

## Ultrastructure of the optic gland of the squid *Sepiotheutis sepioidea* (Cephalopoda: Loliginidae)

Dwight Arrieche

Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente. Cumaná, Sucre, Venezuela.  
Fax 58-93-514023. Tel. 58-93-514185. E-mail: darriech@cumana.sucre.udo.edu.ve

Received. 9- II-1998. Corrected. 07-IV-1999 Accepted. 16-IV-1999.

**Abstract:** I describe the fine structure of the optic gland (OG) in the tropical squid *Sepiotheutis sepioidea* at different sexual maturity stages. Squids were collected in Mochima National Park, Venezuela (10° 20' 22 " – 10° 24' N; 64° 19' – 64° 22' W). Morphometric characteristics and the development of reproductive organs at different sexual maturity stages (female n = 101; male n = 105), were significantly different (ANOVA, p < 0.001). The female gonadal index mean (GI = (ovary weight / body weight) x 100) between 0.1 - 2.95 %, and the male GI mean (GI = (testicle weight / body weight) x 100) oscillated between 0.22 - 1.38 %. The OG are a pair of oval organs located at the posterior edge of the optic tract of the cephalopod brain. The OG showed the same cellular types described in other cephalopods, with slight differences. The secretory cells in young individuals present a dendritic profile with thin cytoplasmic processes, numerous circular mitochondriae with tubular crests and abundant ribosomes. At the last stage of maturity the secretory cells contain rough endoplasmic reticulum in the form of dilated cisternae, with an active Golgi apparatus, secreting electron dense granules. Mitochondriae are scarce and lipofuchsin granules show an increase in size. Secretory cell nucleus length and organelle abundance showed oscillations associated with the life cycle.

**Key words:** Optic gland, cephalopod, *Sepiotheutis sepioidea*, ultrastructure.

In cephalopods the optic glands (OG) form a pair of oval organs located at the posterior edge of the optic brain tract, above the *hilum* of the optic lobes (Boycott & Young 1956). OG histology has been described for *Loligo opalescens*, *Octopus vulgaris*, *O. bimaculatus*, *Eledone moschata* and *Sepia officinalis*. The OG consists of main or secretory cells, blood vessels, nerve cells and support cells (Björkman 1963, Nishioka *et al.* 1966, Barber 1967, Bonichon 1967, Froesch 1974 and Mangold & Froesch 1977).

The role of the OG in the cephalopod life cycle is analogous to that of the hypophysis which controls sexual development in vertebrates (Wells & Wells, 1959, 1969, Wells 1960 and Wells *et al.* 1975). These glands also regulate protein synthesis in the ovaries, stimulate gonadal mitosis, mating behavior, feeding and longevity (Durchon & Richard 1967, O Dor & Wells 1973, Wells *et al.* 1975, Wodinsky 1977 and Mangold 1987). The OG hormone is species specific, not sex specific (Wells & Wells 1975), and glandular secretions have

periodic oscillations associated with temperature and photoperiod (Defretin & Richard 1967 and Richard 1966, 1967 a, b, 1970). The glands are controlled by a reactive peptidergic innervation (Le Gall *et al.* 1988), and their activity varies during the life cycle, associated with the multiplication of reproductive cells (Koueta *et al.* 1995).

The fine structure of the gland suggests that it has several functions. Björkman (1963) suggested that the secretory cells had a high rate of synthesis. The presence of abundant mitochondria implies that the gland may secrete a steroid hormone (Froesch 1979). In *S. officinalis*, however a peptidic like hormone was shown to be present in the OG and hemolymph (Koueta *et al.* 1992). The lipofuchsin granules have been related to the resorption of metabolic residues inside the secretory cells (Froesch & Mangold 1976, Mangold & Froesch 1977 and Froesch *et al.* 1987).

In this study the fine ultrastructure of the tropical squid *Sepiotheutis sepioidea* (Blainville, 1823) (Cephalopoda: Loliginidae) was examined in both sexes at different sexual maturity stages, using Transmission Electron Microscope (TEM) in order to compare secretory cell changes during the life cycle of this specie.

## MATERIALS AND METHODS

206 individuals of *S. sepioidea* were caught (net mesh size 20 mm Ø) in Mochima National Park, Venezuela (10° 20' 22 " -10° 24' N; 64° 19' -64° 22' W). They were acclimatized in tanks (1 500 L) with running sea water, and fed *ad lib.* for at least a week (Estación José G. Hernández, IDEA-Fundaciencias). Each specimen was weighed (body weight BW ± 0.05 g) and the dorsal mantle length (DML ± 0.1 cm) was measured before decapitation, followed by dissection of the OG, with the aid of a stereomicroscope. The OG was preserved by immersion as quoted below for light microscopy (LM) and TEM.

