Spring 1966

ON THE STRUCTURE AND FUNCTION OF THE DIGESTIVE SYSTEM OF THE NUDIBRANCH MOLLUSC ACANTHODORIS PILOSA

M PATRICIA MORSE

MORSE, M. Patricia, 1938-

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University of New Hampshire, Ph.D., 1966
Zoology

ON THE STRUCTURE AND FUNCTION OF THE DIGESTIVE SYSTEM OF THE NUDIBRANCH MOLLUSC *ACANTHODORIS PILOSA*

BY

M. PATRICIA MORSE

B.S., Bates College, 1960

M.S., University of New Hampshire, 1962

A DISSERTATION

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Doctor of Philosophy

Graduate School

Department of Zoology

May, 1966

This dissertation has been examined and approved.

Charlotte G. Mast

George M Moore

Emery F. Swan

Melbourne R. Carruth

Paula Wright

Wilbur L Bullock

May 17, 1966
Date

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Dr. Wilbur L. Bullock who has guided and advised me during the preparation of this dissertation. I am indebted to all the members of my Doctoral Committee for their many helpful suggestions and direction during this investigation, especially to Dr. Melbourne Carriker for his constructive criticism of thesis content. I am most appreciative for the editorial suggestions of Dr. Arthur C. Borror and for the many kindnesses from the faculty and graduate students at the University of New Hampshire.

Thanks are due to the faculty at Northeastern University, especially Dr. Nathan W. Riser for his encouragement toward the completion of this investigation. My sincere appreciation is extended to Dr. Edmund Smith for his suggestions and advice during the initial stages of this investigation, to Dr. Robert S. Friedman and Dr. Burdette Barrett for their help in preparation of the illustrations, and to Mrs. Allan Pollock for her many hours of typing during the preliminary preparation of the text.

Finally I would like to express my deepest appreciation to Dr. Arthur J. West II who has been an inspiration and help not only in preparation of the dissertation but throughout my graduate study.

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SECTION I

INTRODUCTION

One should study both structure and function in order to determine the nature of any system. As Yonge (1926b) wrote, "...it is to the physiological assumptions of the morphologist and the morphological assumptions of the physiologist that the misconceptions of the past are due." In relation to general invertebrate anatomy and physiology Delaunay (1931) reflected, "On peut distinguer, schématiquement, deux périodes dans l'histoire de cette question, la période anatomique (1800-1900) et la période physiologique (1900-1930)."

In the late 1920's C. M. Yonge established the correlation between anatomical and physiological studies on molluscan digestion. Since then there have been excellent accounts of the functional morphology of the digestive tract, i.e., of prosobranchs (Graham 1932, 1939, Fretter and Graham 1962), opisthobranchs (Millott 1937a, Graham 1938, Fretter 1938-39, Howells 1942, Forrest 1953) and pulmonates (Carriker 1946a, 1946b, Carriker and Bilstad 1946). These investigators examined gastropods by careful dissection, reconstruction of serial sections, observation of ciliary currents and experimental feeding cycles. The authors' descriptions combined gross morphology and histology of the digestive tract with a functional correlation of its parts.

The results of these individual investigations have led to a broader understanding of gastropod digestion. This is reflected in the discussions of form and function of the molluscan stomach by Graham (1949), the gastropod stomach by Morton (1953), the section dealing with molluscan digestion in Morton's book (1958) and in the monograph

of prosobranchs by Fretter and Graham (1962).

The major publications on the functional anatomy of the nudibranch alimentary canal are Graham's (1938) account of eolid digestion and Millott's (1937a) investigation of a sponge-eating dorid Jorunna tomentosa. Dr. Edmund Smith (personal communication, 1964) indicated the need for further basic studies on the functional anatomy of the nudibranch digestive system. Consequently, the opisthobranch mollusc Acanthodoris pilosa (Abildgaard, 1789) was chosen for study because there are few accounts of dorid nudibranch digestion and this particular nudibranch is available in a relatively stable inter-tidal population on the northern coast of Maine (Fig. 1).

This research was undertaken to determine the functional morphology and histology of the digestive tract of A. pilosa. Gross structure was correlated with the entire organism by dissection and serial section reconstruction. Microscopic anatomy and function were studied by utilizing a variety of histological fixatives, stains and histochemical reactions. In accordance with the investigations of Yonge (1926a, 1926b), Fretter (1937), Graham (1938) and Millott (1937a), iron saccharate was employed for experimental indication of intracellular digestion.

The observations and results of this investigation have been utilized to correlate form and function in the digestive tract of A. pilosa. Where possible, terminology parallels that used by authors describing closely related animals. However, the characteristic out-pocketings of the molluscan stomach have been given a variety of names by numerous investigators. Yonge (1926b) working with lamellibranchs related the problem as follows:

> Because of the superficial resemblance of the digestive diverticula of the Lamellibranchs, and of many other Invertebrates, to the liver of the Vertebrates, and the discovery in them of glycogen by Bernard (1855), they became known as the "liver." Weber (1880) later introduced the name hepatopancreas as a result of his discovery of the secretory powers of the diverticula in the Crustacea. In spite of the fact that none of the constituents of bile has ever been discovered in the Invertebrates, and that the digestive diverticula are in no way analogous to the liver of the Vertebrates, as Jordan (1912) has shown in his review of the subject, the terms "liver" and "hepatopancreas," as well as the less questionable designation "digestive gland," are still generally used. Moreover, no attempt is made to distinguish between these organs in the different groups of Invertebrates although both their structure and function in, for example, the Lamellibranchs, Gastropods, Cephalopods, and Crustacea are totally different. In some cases they constitute a digestive gland; in others, including the Lamellibranchs, as I hope to show in this paper, their function is that of assimilation, and so they are most suitably designated digestive diverticula.

In gastropods the outpocketings function in intracellular and extracellular digestion and thus constitute a digestive gland (Fretter and Graham 1962). The term "digestive gland" is used in this study of the opisthobranch, Acanthodoris pilosa. In the historical account, terms employed by the investigators are retained unchanged; however, it is recognized that these terms all refer to the same organ.

The purpose of this study has been to increase the available knowledge of opisthobranch digestion. It is intended that this account will lead to a further understanding of the relationships of form and function in the digestive system of opisthobranch molluscs.

SECTION II

HISTORY

Jan Swammerdam (1737) was one of the earliest workers to describe the "liver" of a shelled snail. His descriptions of the alimentary tract of what was probably a pulmonate ("two pairs of tentacles") are suggestive in terminology and functions of the alimentary tract in man. The first significant descriptive work of opisthobranch molluscs, and the most complete account of the anatomy of Doris yet published, was by the French anatomist Cuvier (1817). He dissected and described the digestive system of several dorid nudibranchs.

The first description of the digestive tract of Acanthodoris (= Doris) pilosa appeared in the 1845 classic Ray Society monograph by Alder and Hancock on British nudibranchiate molluscs. They dissected and compared the anatomy of several species of dorids, and included illustrations of the digestive system of A. pilosa. Later, Hancock and Embleton (1852) published a more detailed account of the anatomy of members of the genus "Doris." A. pilosa was described with additional observations on the gross anatomy of the digestive system.

Following the period of anatomical descriptions of molluscs by these early investigators, numerous studies were made on the histology and physiology of the molluscan digestive tract. It became customary to study one particular organ in detail and the digestive gland became a focal point of attention.

Barfurth (1880, 1881, 1883), one of the earlier investigators, described the digestive gland as a "hepatopancreas" and histologically established three cell types, the "Fermentzellen, Leberzellen and

Kalkzellen." Subjecting the cells to various chemicals, he assigned functions based on observations of cell reactions in histological sections. Barfurth worked on a limited number of animals and his conclusions have been questioned by subsequent authors (Faust 1920, Hurst 1927). He did note variation in the form of the digestive gland, "Die Leber hungernder Schnecken nimmt an Volumen und Gewicht bedeutend ab; mit der Untersuchung weiterer Veränderungen während Hunger und Verdauung bin ich noch beschäftigt." Frenzel (1883, 1885, 1886) used morphological characteristics to describe the digestive cells in representatives of all classes of molluscs. Included in the latter's publications are the first histological accounts of an opisthobranch digestive gland. He recognized three cell types. In 1896 Hecht published an extensive account of nudibranch histology and included a section on the digestive system. He examined several dorids in his work and described four cell types within the digestive gland. Hecht used "physiological techniques" (not given in detail) to draw conclusions of functional activities within the digestive gland of nudibranchs. Observations on variation of the digestive gland among eolid nudibranchs were pointed out in Henneguy's (1925) account of the histology of the eolidien "liver." He was the first to describe ciliated cells in the epithelium of this organ.

The next significant studies in opisthobranch histology were the investigations of the French microscopist, Charles Rousseau (1934, 1935). In his papers on the "liver" of eolidien nudibranchs, he recognized four cell types and discussed their appearance in histological sections. He suggested the occurrence of a secretory cycle in the digestive epithelium. In his historical account, Rousseau

reviewed the status of the physiological studies of other investigators and added his own results of experimentation in the physiological function of the cells of the eolid nudibranch liver.

Physiological investigations of the digestive gland and its association with molluscan digestion were initiated by Cuenot (1892, 1899a, 1899b, 1928). He injected vital dyes into the body cavities of gastropods to determine the roles of the digestive gland in the uptake of nutritive materials and as an excretory organ. He attributed the functions of secretion, excretion, absorption and storage of reserve foods to the "liver" of gastropods. Enriques (1902) discussed these processes as they related to the "liver" of tectibranchs. He described intracellular digestion of chloroplasts in the "Leberzellen" as "...une function nouvelle..." According to Potts (1923), Von Bruel, in 1904, was the first to observe a natural occurrence of intracellular digestion in molluscs. In the digestive gland cells of an ascoglossan opisthobranch (Hermea), he noted chloroplasts of Bryopsis which were derived from the food of the animal. Krijgsman (1925, 1928) described the cycle of secretion and resorption in Helix and discussed the lack of intracellular digestion of the "hepatopancreas."

The publications on the anatomy and physiology of the molluscan digestive tract during the 1930's reflected the influence of the English functional morphologist C. M. Yonge. Dealing with digestion in lamellibranchs, Yonge (1926b) pointed out the necessity of studying living animals and their natural physiological functions in order to better understand molluscan digestion. He instituted the use of carmine and carbon particles for the study of ciliated tracts in the digestive tubes, and emphasized the modifications of the tract in relation to

the utilization of various types of foods. In many instances, Yonge (1931, 1936, 1954) discussed extensively the phylogenetic interrelationships and the significant trends of evolutionary development based on studies of digestive processes among the invertebrates.

Yonge's influence in the late 1930's in publications on molluscs was evident in the investigations on opisthobranchs by Millott (1937a), Graham (1938) and Fretter (1938-39). Millott (1937a) investigated the functional anatomy of the digestive system of a sponge-feeding dorid nudibranch. He described in gross anatomical, histological and functional terms various sections of the digestive tract. Millott used fish blood, iron saccharate and olive oil to study the sites of digestion and absorption. Detailed line drawings of digestive cell types, including three cell types of the digestive gland, accompanied the text. The functional morphology of the eolid nudibranch digestive tract was studied in a similar manner by Graham (1938). Graham also discussed the interesting occurrence of nematocysts in the digestive tract of those nudibranchs which feed on hydrozoans.

The section on Opisthobranchia in Bronn's Klassen und Ordnungen des Tierreichs was compiled by H. Hoffman in 1939. The portion dealing with the digestive tract of nudibranchs includes a summary of all the past literature in both anatomy and histology. Included in the numerous illustrations are many gross anatomical descriptions of digestive tracts of nudibranchs collected on various expeditions by Odhner and Bergh. The references to A. pilosa in this series are all from the 1845 monograph by Alder and Hancock.

Howells (1942) studied the structure and function of the digestive canal in the herbivorous tectibranch Aplysia and described four cell types

in the digestive gland.

Gabe (1952) described four cell types for the digestive glands of two heteropods (Pterotrachea coronata and P. mutica). Using histochemical methods he compared function in the digestive tract of heteropods with that of other molluscs. Thiele (1953) used two eolid nudibranchs, among 32 representative molluscs, to study the detailed structure and function of the digestive gland. He recognized and described at length the rhythmic cellular secretions and identified only two cell types, "...den Sekretions-Resorptionszellen (SR-Zellen) und den Kalkzellen."

Forrest (1953) described the functional morphology of several dorid nudibranchs including A. pilosa. He pointed out that A. pilosa is a suction feeder, using a modified buccal cavity as a suctorial pump. He introduced the term "extrusion" to describe the nitrogenous excretory phase of the digestive cycle in the digestive gland cells. Although not giving a detailed histological account, he did differentiate three cell types in a sponge-eating dorid and at least four types in the suctorial feeders. He also discussed the food of the suction feeders (including A. pilosa). Forrest concluded that the alimentary tract throughout the dorids is basically similar in structure and function, but different feeding habits result in certain modifications.

The Japanese investigator Nakazima (1956) studied the "mid-gut gland" of 62 molluscs of all classes, including five opisthobranchs, in an attempt to correlate the structure and function of the digestive gland. He described the gross anatomy, histology and some histochemistry of this gland in each species. Nakazima briefly and superficially correlated form and function within the classes of molluscs.

Several notable books include accounts of molluscan digestion. Morton (1958) published on the form and function of molluscan digestive systems, citing many previous workers who have correlated gut modifications with the diet. Fretter and Graham (1962) wrote an account of prosobranch digestion in their Ray Society monograph on the group. They recognized two types of cells in the digestive gland of herbivorous prosobranchs and three types in carnivores.

Several papers in the early 1960's, although dealing with pulmonates (Sumner 1965), Bani 1963, Abolins-Krogis 1960) and prosobranchs (Robinson 1960, Martoja 1961, Pugh 1963), are historically significant in the methods employed for application to opisthobranch research. Abolins-Krogis (1960), although primarily interested in the relationship of the "hepatopancreas" of Helix pomatia to shell repair, presented one of the first extensive histochemical investigations of digestive gland cells. Pugh (1963) and Sumner (1965) related histochemistry to digestive functions in prosobranchs.

Summarily, early description of the digestive system of A. pilosa was an anatomical account (Hancock and Embleton, 1852), and little reference to this animal was found in the history until the investigation of the buccal pump by Forrest (1953). However, the literature on molluscan digestion has passed through definite phases of study which are important to present investigations. The original anatomical descriptions were followed by cytophysiological determinations. The digestive gland, the largest organ in the molluscan body involved in digestion, became the center of investigation. Disagreements concerning microscopic anatomy and functional activity of the digestive gland cells are found throughout the literature to the present time. From

the 1930's up to the present, functional morphologists have investigated molluscs as integrated organismic entities. Their accounts of molluscan digestion emphasize the variability encountered from one mollusc to another. It was with this background of understanding that this investigation was undertaken. The digestive system of A. pilosa was studied in relationship to its functional anatomy. The literature has established that A. pilosa must be studied as an integrated whole, with its habits of feeding, gross morphology and histological modifications correlated with the process of digestion. The publications of the functional morphologists have paved the way for the present investigation.

SECTION III

MATERIALS AND METHODS

Collection of Animals

Acanthodoris pilosa was collected during the summer months of 1963, 1964 and 1965 from a population on the northern coast of Maine. The majority of animals were taken during July at the Moosehorn Wildlife Refuge, Edmunds, and additional collections were made at Crowe Neck, North Trescott and Starboard Island, Bucksport. In all cases the animals were collected from Ascophyllum and Fucus fronds on the outer edges of rocky areas. In the course of several trips it was noted that the animals were distributed in approximately a two foot horizontal band along the gently sloping rocks just above the mean tide level.

A. pilosa exhibited a variety of color patterns including black, brown, orange, purple, gray and yellow. The black, brown and yellow nudibranchs were the most numerous, closely resembled the surrounding algae and were associated with a brown encrusting leathery ectoproct, Flustrellidra hispida (identified from the paper of Rogick and Croasdale, 1949). On several occasions, removal of a nudibranch from a submerged ectoproct revealed the rasped surface of the ectoproct suggesting a feeding relationship.

All individuals of A. pilosa were approximately 3/4" to 1" long. As they were surrounded by egg ribbons in the seaweed and continued to lay eggs on the sides of the buckets after collection, they were recognized as being sexually mature. The observed lack of small forms of A. pilosa in the Maine collections suggested a migrating breeding population. The animals were collected in styrofoam buckets

and either were studied immediately at the camp site or transported in plastic bags packed in ice in styrofoam buckets to the University of New Hampshire.

