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A transmission Electron Microscopic Study of Cnidae and Mucous Cells in the Tentacles of *Anemonia sulcata*

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ABSTRACT. The sea anemone *Anemonia sulcata* (Pennant), inhabiting marine-waters of Egypt can sting human skin by their numerous adherent tentacles. They cause erythema, urticaria, itching and scattered patches of dermatitis over the abdomen and hands during swimming or collection of specimens. Tentacles of this species are firstly studied by transmission electron microscopy in Egypt. The present paper describes the ultrastructure of two types of cnidae and mucous cells. The cnidae include the adhesive spirocysts and the stinging nematocysts namely microbasic b-mastigophores. The structure and function of these specialized cells are discussed.

Cnidae are unique intracellular organelles of the phylum Cnidaria. Some of these cnidae contains toxins and characterized by their stinging capability. These stinging cells act as cellular weapons directed towards the predators, prey and even man. Numerous marine cnidarians have powerful toxins such as *Pelagia noctiluca, Physalia physalis, Chironex fleckeri, Aiptasia pallida, Anemonia sulcata* (Halstead 1974).

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Previous studies have demonstrated that several investigators are attracted to the study of stinging cnidae in various parts of the world (Westfall 1965, Mariscal 1980, Gooley *et al.* 1984, Hidaka and Miyazaki 1984, Weber *et al.* 1987, Mckay and Anderson 1988, Tardent *et al.* 1990, Weber 1991, Komatsu *et al.* 1992, Pires and Pitombo 1992, Manoleras and Norton 1994). They study these specialized organelles from different views including their structure, mechanism of discharge, toxic properties and biochemical composition.

In Egypt, these studies have not paid the same attention. It is obvious that the cnidarians are considered important members of the marine Egyptian fauna. Medically important cnidarians, as some jelly fishes, fire corals and sea anemones, are nearly dangerous. The sea anemone *Anemonia sulcata,* which has a world wide distribution, is a venomous cnidarian commonly found in the Mediterranean coast of Egypt (Ghobashy *et al.* 1979, Shoukr 1984). The anemone tentacles are capable of producing a clinical sting in human. The stinging habit of this species is recognized in other regions of the world by some authors *e.g.* Southcott 1963, Cariello and D'Aniello 1975, Alsen *et al.* 1978, Schmidt 1982, Godknecht and Tardent 1988, Manoleras and Norton 1994). The present work throws some light on the ultrastructure description of the cnidae, as well as the mucus-secreting cells, found in the tentacles of the sea anemone *A. sulcata.*

Materials and Methods

Anemone specimens, used in this study, were collected from the rocky coasts at Abou kir (Alexandria) on the Mediterranean sea. They were placed in large plastic containers with sea water and transported to invertebrate laboratory aquaria at the University of Tanta. They were maintained in natural filtered sea water under constant aeration in glass aquaria.

Small pieces of tentacles were excised with fine scissors and then fixed for 12 hours in cold 3% glutaraldehyde in phosphate buffered salt solution (pH7.3). Tentacles were washed for 30 minutes in each of three changes of 0.25 M phosphate buffer and then post-fixed for 2-5 hours in 1% osmium tetroxide in the same buffer. After postwashing in buffer, they were dehydrated in a graded ethanol series (50%, 70%, 80%, 90%, 95%, 100% I, 100% II) for 30 minutes at each step. Then, they were embedded in Epon and stained with uranyl acetate and lead citrate at Ain Shams electron microscopy centre in Cairo. Grids were examined with Philips 201 transmission electron microscope.

Observations

The tentacles of the sea anemon *A. sulcata* are hollow structures containing three layers: an outer ectoderm, a middle mesoglea and an inner endoderm. The common specialized cells observed within the ectoderm are the cnidae, the mucous cells, the highly vacuolated musculo-epithelial cells and the pigments usually known as lipofuscin (Figs. 1, 3 and 7).

Cnidae

The cnidae are oriented perpendicularly to the outer surface of the ectodermal epithelial cells of the tentacles. Two distinct types of tentacular cnidae are present: the spirocysts and the nematocysts termed microbasic b-mastigophores (Fig. 2).

1. *Spirocysts*

They are much more abundant than the nematocysts. They have a relatively thin, single-walled capsule with a spirally coiled thread. The capsules of the spirocysts measure about 0.61-16.7 µm in length by 0.29-4.0 µm in width. Transmission electron microscopy shows that the inner surface of the capsular wall appears serrated (Fig. 4) and has transversely oriented parallel ridges. The thread is hollow and regularly coiled within the capsule. It is unspined but has fine hollow specific tubules or microfibrillae. The latter are attached to the inner surface of the undischarged thread (Fig. 5). The uneverted tubules reach to about 0.12-0.35 µm in diameter. The thread is filled with an electron-dense granular material. Diameter of the thread ranges between 0.71-2.1 µm. No Flagellum or ciliary structures appear to be associated with the spirocysts.

2. *Microbasic b-mastigophores*

The capsules are elongated with rounded ends. They are usually double-layered, the inner layer being continuous with the inverted shaft (Fig. 6). The capsules of this type of nematocyst measure about 11.9-24.2 µm in length and 4.5-5.2 µm in width. The inverted shaft is straight and appears as a hollow filament in the center of the undischarged capsule. The nematocyst thread or tubule is coiled about the shaft. It bears spiny folds but lacking the hollow microfibrillae. The stinging capsule is filled with an electron-dense granular material (Venom) and devoid of the internal serrations.