The mantle with the reproductive organs was preserved with 4% formalin (24 h), and

then washed in running tap water and the reproductive organs were removed and measured (± 0.05 cm; ± 0.0001 g). The sexual maturity stage was determined for each specimen as follows: juvenile (I), immature (II), in maturation (III), mature (IV), and post-reproductive (V). This last was identified only in copulating males, and post-reproductive females were not captured. The sexual maturity stages were determined using the measurements of reproductive organs quoted for this species (Robaina & Voglar 1986, Voglar & Robaina 1987), and the female sexual maturity stages were estimated using the nidamental gland index (NGI = (Nidamental Gland length/ Dorsal Mantle length)) (Durward *et al.* 1979). Gonadal index (GI) was estimated as the relationship between gonad weight and body weight, in the females the gonadal index was calculated using the formula (GI = (ovary weight / body weight) x 100), and in the males this was calculated as (GI = (testis weight / body weight) x 100). An analysis of variance (ANOVA) was used to test for significant differences in the biometric parameters between the sexual maturity stages (females) and the dorsal mantle length intervals (males), these data were compared using an *a posteriori* Scheffe test (Sokal & Rohlf 1981).

For LM, samples including the optic lobe and the optic stalk were immersed in Gilson fixative (2 – 6 h), and then well washed in running tap water, dehydrated and embedded in paraffin. Paraffin sections (6 µm) were stained with Hematoxylin-Eosin (HE), Periodic acid Schiff reaction (PAS) and Herlant tetrachromic stain (Humason 1979). LM sections were analyzed using an image analysis system (Javelin Smart Cam) adapted to a light microscope, with a digital micrometer.

For TEM, the brain was prefixed by immersion in either of the following fixatives (30 min.) with some drops of 0.1% (w/v) methylene blue (vital stain)(Bern, H., pers. com.): 2.5% glutaraldehyde (GA) in 0.1M phosphate buffer saline pH 7.8; 2.5 % GA in 0.1M sodium cacodylate buffer pH 7.8; 3% GA, 20% sucrose (w/v), 0.5% CaCl<sub>2</sub>, 1% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, 0.1% picric acid in 0.1 M sodium

cacodylate buffer (Evans *at al.* 1976). The brain was then washed in the same buffer, and the OG dissected from the cerebral tissue followed by one hour fixation in fresh fixative. The OG was washed, post-fixed (1% OsO<sub>4</sub>, 90 min.), *in bloc* stained (2% uranyl acetate, 30 min.), and processed for conventional TEM. Thin sections were contrasted with uranyl acetate and lead citrate (Reynolds 1963), and observed in a H-600 TEM.

## RESULTS

The OG is located over the optic stalk close to the optic lobe, it is covered by a fine connective layer, and has an elongate shape. The OG histology is well differentiated from the cerebral tissue, the OG cells are in a compact mass, whereas cerebral cells are larger, and form layers surrounding eosinophylic nerve fibers (Fig. 1). At the juvenile maturity stage the

OG is almost undifferentiated from the cerebral tissue under a stereoscope, having a reddish coloration in individuals with a larger dorsal mantle length, mainly mature females. The female gonadal index (GI) oscillated between 0.1 - 2.95 %, and the nidamental gland index between 0.9 - 0.28 %, with significant differences between the sexual maturity stages (Table 1). The reproductive organs in the males showed a tendency to increase with dorsal mantle length, but the spermatophore number was associated with reproductive behaviour, GI oscillated between 0.22 - 1.38 % (Table 2). These data had significant differences (ANOVA,  $p < 0.001$ ), but in an *a posteriori* Scheffe test the means did not differ (Table 3, 4).

Paraffin sections give a clear picture of OG organization. The secretory cells are the most numerous cells, they are ovoid with cytoplasmic processes (18.5 - 25.6  $\mu\text{m}$ ), and the nucleus (9.11 - 11.38  $\mu\text{m}$ ) is circular with a dense nucleoli, both showing a tendency to

TABLE 1

*Biometric parameters of the female reproductive organs in the different sexual maturity stages (average  $\pm$  standard deviation; N = sample size). The reproductive organs show a tendency for linear increase in size between the sexual maturity stages, including the diameter of the nucleus. Sample size is indicated below the mean and standard deviation.*