Several intertidal collections were also made at Hilton Park, Dover, New Hampshire, where A. pilosa occurred on the sides and undersurfaces of rocks. There was greater variation of size in members of this population; most appeared smaller than specimens collected in Maine. They were observed feeding on Flustrellidra which encrusts the edges and undersides of rocks.

Fixation

A variety of techniques were utilized to fix parts and preserve whole specimens of Acanthodoris pilosa for further study. In addition, other individuals were used for dissections and observations.

For immediate dissection, living animals were relaxed in 8% magnesium chloride (w/v) and, while still in this solution, dissected under a dissecting microscope. Gross anatomical drawings were made of the whole animal with the digestive tract exposed. To study living cells, pieces of the digestive gland were squashed on slides and observed under the compound microscope.

Animals to be serially sectioned for gross anatomical studies of the digestive tract either were relaxed in 8% magnesium chloride (w/v) prior to fixation, or were placed directly in the fixing fluids. The first method resulted in less distortion. Hilton Park specimens were utilized for serial sectioning.

For study of the digestive gland of A. pilosa nudibranchs were dissected immediately after collection without relaxation. The gland was excised from the body cavity and placed directly in the fixative.

Because of disagreements concerning the best fixative for nudibranch digestive organs (Millott 1937a, Graham 1938), a variety of fixatives was tried to determine the most suitable for A. pilosa. Fixatives were used cold and at room temperature. Most, including Champy's fluid, were most effective under cold conditions. Living animals were placed in containers of salt water resting in an ice-water mixture. Under these conditions, organs were excised and fixed in precooled vials of fixative.

For general histological study, cold Baker's calcium-formalin and Helly's fluid were demonstrably the best. In field collections, Baker's formalin was easier to use because tissues need not be washed, and may be left in the fluid indefinitely.

Helly's fixative was utilized for histological study of the digestive gland and to fix the nudibranchs used in a study of the feeding cycle. Specimens were fixed for 10 to 12 hours and washed thoroughly in tap water for 24 hours. The superiority of this fixative as found in this study agrees with Graham's (1938) contention that Helly's fluid was most "successfully employed." On the other hand in this study of cells of the buccal cavity and esophagus, Bouin's fluid gave less distortion of tissues than Helly's, in disagreement with the conclusions of Millott (1937a) that this fluid is "relatively valueless."

Fixation with Champy's fluid gave excellent cytological detail. Although osmic acid is known for its poor penetration as a fixing component, Champy's proved useful when used under cold conditions.

Smith (personal communication, 1964) suggested the following method using propylene phenoxetol as a preservative. Several nudibranchs were relaxed in 8% magnesium chloride (w/v), placed in Baker's

formalin for 4 hours and transferred to a 1% solution of propylene phenoxetol in distilled water (v/v). This method preserved some of the original colors of the mantle organs and caused less shrinkage and distortion of the digestive organs than other methods. Animals treated in this manner were used for gross anatomical dissections.

Special fixatives were employed for histochemical studies. To show the presence of alkaline phosphatase, the digestive gland was fixed in 80% ethanol. Barka and Anderson (1963) recommend use of Gendre's fixative for preserving glycogen. Tissues were placed in this fluid in a vial and put immediately into a freezer at -20°C for 18 hours. Tissues fixed in Carnoy's fixative and Lillie's neutral buffered formalin were also used.

The animals for the iron saccarate feeding experiment were fixed in an equal mixture of 5% ammonium sulphide in 95% ethanol mixed with Bouin's fixative just prior to use (Millott 1937a). When these solutions were mixed, a flocculent yellow precipitate formed which settled on the tissue and the bottom of the vial during fixation. This washed away with 70% ethanol and did not appear to interfere with sectioning or staining.

After fixation tissues were stored routinely in 70% ethanol. The usual graded ethyl alcohol series was used for dehydration, benzene for clearing, tissues were infiltrated from 4 to 6 hours at 55°C in Tissuemat (Fisher) and embedded in paraffin. Tissue blocks were trimmed during the clearing step. Sections were cut at 5μ or 7μ and mounted on slides. Some sections were mounted serially while in other cases every 5th section was mounted. Albumen was originally employed as an adhesive, using one drop to 30 cc of distilled water. Eventually this

was replaced by distilled water, eliminating any potential staining due to albumen on the slide. Slides were dried on the warming table at 47°C and stored in slide boxes.

Staining Techniques

Histological stains for the digestive tract of A. pilosa were employed to provide 1) general differentiation of many tissues in serial sections of the entire animal, 2) nuclear detail for study and comparison of cell types and 3) contrasting cytoplasmic staining.

For general differentiation of tissues two triple stains were employed, Gomori's trichrome and Masson's trichrome. By combining several dyes, these methods differentiate nuclei, cytoplasm and connective tissue. They were not used for cell detail but were valuable in studying the location of connective tissue relative to the digestive epithelium.

The best stain employed for nuclear detail was Heidenhain's iron hematoxylin. Ehrlich's hematoxylin was routinely used in a 1:9 distilled water dilution with excellent results. Other nuclear stains utilized were Kernechtrot stain and Safranin O both of which stain the nucleus bright red. Orange G., Eosin and Fast Green served as counterstains in combination with one of the nuclear stains.

Extensive histochemical staining was applied to the digestive gland to ascertain the chemical nature of the various cell inclusions. Among inorganic constituents an element of interest was the localization and determination of the presence of calcium in the cells of the digestive gland. The method of Von Kossa was employed.

Tests for localization of organic constituents of cells were divided into several groups. For carbohydrates, the Schiff reaction

was employed using periodic acid oxidation (PAS) and using saliva and acetylation controls. Best's carmine was utilized for glycogen and Mayer's mucicarmine for mucin. For acid mucopolysaccharides the alcian blue and astra blau methods were employed. For carbohydrate metachromasia, thionin, Mallory's phosphotungstic acid hematoxylin and azure eosinate at several different pH levels were used. To determine the general location of protein materials, the mercuric bromphenol reaction according to Mazia et al (1953) was carried out on sections. Lipids were detected by using Oil Red O and Oil Blue N on frozen sections of Baker's formalin fixed material. Gomori's test for the presence of alkaline phosphatase was carried out on material which had been fixed in 80% ethanol.

Ciliary Current Studies

A. pilosa were dissected both in 8% magnesium chloride (w/v) and without relaxation to expose the ciliated areas of the digestive tract. Particulate substances were utilized to follow ciliary currents.

Carbon particles of animal charcoal and of lamp black were ground into a fine powder with a mortar and pestle. Distilled water was added to the powder and the mixture was ground to a paste and diluted to a fine suspension. In addition, a similar suspension of carmine was used. Drops of suspension were dropped in the desired area of the tract by means of a drawn out medicine dropper. Pathways shown by movement of particles were observed under a dissecting microscope and recorded on pre-drawn outlines of the digestive tract.

Feeding Experiments

Natural feeding cycle: Fourteen animals were used in two experiments to determine the natural cycle of the digestive gland cells during feeding. Nudibranchs were collected, stored in the cold room at the University of New Hampshire at a temperature of 12°C and starved for 24 hours. Pieces of Ascophyllum encrusted with Flustrellidra hispida were placed in 3½" finger bowls filled with sea water and one nudibranch put in each container. Time of onset of feeding was recorded and animals were fixed at 15 minute, ½, 1, 2, 3, 6, 8, 10, 20 and 23 hour intervals after feeding. Animals were fixed in cold Helly's for 9-11 hours and washed for 24 hours in running tap water. They were embedded in Tissuemat, sectioned at 7μ and stained in a variety of histological stains.

Experimental feeding cycle: Several British investigators (Yonge 1926a, Millott 1937a, Graham 1938, Fretter 1938-39) utilized a suspension of iron saccharate to study digestion in molluscs. This substance when injected or taken into the gut will be absorbed by the cells and digested (saccharate portion). By fixing animals at desired intervals and applying acidic potassium ferrocyanide to the mounted paraffin sections, the Prussian blue reaction demonstrates the presence of iron. This technique aids in determining the degree of intracellular digestion in nudibranchs and helps to indicate the digestive portions of each structure of the gut.

Six A. pilosa were starved for 24 hours in the coldroom and then relaxed in 8% magnesium chloride (w/v). A suspension of iron saccharate was injected by means of a medicine dropper into the mouth cavity. At intervals of ½, 1, 3, 6, 10, and 16 hours, experimental

animals were fixed in equal parts of Bouin's fixative and a 5% solution of ammonium sulphide in 95% alcohol. The digestive tract of fixed whole animals was excised, embedded, and sectioned. The slides were stained for the Prussian blue reaction and examined for distribution of iron saccharate. As a control, an animal not injected with iron saccharate, was fixed in the prescribed mixture and examined for the natural occurrence of iron in the gut.

SECTION IV

OBSERVATIONS

Gross Morphology

The digestive system of Acanthodoris pilosa is exposed by cutting through the muscular mantle which covers the dorsal surface. The visceral organs are enclosed within a thin tough fibrous membrane which is attached laterally to the edge of the body wall at the junction of the foot and mantle. In the dark nudibranchs, this membrane is heavily pigmented and darker than the external mantle. The membrane binds organs tightly creating an internal pressure causing the internal organs to extrude with force from any opening cut in it.

When the membrane is drawn aside, the major organs of the body are exposed (Fig. 2). The posterior half of the visceral area is covered by the two auricles and ventricle of the heart. Vessels from the ventricle form an extensive covering over the large heart-shaped digestive gland. Typical of dorids, and unlike eolid nudibranchs, Acanthodoris pilosa is grouped by Bergh (1891) as a cladohepatic nudibranch due to the compact anatomy of the digestive gland. The digestive gland is covered externally with hermaphroditic gland tissue and the anterior right section of the space is filled with a series of ducts and glands associated with reproductive activities. The digestive gland is penetrated by numerous blood vessels which are associated with connective tissue and envelop the organ. The exposed portion of the stomach lies in a depression on the mid-dorsal surface of the gland.

The buccal apparatus (Fig. 3) occupies the anterior fourth of the oval visceral area. The dorsal portion is composed of a bilobed

bulb divided mid-sagitally by a white tough strip. On either side of this median strip radiating laterally and in a symmetrical fashion are muscle fibers forming the thick walled buccal pump (Forrest, 1953). Ventral to the buccal pump is the anterior opening into the digestive tract.

On the posterior dorsal surface of the buccal apparatus is the opening of the esophagus. The esophagus enlarges dorsally into a thin-walled crop. In dissections of living animals, the crop appeared either to be collapsed or rigidly distended with a liquid material. The posterior portion of the esophagus is of a uniform diameter and passes directly back from the buccal mass, disappearing into the mid anterior surface of the digestive gland. The radula sac protrudes from the posterior ventral surface of the buccal apparatus.

On either side of the esophagus and emptying into the dorsal lateral walls of the buccal mass are paired salivary glands. The glands are divided into two portions, a proximal follicular portion and a distal thin flattened string-like area. The flattened section courses parallel to the esophagus terminating prior to the disappearance of the esophagus into the digestive gland. There is a nerve connection from the sub-esophageal ganglion that enters the muscle of the buccal mass ventral to the salivary gland opening. Ganglia and commissures of the nervous system form a ring around the anterior tubular portion of the esophagus. On the posterior ventral surface of the buccal mass is the prominent radula sac which extends slightly beyond the posterior dorsal limit of the buccal mass.

In a ventral dissection, the mouth is anterior to the foot and is separated from it by a transverse furrow. Surrounding the mouth

are the oral tentacles which are modified into a veil and drawn out on either side into muscular leaf-like processes (Fig. 4). Depending on the activity of the living animals, these processes are rounded or acutely angled. In fixed nudibranchs, the processes appear triangular and ridged. Around the lateral and anterior border of the veil is a natural grooved depression caused by the overhang of the cloak. Thus the plane of the mouth, because of the position of the fleshy veil is the same as the foot, and when the animal is crawling, is in continual contact with the substrate. The posterior portion of the tentacles is split and the mouth appears as a longitudinal slit surrounded on three sides by the fleshy veil.

Cutting this outer lip away reveals a short tubular connection to the buccal mass. Exposed on the inner end of this short channel is the inner lip which appears ridged. Within the rounded lip is another non-ridged lip called the buccal lip (Fig. 5). This buccal lip is characterized by numerous minute closely set bifid spines on the exposed smooth surface. The more posterior portion of the buccal lip is divided into two hard rudimentary plate-like jaw structures.

Bisection of the buccal mass reveals the morphology and association of the inner organs. The strongly muscular sides leave a small opening between their halves in the buccal pump region. Projecting into the anterior cavity from the floor of the buccal mass is the bilobed odontophore with the toothed radular ribbon in the median groove. The ribbon is in close contact with the opening of the buccal cavity and the teeth protrude between the buccal lips. The odontophore is attached to the lateral ventral walls of the buccal mass in the posterior region by strong muscular bands. The radula disappears posteriorly

into the radular sac. A membranous V-shaped anterior extension of the radular sac covers the dorsal portion of the radula.

The relationship of the esophagus, stomach and digestive gland is apparent in dissections and reconstruction of serial sections. The esophagus enters the anterior face of the digestive gland and empties into the floor of the stomach. This thin-walled portion appears to receive three major ducts of the digestive gland; one large opening is on the right side of the animal and two smaller openings enter on the left side (Fig. 6). The ridged lining of the stomach is continuous with the ducts of the digestive gland. The largest of the three openings receives numerous smaller ducts which communicate with the posterior portion of the digestive gland. The two smaller ducts communicate by a series of minor ducts with the anterior section of the gland. These minor ducts in all cases eventually lead directly into the blind tubules of the digestive gland epithelium.

The dorsal portion of the stomach consists of a highly folded mucosa enveloped by circular muscle. The intestine emerges from the anterior portion of the exposed stomach. The intestine curves to the animal's right on the dorsal surface of the digestive gland and eventually courses posteriorly. There it passes under the heart and terminates at the anus in the center of the non-retractile branchiae postero-dorsally on the mantle.

Also associated with the dorsal stomach and located in the bend at its junction with the intestine is the reduced cecum. It is muscular and pear-shaped in form.

Histology

Buccal mass: The oral veil (Fig. 7) is clothed with ciliated columnar epithelium. On the surface of the epithelium are droplets of secretion which originate from secretory cells. In the submucosa are elongate sac-like cells which stain metachromatically and have light staining oval nuclei. The ducts from these cells pass through the epithelium and empty their secretion on the surface. Additionally there are small multicellular glands scattered through the dermal area with a basophilic secretion within the cell. Their pyramidal nuclei are found at the periphery of the cells. Surrounding these two types of secretory cells are numerous muscle fibers.

Still another type of glandular tissue is well developed in the subepidermal layer on the inner portion of the oral veil. These glands empty their secretion into the cavity formed at the junction of the veil and inner tentacles (Fig. 8). The glands are multicellular with the nuclei located on the outer borders of the cells. The cells are filled with a secretory substance which stains metachromatically (red with thionin and orange with Safranin O).

The inner lips (Fig. 8) are secondarily folded with finger-like projections on the inner and outer surfaces. On the outer surface, the epithelium is ciliated and columnar. However, on the medial edge and on the inner surface there is a homogeneous cuticular layer. The cells have basal oval nuclei and a distinct basement membrane. The submucosa of the inner surface epithilium contains numerous muscle fibers scattered in loose connective tissue. There is a pair of pronounced evaginations at one point in the median edge of the inner lips.

Beyond the inner lip is the buccal lip (Figs. 8 and 9). This

circle of tissue which surrounds the edge of the actual entrance into the buccal cavity is covered (on the outer medial and inner edges) by closely set bifid spines (Fig. 10). The underlying epithelium varies from cuboidal to columnar. The cells also vary in their staining reactions depending in which section of the buccal lip they are found. There is a groove on the inner edge of the lip in which the spines are smaller and are closely associated with the underlying epithelium (Fig. 9). The epithelium is cuboidal and the cytoplasm basophilic. There are numerous well defined intracellular canaliculi and, near the proximal border of the cells, are concentrations of secretion associated with the canaliculi. The canaliculi appear to continue through the border of the cell and pour their secretion into the spine which caps the cell (Fig. 11).

The epithelial cells of the buccal lip have well defined large round nuclei with a distinct nucleolus and irregular patches of chromatin. Beneath the epithelial basement membrane is a thin layer of connective tissue and muscle fibers.

Proceeding from this inner surface around the medial edge to the outer buccal lip, the epithelium becomes irregular in height, the basement membrane indistinct and the epithelial nuclei oval. There are intracellular striations but no well defined canaliculi. Between the outer border of the epithelial cells and the now larger buccal spines is a thick solid material which probably is chitin (Millott 1937a). This cuticular layer appears laminated in some places, especially near the buccal spines, is split at right angles to the laminations. These do not always appear, hence the observed fissures may be the result of mechanical injury due to sectioning through the buccal spines rather

than a natural occurrence.

