A histological study of the food canal of *Strongylocentrotus franciscanus*[1]

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KURZFASSUNG: **Eine histologische Studie des Nahrungskanals von *Strongylocentrotus franciscanus*.** Der Nahrungskanal des Seeigels *S. franciscanus* besteht aus vier verschiedenen Abschnitten: Pharynx, Oesophagus, Darm und Rectum. Die Wandung des Nahrungskanals setzt sich zusammen aus einem äußeren Epithel, zirkulären und longitudinalen Muskelschichten, einer Bindegewebsschicht und einem inneren Epithel. In den Wandungen von Pharynx und Oesophagus gibt es zwei Arten von Sekretionszellen; eine liefert mucoide, die andere acide Sekretionsprodukte. Der Magen (erste Intestinalschlinge) ist mit einem inneren Epithel ausgekleidet, welches gelbliche Körner enthält, die extrazelluläre Enzyme produzieren. Im inneren Epithel von Darm (zweite Schlinge) und Rectum konnten keine Sekretionsgranula nachgewiesen werden. Lipoide und Glykogen scheinen in der hinteren Magenhälfte vorzukommen und in der vorderen Hälfte des Darmes. Die Aktivität der alkalinen Phosphatase ist beschränkt auf die freie Begrenzung des inneren Epithels von Magen, Darm und Rectum.

INTRODUCTION

Anatomical and histological descriptions of the food canal of regular echinoids given by HAMANN (1887), CHADWICK (1900) and STOTT (1955) are not complete. Certain histomorphological components require confirmation. Information concerning the reserve materials and other biochemical elements, which seem necessary in a discussion of the functional aspects of the food canal, is lacking. Even though *Strongylocentrotus franciscanus* is a common subtidal species along the Pacific Coast of North America, no detailed anatomical studies of its food canal have been made.

This report is an account of studies undertaken to clarify the existing gaps in our knowledge of histomorphology of the food canal and to correlate by histochemical techniques the structural and functional aspects of the cells comprising the epithelial lining of the food canal.

METHODS AND MATERIALS

Sea urchins were collected locally with SCUBA equipment. All specimens were adults, ranging in size from 100 to 200 mm in test diameter. The specimens were

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dissected at a temperature of 5^0 C and segments of the food canal were fixed for further study. The tissues were processed and imbedded for use subsequently in histological and histochemical studies. For general orientation of cell structure, tissues were placed in Bouin's fixative, imbedded in paraffin, and sectioned at 8 μ. These sections were stained with Heidenhain's iron alum haematoxylin or Delafield hematoxylin.

To reveal the presence of glycogen and related compounds, fixation was in Gender's Allen-Bouin. Following preparation of paraffin sections, the periodic acid-Schiff (PAS) routine was followed. Control slides exposed to the action of a buffered solution of saliva were used for differentiation between glycogen and other PAS-positive substances. For recognition of mucous and mucous-like substances, paraffin sections of tissues fixed in 10 % formalin were stained overnight in a very dilute aqueous solution of toluidine blue and dehydrated with 50 % alcohol. This procedure results in metachromatic staining of acidopolysaccharide elements.

For lipid recognition, tissues fixed in formal-saline were imbedded in gelatin, and frozen sectioned at 10 μ. Sections were mounted in 1 % gelatin then stained with Sudan III. Alkaline phosphatase activity was ascertained in tissues fixed in 80 % alcohol and carried through the routine of Gomori.

RESULTS

The food canal of regular echinoids has four distinct parts: pharynx, esophagus, gut, and rectum. The gut has two loops; the first loop is clockwise, when viewed orally, and the second doubles back upon the first one. The first loop differs from the second loop in having different nutritive functions and cell contents, as described below. Henceforth these loops are indicated in terms of stomach and intestine respectively.

