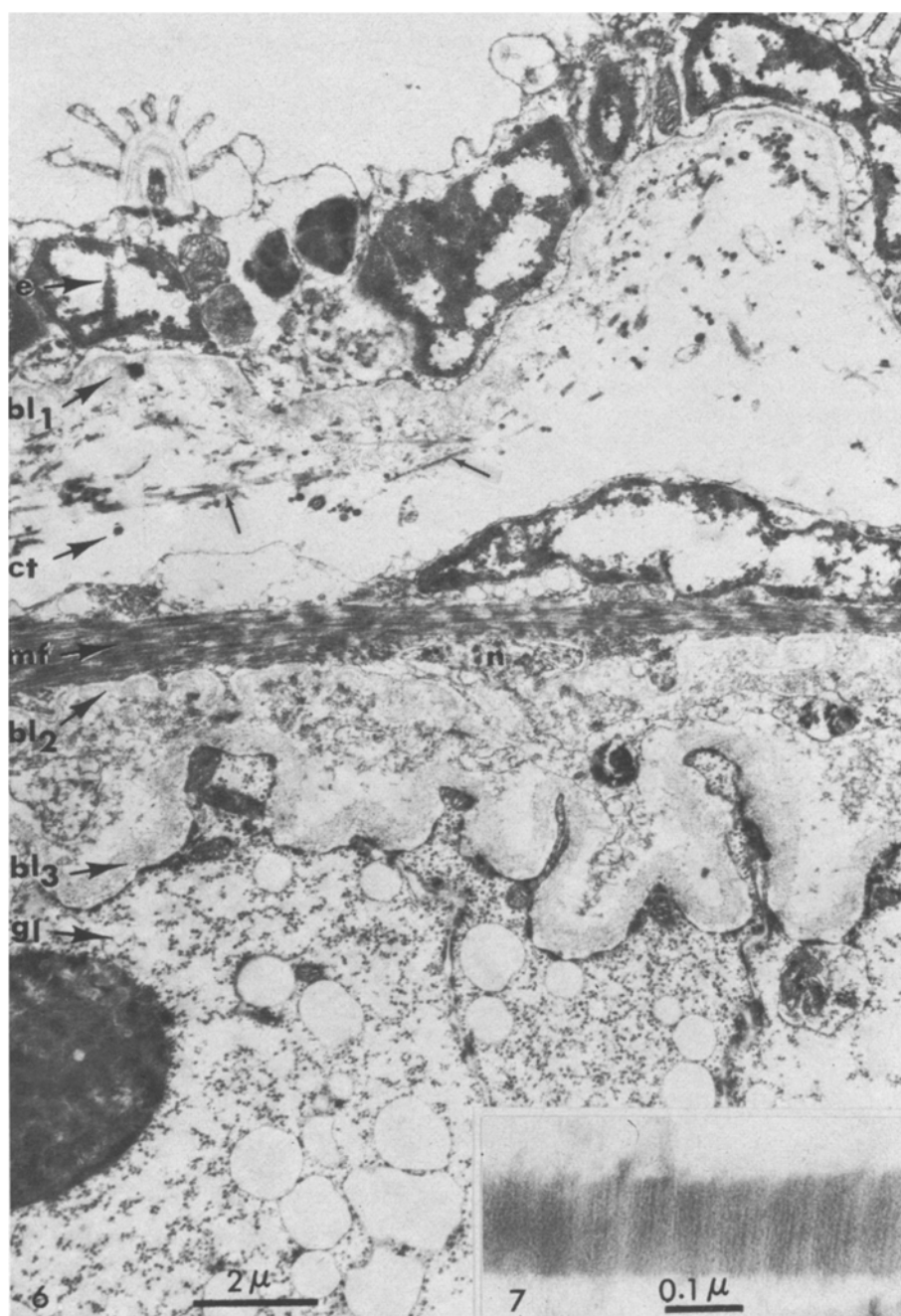


Fig. 6. Low power electron micrograph of an acinus showing the different cell layers. Epithelial layer (e); first basal lamina (bl<sub>1</sub>); connective tissue (ct); muscle fibre (mf); neuron (n); second basal lamina (bl<sub>2</sub>); third basal lamina (bl<sub>3</sub>); germinal layer (gl). Fine arrows indicate collagen fibres. × 8700.

Fig. 7. Magnified view of collagen fibre. × 108,000.

cytoplasm numerous mitochondria and granules, these are often filled with very dark inclusions (figure 2). In the area where the cilium is found projecting, the plasma membrane folds into 12–13 projections (figure 3) and a cylindrical structure surrounding a portion of the cilium outside the cell surface is observed (figure 4).