The muscles in the dermal layer insert near the epithelial cells in the area where the basement membrane is indistinct. The muscles occur in bundles and attach at the edges of the buccal mass.

The spines on the inner groove point toward the lumen and are slightly recurved. On the outer surface of the lip the spines point toward the outside edge of the cavity and are slightly curved outward. They are stout structures and are bifid at the tip.

From just inside the lateral edges of the buccal lips are a pair of projections of the chitin-like layer. They originate above the spine-forming groove of the buccal lip and extend ventrally toward the mouth cavity (Figs. 8 and 9). This material is stratified and stains like the cuticle of the buccal cavity. The projections are probably of a similar chitin-like material. These structures have been named the rudimentary jaws by Hancock and Embleton (1852). The epithelium underlying this area consists of extremely tall columnar cells which contain numerous intracellular canaliculi and appear continuous with the underlying muscle layers. The basement membrane is indistinct.

There is a differential staining of these rudimentary jaws; outer edges are basophilic while the central portion stains acidophilic. Canaliculi from the cells appear to force their secretion out to form these jaws, giving a stratified secretion just above each cell border.

The mouth cavity is continuous with the anterior cavity of the buccal mass. This area anterior to the odontophore and radular ribbon is lined by columnar epithelium covered by a thick cuticle on the surface (Fig. 12 and 13). The cuticle is further stratified into two distinct sections which stain differentially in the same manner as the

rudimentary jaws. The layer nearest the epithelium is acidophilic, staining evenly and intensely with cytoplasmic stains and the outer layer stains unevenly basophilic.

The dorsal epithelium in the buccal pump region is evaginated to form a groove along the median line. The character of the epithelium in the groove changes from cuticle covered cells to ciliated cells (Fig. 14). The groove deepens as it passes posteriorly toward the esophagus. The position of the groove in dissections of living and fixed animals is just dorsal to the radular ribbon on the protruding odontophore.

Glandular epithelium (Fig. 15) lines the walls of the buccal cavity lateral to the ciliated dorsal groove and above the odontophoral muscle connections to the buccal wall. The cells are not ciliated and are distended by a secretion that is metachromatic and PAS positive. The ciliated salivary ducts pass through the lateral walls of the buccal cavity and empty on the surface of the glandular epithelium (Fig. 16).

The odontophore and radular ribbon fill the central region of the buccal cavity. The epithelium covering the muscle bundles of the odontophore resembles that lining the anterior buccal cavity. The radular ribbon is adequately described by Hancock and Embleton (1852) and Alder and Hancock (1845). Its formation was not studied here as accounts of radular formation and replacement are reported by Sollas (1907), Gabe and Prenant (1952) and Runham (1961).

Salivary glands: The paired salivary glands in A. pilosa are differentiated into two areas morphologically but not histologically. In both parts the epithelial lining is characterized by large cuboidal epithelial cells. These cells stain strongly basophilic and have

rounded nuclei and distinct nucleoli (Figs. 17 and 18). In the follicular portion of the gland some of the epithelial cells are ciliated. The duct leading from the gland to the buccal mass is lined by thickly ciliated epithelium. The entire structure is covered by a thin layer of connective tissue. There is evidence of secretion in the lumen of the gland.

Esophagus: There is an area of transition in the dorsal posterior limits of the buccal cavity and the beginning of the esophagus. The dorsal wall of the deepened groove changes typically to eleven, close-set, longitudinal ridges of unciliated cuboidal epithelium. The nuclei of the epithelium are oval to round and have little chromatin material. There is a thin layer of cuticular material on the cell border and the epithelium in this region is very thin. More posteriorly in the esophagus, the ridges decrease in number and the height of the epithelium increases. There is very little connective tissue outside this epithelium. In the area where the commissures of nerve fibers join the two supraesophageal ganglia, the epithelium coalesces to six major ridges with indications of secondary folding. The nuclei are oval and distinct. Posterior to the area of the commissures, the dorsal epithelium progressively alters to ciliated columnar epithelium.

The dorsal ciliated ridges of the esophagus expand into the crop posterior to the cerebral ganglion (Fig. 19). At the point of expansion there are five deep ciliated longitudinal grooves leading into the crop. There are valve-like invaginations of the epithelium at the entrance of the crop (Fig. 20).

The epithelial lining of the crop alternates in appearance according to distension due to the presence of food. In the collapsed

state the glandular nature of the lining is evident and there is folding of the epithelium (Fig. 21). Surrounding the crop is a thin layer of connective tissue with scattered muscle fibers. In the distended crop, the dorsal and lateral walls are drawn out into irregular clumps of glandular epithelium. Their nuclei are located near the edges of the glandular epithelial cells and the sites of secretion cause tremendous distortion rendering difficult delineation of cell boundaries. The basal portion of the crop is lined by ciliated low columnar epithelium with patches of secretory epithelium (Fig. 22). The glandular portions show metachromasia and in many cases are basophilic.

It is relatively simple to follow the crop posteriorly in serial cross-sections which possess a homogeneous mass of food. Scattered in this food are rounded cells corresponding to the wandering cells described by Millott (1937b).

Posteriorly beyond the expanded crop region the esophagus is tubular and is lined by ciliated columnar epithelium. There are rounded glandular epithelial cells scattered throughout the tubular portion of the esophagus (Fig. 23).

Stomach and hepatic ducts: A layer of ciliated columnar epithelium lines the thin-walled floor of the stomach cavity. The mucosa is ridged and the ridges are continuous with the ducts of the large digestive gland. The epithelial cells vary in height with low columnar cells in the crypts and tall columnar cells on the crests of the ridges. There is a large glandular area associated with the lower section of the stomach above the digestive gland ducts (Fig. 24). There are no folds in this area, the epithelium is uniform in height and consists of two types of cells. One is tall ciliated columnar and the other is

glandular with a long neck emptying into the lumen of the stomach. The presence of the neck of the gland cell gives the overall border of this area an uneven appearance, that is, the ciliated cells stand apart (Fig. 25). Nuclei of the glandular cells are basal, irregular in shape, and stain darkly with nuclear stains. The cytoplasm immediately surrounding the nucleus is homogeneous. The broad base of the cell rests on the basement membrane. Above the nucleus is a large vacuolated area, very irregular in shape and appearing as if a secretion had been present which distends the cell. Columnar cells bearing cilia appear wedged in between the gland cells.

The major hepatic ducts are lined with tall ciliated columnar epithelial cells arranged in distinct longitudinal folds with very little intercellular space (Figs. 26 and 27). There is a well delineated ciliated border and the cilia are closely set. The nuclei are situated basally and the cells rest on the basement membrane. The underlying connective tissue extends into the longitudinal folds. There are simple thin exocrine glands dispersed in the ciliated epithelium. There are numerous amoebocytes in the connective tissue whose nuclei characteristically stain dark with nuclear stains. Giant elongate-oval wandering cells (Fig. 25) are found also in the connective tissue. The wandering cells appear to be the same as those in the lumen of the cecum and intestine where the cells are always round. The connective tissue is dense and fibrous, staining light blue after Masson's trichrome stain. The crypt cells in the folds of the ducts are slightly smaller than the cells out in the lumen although they are similar in appearance.

Distally from the major hepatic folds in the minor folds the glandular cells are more numerous. They secrete rounded droplets of

homogeneous secretion into the lumen of the ducts (Fig. 28).

The dorsal mucosa folds of the stomach (Figs. 29 and 30) are more pronounced and project into the reduced lumen. Major folds are secondarily ridged and exposing a large amount of cellular area to the lumen. This portion of the stomach is distinguished from the ventral area by the increase of connective tissue in the submucosa and the well developed surrounding circular muscles. The epithelial cells are all the same height and possess basal oval nuclei (Fig. 31). The ciliated border of the dorsal stomach epithelial cells is even and stains prominently. The cells all rest on a conspicuous basement membrane. There are intercellular spaces between the middle portions of the cells lining the stomach. Wandering cells, although rare, are present in the lumen and in the connective tissue around the stomach.

Digestive gland: The epithelium of the digestive gland is differentiated into two general cell types, the crypt cells and the more numerous tubule cells. The tubule cells are designated as tubule cell A and tubule cell B (Fig. 32).

The crypt cells are named on the basis of their characteristic position within the digestive gland. They are generally triangular in shape and are located in the crypts of the folded hepatic epithelium with the broad base of the triangle resting on the basement membrane. There are several of these cells adjacent to each other in the crypts. The nucleus of the crypt cell is characteristically spherical and large (Fig. 33). There is a prominent rounded nucleolus and the chromatin is scattered in a reticular pattern around the nucleolus. In stained material fixed in neutral buffered formalin, the crypt cell cytoplasm appears reticular. However in the majority of fixatives the cytoplasm

is homogeneous and has a strong affinity for all basophilic dyes. The cells do not show any spherules of concretion, granules or other detectable cytoplasmic inclusions.

The must abundant cells lining the gland are the tubule cells. They are club-shaped in appearance, form the invaginated folds of the tubules and have basal nuclei. The nuclei are oval in shape with discernable nucleoli and very little chromatin in the nucleoplasm.

Tubule cells are differentiated into tubule cell A and tubule cell B due to the characteristic cell inclusions, the height of the cells and their relative position in the epithelium (Figs. 32 and 34).

Tubule cell A is generally a tall narrow cell located on the inner edge of the invaginations of the hepatic epithelium. The cell is narrow at its attachment to the basement membrane and broadens as it projects into the lumen of the tubule. The free border of the cell varies in shape from rounded to an uneven border with protoplasmic extensions. The cells often appear in different stages of constricting "fragmentation phagocytes" (Morton 1955) into the lumen of the tubule (Fig. 35). In these cases just beneath the rounded border of the cell there appears a layer of homogeneous cytoplasm which stains basophilically. Under this layer is an area packed with small closely set vacuolar-like spaces. Many of the spaces are devoid of any particulate matter while others are partially filled with small inclusions.

When the glands are fixed in Helly's and Susa's fluids, there is an area of light brown fine granulation present in the middle and upper portions of the tubule cells A. This material does not stain readily and appears to vary in amount among the A form of the tubule cells. In some cases it extends the entire length of the cell. In cells giving

off fragmentation phagocytes or rounded with vacuolar like spaces at the tips, the brown granular mass is restricted to an area just above the nucleus or is absent. Evidence from squash preparations of the tubule cells indicates that the pigment of these granular masses produces the light brown color of the living digestive gland. In glands fixed with osmic acid, there are larger round black staining granules scattered through these cells. When Perenyi's fixative is employed, the tubule cells take on some different characteristics. There are many large particles or granules in the vacuoles at the tips of the tubule cells nearest the center of the lumen. With thionin they stain metachromatically and are below the outer border of homogeneous cytoplasm. The particles appear larger than the fine brown granulation so prominent in the Helly's and Susa's fixed material. In these sections there is evidence of smaller fragmentation phagocytes being given off at the tips. There are no particles in the vacuoles and the abstriction involves only a small portion of the cell border as in an apocrine secretion.

The type B tubule cells differ from type A tubule cells in morphological position and granular cell inclusions. Type B cells are found along the sides of the hepatic folds between type A tubule cells and the crypt cells. They are slightly narrower at the base and not as tall as type A cells but resemble them, being columnar and having basal nuclei of similar staining reaction and shape. Again these are variable according to fixative and undoubtedly associated with the feeding cycle. However, generally the type B cell border is not lobed and without a terminal layer of dense basophilic cytoplasm. These cells are characteristically filled with distinct round granules that stain bright red

with Masson's trichrome, blue with Mallory's phosophotungstic acid hematoxylin and deep blue with azure eosinate at pH 6.0. In several cases the granules were seen breaking out of the cells into the lumen of the gland in fixed sections. In these same sections granules of the same dimension and staining properties were found in the hepatic ducts and the intestine.

Perenyi's fixed material stained in Safranin O and Fast green emphasizes the different granules in the tubule cells A and B. In tubule cell A the granules stain red while in tubule cells B along the sides of the folds the majority of the granules are larger and stain green.

In both tubule cells A and B it is common to find large vacuoles which fill the distal two thirds of the cells and often distort the cell outline (Fig. 36). These vacuoles contain rounded spherules of material which vary in number and size. Usually if the spherule is large there is only one in the vacuole where as if the spherules are small, there are commonly two, three or four found in one vacuole and closely clumped. These spherules are very conspicuous in formalin fixed material and are somewhat shrunken after Helly's fixative. They do not show an affinity for most stains although they do stain darkly with Mallory's phosphotungstic acid hematoxylin and iron alum hematoxylin. These spherules often vary in structure internally. In sections fixed in Susa's they are ring shaped around the border and contain a homogeneous center. On numerous occasions these spherules have been seen in the hepatic ducts and in the lumen of the intestine, appearing to have broken out of their vacuoles completely.

The connective tissue underlying the mucosa contains a variety

of cells. The most numerous cells are the amoebocytes with very little cytoplasm and rounded nuclei with such an intense affinity for nuclear stains that nuclear detail is lost. The cytoplasm may be drawn out into two horns, or form a stellate pattern around the nucleus. The amoebocytes vary in density in the connective tissue but are most numerous just beneath the mucosa of the hepatic ducts. They are especially prominent in glands stained with iron-alum hematoxylin.

There are oval lightly staining nuclei under the hepatic epithelium which are associated with the reticular and fibrous strands of cytoplasm. They may represent the nuclei of drastically shrunken Leydig cells of the connective tissue. There are numerous blood cells associated with the connective tissue. In many parts of the gland the blood sinuses envelop the hermaphroditic gland and send branched channels into the tissues surrounding the digestive gland tubules. Andrew (1959) noted, "The blood sinuses of molluscs are very thin-walled and often appear to be simple blood-filled tissue spaces." This condition was observed in the postero-dorsal area of the gland where the blood appears to communicate directly with the gland tubules. The blood sinuses contain many amebocytes. In the sinus areas the blood plasma stains acidophilic with cytoplasmic stains.

The contents of the digestive tubule lumen is variable. Fragmentation phagocytes are most frequently observed in glands where tubule cell A is drawn out into a long thin cell with a vacuolar top. This poses the question whether the observed bodies in the lumen are due to the sectioning procedure or whether they are truly separated phagocytic bodies abstricted from the tips of the type A tubule cells. Evidence of complete separation of these bodies supports opinions of Millott

(1937a) and Morton (1955). Serial sections show that these bodies are not connected in all cases to the tubule cells. Single phagocytes may appear in the center of an extensive lumen. Cells often occur in the lower part of the stomach and in the hepatic ducts where there is no digestive gland epithelium present. Thus the presence of phagocytes is considered to be a natural occurrence and not the result of sectioning. The cells have been observed in various stages of abstricting from the digestive epithelium (Fig. 35). The vacuolar morphology is still evident in the phagocytes when they are found in the lumen. They are often seen with nuclei. The periphery of the fragmentation phagocytes has a layer of basophilic cytoplasm just as is found at the tip of the tubule cell before abstriction.

Another inclusion in the lumen, and present more often near the stomach, are the small rounded non-nucleated bodies which stain basophilically. These appear to be droplets of secretion given off by glands in the epithelium lining the stomach.

In the majority of digestive glands the lumen contains food. This appears either as a homogeneous or as a reticular mass of material. Often when the homogeneous mass is present, the cells are in an extended phase and there are numerous fragmentation phagocytes in the lumen. In several digestive glands small pieces of brown residue were seen in the lumen of the stomach, and these were identified as fragments of the zooecium of the ectoproct Flustrellidra. Occasionally a diatom was also seen.

Spherules which occur in the large vacuoles in both tubule cells A and B are encountered also in the lumina of the digestive tubules. They are often found in conjunction with the rounded granules from

tubule cell B.

A careful search was made for evidence of ciliation on the epithelium of digestive lumina. The majority of preparations showed no evidence of cilia. However, in two cases cilia-like structures were observed. A gland fixed by the freeze-substitution method using an osmic acid-acetone mixture showed strands of filamentous material extending from the epithelium into the lumen of the digestive tubules. In sections fixed in Perenyi's fluid tubule cells appeared striated in several areas. It is possible that these striations were retracted cilia, but further investigation perhaps utilizing the electron microscope is necessary to aid in this determination.

Cecum: The cecum is an evagination of the dorsal stomach wall. It is lined by tall ciliated columnar epithelium which is variable in height. There are no folds of the epithelium but because of the variation in height, the taller cells form finger-like projections into the lumen (Figs. 37 and 38). Nuclei are in the basal third of the cell. The ciliated border is different from any other part of the tract in that it is not distinct where the cilia project from the cell borders. Instead the border gives a fuzzy appearance of dense closely-set cilia. The cilia project far into the lumen of the cecum (Fig. 39).

