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The Anterior Salivary Gland of *Octopus vulgaris* Secret Protein and does not Secrete the Mucus, from the Red Sea, Egypt.

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ABSTRACT

The cephalopods in the Red Sea are active predators of animals, they capture their prey from open water. Because they are fast-swimming animals, they have some modifications in their bodies, such as a fusiform shape and fast swimming, and they transform into the water column by a special structure known as a siphon. Octopus vulgaris is one member of the Cephalopoda present in the Red Sea and has special characteristics, such as the high activity of predators for its prey, such as crustaceans and small fishes. Octopus vulgaris has a special variety of defense systems such as the ink gland, posterior salivary gland, which secretes the protein, and anterior salivary gland, which is the aim of the present study. The anterior salivary gland in *Octopus* vulgaris is a member of the digestive system, and has a role in protein secretion, not secreting mucus as believed until now. The anterior salivary gland in Octopus has bioactive components that are very important in pharmaceutical and biomedical applications such as anticoagulant, anesthetic, and hypotensive activities. Histochemical studies of sections of the anterior salivary gland of Octopus vulgaris show the presence of three types of secretion cells in this gland, all secreting the pure Octopoda saliva and its protein secretion.

INTRODUCTION

In Cephalopods, three types of salivary glands are associated with the buccal mass: the submandibular gland, and the pair of anterior and the posterior salivary glands **(Mangold *et al*., 1989; Budelmann *et al*., 1997; Ponte & Modica, 2017; Dill-Okubo *et al.*, 2021)**. The anterior and posterior salivary glands produce secretions used to paralyze the prey within a few seconds after capture while the submandibular gland contributes to lubricating the passage of the food. The anterior salivary gland of Octopus vulgaris consists of two pairs of right and left lobes that lie under the buccal mass on the ventral side, both in contact with each other by a transverse duct on both sides of the buccal mass (**Ponte & Modica, 2017**; Leite ***et al***., 2021; Ziegler **& Gorny,** 2021). Also, the posterior salivary gland has two lobes and lies above the crop of Octopus, both salivary glands discharge their secretions and are stored in a salivary papilla under the regular ribbon (Moustafa ***et al***., 2010). In both glands, the two lobes are part of every single

gland. The right anterior salivary gland is generally equal to the left lobe in size and has a tubular or acinus structure. The anterior salivary glands in transverse sections are made up of branched ducts and arranged in tubules and variable in cell types. The salivary glands (anterior and posterior) play different roles in feeding by secretion of amylase by the anterior salivary gland and protein by the posterior **(**Pech-Puch ***et al***., 2016). The histochemical study of the anterior salivary gland of Octopus vulgaris refers to the presence of three types of cells: (TA, TB, and TC). By the histochemical stains of many stains, all types of cells of this gland secrete the protein only, and no evidence to date confirms that the anterior salivary gland secretes the mucus. All published papers about the structure of the anterior salivary gland in Cephalopoda indicate the presence of two types of cells only: type A and type B (**Fernández-Gago *et al***., 2018)**.** Some literature published about the anterior salivary gland structure, but indicated one type of cell only, because using a single stain may the presence of some modifications in structure between different species (**Dill-Okubo *et al.,* 2021**). Some authors (Tucker, 2010; **Ponte & Modica, 2017**) suggest the anterior salivary gland of *O*ctopus vulgaris releases a large amount of mucus but staining by PAS gives a negative reaction. From the above, we can say that the goal of this study is to discuss in detail the anterior salivary gland structure and its protein secretions from Octopus vulgaris caught in the Red Sea, Egypt.

MATERIALS AND METHODS

Collection

Samples of *Octopus vulgaris* were collected from the site on the Western Coast of the Red Sea. This site is located 17 km south of Safaga (latitude 26° 38' N longitude 33° 59' E) (Fig., 1). The collection site is rocky shores and samples were collected from the intertidal zone at the time of low tide. The collection was done by using the hand and packing it up. One specimen was collected and put in plastic containers containing seawater. Specimens were narcotic by adding menthol crystal from (El -Naser chemicals company) by water surface of the jar and wait to until relaxation. Specimen dissected in the field to get the studied organs were fixed and put in Blouin's solution for 24 hours for histological preparations.