Sexual Maturity Stage	I	II	III	IV
Dorsal Mantle length	71.43 $\pm$ 16.24 N = 35	106.27 $\pm$ 12.07 N = 34	116.59 $\pm$ 12.07 N = 15	135.80 $\pm$ 13.62 N = 17
Body weight	33.90 $\pm$ 18.87 N = 36	84.73 $\pm$ 24.98 N = 35	115.95 $\pm$ 48.03 N = 15	166.54 $\pm$ 43.37 N = 17
Ovary length	7.41 $\pm$ 3.02 N = 31	11.80 $\pm$ 3.69 N = 34	16.10 $\pm$ 5.18 N = 15	36.01 $\pm$ 6.38 N = 16
Ovary weight	0.03 $\pm$ 0.02 N = 34	0.09 $\pm$ 0.04 N = 35	0.35 $\pm$ 0.30 N = 15	4.06 $\pm$ 2.83 N = 17
Oviducal Gland length	11.59 $\pm$ 3.78 N = 30	18.21 $\pm$ 4.88 N = 33	24.63 $\pm$ 7.07 N = 15	45.91 $\pm$ 6.61 N = 14
Nidamental Gland length	7.59 $\pm$ 1.29 N = 22	11.99 $\pm$ 1.58 N = 30	19.01 $\pm$ 3.09 N = 13	37.74 $\pm$ 4.37 N = 17
Nidamental Gland Index %	0.09 $\pm$ 0.02 N = 22	0.11 $\pm$ 0.014 N = 14	0.16 $\pm$ 0.04 N = 13	0.28 $\pm$ 0.037 N = 17
Gonadal Index %	0.07 $\pm$ 0.03 N = 34	0.11 $\pm$ 0.04 N = 35	0.34 $\pm$ 0.37 N = 15	2.95 $\pm$ 2.35 N = 17
Nucleus diameter $\mu\text{m}$	9.11 $\pm$ 1.11	9.33 $\pm$ 1.12	9.74 $\pm$ 1.50	11.38 $\pm$ 1.24

TABLE 2

*Biometric parameters of the male reproductive organs in the different sexual maturity stages. The individuals were grouped in mantle length intervals, the nucleus diameter was averaged at the fourth maturity stage. The oscillation of the espermatophores at the fourth maturity stage is associated with copulation. Sample size is indicated below the mean and standard deviation.*

Sexual Maturity Stage	II	III	IV	IV	IV	IV
Mantle length interval	40 - 59	60 - 79	80 - 99	100 - 119	120 - 139	140 - 159
Dorsal Mantle length	53.14 ± 3.88 N = 4	71.27 ± 5.66 N = 22	92.77 ± 4.78 N = 29	110.48 ± 5.58 N = 29	127.84 ± 4.62 N = 15	145.67 ± 7.20 N = 5
Body weight	16.13 ± 2.09 N = 4	30.77 ± 6.11 N = 29	62.19 ± 13.98 N = 29	89.56 ± 11.40 N = 29	125.72 ± 14.31 N = 15	176.50 ± 23.91 N = 5
Testicule length	5.50 ± 1.96 N = 4	11.53 ± 4.08 N = 23	20.85 ± 3.73 N = 26	23.05 ± 2.35 N = 29	27.89 ± 2.81 N = 12	31.13 ± 6.28 N = 4
Espermatophoric Organ length	4.42 ± 0.95 N = 3	7.85 ± 2.46 N = 24	12.44 ± 1.66 N = 26	13.35 ± 1.82 N = 29	15.44 ± 2.54 N = 13	18.65 ± 1.80 N = 5
Espermatophores	-	43 ± 50 N = 13	143 ± 73 N = 24	212 ± 87 N = 28	223 ± 92 N = 13	203 ± 44 N = 5
min. - max. Espermatophore length	-	2 - 152 4.78 ± 1.91 N = 6	40 - 279 8.91 ± 1.06 N = 23	47 - 367 10.46 ± 0.66 N = 29	94 - 376 11.06 ± 0.39 N = 13	155 - 264 12.37 ± 0.48 N = 5
Gonadal index %	0.22 ± 0.25 N = 3	0.78 ± 0.63 N = 23	1.53 ± 0.47 N = 26	1.42 ± 0.24 N = 28	1.46 ± 0.28 N = 12	1.38 ± 0.18 N = 5
Nucleus diameter $\mu\text{m}$	9.25 ± 1.50	10.24 ± 1.19	10.93 ± 1.12	-	-	-

TABLE 3

*Analysis of variance (ANOVA) and a posteriori Scheffe test of female morphometric parameters at the different sexual maturity stages.*

Variable	ANOVA	Fs	Degrees of Freedom	Scheffe	Means
Dorsal Mantle length	***	91.729	3/97	9.74	*
Body weight	***	76.052	3/99	21.03	*
Ovary length	***	165.231	3/92	3.05	*
Ovary weight	***	55.319	3/97	0.79	I - III; IV
Oviducal Gland length	***	141.538	3/88	3.78	*
Nidamental Gland length	***	585.428	3/81		
Nidamental Gland Index	***	205.903	3/79	0.02	*
Gonadal index	***	40.749	3/97	0.99	I - III; IV

\* : 0.01 < p < 0.5). \*\*\* : p < 0.001

TABLE 4

Analysis of variance (ANOVA) and a posteriori Scheffe test of the males morphometric parameters at the different sexual maturity stages.