In the cecum of one nudibranch, prominent intercellular cysts are found enclosing small nucleate organisms (Figs. 39 and 40). They appear to be the asexual phase in the life cycle of a sporozoan parasite. With Gomori's trichrome stain the organisms stain bright red and the cyst walls stain light green.

The epithelium of the cecum rests on a layer of circular muscle with an indistinct basement membrane. It appears that the muscles are

attached to the epithelial cells.

The epithelium of the cecum proximal to the stomach is not as variable in height as the section distal to the stomach, thus there is a larger lumen there.

Intestine: The intestine is lined by ciliated columnar cells of relatively uniform size and shape (Figs. 41 and 44). The cilia are closely set together and cover the entire length of the intestinal lumen. The nuclei are basal in position and oval in shape.

The epithelium of the intestine is arranged in distinct longitudinal folds which do not change in size appreciably from the beginning of the intestine to the anus. However, at the anal opening of the intestine there is an increase in the size of the folds, the cells become very regular in outline and the cilia are longer (Figs. 42 and 45).

Glandular epithelial cells are present in the intestine. They are long and slender in shape and empty their secretion into the intestinal lumen. Glandular areas are present in the submucosa of the entire intestine but they are more prominent at the terminal end.

Wandering cells occur in the lumen of the intestine and in the submucosa (Fig. 43). In the submucosa they are oval, and in the lumen, they always appear spherical. In all cases the nucleus has a strong affinity for basic stains and no detail of nuclear inclusions can be seen. The shape of the nucleus varies from oval to horseshoe-shaped and in some cases appears to be lobed. Usually the cytoplasm is basophilic and homogeneous. However vacuoles have been seen in the wandering cells in the lumen of the digestive tract. This description applies to all the wandering cells wherever they are found in the digestive tract.

Ciliary Currents

Utilization of particulate matter to study ciliary currents aids in following the major flow of substances introduced into the digestive system. However, clumping of particles used in this study made it difficult to establish detailed current patterns.

Carbon particles placed on the anterior portion of the buccal mass are swept dorsally and posteriorly along the dorsal ciliated groove of the epithelium of the buccal region. Particles are carried along the longitudinal folds of the ciliated esophagus to the valve at the junction of the buccal cavity with the crop and tubular portion of the esophagus.

The anterior dorsal section of the digestive gland was removed and carbon particles were placed in the region of the esophageal junction. Particles were carried directly along the floor of the stomach, through the major ducts and into the minor ducts. Even under a dissecting microscope it was impossible to follow the particles further than the minor ducts.

The exposed dorsal stomach mucosa possesses six major ciliated folds and particles of carbon flow in the crypts of these folds toward the intestinal openings.

SECTION V

DISCUSSION

The digestive tract of A. pilosa can be divided into four areas of functional significance. The most anterior area is the mouth, buccal cavity and associated parts and functions in reception of food.

Conduction and storage takes place in the next area of the digestive tract which in A. pilosa includes the esophagus and its expanded portion, the crop. This is followed by the stomach, digestive glands and connecting ducts forming the area concerned with digestion and absorption. Finally the cecum and intestine are the area of feces formation.

Area of Reception

A. pilosa is classified by Forrest (1953) as a suction feeder. Although A. pilosa may feed on a variety of foods, it is always found closely associated with Flustrellidra hispida and has been observed feeding on this ectoproct. The nudibranch does not ingest the entire ectoproct but instead sucks out the soft contents of the zooecium.

Anatomical modifications of the area of reception can be correlated with feeding. The oral veil is thrown from side to side as the animal crawls along. When the animal stops to feed, the oral veil is appressed tightly to the substrate. Within the initial opening of the mouth cavity, the inner lip is provided with numerous subepidermal glands which empty their secretion into the cavity. Thus, food entering the mouth is immediately mixed with a glandular secretion. Beyond this is the spined buccal lip. On the outer edges of the buccal lip are numerous folds of the epithelial lining and the submucosa here is richly

supplied with muscles which attach to the buccal wall giving the buccal area wide freedom of movement. The radula is projected through the cuticularized rudimentary jaws and scrapes against the surface of the ectoproct zooecium. The spined buccal lip may serve to anchor the animal to the ectoproct during the scraping by the radula and sucking of the contents from the zooecium. Buccal spines are often broken off on the outer surface of the lip, being replaced from the inner groove of the lip. These broken spines evidently pass through the digestive tract as they were found in feces.

Once the radula has rasped an opening in the zooecium, the soft contents are withdrawn from the animal. The reception area of A. pilosa is greatly modified for this function. The dorsal anterior section of the buccal mass is richly supplied with muscles and forms a bulb. This can be seen moving under the dorsal muscular mantle in some feeding animals. The buccal bulb acts as a pump drawing in the soft food from the zooecium. The material is taken into the small cavity of the buccal pump above the radula and up into the dorsal ciliated groove leading back to the esophagus. Although the animal does not take in large amounts of zooecium, smaller pieces have been found in various regions of the gut. Numerous diatoms, which may be scraped off by the action of the radula, are also encountered in the gut.

The paired salivary glands empty their secretion onto the lateral glandular walls of the buccal mass. Secretion of the salivary gland probably mixes with the secretion of the glandular wall and aids in passage of food along the ciliated food groove. Whether the salivary glands produce enzymes has not been determined.

Area of Conduction and Storage

The anterior section of the esophagus is expanded into a thin-walled crop. An animal can store large quantities of food during prolonged periods of feeding. There is a valve at the junction of the crop and the buccal mass which controls the passage of material into the crop. The crop is provided with distinct glandular areas whose secretions mix with the food. Food in the crop is monogeneous and contains a minimum of particulate matter. No diatoms or pieces of zooecium were found in the crop and it could be that these materials bypass the crop through the heavier ciliated tubular portion of the esophagus.

The tubular portion of the esophagus is muscular and capable of distension. The longitudinal folds vary in their cross-sectional form according to the amount of food present. The cilia are well developed in the esophagus and undoubtedly play a most important role in conducting food from the buccal cavity and the crop to the stomach. However, there are muscles surrounding the esophagus which may aid in conducting food. The tubular portion of the esophagus disappears into the front of the digestive gland and empties directly on to the floor of the stomach and thus assures distribution of food throughout the major ducts.

Region of Distribution, Digestion and Absorption

Food introduced into the floor of the stomach is immediately distributed to the large major hepatic ducts due to the strong ciliary currents created by the ciliated tracts of the epithelium. This was observed when suspensions of carbon particles were used. Ciliary action is unidirectional, leading into the tubules of the digestive gland. The pathway taken by food in the digestive gland has not been directly observed.

Pathways discussed below are based on the study of serial sections, the results of iron saccharate feeding experiments, analysis of the contents of various sections of the digestive tract and correlation of these findings with previous studies.

It has been established (Krijgsman 1928, Thiele 1953) that there is a rhythmic cycle of secretion, digestion and absorption of food materials in the digestive tract of molluscs. This accounts for the tremendous variety of cellular inclusions in the digestive gland, and the past confusion in the literature regarding cell types. Once food is in the digestive tubules, tubule cells become elongate and club-shaped especially in cell A, near the lumen of the tubule. There are numerous stages of the gland abstricting fragmentation phagocytes. These phagocytes are nucleated in some cases; in others they appear to be an apocrine secretion and are small and non-nucleated. In nudibranchs that have nucleated fragmentation phagocytes in the lumen, the nuclei of the tubule cells vary in their position in the cells; in many cases they are very near the lumen at the distal ends of the tubule cells suggesting a holocrine secretion. In other glands the fragmentation phagocytes often do not have nuclei and the nuclei of the epithelial cells are basally located showing no evidence of holocrine secretion. The cytoplasm of the fragmentation phagocytes is vacuolated, only some of the vacuoles containing particles. In animals injected with iron saccharate and fixed 10 and 16 hours after introduction of iron saccharate to the digestive tract, there were numerous large fragmentation phagocytes with distinct particles of iron in the vacuoles. This suggests an initial ingestion of particulate matter into the rounded tips of the tubule cells followed by an abstriction of the fragmentation phagocytes into the lumen. The

iron saccharate experiments were not designed to determine timing of the cycle. The results indicate a correlation between the fragmentation phagocytes and a phase of intracellular digestion. Abstriction of these fragmentation phagocytes provides an increased surface area for ingesting material for intracellular digestion.

It is difficult to ascertain whether any enzyme granules are given off by the tubule or crypt cells during the digestive cycle. Present studies indicate that the main type of digestion in A. pilosa is intracellular but do not exclude the possibility that enzymes are secreted by the salivary glands or the cells of the digestive gland. In alkaline phosphatase experimental sections, there is activity indicated at the border of the crypt cells and tubule cells of the B type. This may be due to a movement of secretion molecules across the cell border. Thiele (1953) has suggested that pre-secretory granules in the tubule cells (S-R cells of Thiele) dissolve during the feeding cycle of Helix and that the secretion binds with protein and that this secretion is undetectable in stained sections. Yonge (1931) discusses a phylogenetic development of extracellular digestion in gastropods correlated with a change from a herbivorous diet to a carnivorous diet. This is yet to be shown for A. pilosa. Due to the watery nature of animals consumed in the diet there appears to be retention of intracellular digestion and lack of specialization of the gut to produce elaborate enzymes for extracellular digestion of grossly particulate foods.

The role of the crypt cells in the digestive gland of A. pilosa is not clear.. These cells contain calcium spherules in many pulmonates and some prosobranchs. Wagge (1951) and Abolins-Krogis (1960) have

indicated their importance as a supply of calcium for shell repair. In pulmonates the calcium is transported to the site of repair via the connective tissue and blood cells. In A. pilosa there is no indication of the presence of calcium and this is correlated with the absence of a shell.

Crypt cells have been shown to be areas of storage (Wagge 1951). This function may occur in A. pilosa, since the cells stain positively for protein material with bromphenol blue and are slightly PAS positive. Additionally there are indications of alkaline phosphatase activity at the cell borders due possibly to absorption of digested material.

A part of the rhythmic digestive cycle of A. pilosa involves "extrusion" (Morton 1953) of a variety of cell inclusions. The digestive epithelium during this period appears irregular with protoplasmic strands extending from the borders and a variety of inclusions are present in the lumina of the tubules, stomach and intestine. There are four types of material which appear to be extruded. The exact nature of these materials and their origin are not understood. All four materials appear together in the lumina of the tubules in a single gland. Furthermore, similar granules are found in fecal strings given off by living animals.

The round granules typically associated with tubule cell B, which stain bright red with Masson's trichrome and blue with Mallory's phosphotungstic acid are also found in the lumina of the digestive tract. In fecal strings from living animals, granules of approximately the same shape and size appear reddish in color. Just what these granules are is not known. Abolins-Krogis (1960) indicated these cells as being excretory cells. She maintains that these excretory cells and the digestive

cells (corresponding to tubule cell A) are variants of the same cell type.

The granular material associated with tubule cell A which is so evident in Helly's and Susa's fixed material is also considered a waste material. The evidence for this is not complete. The coloration of this material is yellow-brown in living animals and a fine granular mass of yellow-brown material is egested in the feces.

Small rounded, somewhat vacuolated cell fragments are often encountered in intestinal contents. It is suggested that these are the end products of the fragmentation phagocytes. The phagocytes have completed their breakdown of food materials, the soluble materials are given off into the lumen and in some way reabsorbed by the lining cells, and the spent fragment of a cell is egested.

Finally, the large yellow round concretions of the large vacuoles in the tubule cells are also found in the lumina of the tubules, stomach and intestine, as well as in fecal strings of living animals. Regarding these concretions in prosobranchs, Fretter and Graham (1962) state "Their real nature is unknown, but as they may often be found, apparently unaltered, in the feces, they appear to be excretory matter of some sort." In prosobranchs and opisthobranchs these concretions are formed in different cells. In some prosobranchs (Fretter and Graham 1962) they are associated with the large "triangular-shaped cells" (crypt cells in A. pilosa). In A. pilosa they are confined to the tubule cells and have not been observed in the crypt cells. Their shape is variable due perhaps to their stage of formation. In some cases they are in a concentric ring form, indicating formation by accretion. These cells were extensively pictured by Henneguy (1925) and he stated

they were composed of xanthin, a waste product of nitrogenous metabolism.

The digestive gland is a complex site of digestive processes. The complexity is increased by the reaction of the cells and their contents to different fixatives. These problems along with the obvious rhythmic cycle involving secretion, absorption and extrusion render it difficult to establish a well defined description of the gland for any one animal. Previously a variety of names and assigned functions have been designated for the cells of the digestive gland. On the basis of studies of A. pilosa it is impossible to designate cells of the digestive gland tubules as either digestive or excretory. With studies of enzymes and further studies in the feeding cycle, these characterizations may become possible. For these reasons these cells are tentatively named tubule cells (variants A and B). and crypt cells.

There are two blood cells that are associated with various organs throughout the digestive system. One is the small amoebocyte generally located in the connective tissue. They are most numerous in the submucosa of the hepatic ducts. In some forms the small cytoplasmic areas show a positive glycogen reaction to Best's carmine stain. Amoebocytes may play a role in the transfer of food materials from the fragmentation phagocytes to the crypt cells for storage as they are often numerous at the edges of the crypt cells in the digestive gland.

The other type of cell is the wandering cell. Millott (1937b) discusses the occurrence of wandering cells in the digestive epithelium of a variety of nudibranchs including A. pilosa. Utilizing the Prussian blue reaction, he injected iron saccharate into the haemocoel. By fixation at intervals and studying sections of the animals, he studied the subsequent distribution of iron. He concluded these cells were

wandering cells, amoeboid when living, and that they were actually a special type of blood cell. He further states that "evidence indicated that the wandering cells of nudibranchs are excretory taking up effete matter from the haemocoele and discharging it into the lumen of the gut." In A. pilosa these cells have been observed throughout the gut lumina. They are rare in the esophagus and most numerous in the intestine and cecum. They are capable of moving freely between the connective tissue of the submucosa and the lumina of the organs and they are seen in several cases passing between the epithelial cells. Millott (1937b) suggests the cells discharge their products into the lumen of the gut and then migrate back through the wall. Observations in A. pilosa confirmed the wandering behavior of these cells although the nature of their inclusions is not known. The cells are not seen in the blood sinuses. This may be due to a different staining reaction of the cell when it is being formed, or there may be a different origin of the cells in A. pilosa.

It is difficult to understand how extrusible material in the hepatic ducts moves into the stomach cavity. Millott (1937a) describes the cilia of the hepatic ducts as beating both in the direction of the blind tubules and toward the dorsal stomach in separate tracts. This was not seen in A. pilosa although this may be due to the clumping of the carbon particles rendering a reverse current invisible. It would seem logical that the contents of the tubules are caught in an ascending current and swept into the muscular stomach.

Area of Feces Formation

Ciliation of the hindgut is similar to that of the tubular esophagus. There are major tracts in the intestine which conduct the feces to the outside. Because of the thickening of the folds at the anus, the feces is ejected forcefully from the opening. The feces comes out in spurts which aids in prevention of fouling of the branchial area.

Yonge (1931) has suggested that in gastropods there is a correlation between the compactness of the fecal string and the position of the anus relative to the mantle cavity. Prosobranchs which have undergone torsion have elaborate mechanisms which compact undigested material thus preventing fouling of the closely associated mantle cavity. Opisthobranchs on the other hand have undergone detorsion and the anus is located on the postero-dorsal surface. In A. pilosa, feces drop off the sides of the animal, the fecal string is not well compacted and the cecum is reduced in size. This functional morphology in A. pilosa supports Yonge's theory.

The cecum in A. pilosa is a small structure which communicates with the stomach. A ciliated groove leads into the narrow opening of the cecum. In one animal, a fecal string was found in the intestine. This consisted of compacted detritus surrounded by a thick clear layer of mucous. Recognizable in the pellet were diatoms, teeth and sections of ectoproct zooecium. There were many wandering cells in the lumen. This was not seen in the majority of animals; the cecum is generally devoid of any material and only occasionally are diatoms and pieces of teeth found in the intestine. The glands in the cecum give rise to the mucous which covers the large particulate waste materials and the cilia function to compact any pieces of hard parts into small pellets.

The rare presence of hard parts of the animal's food would account for the small cecum. Millott (1937a) reports a well developed cecum in the sponge eating dorid Jorunna tomentosa. In this instance the cecum serves to coat sponge spicules with mucous and compact them into a fecal string. The cecum of A. pilosa only forms small fecal pellets.