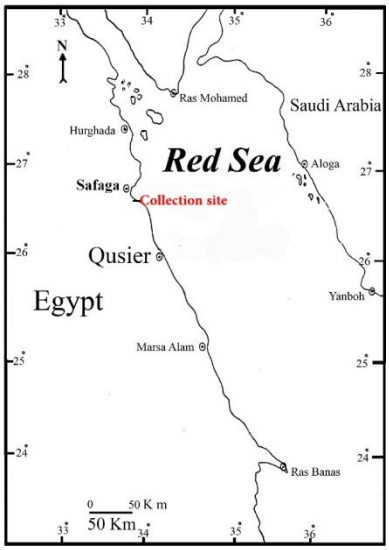


Fig. 1. A map of the Red Sea showing the collecting site.

HISTOPATHOLOGICAL STUDIES

Organs sectioned were cut off from the body and placed into Blouin's solution in seawater for 24 hours. Fixed parts were then passed to the graded series of alcohol from 100%. They were cleaned in toluene three times each for five minutes, then embedded in paraffin wax. Sectioning was made in the National Institute of Oceanography and fisheries by microtome (Leica RM 2125 – Germany) at 5-7 µm thickness. Sections were stained with the following stains:

1. Harris hematoxylin and eosin combination (H&E) (Steedman, 1950).
2. Bromophenol blue stain to demonstrate the general proteins (**Mazia *et al.*, 1953**).
3. Periodic acid Sheff's reaction (PAS) for the demonstration of polysaccharides in various cells and tissues **(McManus, 1948).**

The slides were dehydrated through an ascending series of ethanol after staining. They were then passed through xylene to mounting medium and covered with coverslips. The section was photographed by (Leica cameras and stereo microscopes Leica ICC50 HD Japan 2014).

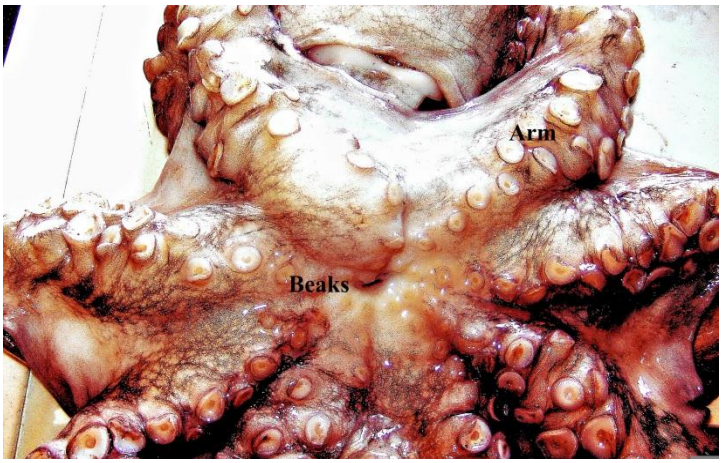


Fig. 2. The body plan of a marine (*Octopus vulgaris* Cuvier, 1797): (Ventral view)

RESULTS

Octopus vulgaris anterior salivary gland is only two pairs, small in size compared with the posterior salivary gland. The anterior salivary gland is located on each side of the buccal mass on the posterior side and consists of two lobes (right and left). The right lobe of the anterior salivary glands leads to three ducts, the first duct extends towards the salivary papilla, the second duct joins the left lobe of the same gland, and the third one extends backward to the right lobe of the posterior salivary gland. The secretions of the posterior pair are joined to the buccal cavity by a single duct that enters the posterior aspect of the buccal mass (**Figs. 3, A, B**). Histologically, the anterior salivary glands of Octopus vulgaris are formed of two layers composed of connective tissue consisting of several tubular and tubular-acinar units bound together. Each tubule is lined with secretory cells, which secrete matter according to the type of cells.

The secretory granules in the first type are densely distributed all over the cytoplasm and were deeply stained with hematoxylin-eosin as compared with the second one. The first type of secretory cell is fewer in number than the second one. The secretory granules in the first type are densely distributed all over the cytoplasm and were deeply stained with hematoxylin-eosin (**Figs. 4 A-E**). The first type of secretory cells is larger in number than the second one, but Type B is common in section and stained with bromophenol blue (protein stain). While the Type C is few and contains many protein granules which Also, secret the protein (**Figs. 4 F, G**). For the present study, no evidence for mucus of secreting cells was present in the anterior salivary gland of Octopus vulgaris as in previous studies, because the PAS stain gave a negative reaction (Figure H).