Variable	ANOVA	Fs	Degrees of Freedom	Scheffe	Means
Dorsal Mantle length	***	397.153	5/99	4.91	*
Body weight	***	220.758	5/105	11.38	*
Testicule length	***	68.134	5/92	3.22	40-59; 60-79; 80-119; 120-139; 140-159.
Espermatophores	***	11.736	5/78	72	60-79; 80-119; 100-119.
Espermatophore length	***	81.11	5/71	0.85	60-79; 80-99; 100-139; 140-159.
Gonadal index	***	12.849	5/91	0.60	40-79; 80-159.

\* :  $0.01 < p < 0.5$ . \*\*\* :  $p < 0.001$

increase in size with the maturity stages (Table 1, 2). The blood vessels are prominently dispersed throughout the OG. Support cells are scarce, in some sections they are seen to be aggregated, with a characteristic round and basophilic nucleus (Figs. 1, 2, 3, 4).

At the ultrastructural level OG cytoarchitecture denotes a complex array of fine cell processes intermingled between the main cells, blood vessels, nerve cells and support cells (Figs. 5, 6). The main cell profile is oval with thin cytoplasmic processes, its organelles are very numerous giving a dense appearance. The nucleus is euchromatinic with scarce heterochromatin, the nucleoli have a clear *pars amorpha* surrounding a darker *nucleolonem* (Figs. 5, 7). Ribosomes are numerous, and are found dispersed throughout the cell, associated with polysomes dispersed in the cytoplasm, rough endoplasmic reticulum, and covering clear cytoplasmic vesicles (Figs. 8, 10, 11). The rough endoplasmic reticulum cisterns are thin, and extend from the nuclear envelope to the cellular membrane (Figs. 8, 9, 19), at the secretory stage the endoplasmic reticulum cisterns are aggregated in swollen concentric sacules (Figs. 12, 13). The Golgi apparatus is active, it secretes clear and electron dense vesicles and there are at least two Golgi complexes in each cell associated with rough endoplasmic reticulum, and lipofuchsin granules. At the mature sexual stage (IV) the Golgi is very

active secreting numerous electron dense granules (Fig. 12). Mitochondriae are elongated or oval with tubular cristae, they are very abundant in the I, II and III stages, whilst in the IV stage there are few mitochondriae. These reductions could be associated with mitochondrial matrix desorganization and destruction by autophagic lysosomes (Figs. 8, 9, 12). Lipofuchsin granules are found from the third sexual maturity stage onwards, and are associated with the Golgi stacks, these profiles are electron dense with clear inclusions, and in the larger mantle length specimens they shown an increase in size (Figs. 7, 9, 12).

This gland is well vascularized with large blood vessels. The pericyte cells cover the endothelial cells, and have a double basal membrane. The cellular membranes are tightly joined in an irregular fashion, and there are collagen fibers in the cytoplasm. The cytoplasm of the endothelial cells is not easily identified because the lumen has an electron dense hemolymph which occupies nearly the entire space. Nevertheless the organelles are restricted mainly to the area close to the nucleus (Fig. 13, 14, 15).

The nerve cells are distributed between the principal cells and blood vessels in long axon bundles with terminal synaptic buttons with different kinds of neurosecretory vesicles. The axon bundles are tightly packed, they have long microtubules, and some microvesicles.

The synaptic buttons are in contact with axon bundles, secretory cells and blood vessels, and there were no synaptic electron dense contacts identified. The neurosecretory vesicles were classified according to electron density and size in: small agranular (35 – 70 nm), agranular (90 – 150 nm), moderate to very dense (95

– 130 nm) and dense with a smooth limiting membrane (90 – 170 nm) (Figs. 6, 11, 14, 16). Glial cells are intermingled between the OG cells, with long cytoplasmic processes, their cytoplasm is clear with a particulate material and few organelles, some mitochondriae, vacuoles and vesicles (Figs. 6, 11, 16).

