

electrodes had resistances in the range of 50–100  $M\Omega$  and recorded larger resting and secretory potentials than the other. The methods of recording membrane potential have been described previously<sup>8,9</sup>.

The experiments were performed in the following manner. An acinus was impaled with a Procion electrode and, provided the resting potential did not decline, the salivary duct nerves were stimulated to elicit a secretory potential. Procion was injected into the acinus by passing hyperpolarizing current pulses (10–20 nA, 100 msec) at 5 Hz through the microelectrode. Procion injection was continued for periods of 5 min, interrupted for about 1 min solely to monitor resting and secretory potentials. The microelectrode remained inside the cell for up to 30 min as judged by these criteria. The salivary gland was removed from the chamber about 30 min after the end of the experiment and fixed in 10% formalin. After wax embedding, 10  $\mu$ m sections were prepared and mounted in Gurr's Uvinert mountant for examination by fluorescence microscopy and then photographed. The cover slips were floated off the slides by prolonged immersion in xylene and the tissue sections stained with Ehrlich's Haematoxylin and Eosin and finally mounted in Gurr's XAM for examination by light microscopy.

**Results and discussion.** In 11 experiments it was demonstrated that the site of microelectrode recording of secretory potentials was the interior of gland cells. The Figure shows representative results from 2 experiments where responses were obtained from each of the two cell types present in the acinus. Examination with light microscopy (d) established that the cells stained with Procion under fluorescence microscopy (a) were peripheral and central cells. In sections stained with Haematoxylin and Eosin the peripheral cells appear pink, are generally pyramidal in shape and show a prominent brush border lining the lumen of an intracellular ductule. In Figure a) (peripheral cell) the lumen of this ductule can be seen to be distended and free of Procion Yellow. Some of the dye appears to have spread from the peripheral cell across its apical margins to neighbouring central cells. The central cells stain purple to varying degrees, show the presence of large granules and do not contain an intracellular

ductule since they discharge their secretion into the central lumen of the acinus<sup>8</sup>. It can be seen in Figure a) (central cell) that Procion Yellow has been deposited preferentially in the nucleus, although this may be fortuitous.

In 9 other experiments it was found that 3 of the cells were peripheral and 6 were central. Thus it seems that responses can be recorded from either kind of cell and therefore, in the light of the previous evidence<sup>8</sup>, from any cell in an acinus. It seems unlikely that each cell has its own innervation in view of the sparse number of nerve fibres running to acini<sup>10</sup>. Although this possibility cannot be excluded by the present experiments, it seems more probable that acinar cells, even of different kinds, are coupled electrically. It might be that a secretory potential is evoked in a single cell, or perhaps a few cells, and spreads electrotonically. Electrophysiological evidence for electrical coupling in this gland has already been reported<sup>9</sup> and is consistent with the presence of septate desmosomes<sup>8</sup> between acinar cells. Also consistent with this suggestion is the observation that in the experiments described above (peripheral cell, a), Procion Yellow appears able to spread from one cell to another; however, further experiments are required to investigate this point.

**Summary.** Secretory potentials evoked by nerve stimulation have been recorded from both types of cell (peripheral and central) present in the acini of cockroach salivary glands.

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<sup>12</sup> J. N. BARRETT, in *Intracellular Staining in Neurobiology* (Eds. S. B. KATER and C. NICHOLSON; Springer-Verlag, New York 1973), p. 283.

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## A Preliminary Report on the Fine Structure of *Tripneustes esculentus* Eggs<sup>1</sup>

The past decade has given us much information about the structural organization of unfertilized, fertilized and centrifuged sea urchin eggs<sup>2–4</sup>, but the methods of fixation employed in those studies resulted mostly in general coarse preservation of cytoplasmic organelles.

It is essentially because of the importance of the sea urchin egg in the study of embryology that work involving modern fixation methods of this material for electron microscopy observation has been in progress in our laboratory for some time. Our aim was to arrive at a suitable fixation procedure that enable us to make more meaningful interpretations of the ultrastructure of the eggs in normal or experimental development.

The purpose of this communication is to report the best preservation for eggs of the sea urchin *Tripneustes esculentus* so far obtained through extensive screening and modification of various methods.