SECTION VI

CONCLUSIONS

This investigation has demonstrated that Acanthodoris pilosa feeds on the ectoproct, Flustrellidra hispida and the functional anatomy of the digestive system reflects modifications for manipulation of semi-liquid food. Morphological investigations correlated with iron saccharate experimentation and ciliary current studies have elucidated more fully the functions of the digestive tract of A. pilosa.

The following major alimentary tract modifications have been identified for A. pilosa.

1. The anterior buccal mass is enlarged forming a muscular buccal pump which is utilized to suck the contents of the ectoproct zooecium into the buccal cavity once the radula has rasped a hole in the zooecium.

2. A spined buccal lip at the entrance of the buccal cavity probably functions to anchor the muscular buccal mass during pumping and/or scraping activities.

3. An enlargement of the dorsal anterior esophagus forms a thin-walled crop capable of distension and functioning for storage of semi-liquid food.

4. Fragmentation phagocytes are formed by apocrine and holocrine secretions of the tubule cells of the digestive gland. They serve as sites of intracellular digestion as was concluded by iron saccharate experimentation.

5. There is a reduced cecal evagination of the dorsal stomach which probably functions to form small fecal pellets.

The digestive gland epithelium of A. pilosa consists of two cell types, the pyramidal-shaped crypt cells and the more numerous tubule cells with their variants A and B. The rhythmic nature of the digestive epithelium involves: 1) a period of ingestion of food particles by the fragmentation phagocytes and the tips of the tubule cells, 2) a concentration of undigested materials in the tubule cells and 3) extrusion of these waste products into the lumina of the digestive gland. The function of the crypt cells is not fully known although histochemical results indicate they may be sites of storage of reserve foods.

Amoebocytes are found in the connective tissue of the digestive tract and are most numerous in the area of the ventral stomach and hepatic ducts. Less frequently, large wandering cells are found in the connective tissue, between epithelial cells and in the lumina of the tract. Whether these two blood cells have a common origin is still undetermined.

The primary method for food conduction through the digestive tract is by ciliary currents. For this function the mucosal lining of the various parts of the tract is well supplied with gland cells and ciliated epithelial cells. In addition to drawing food into the buccal cavity, the buccal pump probably serves to propel food through the esophagus.

The intestine functions to conduct undigested food to the outside. Due to detorsion, the anus opens on the postero-dorsal surface of the nudibranch. The fecal string is of a loose nature and there is a conspicuous absence of hard parts in the diet of this suction feeder.

In summary, Acanthodoris pilosa is a carnivorous opisthobranch and the functional morphology of the digestive system shows modifications for suction feeding. Subsequent investigations should be directed toward

specific digestive organs of A. pilosa and comparisons of this nudibranch with other suction feeders. Such studies must correlate the functional anatomy of the entire digestive tract for greatest value in contributing to the general understanding of nudibranch digestion.

SECTION VII

FIGURES

Figure 1. Acanthodoris pilosa. The oval-shaped nudibranch is characterized by the bipinnate branchiae encircling the anal opening and soft conical papillae covering the dorsal mantle. (x 7)

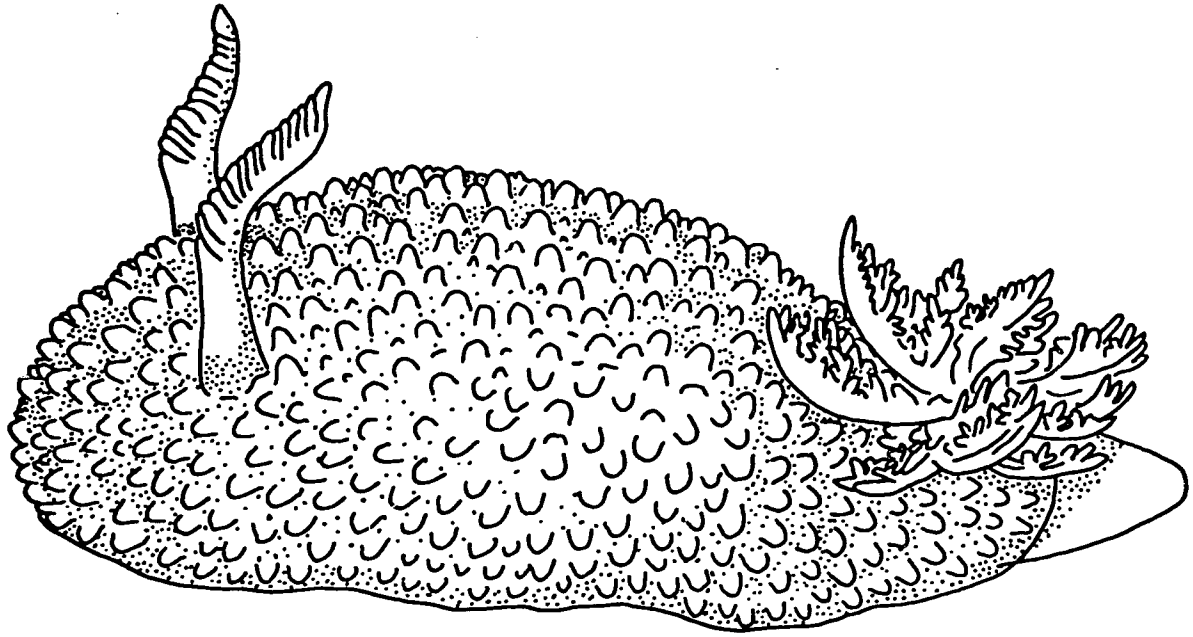

Figure I

Figure 2. Gross morphology of the digestive organs of A. pilosa exposed by cutting through the dorsal mantle and tough thin membrane coverings. Drawn from nudibranch fixed in Baker's formalin and stored in 1% propylene phenoxetol. A.O., anal opening; B.R.M., buccal retractor muscle; B.S., blood sinus; C., cecum; CR., crop; D.S., dorsal stomach; E., tubular portion of esophagus; I., intestine; M.S., median strip; S.G.A., follicular portion of salivary gland; S.G.B., thin flattened portion of salivary gland. (x 9)

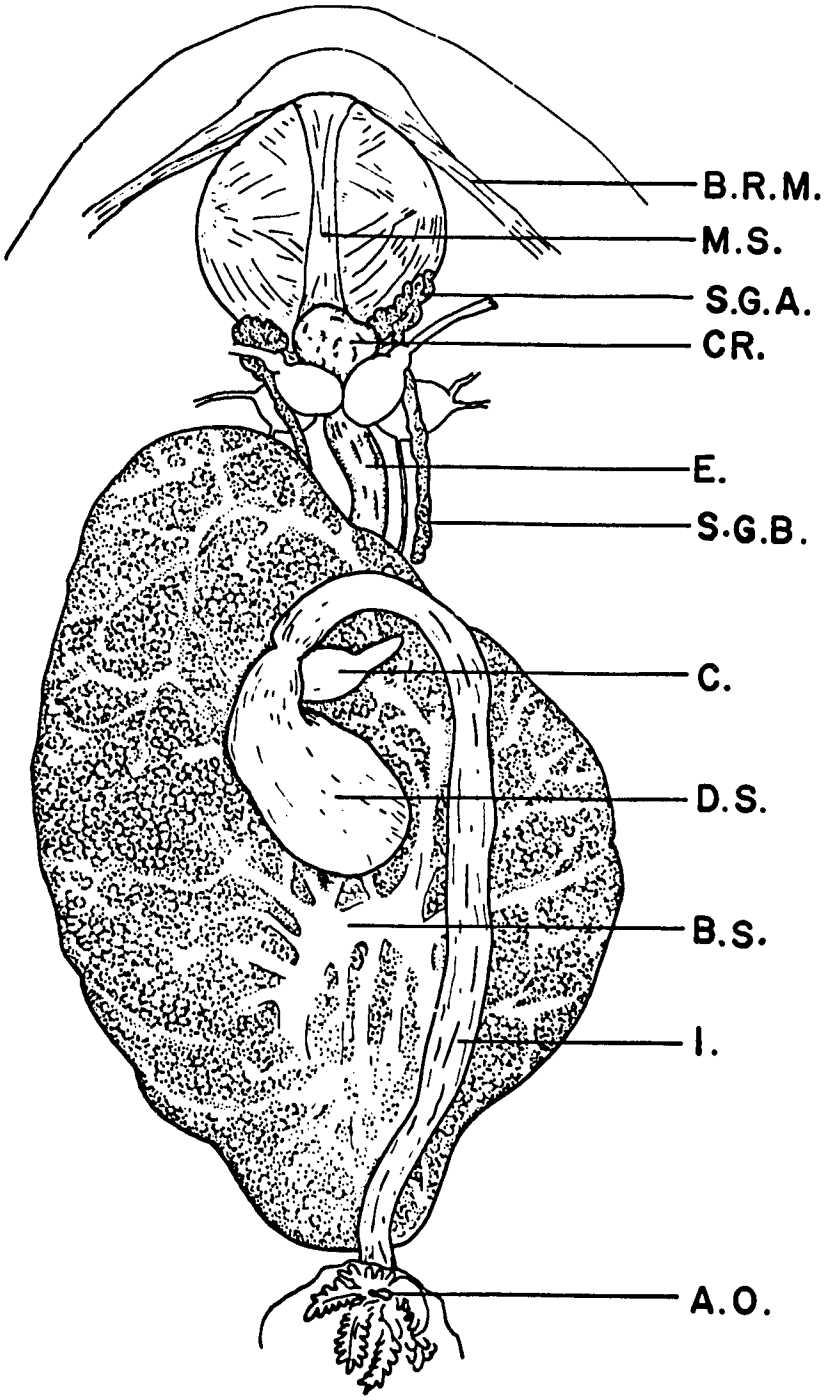

Figure 2

Figure 3. Lateral view of the buccal mass and associated parts of A. pilosa. Note the anterior dorsal enlarged buccal pump. B.P., buccal pump; CR., crop; E., tubular portion of esophagus; S.G.A., follicular portion of salivary gland; S.G.B., thin flattened portion of salivary gland; R.S., Radular sac. (x about 10)

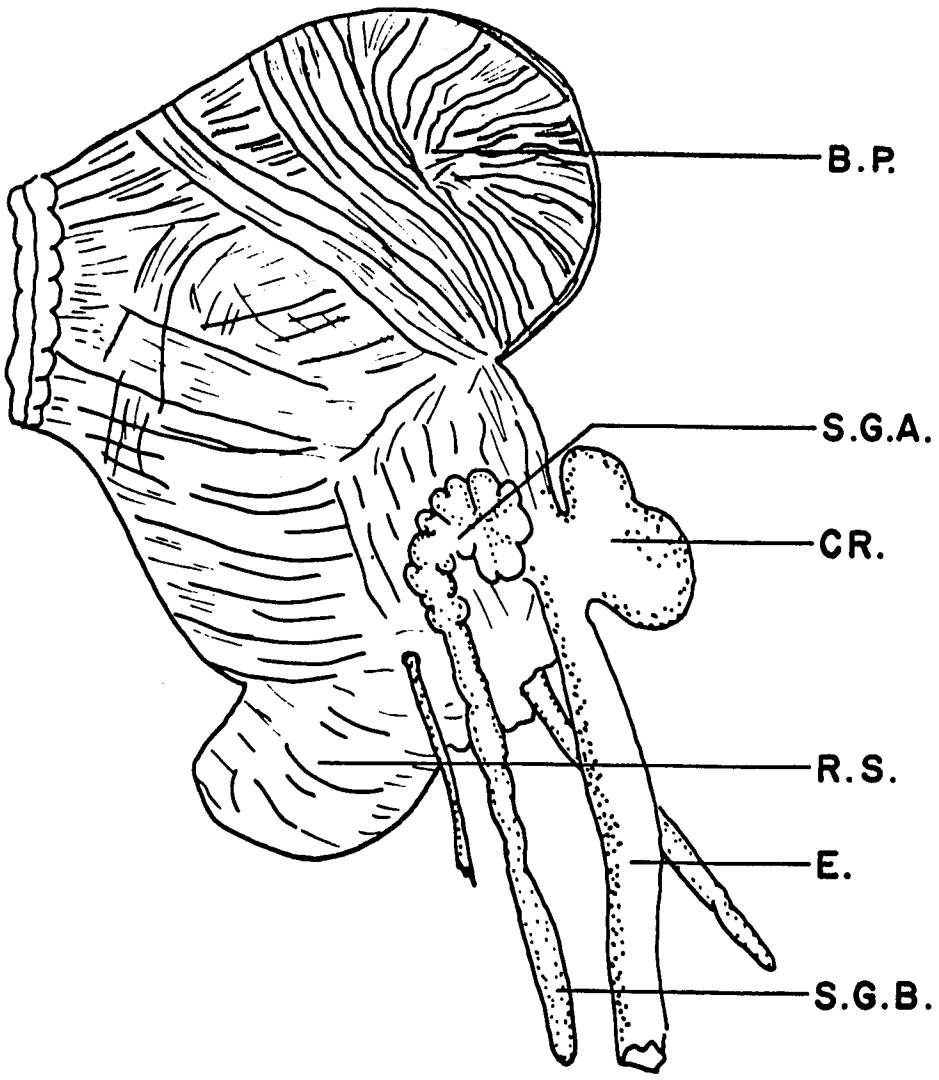

Figure 3

Figure 4. Anterior ventral view of A. pilosa with the oral veil drawn out laterally into triangular processes. The longitudinal slit in the center of the oral veil leads into the oral cavity. Fixed in Baker's formalin. F., foot; M.O., mouth opening; O.V., oral veil. (x about 6)

Figure 5. Anterior ventral view of ridged inner lip and spined buccal lip exposed by cutting away the oral veil of A. pilosa. The radula protrudes from the center opening. B.L., buccal lip; B.P., buccal pump; B.R.M., buccal retractor muscle; I.L., inner lip; M.S., median strip; R., radula. (x about 14)

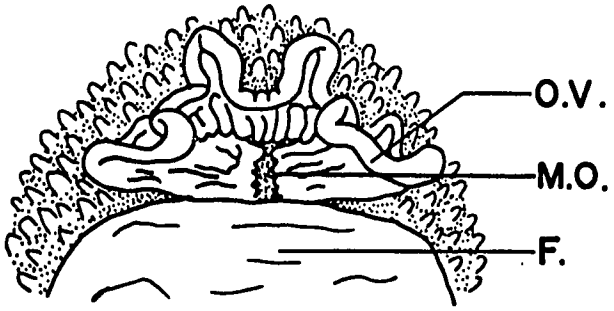

Figure 4

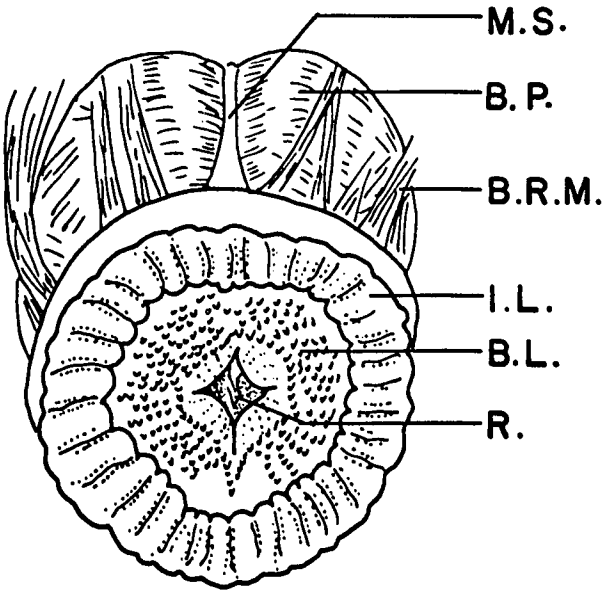

Figure 5

Figure 6. Major hepatic ducts of A. pilosa leading from the ventral floor of the stomach, exposed by reflecting the dorsal portion of the stomach to one side. Fixed in Baker's formalin. D.S., dorsal stomach; E., esophagus; H.D., hepatic ducts; I., intestine; V.S., ventral stomach. (x about 13)