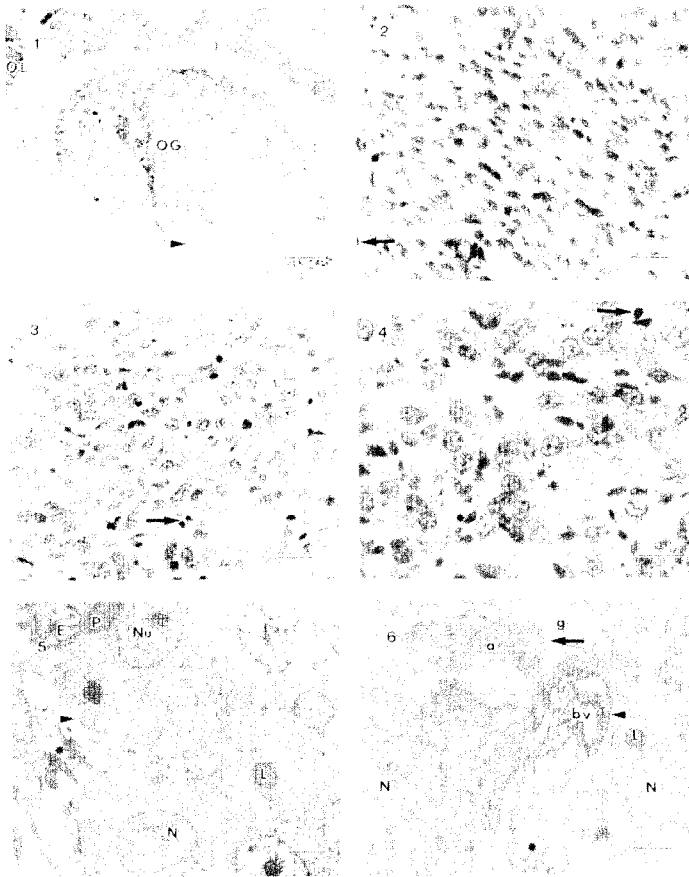


Fig. 1. The optic gland (OG) is covered by a fine connective cell layer ( $\triangleright$ ), and is close to the optic lobe (OL). Sexual maturity stage II, female, 94.9 mm mantle length, 63.6 g body weight. Paraffin section, Hematoxylin-Eosin stain. Bar 25  $\mu$ m.

Fig. 2. Sexual maturity stage II, female 94.9 mm mantle length, 63.6 g body weight. Hematoxylin-Eosin stain.

Fig. 3. Sexual maturity stage III, male 76.4 mm mantle length, 36.04 g body weight. Herlant tetrachromic stain.

Fig. 4. Sexual maturity stage IV, male 123.35 mm mantle length, 111.71 g body weight. Periodic acid Schiff reaction.

Figs. 2, 3, 4. The OG is made up of the principal cells ( $\triangleright$ ) the most numerous cells in the OG, with prominent nuclei, and support cells in which the nuclei are very dense with a smaller diameter ( $\blacklozenge$ ). Running through the tissue are the blood vessels (\*). Figures 2, 3 and 4 show the increase in cell size. Bar 10  $\mu$ m.

Fig. 5. TEM low magnification view. The secretory cells present a dense cytoplasm with a central round nucleus (N), and lipofuchsin (L) granule. Blood vessel (\*) in a transverse section showing the electrondense pericyte nucleus (P) and endothelial nucleus (E). Clear areas correspond to glial processes ( $\triangleright$ ). Nu – nucleoli. Bar 5.5  $\mu$ m.

Fig. 6. Section with the OG cellular types, that shows the characteristic array of blood vessels (bv) in longitudinal section and axon bundles (a) with terminal synaptic buttons ( $\blacklozenge$ ). Glial (g) cell cytoplasm is clear with dispersed dense material. Main cell nuclei (N) are euchromatinic, and the cytoplasmic processes (\*) are in contact with the blood vessels. N – nucleus. Basal membrane ( $\triangleright$ ). Bar 2.5  $\mu$ m.