Mucous cells

They are elongated in shape (reach to about 27.7 µm long and 14.2 µm wide) and numerous in the ectoderm of tentacles. Two types of mucus-secreting cells were noticed among the musculo-epithelial cells with electron-dense or electron-opaque matrix (Fig. 1). The tip of the mature mucous cell is exposed to the external environment through a short neck (Fig. 7). Mucous granules (reach to about 5.7 μm in diameter) appear rounded or oval and are limited by a distinct membrane (Fig. 8).

Discussion

In the Egyptian fauna, contact with the sea anemone *A. sulcata* tentacles results in a painful cutaneous erythema and urticaria. Its sting is a nuisance during swimming of children and collection of specimens. This is the first record of the sting of this species for the Egyptian waters. Moreover, other stinging cnidarians were observed during some journeies to the Egyptian coasts. Severe stings have recognized by the fire coral *Millepora* of the Red sea near Al-Ghardaqa and Safaga. Also, large forms of Jelly fishes *e.g. Cyanea,* which were common in the Mediterranean coast as in Ras el-Bar, causing painful stings on touching their tentacles.

The ultrastructural studies showed that the tentacles of *A. sulcata* are equipped with two types of cnidae: the spirocysts and the nematocysts termed microbasic b-mastigophores. Recently, cnidae are divided into three major categories: spirocysts, nematocysts or stinging capsules and ptychocysts (Mariscal *et al.* 1977a). There are about 30 different types of stinging cnidae. Types and sizes of these cnidae are important taxonomic characters in sea anemones. Some confusion appears in the terminology of nematocysts and several names may exist. Different types of nematocysts described in the literature proved to be the same. As for example, microbasic b-mastigophores have several terms for the same structure as basitrichs, b-rhabdoids and spirula (England 1987).

The transmission electron microscopy has demonstrated that the spirocysts differ from microbasic b-mastigophores in many respects. The spirocyst capsule seems to be single instead of double-walled. The internal surface of the spirocyst capsule has a serrated appearance as described by Picken and Skaer (1966) and Mariscal and McLean (1976). The thread of the spirocyst is easily identified by the spiral coiling inside the capsule. Moreover, the spirocyst threads are devoid of spines but containing tiny tubules or microfibrillae. Spirocysts are characterized by the absence of toxins (Mariscal *et al.* 1977b) and presence of an electron-dense material (sticky substance) inside the threads. The spirocysts have microvilli which serve as receptor sites for discharge of threads (Mariscal *et al.* 1978) but lacking a flagellum or ciliary structures which may be typical of microbasic b-mastigophores (Hidaka and Miyazaki 1984).

The location of the cnidae, in addition to the mucous cells, in the outer ectodermal epithelium of the tentacles suggests interesting functions. With regard to spirocysts, the thread containing electron-dense material (sticky substance) and bundles of fine tubules or microfibrillae. When the spirocyst thread discharges, the tubules probably evert like the fingers of the gloves. Mariscal *et al.* (1976 and 1977b) have described fine microfibrillae on the discharged thread of the spirocyst from the tentacles of the solitary scleractinian coral *Paracyathus stearnsii.* They stated that these discharged tubules solubilize on contact with sea water and adhere to various substances. Similarly, Westfall (1965) and Goldbeg and Taylor (1989) have demonstrated that the spirocyst thread has the ability to form adherent microfibrils when expelled into a watery medium. Moreover, the microfibrillae increase the surface area and the adhesive character of the spirocyst thread.

In the present study, the tentacles of *A. sulcata* adhere firmly to human skin. This is frequently happened during collection of specimens from Alexandria coasts. Therefore, it is commonly difficult to dislodge the tentacles without damage to the anemone. It appears that the spirocysts are the primary adhesive organelles which are responsible for stickiness of the tentacles of *A. sulcata.* On the other hand, spirocyts enable sea anemones in food capture and attachment to the substratum (Mariscal *et al.* 1977b). Furthermore, the mucous secretions of the mucous cells help the anemone tentacles for adhesion to human skin or trapping small prey during feeding.

Microbasic b-mastigophores have a defensive function and adapted for penetration (Godknecht and Tardent 1988). The thread of these stinging capsules is spined and lacking the hollow tubules or microfibrillae. On discharge, the threads penetrate the human skin or the prey. The spines aid in attachment and the capsular contents (poisonous substance) are ejected from the open tip of the thread. These toxins are responsible for the erythema, urticaria and itching appeared on the human skin. The toxicology of nematocysts of the venomous anemone *A. sulcata* needs future investigations in the Egyptian fauna. In addition, the stinging cnidarians and their treatment await some co-operation from toxicologists and physicians.

Acknowledgements

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References

Alsen, C., Beress, L. and **Tesseraus, I.** (1978) Toxicities of sea anemone (*Anemonia sulcata*) polypeptides in mammals. *Toxicon,* **16:** 561.

Cariello, L. and **D'Aniello, A.** (1975) Isolation and characterization of four toxic protein fractions from the sea anemone *Anemonia sulcata. Toxicon,* **13:** 353.