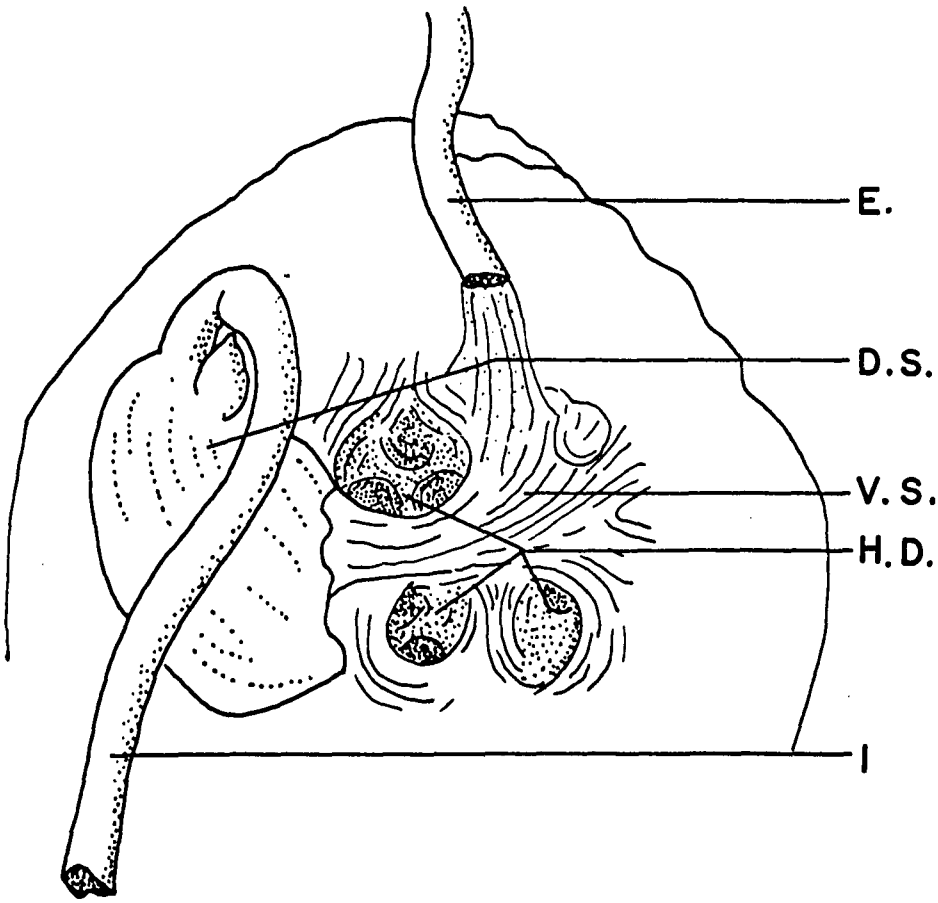

Figure 6

Figure 7. Transverse section of A. pilosa in the region of the oral veil. Fixed in Bouins, stained with Safranin O and Fast Green. (x 70)

Figure 8. Transverse section of the inner lip and spined buccal lip of A. pilosa. The multicellular glands are shown darkened with stain and distributed in the subepidermis on the inner side of the oral veil and the outer edge of the inner lip. The rudimentary jaws originate just inside the buccal lip. Fixed in Bouins, stained with Safranin O and Fast Green. (x 60)

Figure 7

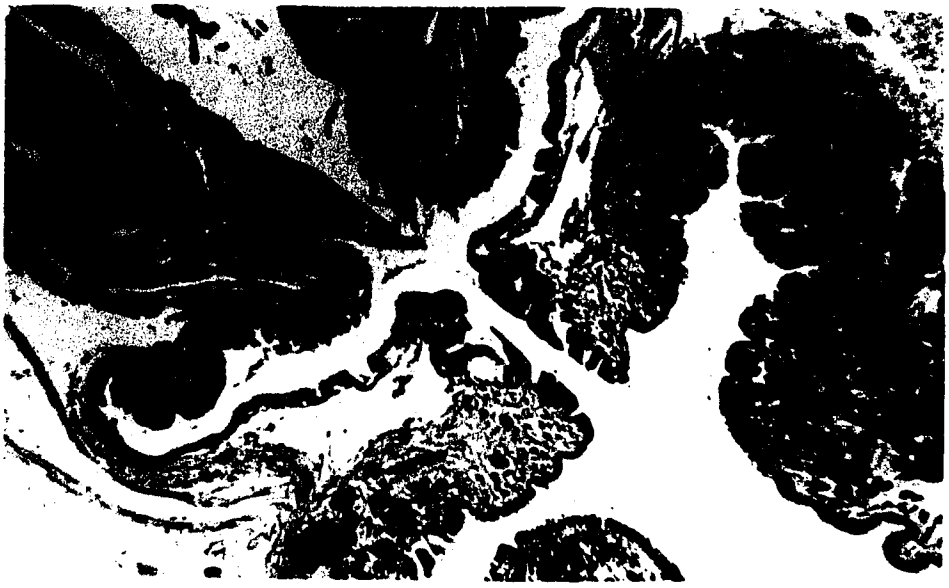

Figure 8

Figure 9. Transverse section of the buccal lip of A. pilosa showing the relationship of the buccal spines to the underlying epithelium. The inner groove is where the spines originate and the rudimentary jaws are extensions of the cuticular buccal lining. Camera lucida drawing from Bouins Fixed material. B.S., buccal spine; I.G., inner groove; R.J., rudimentary jaw.

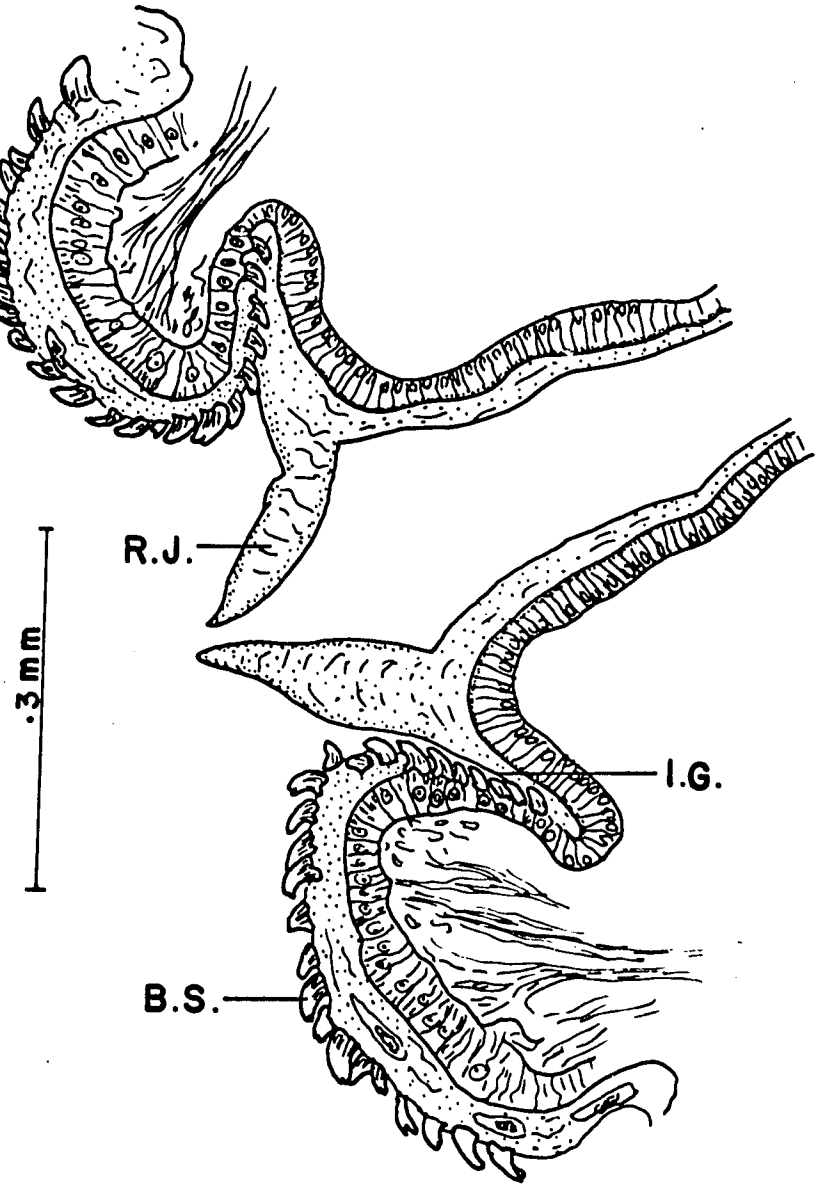

Figure 9

Figure 10. Photomicrograph of buccal spines of A. pilosa. Fixed in Bouins, stained with thionin. (x 2300)

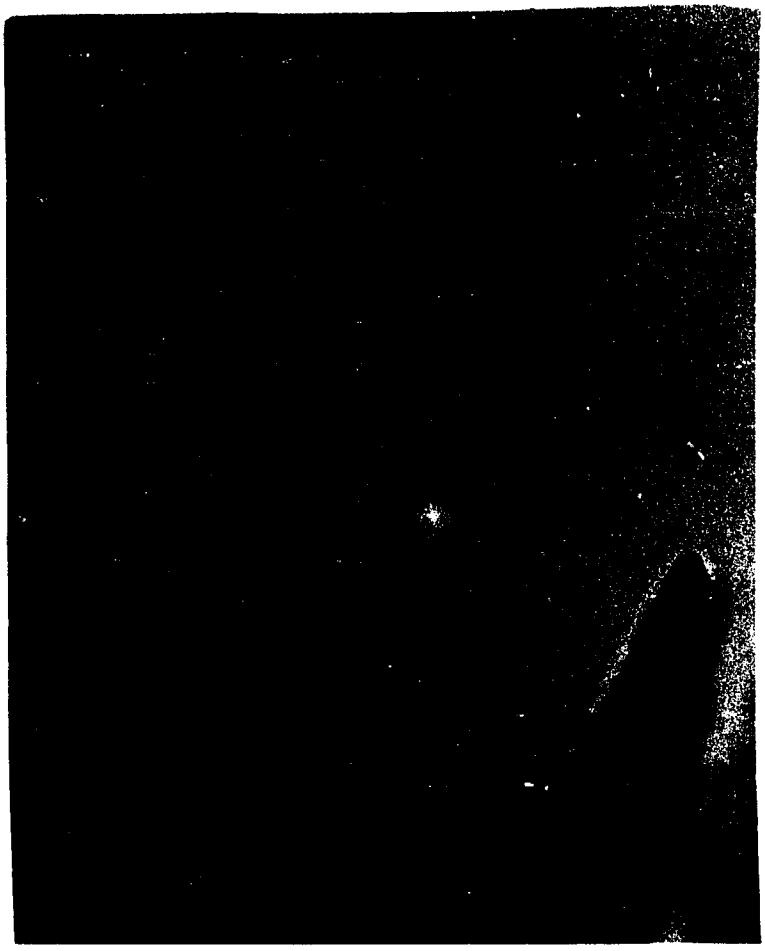

Figure 10

Figure 11. Portion of a section through the groove along the inner edge of the buccal lip. The intracellular canaliculi pass from the epithelial cell into the buccal spine capping the cell. Camera lucida drawing from Bouins fixed material. B.S., buccal spine; I.C., intracellular canaliculi.

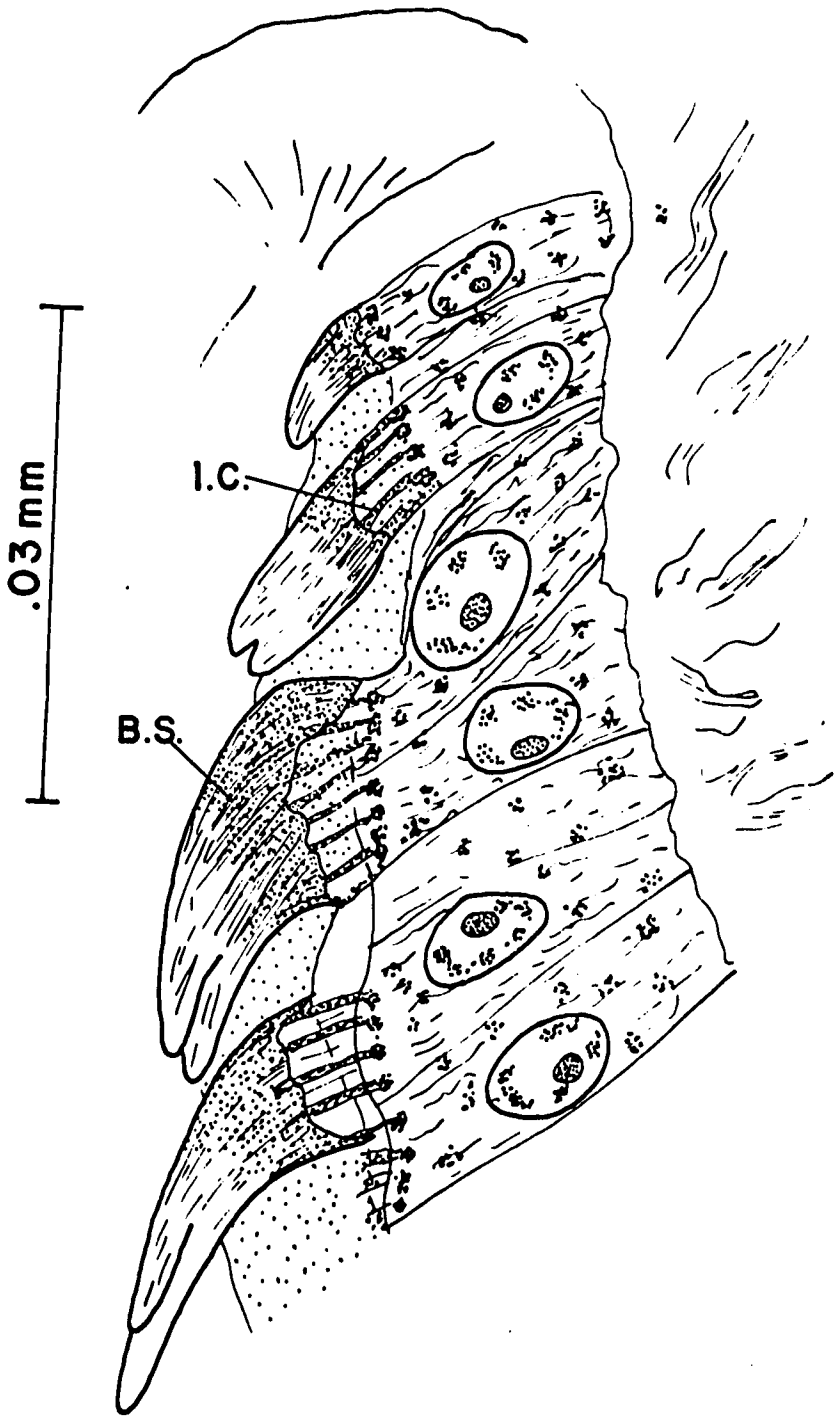

Figure 11

Figure 12. Portion of a transverse section through the cuticular lining of the buccal cavity showing the relationship of the intracellular canaliculi of the epithelial cells to the secreted cuticle. Camera lucida drawing from Bouins fixed material.