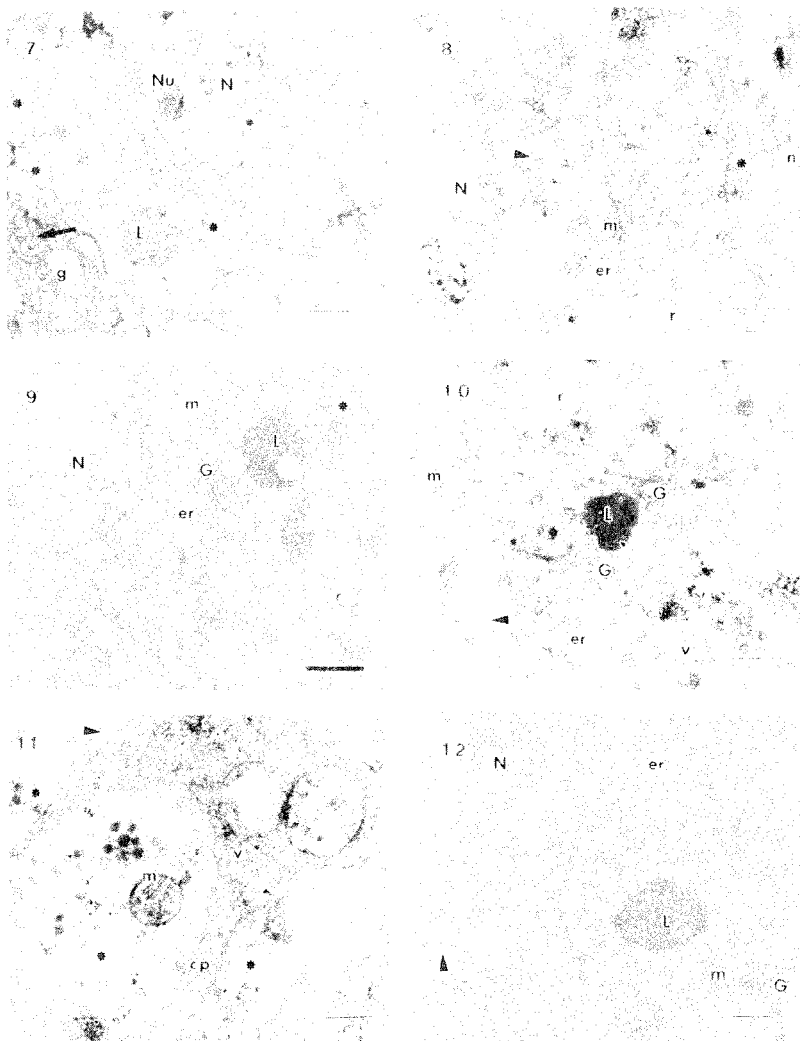


Fig. 7. Section with some main cell processes (\*). The cytoplasm is filled with abundant organelles. Within the nucleoli the *pars amorpha* and *nucleolonem* can be observed. Nerve cell bundle (↔) and glial (g) processes. Bar 2.5  $\mu\text{m}$ .

Fig. 8. Main cell profile that shows abundant mitochondria (m) with tubular crests, endoplasmic reticulum (er) cisterns are thin and dispersed between organelles, ribosomes (r) are observed throughout the cell, and there are some clear cytoplasmic vesicles (v). Axon bundles (n) are in transversal section. Main cell cytoplasmic process (\*). Bar 0.8  $\mu\text{m}$ .

Fig. 9. Within the secretory cell cytoplasm organelles are abundant and show high activity. Circular mitochondria (m) are located close to the Golgi (G) apparatus and autophagic lysosomes (L). Endoplasmic reticulum (er) is scarce, but there are abundant ribosomes (r). Bar 0.8  $\mu\text{m}$ .

Fig. 10. Main cell that shows Golgi complex (G) secreting electron dense granules with a lipofuchsin (L) granule. Within the cytoplasm there are some clear vesicles (v), free ribosomes (r), mitochondria (m), endoplasmic reticulum (er). The cytoskeleton (v) is composed of thin microtubules. Bar 0.4  $\mu\text{m}$ .

Fig. 11. Section of a main cell process (cp) in contact with synaptic buttons (\*) filled with different kinds of neurosecretory vesicles and a single circular mitochondria (m) with tubular crests. The main cell (cp) contains clear vesicles (v), one of which can be observed to extrude from the cellular membrane. Bar 0.4  $\mu\text{m}$ .

Figs. 12 and 13. The cytoplasm of a main cell at the mature stage has features different to those of previous stages. The mitochondria (m) are scarce, the Golgi (G) apparatus profile is extended (compare with figures 9, 10), secreting numerous dense granules (v), and the rough endoplasmic reticulum (er) cisterns are swollen, and aggregated occupying a considerable part of the cell volume. Bar 1.25  $\mu\text{m}$ .