England, K.W. (1987) Certain Actiniaria (Cnidaria, Anthozoa) from the Red sea and tropical Indo-Pacific Ocean. *Bull. Br. Mus. Nat. Hist (Zool.).* **53**(4): 205-292.

Ghobashy, A.F., Abdel-Hamid, M.E. and **Shoukr, F.A.** (1979) Sea anemones in Alexandria water. *Bull. Fac. Sci. Assiut Univ.,* **8**(2): 77-98.

Godknecht, A. and **Tardent, P.** (1988) Discharge and mode of action of the tentacular nematocysts of *Anemonia sulcata* (Anthozoa: Cnidaria). *Mar. Biol. (Berl).,* **100**(1): 83-92.

Goldberg, W.M. and **Taylor, G.T.** (1989) Cellular structure and ultrastructure of the black coral *Antpathes aperta.* 2. The gastrodermis and its collar cells. *J. Morph.,* **202:** 255-269.

Gooley, P.R., Beress, L. and **Norton, R.S.** (1984) ^{1}H nuclear magnetic resonance spectroscopic study of the polypeptide toxin I from *Anemonia sulcata. Biochemistry,* **23:** 2144-2152.

Halstead, B.W. (1974) *Venomous coleenterates: hydroids, Jelly fishes, corals and sea anemones, In:* **Bücher, W.** and **Buckley, E. (eds.).** Venomous animals and their venoms, Academic press, New York, 395 p.

Hidaka, M. and **Miyazaki, I.** (1984) Nematocyst discharge and surface structure of the ordinary and sweeper tentacles of scleractinian coral, *Galaxea fascicularis. Galaxea,* **3:** 119-130.

Komatsu, S., Furukawa, K., Abe, K., Hirano, H. and **Ueda, M.** (1992) Isolation and characterization of equinatoxins from the sea anemone *Actinia equina.* L. *Chem. Pharm. Bull* (Tokyo), **40**(10): 2873-2875.

Manoleras, N. and **Norton, R.S.** (1994) Three-dimensional structure in solution of Neurotoxin III from the sea anemone *Anemonia sulcata, Biochemistry,* **33:** 11051-11061.

Mariscal, R.N. (1980) The elemental compostition of nematocysts as determined by X-ray microanalysis, *In:* **Tardent, P.** and **Tardent, R. (eds.)** Developmental and cellular biology of coelenterates, Elsevier North-Holland Biomedical press, Amsterdam, New York, 337-342 pp.

Mariscal, R.N. and **McLean, R.B.** (1976) The form and function of cnidarian spirocysts. 2. Ultrastructure of the capsule tip and wall and mechanism of discharge. *Cell Tiss. Res.* **169:** 313-321.

Mariscal, R.N., Conklin, E.J. and **Bigger, C.H.** (1977a) The Ptychocyst, a major new category of cnidae used in tube construction by a cerianthid anemone. *Biol. Bull.,* **152:** 392-405.

Mariscal, R.N., McLean, R.B. and **Hand, C.** (1977b) The form and function of cnidarian spirocysts. 3. Ultrastructure of the thread and the function of spirocysts. *Cell Tiss. Res.,* **178:** 427-433.

Mariscal, R.N., Conklin, E.J. and **Bigger, C.H.** (1978) The putative sensory receptors associated with the cnidae of cnidarians. *Scan. Elec. Micro.,* 959-966.

Mckay, M.C. and **Anderson, P.A.** (1988) Preparation and properties of cnidocytes from the sea anemone *Anthopleura elegantissima. Biol. Bull.,* **174:** 47-53.

Picken, L.E. and **Skaer, R.J.** (1966) A review of researches on nematocysts. *Symp. Zool. Soc. Lond.,* **16:** 19-50.

Pires, D.O. and **Pitombo, F.B.** (1992) Cnidae of the Brazilian Mussidae (Cnidaria: Scleractinia) and their value in taxonomy. *Bull Mar. Sci.* **51**(2): 231-244.

Schmidt, G.H. (1982) Replacement of discharged cnidae in the tentacles of *Anemonia sulcata. J. Mar. Biol. Ass.* U.K., **62:** 685-691.

Shoukr, F.A. (1984) A Field guide to identification of Egyptian members of Actiniaria. *Bull. Fac. Sci. Zagazig Univ.*, **6:** 683-703.

Southcott, R.V. (1963) Coelenterates of medical importance, *In:* **Keegan** and **Macfarlan (eds.).** Venomous and poisonous animals and noxious plants of the pacific region, pergamon press Inc., New York, 41-65 pp.

Tardent, P., Zierold, K., Klug, M. and **Weber, J.** (1990) X-ray microanalysis of elements present in the matrix of cnidarian nematocysts. *Tissue Cell,* **22**(5): 629-643.

Weber, J. (1991) A novel Kind of polyanions as principal components of cnidarian nematocysts. *Comp. Biochem. Physiol.*, **98**(A): 285-291.

Weber, J., Klug, M. and **Tardent, P.** (1987) Detection of high concentration of Mg and Ca in the nematocysts of various cnidarians. *Experientia,* **43:** 1022-1025.