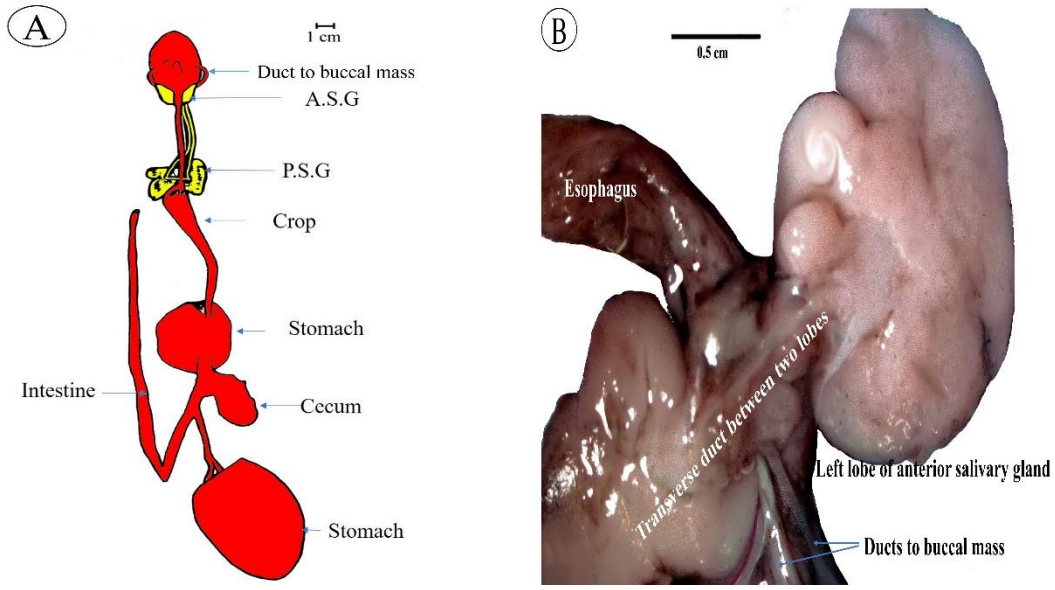
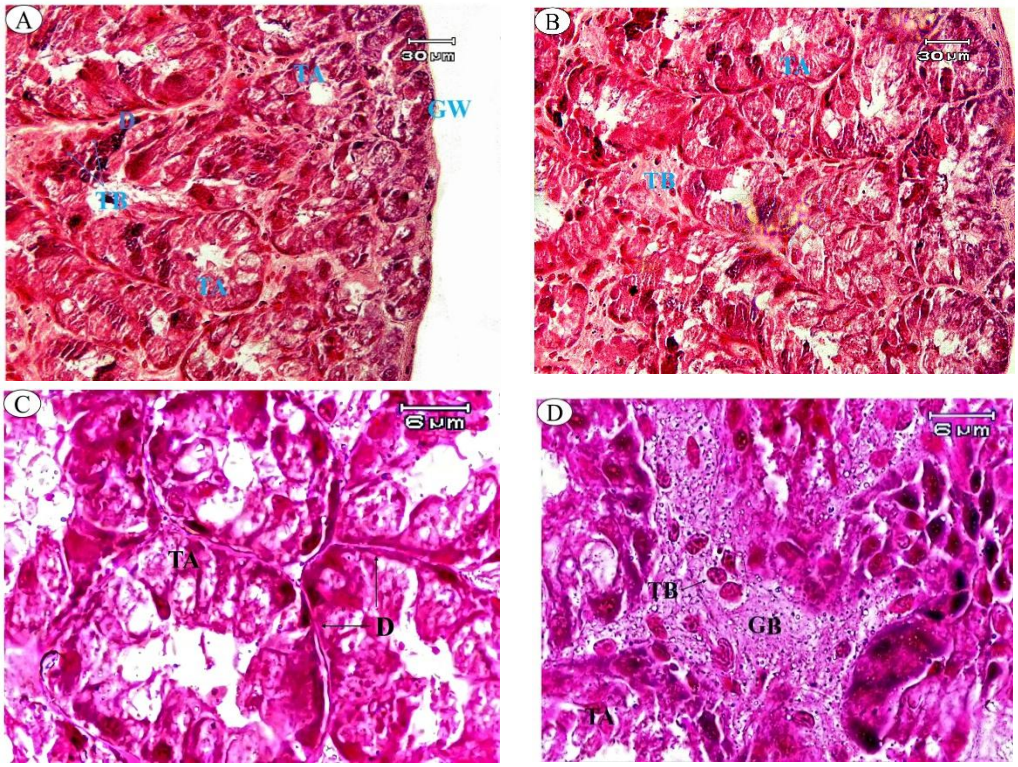


Fig. 3. (A): Manual diagrammatic for the complete digestive system of Octopus vulgaris.
(B): Ventral side of the anterior salivary gland of Octopus vulgaris.