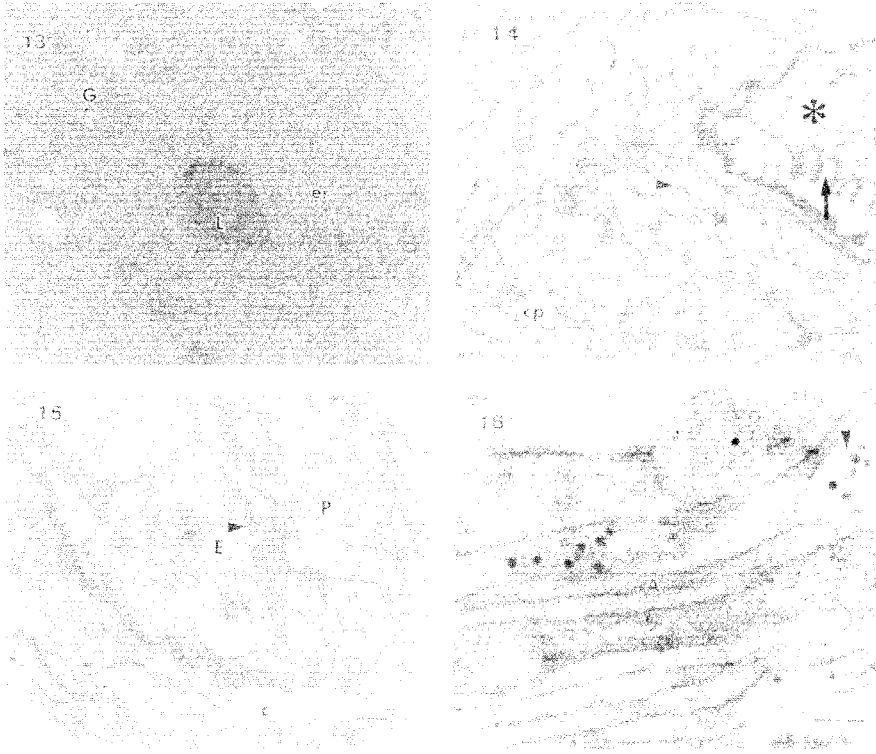


Fig. 14. Section that shows the complex array of main cell processes (cp) nearby a blood vessel (\*) with fenestrations (◄). The nerve cells (►) are associated with the blood vessels. Bar 1  $\mu\text{m}$ .

Fig. 15. Transverse section through a blood vessel. At the pericyte (P) there are collagen fibers (c), the cellular membranes are very tightly joined, and delimited by a double basal membrane, and the cytoplasm contains some organelles. The cytoplasm of the endothelial cell (E) is in contact with the hemolymph (►), which is apparently separated by a membrane. Bar 0.8  $\mu\text{m}$ .

Fig. 16. Axon bundles (A) in longitudinal section, with terminal synaptic buttons (\*) filled with different kinds of neurosecretory vesicles (►). The axon contains some vesicles, but its distinctive feature is the presence of microtubules. Bar 0.8  $\mu\text{m}$ .

## DISCUSSION

The morphological characteristics (mantle length and reproductive organs) in males and females of *S. sepioidea* were good indicators of the sexual maturity stage, although some individuals reached the same maturity stage at different mantle length intervals. Robaina & Voglar (1986) and Voglar & Robaina (1987) found squids at different stages of sexual maturity with the same mantle lengths. In this study it was not possible to

examine the OG of females during the spawning period or at the post-spawning stage, because their reproductive behavior shows care of eggs until hatching, this is followed by a short resting period in which the female broods most of the mature eggs and finally dies. Wodinsky (1977) quoted in *Octopus humeelincki* an apparently self destructive system of the OG which inhibits feeding in brooding females, preventing the predatory female from eating the eggs, and thus ensuring juvenile survival.



The OG shows a highly vascularized tissue and fine structure sections from *S. sepioidea* were improved by modifying some combinations of fixatives and schedules. The fixer quoted by Evans *et al.* (1976) followed by extensive washings gave the best tissue preservation, while the aldehyde prepared in phosphate buffer saline with filtered sea water resulted in a bad preservation. Problems were also reported with *O. vulgaris* and *S. officinalis* OG fine structure by Defretin & Richard (1967) and Nishioka *et al.* (1970). In *S. sepioidea* a hematonervous barrier built by the cellular wall, and gap junctions (*zonula occludens*) between the endothelial cell and the pericyte may halt the diffusion of the fixative in the squid giant axon (Villegas & Villegas 1968). Villegas (pers. com.) obtained the best preservation by immersion instead of perfusion, this latter being the suggested procedure for delicate tissues.

The fine structure of the tropical squid *S. sepioidea* OG, showed the same cellular types described in other cephalopods, with some distinctive features. *S. sepioidea* secretory cells showed differences in organelle abundance between the sexual maturity stages: at the juvenile and immature stages there are numerous mitochondriae, endoplasmic reticulum cisterns are thin and dispersed in the cytoplasm, the Golgi apparatus has a curved profile and actively secretes granules of different densities, whilst in the IV maturation stage (females) and reproductive individuals (males) secretory cells are almost depleted of mitochondriae, endoplasmic reticulum cisterns are dilated and well developed in a concentric fashion, and the Golgi complex actively secretes electron dense granules which are dispersed in the cytoplasm. Some of these are close to the lipofuchsin granules and to the cellular membrane, suggesting a possible secretory mechanism through the blood vessels.

*S. sepioidea* OG secretory activity was demonstrated by the production of electron dense secretory granules in the sexually mature stage (Figs. 12, 13). However, the presence of clear vesicles, some of them associat-

ed with polysomes, could indicate the existence of other secretory processes in the life cycle, as seen by the extrusion of a clear vesicle close to a synaptic button (Fig. 11). Moreover, the cellular characteristics indicate active cells, with euchromatinic nuclei, an abundance of organelles, and an increase in lipofuchsin volume and cell size with dorsal mantle length.