Westfall, J.A. (1965) Nematocysts of the sea anemone *Metridium, Am. Zool.,* **5:** 377-393.

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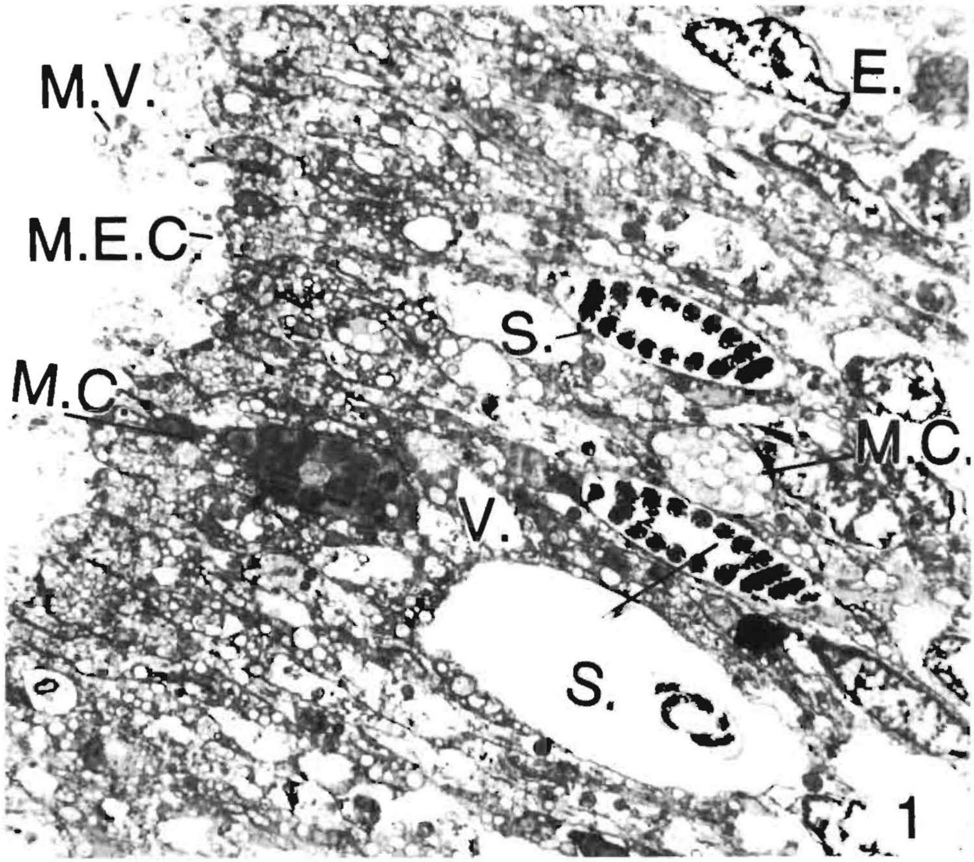

Fig. 1. Transmission electron micrograph of the tentacle ectoderm of *A. sulcata* illustrating spirocysts and mucous cells in the outer region (X 2,576). E., ectoderm; M.C., mucous cell; M.V., microvilli: M.E.C., musculo-epithelial cell; S., spirocyst; V., vacuole.