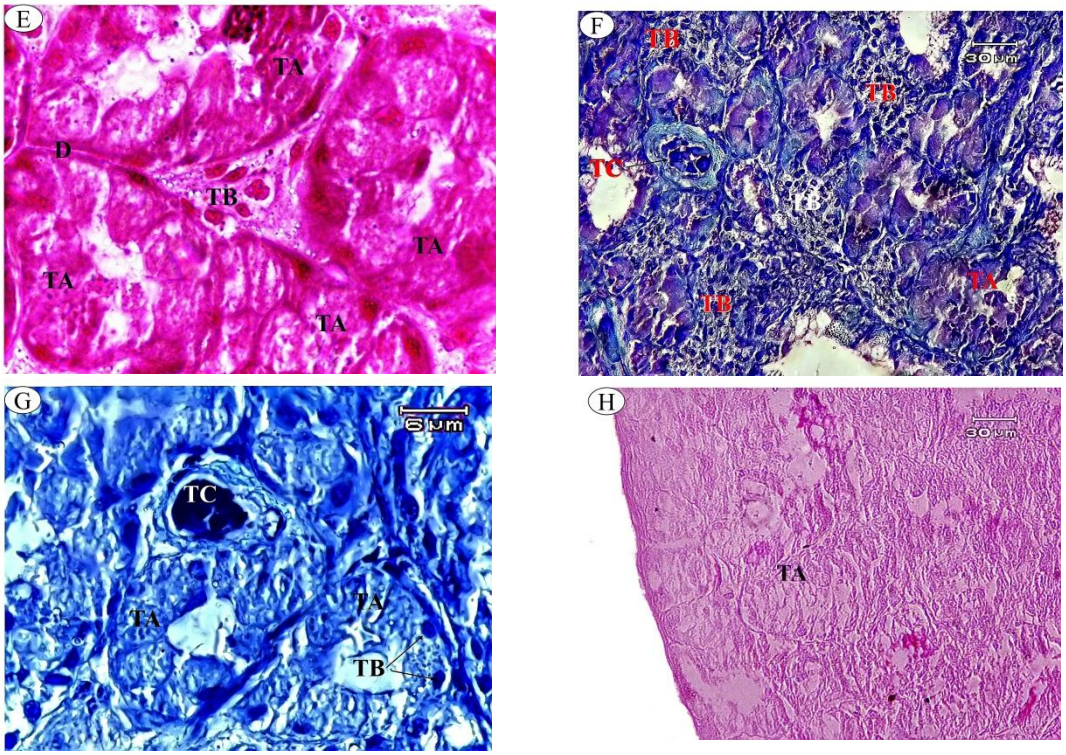


Fig.4. (A-H). All are high magnification (30-100 µ) shows: the structure of the anterior salivary gland of *Octopus vulgaris* from (A- E) stained with H&E see the type of TA, TB cells and GW gland wall, D, Ductulus between cells, GB, Gland parenchyma. (F-G) stained by protein stain (Bromophenol blue stain) shows both types (TC and TB) which are secreted proteins. (H) Stained by the periodic acid chief (Carbohydrates stain) and give a negative reaction.

DISCUSSION

The anterior salivary gland of Octopus vulgaris increases the predation efficiency of the animal through its protein secretions (**Ponte & Modica, 2017**). The anterior salivary gland secretes the protein only and the secretion causes the paralyzing of prey (**Gonçalves & Costa,** 2021). The anterior and posterior salivary glands of O. vulgaris consist of tubules with three types of secretory cells. The present study revealed that type A cells are associated with two types of cells, B and C, all responsible for protein secretion. The previous types give a positive reaction to the bromophenol blue stain. This agrees with (**Fernández-Gago** *et al*., 2018; Yamate *et al*., 2021). Some authors (Ramachandiran ***et al.*, 2020; Dill-Okubo *et al.*, 2021 Gonçalves & Costa,** 2021) suggested the anterior salivary gland of Octopus vulgaris secretes the mucus, but this is not accurate evidence because the anterior salivary gland gives a negative reaction to PAS (Periodic acid Schiff) stain. There is relatively no evidence about the secretion of mucopolysaccharide extractable from the anterior gland tissue or its mucous secretion. Further observations of this kind, detailing the biochemical nature of the type of protein produced in these tissues, will clarify this situation in the future through more study.

CONCLUSION

The anterior salivary gland of Octopus vulgaris secretes proteins but does not secrete mucus. The author has estimated its secretion by using two types of stains, protein stains (Bromophenol blue) and (PAS) stains. The author found the protein cells of this gland are stained by protein stain, indicating the natural secretions of these cells.

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