**Methodology.** Sea urchins of the species *Tripneustes esculentus* and sea water were obtained from the Caribbean Research Centre, Martinique, and kept at 22°C for a few hours. The eggs, obtained by injection of 0.53  $M$  KCl in the vicinity of the oral region, were gently filtered through

cheese-cloth and repeatedly washed with millipore-filtered sea water. The eggs were then fixed in the following pH 7.4 mixture: Glutaraldehyde-paraformaldehyde (50%), prepared in Martinique sea water. The chemicals were mixed previous to use, and the washed cells were suspended in this solution for only 5 min at room temperature approximately (23°C).

Since longer fixation periods can result in leaching out of the pigment granules, the fixation time was kept at a minimum, and 5 min was found to be most appropriate. This was followed by treatment of the eggs with a 1% solution of osmium tetroxide ( $OsO_4$ ) in sea water at 4°C. The eggs were then dehydrated in Epon using standard techniques. To improve contrast, the sections were double-stained with uranyl nitrate and citrate. The preparations were examined with a JEM 7-A electron microscope.

<sup>1</sup> This work was supported by a grant from the National Research Council of Canada.

<sup>2</sup> F. W. FLITNEY, J. R. microsc. Soc. 85, 353 (1966).

<sup>3</sup> M. GOUSKENS, Expl Cell Res. 39, 413 (1965).

<sup>4</sup> J. J. PASTEELS, P. CASTIAUX and G. VANDERNEERSCHKE, Arch. Biol. 69, 627 (1958).

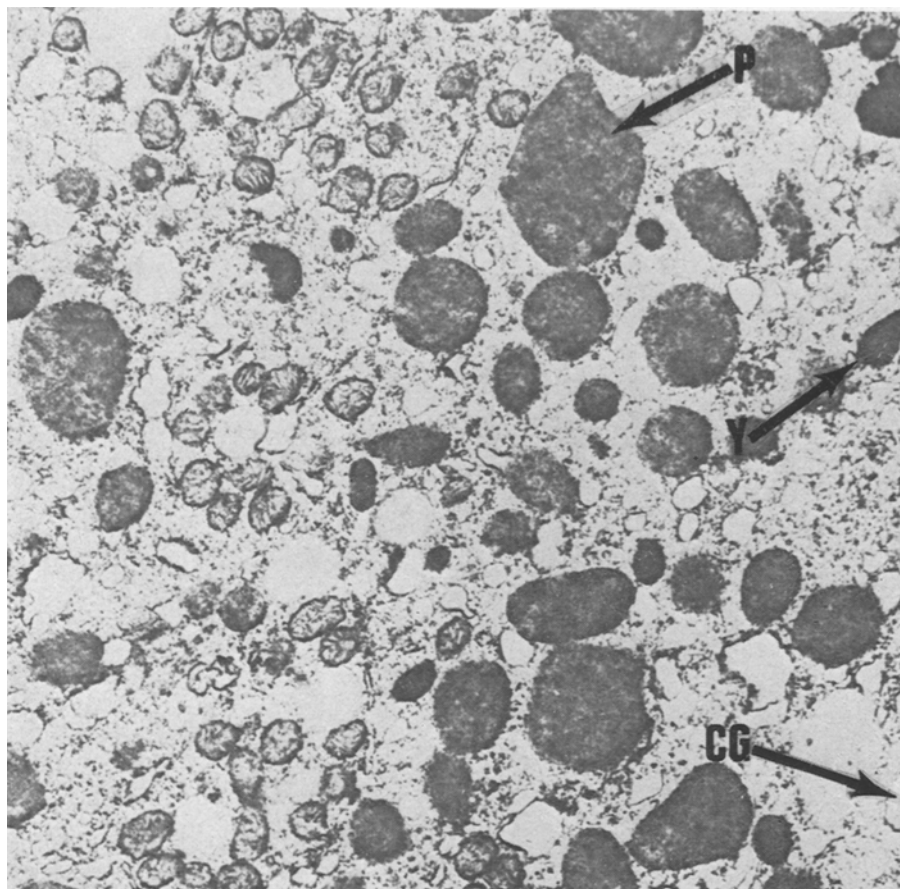


Fig. 1. Low power view of an unfertilized sea urchin egg (*Tripneustes esculentus*) fixed for 5 min in a mixture of paraformaldehyde and sea water at pH 7.4. Y, Yolk granule; P, pigment granule and CG, cortical granule.  $\times 10,000$ . Further details in text.