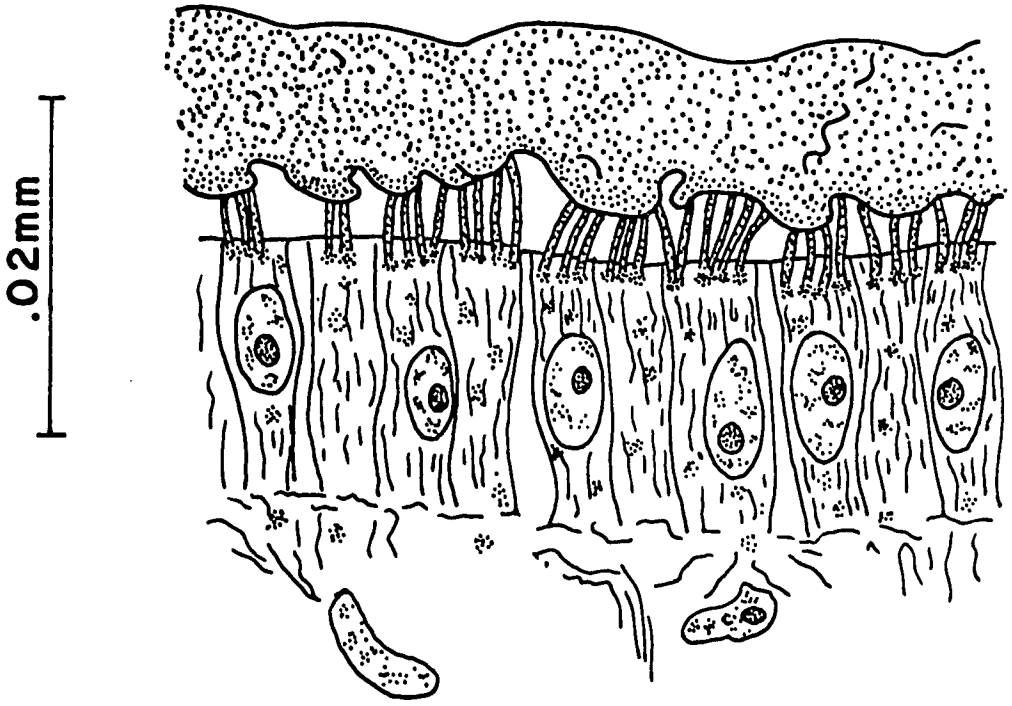

Figure 12

Figure 13. Photomicrograph of a transverse section through the buccal cavity of A. pilosa in the region lined by dark staining cuticle. Fixed in Bouins, stained with Gomori's trichrome. (x 1700)

Figure 14. Ciliated epithelium of the buccal cavity of A. pilosa. A wandering cell is observed in the epithelium. Fixed in Bouins, stained with Hematoxylin and Eosin. (x 600)

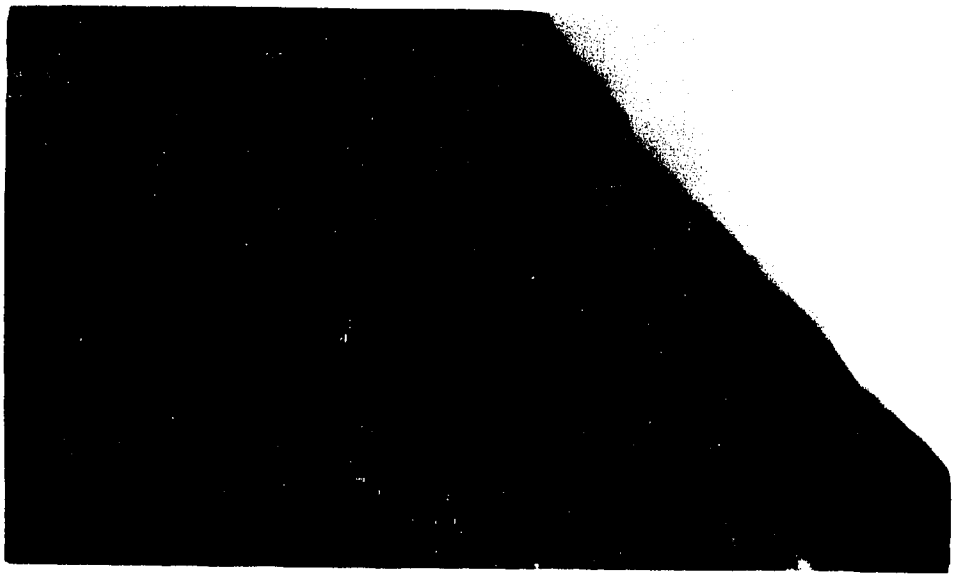

Figure 13

Figure 14

Figure 15. Transverse section of the glandular ridge on the lateral buccal wall of A. pilosa. The uneven appearance of the epithelium is due to glandular secretions distending the cells. Fixed in Bouins, stained with Hematoxylin and Eosin. (x 200)

Figure 16. Transverse section through the posterior portion of the buccal mass of A. pilosa. The ciliated duct of the dark staining salivary gland is seen just before it enters the buccal cavity on the lateral wall in the region of the glandular ridge. Fixed in Bouins, stained with Hematoxylin and Eosin. (x 60)

Figure 15

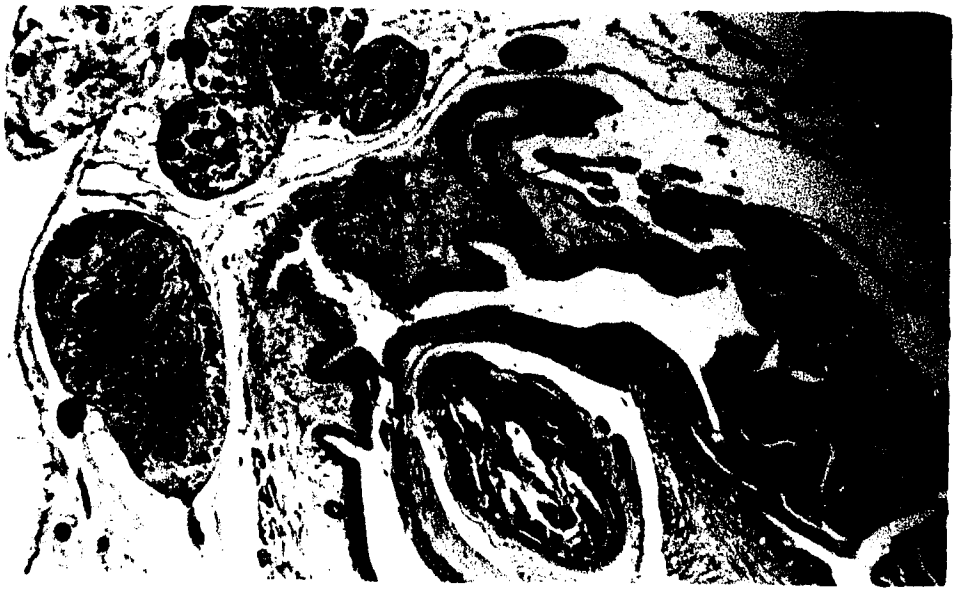

Figure 16

Figure 17. Longitudinal section through the distal portion of the salivary gland of A. pilosa. Camera lucida drawing from Bouins fixed material.

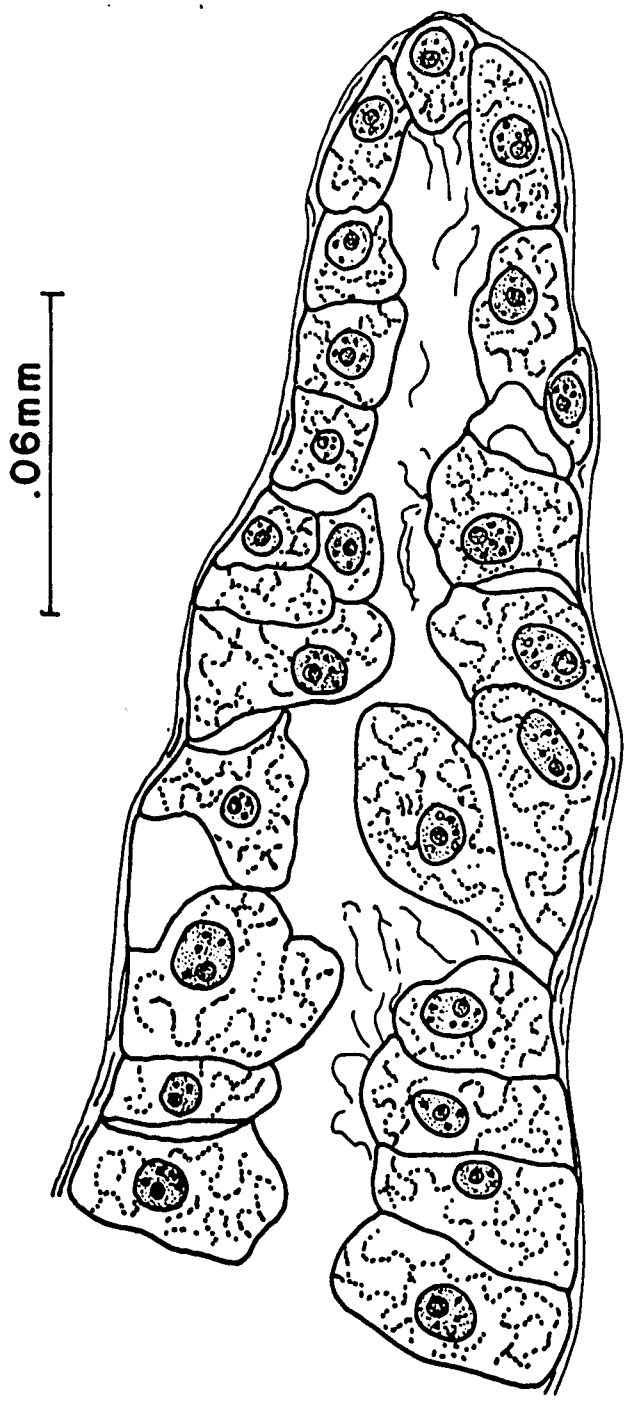

Figure 17

Figure 18. Photomicrograph of a longitudinal section through the distal portion of the salivary gland of A. pilosa. Fixed in Bouins, stained with Thionin. (x 700)

Figure 19. Transverse section of A. pilosa through the buccal mass at the opening of the buccal cavity into the modified crop of the esophagus. There are large ganglia on either side of the esophagus. Fixed in Bouins, stained with Thionin. (x 60)

Figure 18

Figure 19

Figure 20. Transverse section through the posterior part of the buccal mass of A. pilosa showing the valve which controls entrance of food into the crop. Fixed in Bouins, stained with Thionin. (x 60)

Figure 21. Longitudinal section through A. pilosa showing the crop in a collapsed state. Fixed in Helly's, stained with Gomori's Trichrome. (x 60)

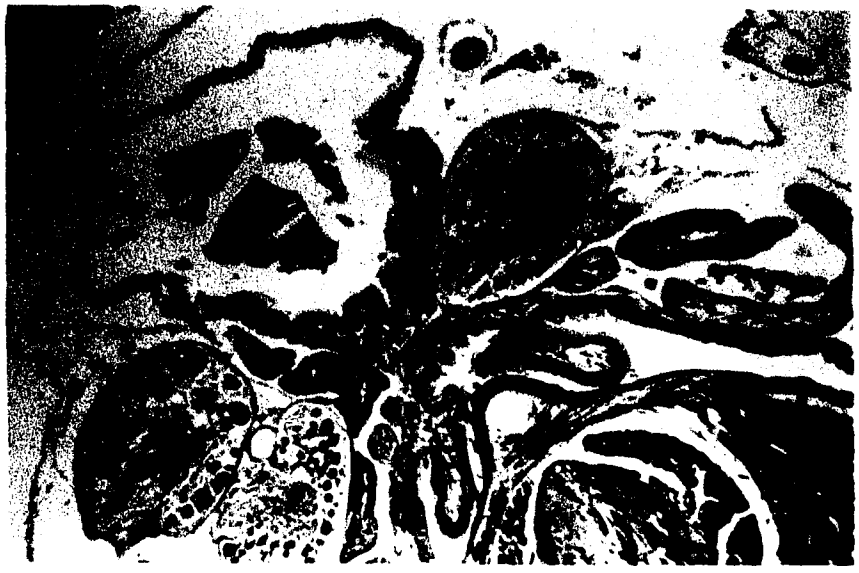

Figure 20

Figure 21

Figure 22. Transverse section through ventral portion of the crop of A. pilosa showing ciliated columnar epithelium with patches of glandular cells. Fixed in Bouins, stained with Gomori's Trichrome. (x 200)

Figure 22

Figure 23. Portion of a longitudinal section through the tubular esophagus of A. pilosa. A rounded glandular epithelial cell is seen between the ciliated columnar cells. Camera lucida drawing from Bouins fixed material.

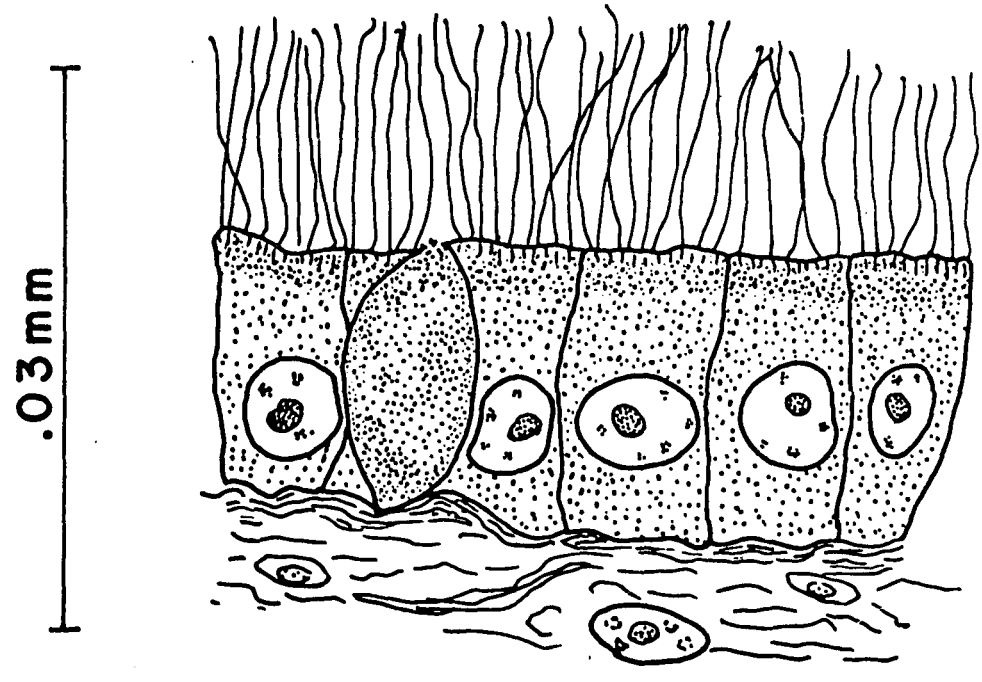

Figure 23

Figure 24. Section through glandular area of ventral stomach in A. pilosa. Fixed in Bouin-DuBoscq, stained with Masson's Trichrome. (x 150)

Figure 25. Enlargement of glandular area in ventral stomach of A. pilosa showing the ciliated cells wedged between the glandular cells of the epithelium. A darkened oblong wandering cell is in the underlying connective tissue. Fixed in Bouin-DuBoscq, stained with Masson's Trichrome. (x 550)

Figure 24

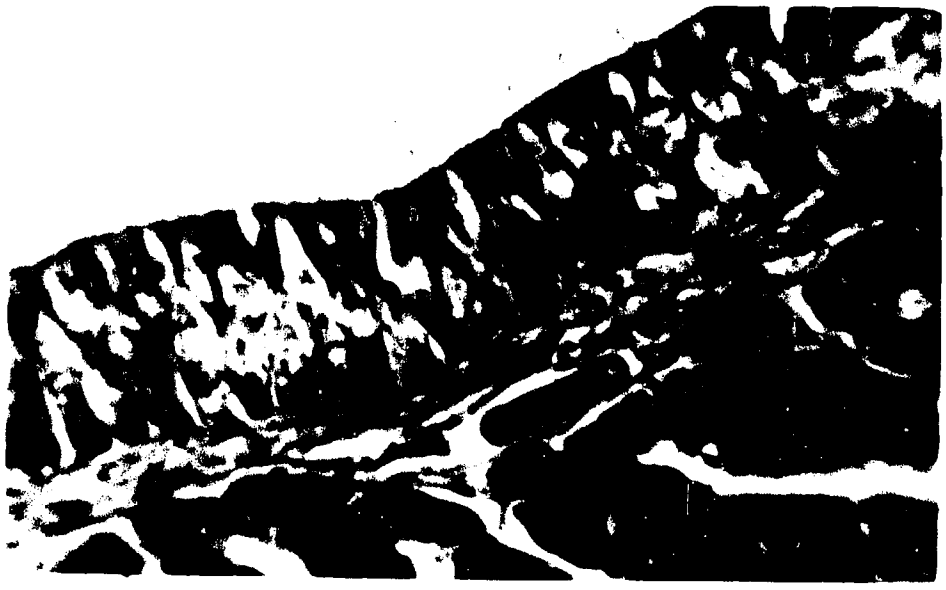

Figure 25

Figure 26. Section through the hepatic duct of A. pilosa. The thin secretory cells are dispersed among the ciliated columnar cells of the ducts. A wandering cell with its characteristic bean-shaped nucleus is in the epithelium. Camera lucida drawing from Helly's fixed material. E.C., epithelial cell; G.C., gland cell; W.C., wandering cell.