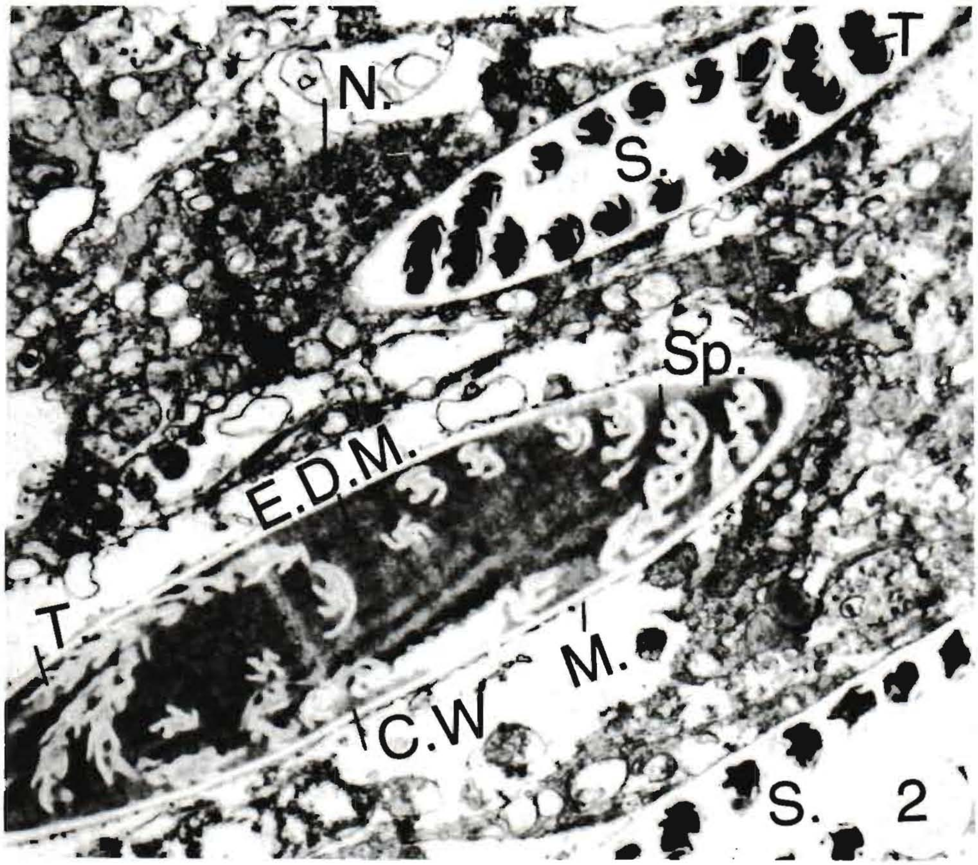

Fig. 2. Transmission electron micrograph of two types of cnidae from the tentacle ectoderm showing a spirocyst with single-walled capsule and a microbasic b-mastigophore with double-walled capsule (X 5,520). C.W., capsule wall: E.D.M., electron-dense material; M., microbasic b-mastigophore; N., nucleus; S., spirocyst: T., thread.

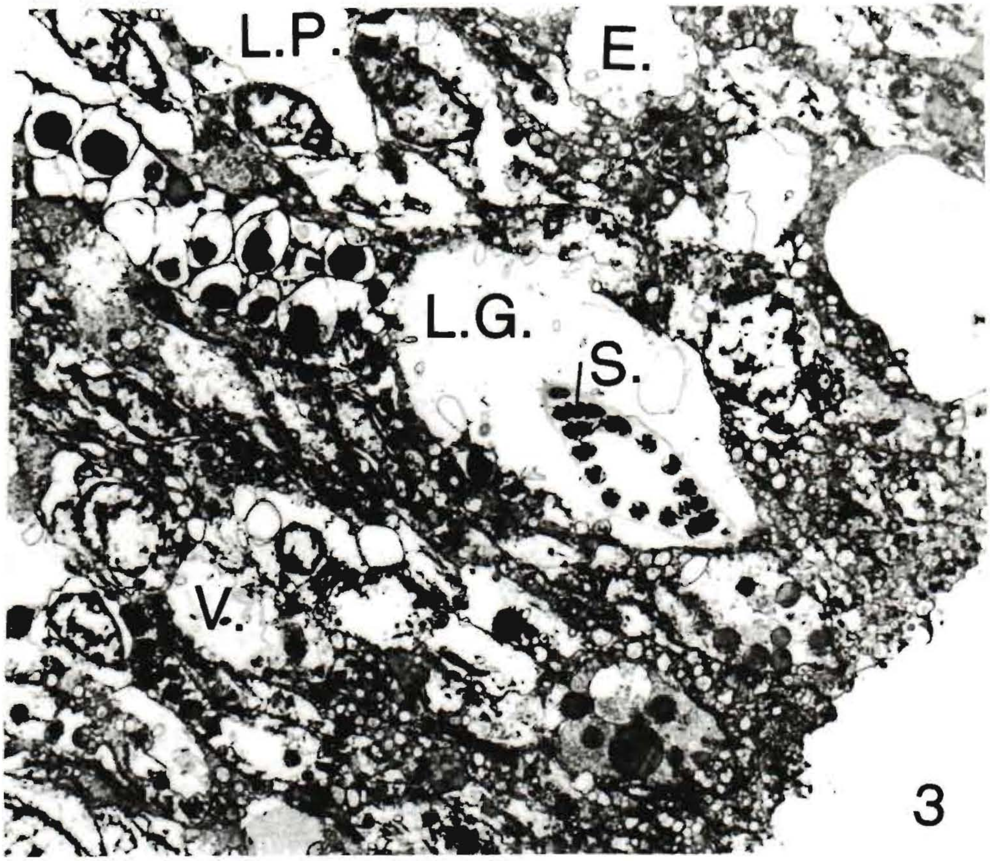

Fig. 3. Transmission electron micrograph of the tentacle ectoderm showing lipofuscin pigments and a spirocyst (X 2,576). E., ectoderm; L.G., lipid granules; L.P., lipofuscin pigments; S., spirocyst; V., vacuole.