The wall of the food canal is simple; the presence of four main layers of tissue is observed throughout the entire length of the gut. Histologically they consist of an outer epithelium of flat cells whose distal surface is bathed by coelomic fluid, a layer of definite bands of circular muscle and longitudinal muscle cells, a layer of connective tissue which varies in thickness, and an inner epithelium of a single layer of extremely tall and slender cells. The inner epithelial lining is chiefly responsible for the thickness of the wall. However, the thickness of the epithelium varies markedly, ranging from 45 to 210 μ.

The descriptions presented below elucidate both histologically and histochemically the cellular constituents of the lining epithelium of the various parts of the food canal.

P h a r y n x : The pharyngeal wall is deeply grooved; hence it may enlarge and contract during food passage. There are attachments of connective tissue running from the lips to the compasses of Aristotle's lantern. In the mid-pharyngeal segment the well-marked grooves in the radial regions are exclusively lined with many mucous gland cells, and these, together with secretory cells with strongly basophilic granules, are distributed randomly throughout the simple epithelial cells.

Mucous gland cells average 5 μ in diameter and vary in height from 111 μ to a maximum of 221 μ depending on their location and animal size. Their nuclei are broadly oval-shaped, measuring 3.7 μ to 6.1 μ and are approximately positioned at the middle third of the cells. The space is filled with granules, which stain slightly with haematoxylin. At a variable distance from the base, the cells narrow and finally open into the lumen of the pharynx. The basophilic granules are comparatively larger and more numerous in the basal portions of the cells, above the nuclear region they become smaller and more sparse. These granules react to the Schiff reagent after treatment with buffered saliva, but do not stain metachromatically with dilute toluidine blue. The cells covering the grooved region consist of granules with a strongly positive reaction to the Schiff reagent after salivary digestion, while they do not stain metachromatically with dilute toluidine blue in acid solution. From these data I conclude that the content of these cells is neutral mucopolysaccharides. The secretory cells with strongly basophilic granules were observed over the free ends of the epithelium; these cells were usually expanded to a diameter many times that of the neighboring cells. The granules of the cells exhibited PAS-negative reaction but stained strongly with haematoxylin. The assumption here is that these cells discharge fluid from the basophilic granules within them, and that the fluid is mainly responsible for the relatively greater acidity of the pharynx as compared with the rest of the food canal.

In non-stained materials, a larger number of reddish colored granulocytes, measuring 18 μ, are notable in the connective tissue layer and the basal region of the inner epithelium. These are the echinochrome-containing ameboid cells. They move about actively between the intercellular spaces and have been seen to move into hemal vessels. Abundant agranulocytes have been found in association with these red ameboid cells. Their size measures 8 μ in diameter. When stained with haematoxylin, their cytoplasm appears clear and faintly basophilic in character.

Esophagus: The esophagus has well-developed circular and longitudinal muscles in its wall and its inner epithelium is a continuous layer of mucous cells. Two types of secretory gland cells are responsible for the production of secretion. One type of secretory cell possess the strongly basophilic granules situated from a region near the basement membrane to the free edge of the cell. These granules are larger and more numerous in the apical region of the cell. They stain strongly with haematoxylin, and are faintly PAS-positive. The other type of secretory cell contains numerous granules which are restricted to the apical segment of the cell. In addition to being PAS-positive, their materials are basophilic and do not stain metachromatically with dilute toluidine blue. In all secretory cells, the nuclei lie at various depths in the epithelium.

Stomach (Fig. 1): The inner epithelium of the stomach region is composed of cylindrical cells, which vary in length (90–162 μ) and give rise to ridges on the inner wall of the stomach. Their nuclei are similar in size and shape to those of the secretory cells in the pharynx, and lie at various depths in the tall cells. The outstanding feature of the secretory cells is their granular content. These small, yellowish, refringent granules lie in clumps in the expanded region adjacent to the nucleus, but they have not been observed below the nucleus. They may extend in one or many rows to the free end of the cell. These secretory spherules are encountered frequently in the stomach epithelium alone. However, their conspicuous absence is noted in the rest of the gut.