Filaments can also be seen on the cell surface, and these appear to project in the coelomic space and to be continuous with the outer leaflet of the unit membrane (figure 5). Below and lining the epithelial layer (figure 6) are found the basal lamina 1 and a connective tissue layer containing collagen fibres. Fibroblasts immediately follow this basal lamina 1 layer. The next layer is made up of muscle fibres and neurons followed by a second basal lamina, a connective tissue layer and a third basal lamina

(the thickness of the laminae 1, 2, and 3 are, approximately and respectively, 300, 200 and 400 nm).

The germinal layer that follows the third basal lamina is composed of cells undergoing spermiogenesis. The mitochondria of early spermatids are round and quite typical and appear as a unique, conspicuous, mitochondrion in late spermatids. Also, the distal centriole is found in the articular fossa. The proximal centriole is also seen and its open side faces the nucleus. It is thus apparent from these observations that the ultrastructure of sea urchin testes shows no obviously important differences with the fine structure of testes reported for other organisms, and that the glutaraldehyde-paraformaldehyde mixture appears to be the most useful fixative for these preparations.

### Morphological evidence of a polypeptide-like secretory function of the B cells in the mouse synovial membrane<sup>1</sup>

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**Summary.** In the synovial membrane of the mouse, morphological features associated with active secretion are unusually well developed in cells immediately subjacent to the lining layer (in the position of B cells), comparable to those of cells known to elaborate polypeptides.

The intimal lining of the synovial membrane is usually described as comprising 2 cell types, designated A and B cells, although distinctive morphological characteristics of these 2 types are not well defined<sup>3–8</sup>. The A cells form the lining layer of the synovial cavity. B cells, which lie under the A cells, have a more highly developed rough endoplasmic reticulum, which suggests that they may secrete proteins<sup>4</sup>. Our electronmicroscopic observations

of synovial membranes in the mouse have revealed that, in this species, ultrastructural evidence of polypeptide synthesis is striking in cells occupying the position of B cells. Cells which show such morphological specialization characteristic of cells primarily involved in polypeptide secretion have not, to our knowledge, been described in any other type of connective tissue.

**Methods.** The phalangeal, metatarso-phalangeal and knee joints were taken from female and male mice after intracardiac perfusion with 5% glutaraldehyde solution (phosphate buffer 0.1 M, pH 7.4). After 24 h in the same fixative, followed by 5–8 days decalcification in fixative with 0.1 M EDTA, they were divided into sagittal sections, post-fixed in 2% osmic acid and embedded in araldite-epon. Semi-thin sections (1  $\mu$ m) were colored using several techniques for histological studies. Thin sections were contrasted with uranyl acetate and lead citrate.

**Results and Discussion.** Under the layer of interdigitated processes of A cells which forms the lining of the synovial cavity, there are many cells, clustered in islets or dispersed, which are characterized by an abundance of small, dense cytoplasmic granules. These cells were never observed in direct contact with the synovial cavity. They are separated from the fibrous stroma by a basal lamina which does not extend into the islets. Although they sometimes present an epithelioid arrangement, individual cells are always separated by a narrow connective space

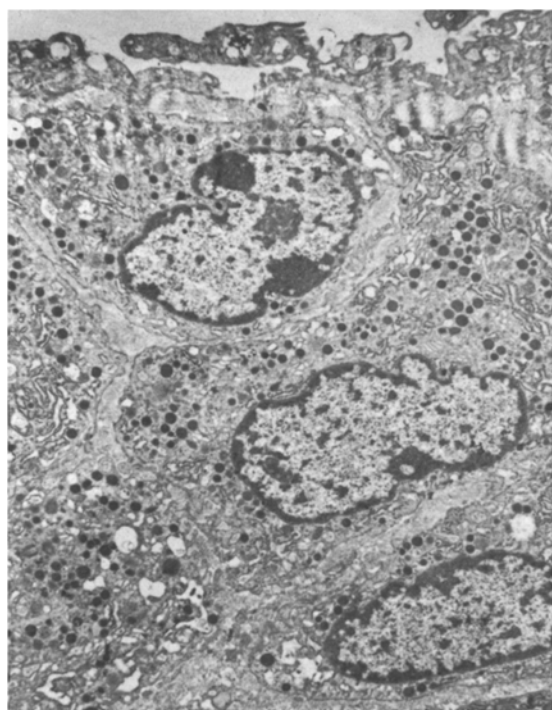


Fig. 1. Cluster of B cells with dense secretion granules under the epithelioid layer lining the synovial cavity.  $\times 6000$ .

- 1 This work was supported by grant No 74.1.133.4, Institut National de la Santé et de la Recherche Médicale, France.
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