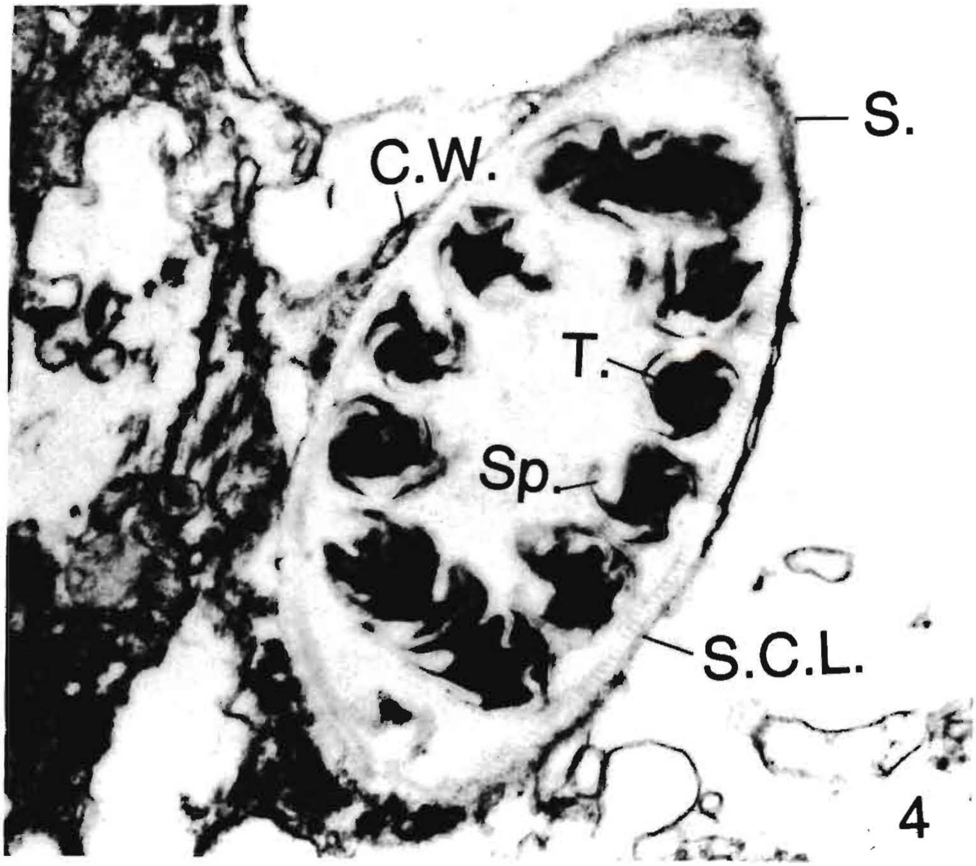

Fig. 4. Transmission electron micrograph of a longitudinal section through a spirocyst illustrating serrated capsule lining and undischarged thread (X 15,640). C.W., capsule wall; S., spirocyst; SP., spine; S.C.L., serrated capsule lining; T., thread.

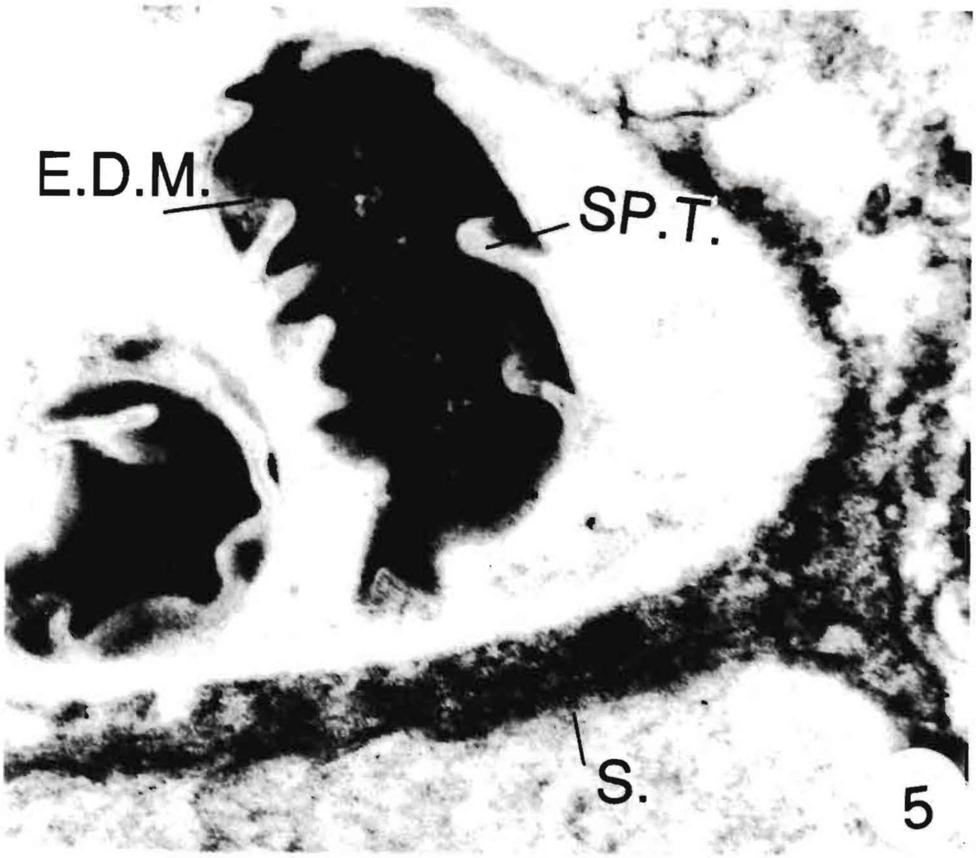

Fig. 5. Transmission electron micrograph through undischarged spirocyst showing electron-dense material (sticky substance) inside the thread as well as specific tubules or microfibrillae attached to the inner surface (X 33,120). E.D.M., electron-dense material; S., spirocyst; SP. T., specific tubules (microfibrillae).