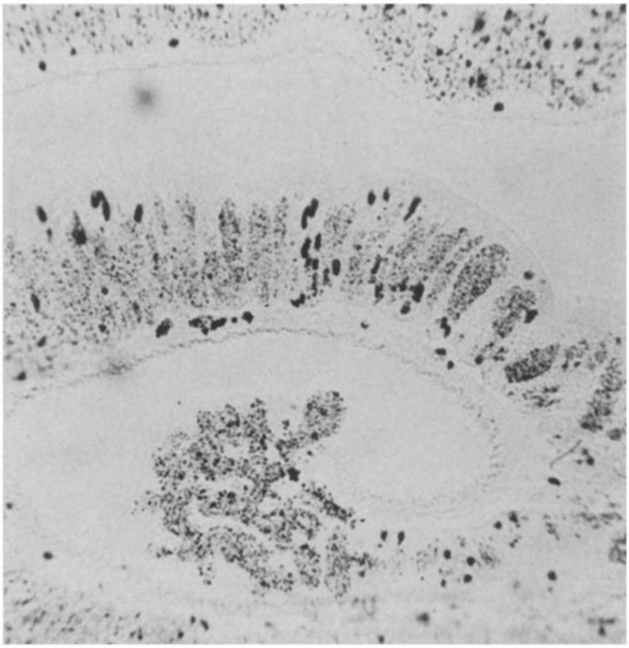

Fig. 1: The echinochrome-containing amoebocytes in basement layer, intercellular spaces, and connective tissue layer of the stomach. Bouin-Haematoxylin. × 140

Small deposits of glycogen are distributed in the basal portions of the cells, particularly in the hinder half of the stomach. Salivary digestion removes all PAS-reactivity attributable to glycogen; however, several sites remain strongly positive. These sites are limited to the connective tissue and the surface region of the inner epithelium of the stomach wall. In addition to this reaction, these sites are faintly basophilic and do not stain metachromatically with toluidine blue. In a well-fed animal these cells contain deposits of lipids, which stain pink with Sudan III. The lipids forming moderate-size droplets, are distributed throughout the basal portions and are lacking from the distal half.

Alkaline phosphatase activity is largely limited to the free edge of the inner epithelial region, but it is weak or lacking in the deeper portions of the epithelium. This enzymatic activity is stronger in the fore half region than in the hinder half. Eosinophilic granulocytes, measuring 9.5 to 18 $\mu \times 16 \times 29.3$ μ are sometimes found imbedded within the inner epithelium. Such granulocytes exhibit yellowish-color in non-stained preparations.

The absorption of ingested material was tested by feeding the animals on starch solution containing carbon particles (under 5 μ in size). Fed individuals were left for definite periods after which the food canal was fixed with Bouin's. Paraffin imbedded sections were used. In the stomach region, within the epithelium one or more carbon particles were found inside the granulocytes, which were distributed heavily near the free edges of the epithelium (Fig. 2). However, cross-sections from all parts of the