Defretin & Richard (1967) demonstrated that when *S. officinalis* is kept in a dark photoperiod the OG secretory cells produce three kinds of secretory granules, suggesting that they are filled with the same product in different stages of synthesis. *Octopus vulgaris* OG enlargement occurred both in the nucleus and secretory cell cytoplasm after cerebral surgery (Wells 1964). *Eledone cirrhosa* OG enlargement was statistically associated with female gonad maturation, and it is suspected that this process occurs normally (Boyle & Thorpe 1984). Koueta *et al.* (1995) described cellular changes in *S. officinalis* OG histology during sexual maturation; and found that main cell secretory activity was correlated with a peak of mitogenic activity in both sexes.

Lipofuchsin granules in the OG of some octopods are numerous, and measured up to several micrometers in diameter. Mangold & Froesch (1977) did not find a correlation between the size of lipofuchsin granules and sexual development in females. Lipofuchsin granules are implicated in the defense mechanism of *O. vulgaris* in the presence of high molecular weight molecules in the blood stream (Froesch & Mangold 1976, Froesch *et al.* 1978 and Froesch 1979). In *S. sepioidea* a lipofuchsin granule engulfing a degenerated mitochondriae was observed.

The presence of different types of neurosecretory microvesicles in the axon bundles and synaptic buttons in the OG of *S. sepioidea* indicates the existence of different stimuli controlling OG activity. These stimuli were reported as excitatory or inhibitory (Nishioka *et al.* 1966 and Froesch 1974). In the stellate ganglion of *S. sepioidea* the neurosecretory microvesicles are small and agranular (50 - 80 nm) (Castejón &

Villegas 1964), in the stellate ganglion of *Loligo vulgaris* there are both small agranular vesicles (40 - 60 nm) and large agranular vesicles (average 65 nm) (Gervasio *et al.* 1971). In the OG of *Loligo opalescens* the more common granular vesicles have a loose limiting membrane (80 - 160 nm), and a few axon-like processes containing dense vesicles (100 - 300 nm) with a smooth limiting membrane (Nishioka *et al.* 1970).

Field populations of *S. sepioidea* have a steady increase of the OG secretory cell diameter in both sexes. Sexual maturation in octopus was associated with a gross enlargement of the OG and a change in colour from cream to bright orange after optic nerve surgery (Wells & Wells 1959). In *S. officinalis* growth and sexual development was stimulated by a dark photoperiod and high temperature (Richard 1966, 1967 a, b, Defretin & Richard 1967). Likewise *O. vulgaris* mature individuals showed more intense histological reactions, and an increase in size in cytoplasm, nucleus and granules in the principal cells (Bonichon 1967). *Eledone cirrhosa* OG enlargement was statistically associated with the weight of the ovary and oviducal glands (Boyle & Thorpe 1984). Lipofuchsin granules and octopus body weight were well correlated, but the secretory mechanism was not demonstrated (Mangold & Froesch 1977).

#### ACKNOWLEDGEMENTS

I thank "Estación Experimental José Gregorio Hernández, Fundaciencias - IDEA", Jesús Rodríguez, Susan Tai, Gilma Hernández de A., Frances Osborn and Osmar Nusetti.

#### RESUMEN

Se examinó la ultraestructura fina de la glándula óptica (GO) en varios estadios de desarrollo sexual del calamar tropical *Sepiotheutis sepioidea*, proveniente de las costas de la Bahía de Mochima, Venezuela (10° 20' 22" - 10° 24' N; 64° 19' - 64° 22' W). Los parámetros morfométricos y de los órganos reproductivos (hembras n =

101; machos n = 105), presentaron diferencias significativas entre los estados de maduración sexual (ANOVA,  $p < 0.001$ ), el índice gonadal promedio en las hembras osciló entre 0.1 - 2.95 %, y en los machos entre 0.22 - 1.38 %. La GO son un par de órganos ovoides ubicados en el borde posterior del tracto óptico en el cerebro de los cefalópodos, ésta posee los mismos tipos de células descritos en otros cefalópodos con algunas leves diferencias. La célula principal en el estadio de maduración juvenil posee un perfil dendrítico con delgados procesos del citoplasma, abundantes ribosomas, mitocondrias circulares con crestas tubulares, y un núcleo prominente eucromatínico; en el estadio de adulto las células principales presentan las cisternas del retículo endoplasmático rugoso dilatadas concéntricamente en la célula, el aparato de Golgi es activo con abundantes gránulos electron densos de secreción, las mitocondrias son escasas, y el gránulo de lipofucsina es de mayor tamaño. La dimensión del núcleo de la célula principal y la abundancia de organelos, presentaron oscilaciones en las etapas del ciclo de vida.

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