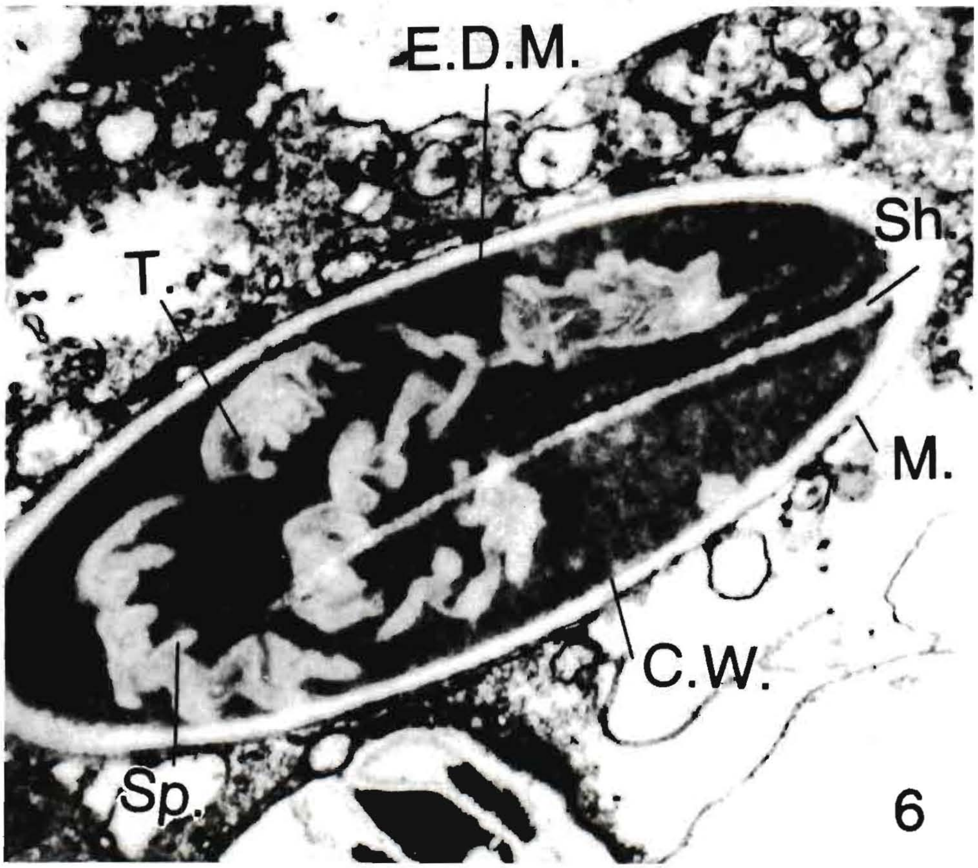

Fig. 6. Transmission electron micrograph of a longitudinal section through a microbasic b-mastigophore illustrating inverted shaft, undischarged capsule with electron-dense material (venom) and thread bearing spiny folds (X 11,960). C.W., capsule wall; E.D.M., electron-dense material; M., microbasic b-mastigophore; Sh., shaft; SP., spine; T., thread.

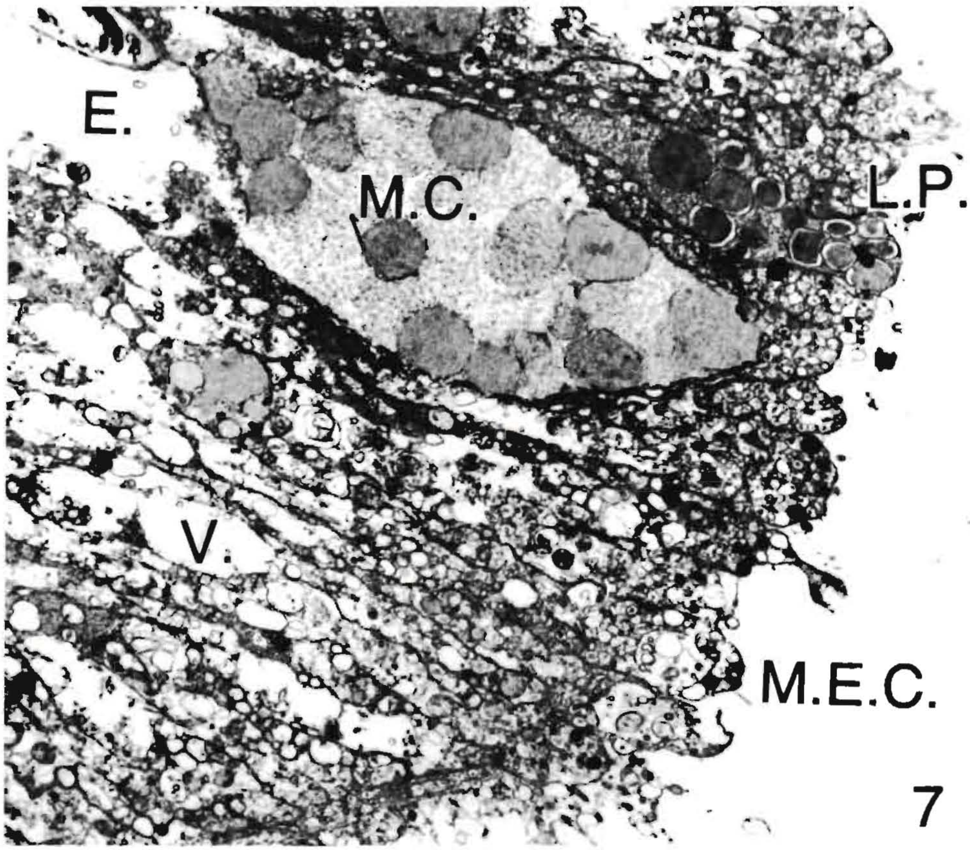

Fig. 7. Transmission electron micrograph through musculo-epithelial cells showing mucous cell at their periphery (X 3,312). E., ectoderm; L.P., lipofuscin pigments; M.C., mucous cell; M.E.C., musculo-epithelial cell; V. vacuole.