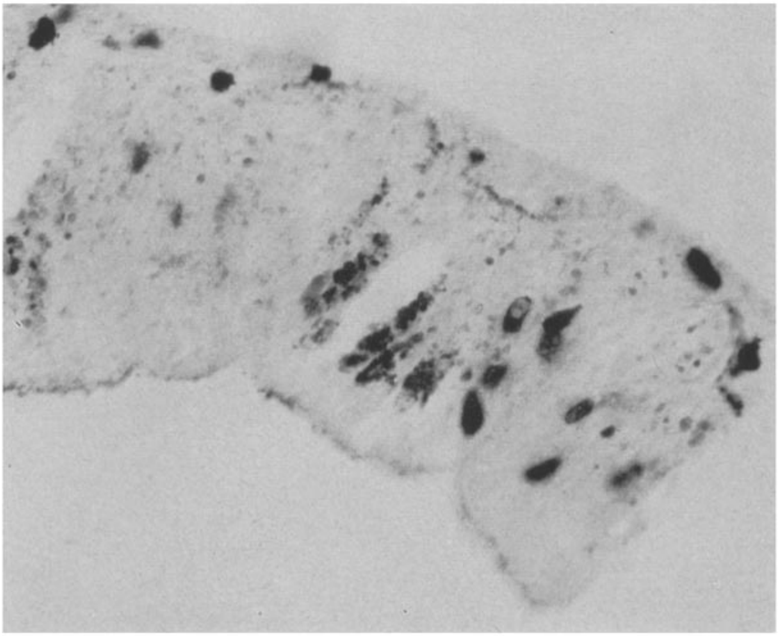

Fig. 2: Cross-section through the stomach showing large granulocytes, echinochrome-containing amoebocyte and carbon particles. × 384

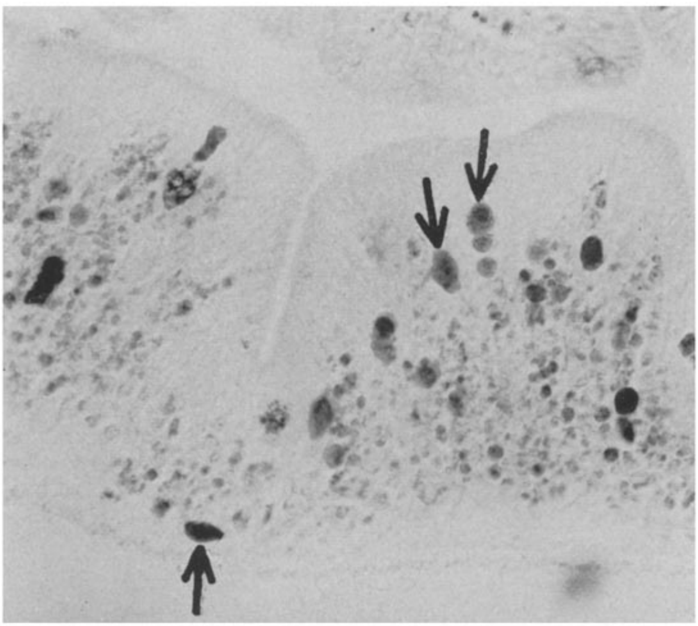

Fig. 3: Cross-section through intestine showing distribution of large granulocytes. The large, dark, oval-shaped structures are the echinochrome-containing amoebocytes (see arrows). × 384

Fig. 4: Cross-section through rectum; peritoneum at left. Bouin-Haematoxylin. × 140

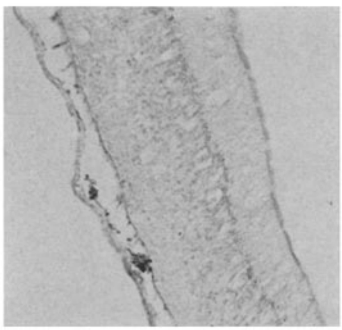

Fig. 5: Section of intestine showing distribution and deposits of glycogen granules. Two echinochrome-containing amoebocytes are shown in the hemal channel (lower left). PAS technique. × 576

pharynx and esophagus of specimens fed on carbon particles did not show the presence of any granulocytes.

Intestine and Rectum: The intestine and rectum of animals fed with carbon particles showed absence of the granulocytes from the epithelial layer. The intestine (Fig. 3) differs from the stomach in its relatively thinner walls, and lack of yellowish refringent granules and of any basophilic granules. Detailed examination shows the intestine to have an inner epithelium composed of cylindrical cells measuring