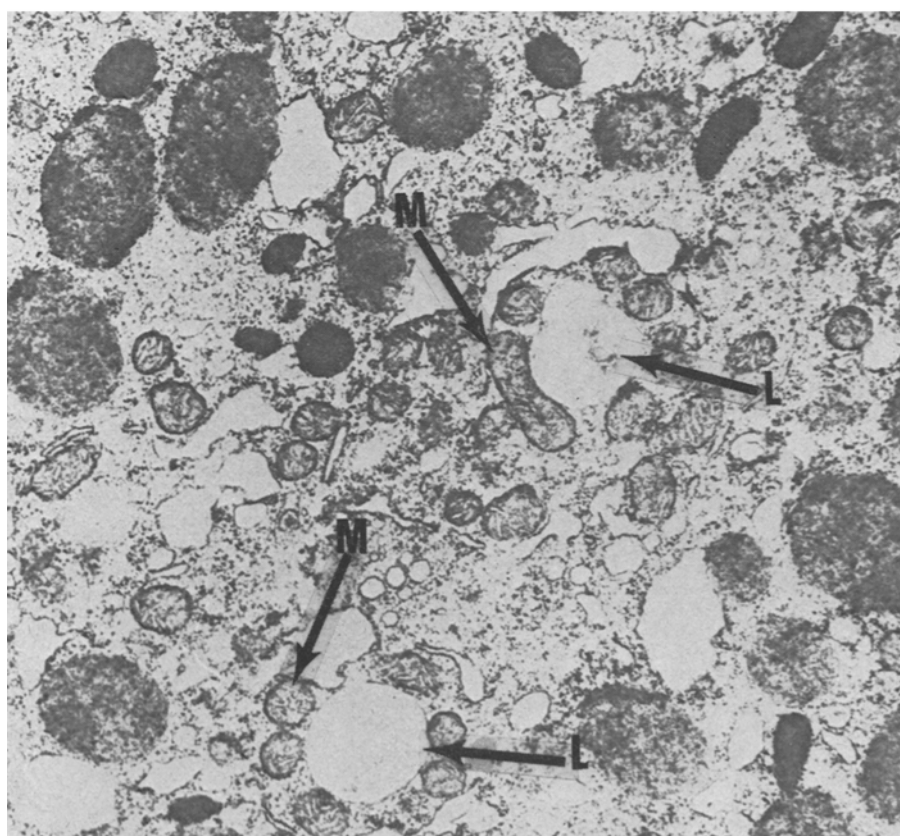


Fig. 2. Internal region of a sea urchin egg fixed as for Figure 1 and showing a rosette-like structure composed of mitochondria (M) surrounding a lipid granule (L).  $\times 24,000$ .