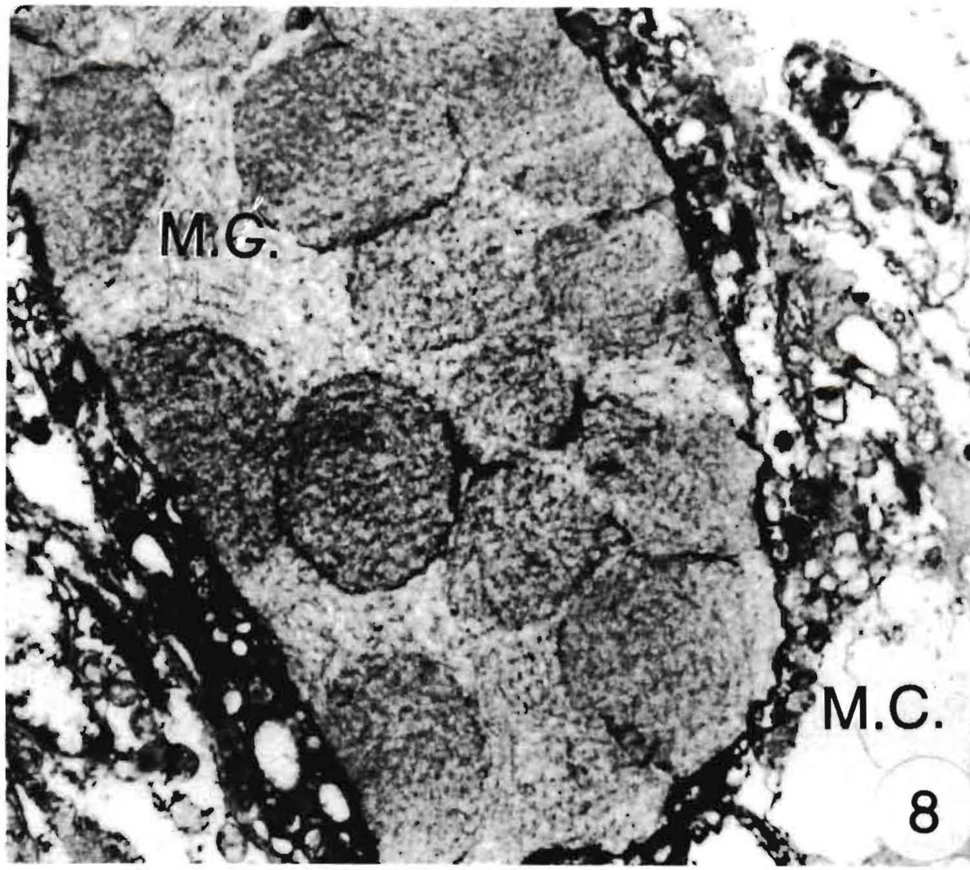

Fig. 8. Transmission electron micrograph through a mature mucous cell showing aggregations of mucous granules (X 5,520). M.C., mucous cell; M.G., mucous granules.

دراسة بالمجهر الالكتروني النافذ للخلايا اللاسعة والخلايا المفرزة للمخاط في لوامس شقائق النعمان الثعبانية (*Anemonia sulcata*)

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تنتشر شقائق النعمان الثعبانية (*Anemonia sulcata*) بكثرة على ساحل البحر الابيض المتوسط بمنطقة الاسكندرية ، وقد لوحظ ظهور بعض الاضرار عند إلتصاق لوامسها العديدة ذات الحركة الثعبانية بأجسام الاطفال اثناء السباحة واللهو في الشواطىء الضحلة الصخرية ، وكذلك عند جمعها للدراسة المعملية ، وتسبب هذه اللوامس احمراراً بالجلد ، وحساسية ، وهرش ، والتهابات تظهر على هيئة طفح جلدي . ويعتبر هذا البحث أول تسجيل لاظهار القدرة اللاسعة لهذه الاحياء البحرية في المياه المصرية ، كما أنه يلقي الضوء الى الاهمية الطبية لبعض اللاسعات مثل قناديل البحر والمرجان الناري وغيرها حتى تحظى بالاهتمام وخاصة من علماء السموم والاطباء .

وفي هذا البحث تم دراسة تركيب اللوامس في شقائق النعمان (*Anemonia sulcata*) بواسطة المجهر الالكتروني النافذ ، وقد تم وصف التركيب الدقيق لنوعين من الخلايا اللاسعة والخلايا المفرزة للمخاط .

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واشتملت الخلايا اللاسعة على الحوصلات اللولبية اللاصقة والمسؤولة عن الالتصاق الشديد بجلد الانسان والحوصلات السوطية المخترقة والمسؤولة عن اختراق المادة السامة لجسم الانسان وظهور الالتهابات الجلدية . ويناقش البحث أوجه الاختلاف في التركيب والوظيفة لهذه الخلايا .