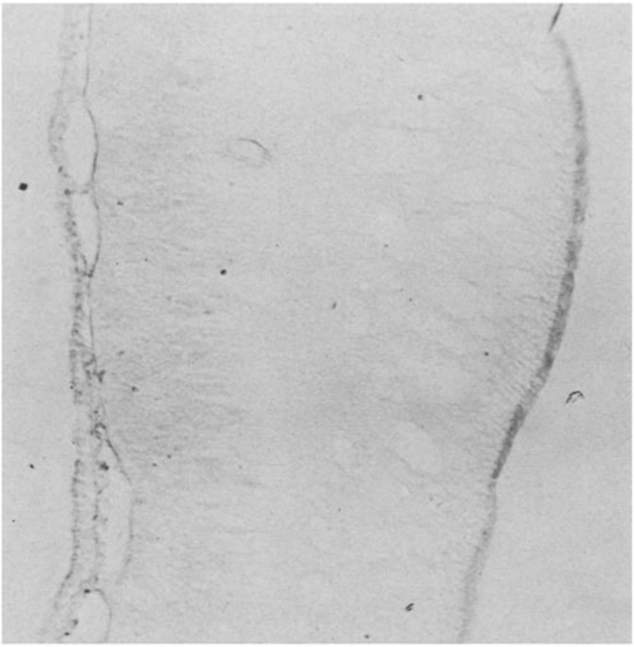

Fig. 6: Cross-section of intestine showing deposits of PAS-positive material after salivary digestion. Vacuoles to the left are segments of the hemal system. PAS control slide. × 576

73 × 162 μ in height. The cytoplasm is clear, homogeneous, and stains faintly with haematoxylin. The nuclei lie at various depths in the epithelium. Histologically the rectum (Fig. 4) is similar to the intestine except that the inner epithelium is thicker, measuring 37–82 μ in height of cells.

In the fore half on the intestine, glycogen (Fig. 5, 6) and lipid deposits are shown in the mid-region of the inner epithelium, while alkaline phosphatase activity is limited at the free end of the epithelium. Glycogen and lipid deposits are not found in the rectum. Alkaline phosphatase activity, however, appears restricted to the marginal region of the inner epithelium, although the activity is very weak.

DISCUSSION

The histomorphology and histophysiology of the food canal of echinoids has been the subject of many investigations. Fürth (1903) provided a brief description of the food canal of a sea urchin. The account of Lasker & Giese (1954) concerning the nutrition of the purple urchin *Strongylocentrotus purpuratus* included the food canal, but histological information was lacking. Concerning the cell density of the epithelium, there is no analysis. Stott (1955) published in some detail the structure and function of the food canal and associated hemal canals in *Echinus esculentus*.

Stott's findings on the secretory cells in the pharyngeal epithelium agree with the findings here presented on *Strongylocentrotus franciscanus* insofar as the presence of two kinds of secretory cells is concerned. However, differences regarding histomorphological descriptions of these secretory cells exist. In *Echinus esculentus*, the basophilic granules and mucous granules in the secretory cells are localized uniformly over the whole of the cells, but the location of these cells was not stated. In *S. franciscanus*, the mucous gland cells contain slightly basophilic granules, which are larger and more numerous in the basal portion than above the nuclear region of the cell. Moreover, secretory cells with strong basophilic granules are observed over the free ends of the epithelium and they are located primarily near the grooved region. Stott's (1955) description and figures show the wall of the esophagus to be composed simply of a continuous layer of tall, narrow, cylindrical mucous secreting cells. In this study, however, two secretory cell types were noted: one type for the secretion of neutral mucopolysaccharides, the other type for the secretion of basophilic granules. I conclude that the latter type produce an acid secretion, which may be responsible for the relatively greater acidity (above pH 6.4) of the esophagus, like that in the pharyngeal region.

The inner epithelium of the stomach consists of long columnar cells which contain chains of small yellowish granules. These granules are distributed in the stomach epithelium proper. Digestive enzymes of the stomach extract predominate in their activity over those in the rest of the food canal. On the basis of gross gut morphology, without any observation of enzymatic activity, Delanuay (1931) suggested that the first half of the gut itself possesses an active digestive function. From the above, it seems likely that these granules located in the stomach epithelium are discharged through the free surface and break down presumably to provide the extracellular exzymes of the lumen.