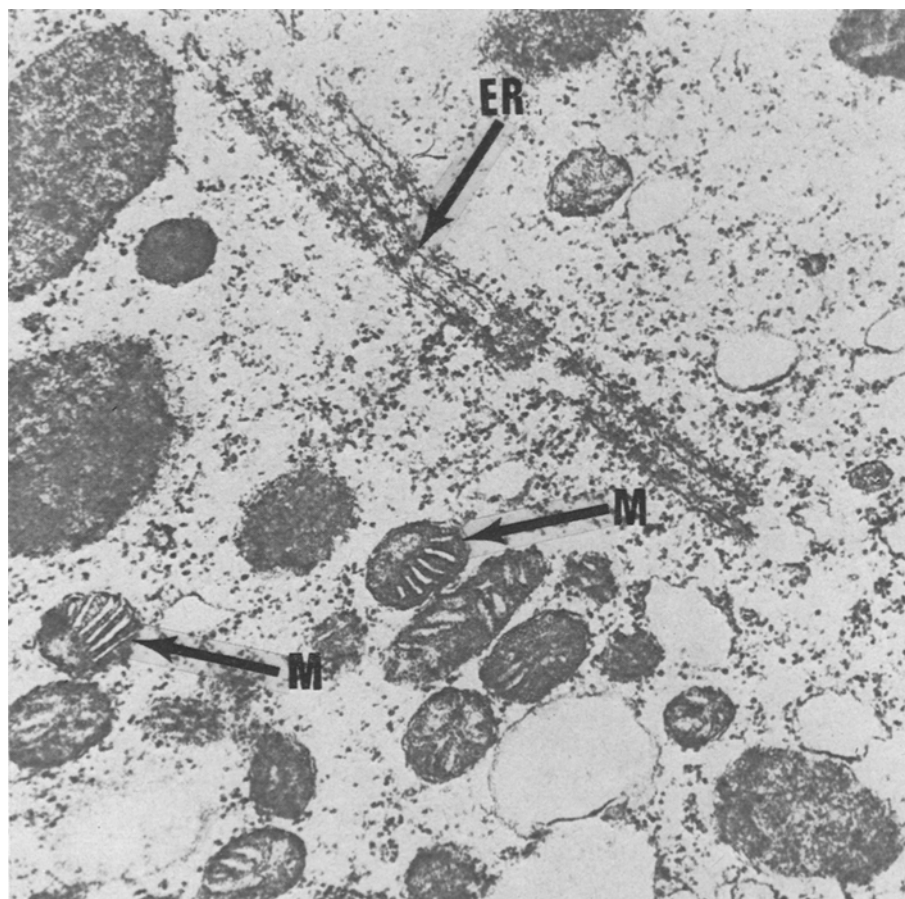


Fig. 3. Enlarged view of mitochondria (M) and endoplasmic reticulum (ER).  $\times 44,000$ .

**Results.** Figure 1 represents a low power view of the egg and contains a portion of the nucleus and various cytoplasmic organelles. The debris seen at the outer surface of the membrane probably represents remnants of the jelly coat. The overall smoothness of fixation is well demonstrated in the micrograph as evidenced by a reduction of cellular contraction following fixation. Although the preparations show some undulations at the egg surface these are not as pronounced as the classical microvilli-like projections. The section of the membrane measures about 200 Å in thickness. The pigment granules are well scattered all over the cytoplasm, some lying between cortical granules in the periphery of the egg. They can be distinguished from the yolk granules by their dark appearance and generally smaller size. The yolk granules are characterized by a granular sub-structure, and often contain somewhat electron opaque areas of 130–150 Å in diameter.

The lack of microvilli-like projections and the spacing of the cortical granules thus suggest that there has been very little shrinkage during fixation, and the inner morphology of the cortical granules seems to have been well preserved.

Mitochondria are often found in the form of a rosette surrounding a lipid granule (Figure 2) and display a peculiar orientation of cristae. Rosettes have also been reported by PASTEELS<sup>4</sup> in sea urchin eggs, and the appearance of the lipid granules, when compared with osmium-fixed cells, indicate some solubilization of lipids<sup>2</sup>.

Other structures of the cytoplasm are nevertheless quite well preserved, e.g., endoplasmic reticula, Golgi apparatus, annulated lamellae and many small vesicles (Figure 3). The cytoplasmic matrix is loaded with ribo-

somal-like particles 230–290 Å in diameter. However, some of these could represent the  $\beta$ -type glycogen granules. The nuclear membrane shows its characteristic porous nature and the nucleus is filled with a fine fibrillar material about 40 Å in thickness. No chromosomal differentiation is apparent in this stage. In all cases observed, *Tripneustes esculentus* egg fine structure appears to be quite similar to eggs obtained from the sea urchin *Arbacia punctulata*. Although we are still far from the ideal sea urchin egg fixatives, it appears evident, from this work, that the glutaraldehyde-paraformaldehyde mixture (when this is used for a *short* period of time) shows the greatest promise for an electron microscope study of *Tripneustes esculentus*.

**Summary.** The fine structure of *Tripneustes esculentus* eggs was studied with the aid of an electron microscope. Cells obtained from this West Indies sea urchin showed cortical granules, mitochondria forming a rosette around lipid granules, endoplasmic reticula, Golgi apparatus and annulated lamellae. These structures appear identical to those seen in eggs of the Atlantic sea urchin: *Arbacia punctulata*.

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