According to Hilts & Giese (1949), chemical analysis demonstrated an appearance of small quantities of glycogen in various tissues of the sea urchin *S. purpuratus* but most appear to be localized in the stomach. Lasker & Giese (1954) pointed out that a large store of glycogen exists in the food canal. Glycogen and lipid deposits are histochemically demonstrable in the hinder half of the stomach and in the fore half of the intestine of the species used for this study. The conclusion that these substances are food reserves is based on the fact that a significant decrease in glycogen and lipid concentrations can be measured when animals are starved for five weeks or more.

The presence of alkaline phosphatase activity at the free end of the gut epithelium strongly suggests that the gut possesses an absorptive function. In the case of feeding urchins with carbon granules, the inner epithelium of the stomach contains more numerous granulocytes in comparison with that of an animal collected from the natural habitat. The results of the feeding experiments with carbon granules suggests that absorption of carbon takes the form of migration of granulocytes loaded with carbon through the stomach wall into the hemal canals. Kindred (1924, 1926) observed that certain granulocytes of the sea urchin *Arbacia* liberate their protein or lipid inclusions throughout the organism and that they take up nutrient matter from the digestive system and transform it into the cytoplasmic granules. In the light of these data it may be that amoebocytes in the stomach epithelium participate in nutrition to a greater or lesser degree by ingesting particles and absorbing dissolved nutrients.

The observations concerning the histomorphological and histochemical complement of the food canal may be summarized as follows. The esophagus has well-developed circular and longitudinal muscle in its wall; its inner epithelium is a continuous layer of mucous cells. Consequently, it is assumed that food materials ingested are carried to the stomach along the esophageal region by peristaltic activity of those muscles. Furthermore, the mucous secreted from its inner epithelium may assist in the movement of the food by providing lubrication. Proteins and carbohydrates in the ingested material are digested and absorbed in the stomach region. Some of the nutritive substances are elaborated into reserves of polysaccharides and lipids, while others are transported to other organs for storage and use. The intestine and the rectum act as absorptive organs and a conduction tube for the undigested food material.

Few studies have been made on the hemal system which may serve as an avenue for nutritive substances between the gut and other organs. FARMANFARMAIÁN et al. (1962), GIESE et al. (1959), GREENFIELD et al. (1958), and STOTT (1955) visualize the perivisceral fluid as a nutritive pathway.

The physiology of the hemal system and of the coelomic fluid system and their role in the transport of nutrients remains unknown.

SUMMARY

1. The wall of the food canal is composed of an outer epithelium, layers of circular and longitudinal muscles, a layer of connective tissue of varying thickness, and an inner epithelium generally composed of very tall, slender cells.
2. Two kinds of secretory cells are recognized in the pharyngeal and esophageal wall; one produces a mucoid secretion and the other an acid secretion. Numerous echinochrome containing amoebocytes and agranulocytes are distributed in the connective tissue layer and the basal region of the pharyngeal inner epithelium.
3. The stomach is lined with an inner epithelium containing yellowish granules, which are discharged from its surface to provide extracellular enzymes. Large eosinophilic granulocytes, which are assumed to participate in digestion by ingesting particles and absorbing dissolved nutrient substances, are sometimes found to be imbedded within the stomach inner epithelium. The intestine and rectum, however, have never been found to contain any secretory granules nor any amoebocytes in the inner epithelium. Echinochrome-containing amoebocytes, however, are found in all tissues of the food canal.
4. Lipids and glycogen appear to be deposited in the hinder half of the stomach and fore half of the intestine. Starvation for five weeks or more results in a significant decrease of these reserves.
5. Alkaline phosphatase activity is restricted to the free border of the inner epithelium of the stomach, intestine, and rectum.
6. It is concluded that the stomach functions in the digestion of foods, in absorption of the products of digestion, and that the intestine and rectum function as absorptive organs and a conductive tube for the elimination of undigested food materials.

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