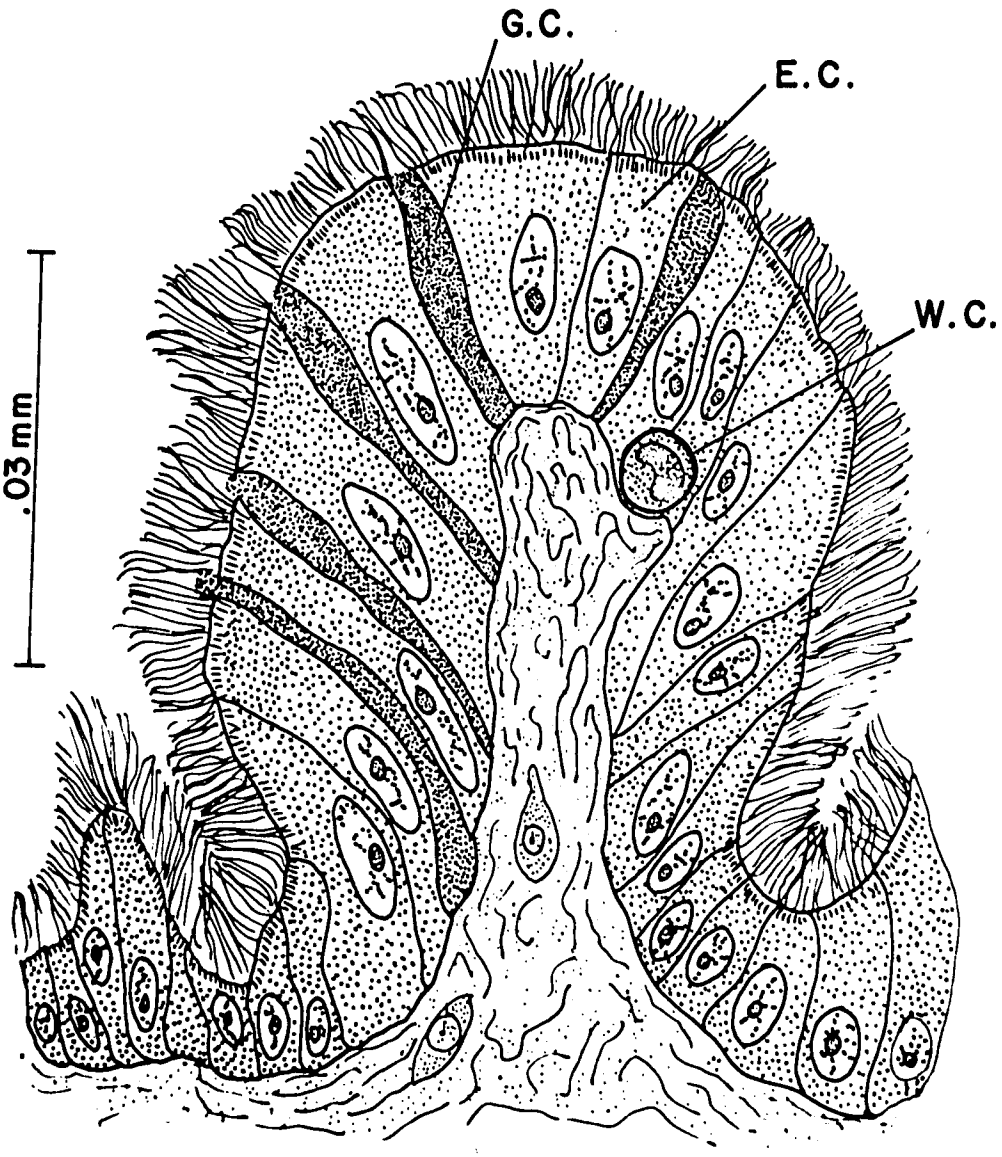

Figure 26

Figure 27. Photomicrograph of the hepatic duct of A. pilosa showing the epithelial folds of the mucosa. Fixed in Helly's, stained with Gomori's Trichrome. (x 700)

Figure 28. Section through folds of a minor hepatic duct of A. pilosa showing droplets of glandular material being secreted into the lumen. Fixed in Bouin-DuBoscq, stained with Masson's Trichrome. (x 1000)

Figure 27

Figure 28

Figure 29. Transverse section through the mucosal folds of the dorsal stomach of A. pilosa. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 100)

Figure 30. Section through the dorsal portion of the stomach of A. pilosa, the layer of circular muscle surrounds the prominent mucosal folds. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 150)

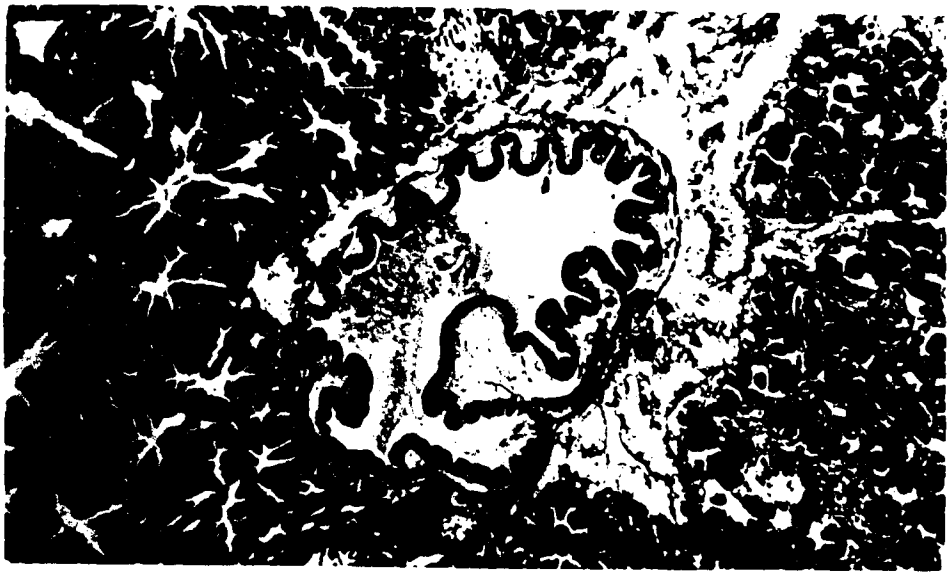

Figure 29

Figure 30

Figure 31. Ciliated columnar epithelium of the dorsal stomach of A. pilosa. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 600)

Figure 31

Figure 32. Section through a fold of the digestive gland of A. pilosa. The large round granules are associated with Type B tubule cells. Type A tubule cells are vacuolated at the distal ends. Camera lucida drawing from Perenyi's fixed material. T.C.A., tubule cell A; T.C.B., tubule cell B; C.C., crypt cell.

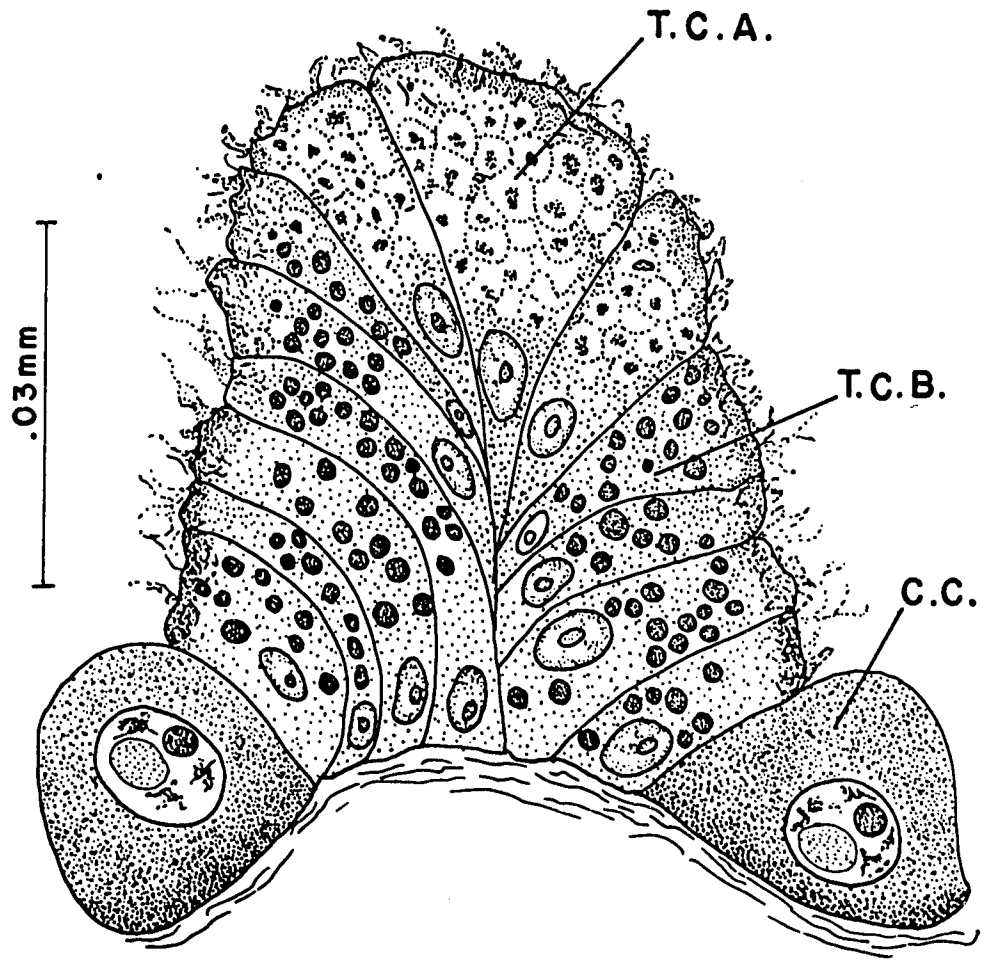

Figure 32

Figure 33. Section through a crypt cell of the digestive gland tubule of A. pilosa. The nucleus shows the large dark staining round nucleolus and numerous chromatin granules. Fixed in Bouin-DuBoscq, stained with Safranin O and Fast Green. (x 1750)

Figure 34. Section through a tubule of the digestive gland of A. pilosa. The dark staining granules are in type B tubule cells. There is a large round dark staining wandering cell in the connective tissue underlying the hepatic epithelium. Fixed in Susa's, stained with Safranin O and Fast Green. (x 400)

Figure 33

Figure 34

Figure 35. Section through a portion of a digestive gland tubule of A. pilosa. The gland appears in different stages of forming fragmentation phagocytes. Camera lucida drawing from Helly's fixed material. F.P., fragmentation phagocyte.

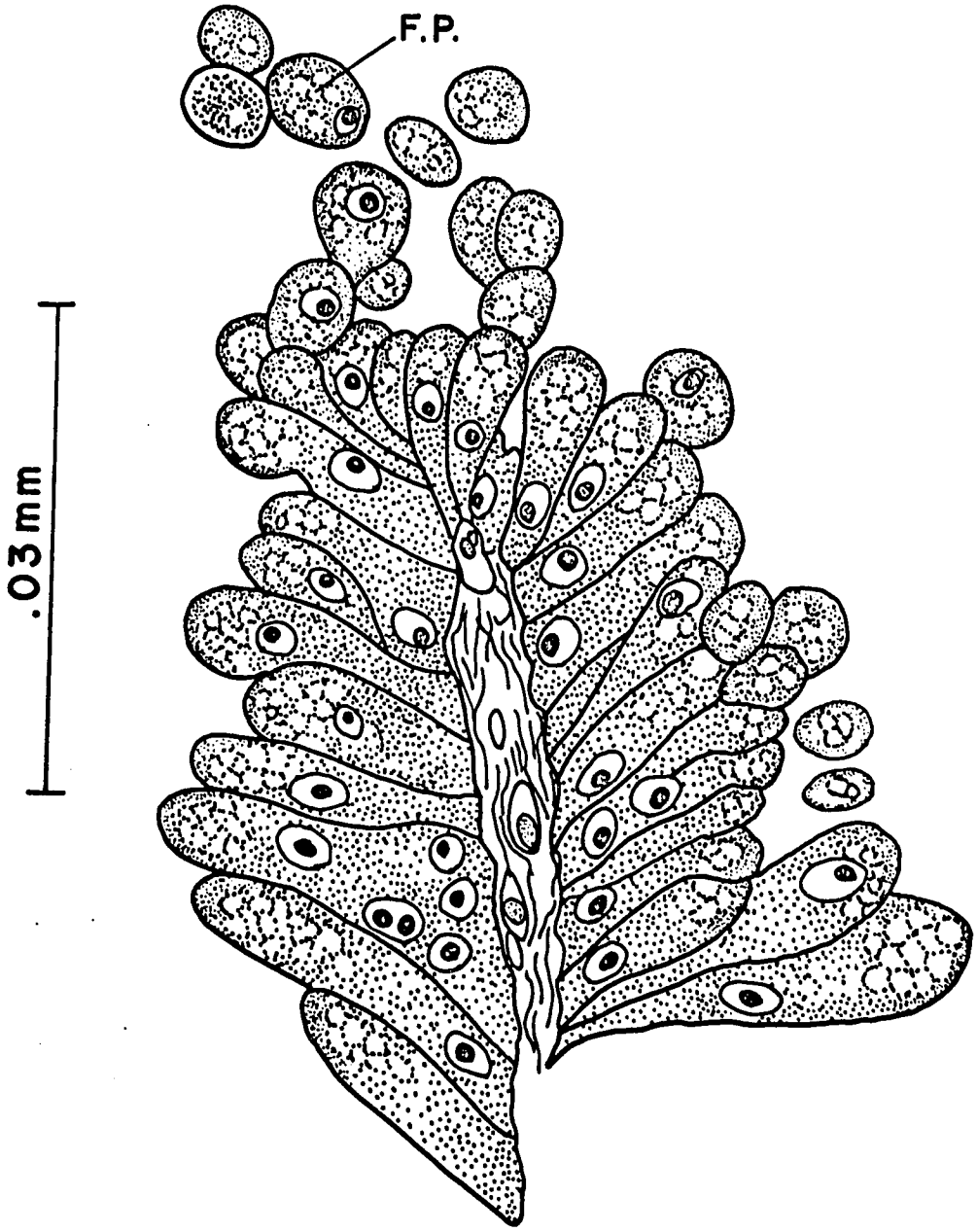

Figure 35

Figure 36. Two tubule cells of the digestive gland of A. pilosa with vacuoles containing one and four rounded spherules. Camera lucida drawing from Baker's Formalin fixed material.

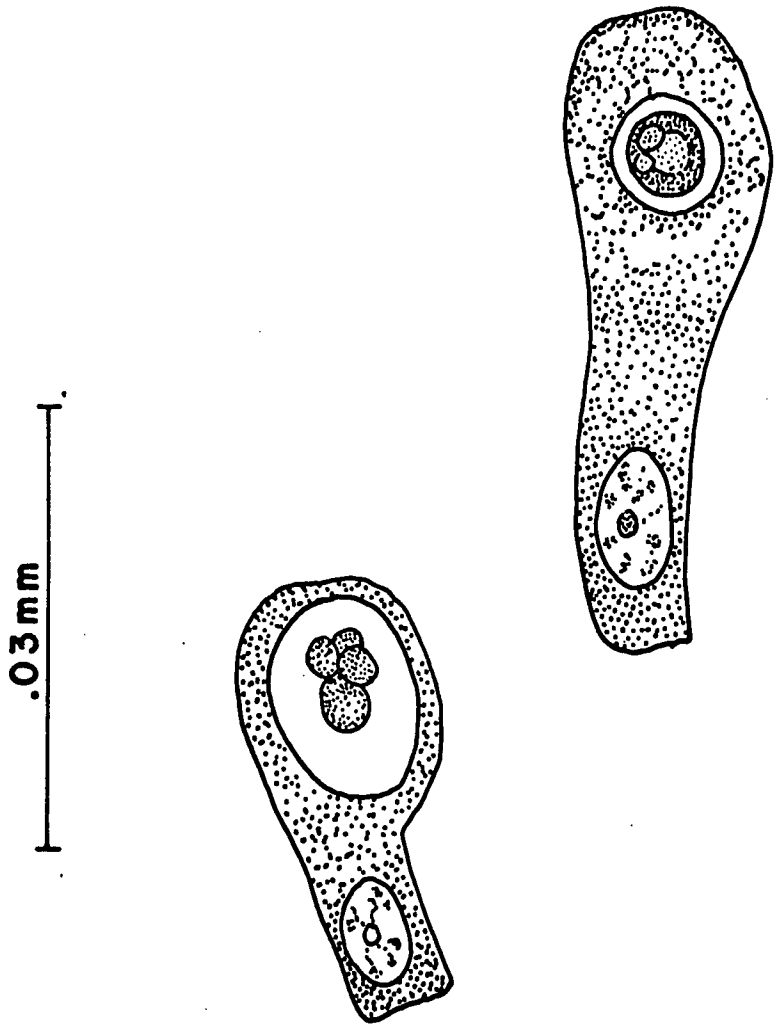

Figure 36

Figure 37. Section through the U-shaped junction of the stomach and intestine with the cecum located in the bend. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 400)

Figure 38. Section through the cecum of A. pilosa. The epithelial cells vary in height, especially in the portion of the cecum distal to the stomach. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 1,000)

Figure 37

Figure 38

Figure 39. Portion of a section through the ciliated columnar epithelium of the cecum of A. pilosa with two intercellular cysts of an unidentified sporozoan parasite. Camera lucida drawing from Bouin-DuBoscq fixed material.

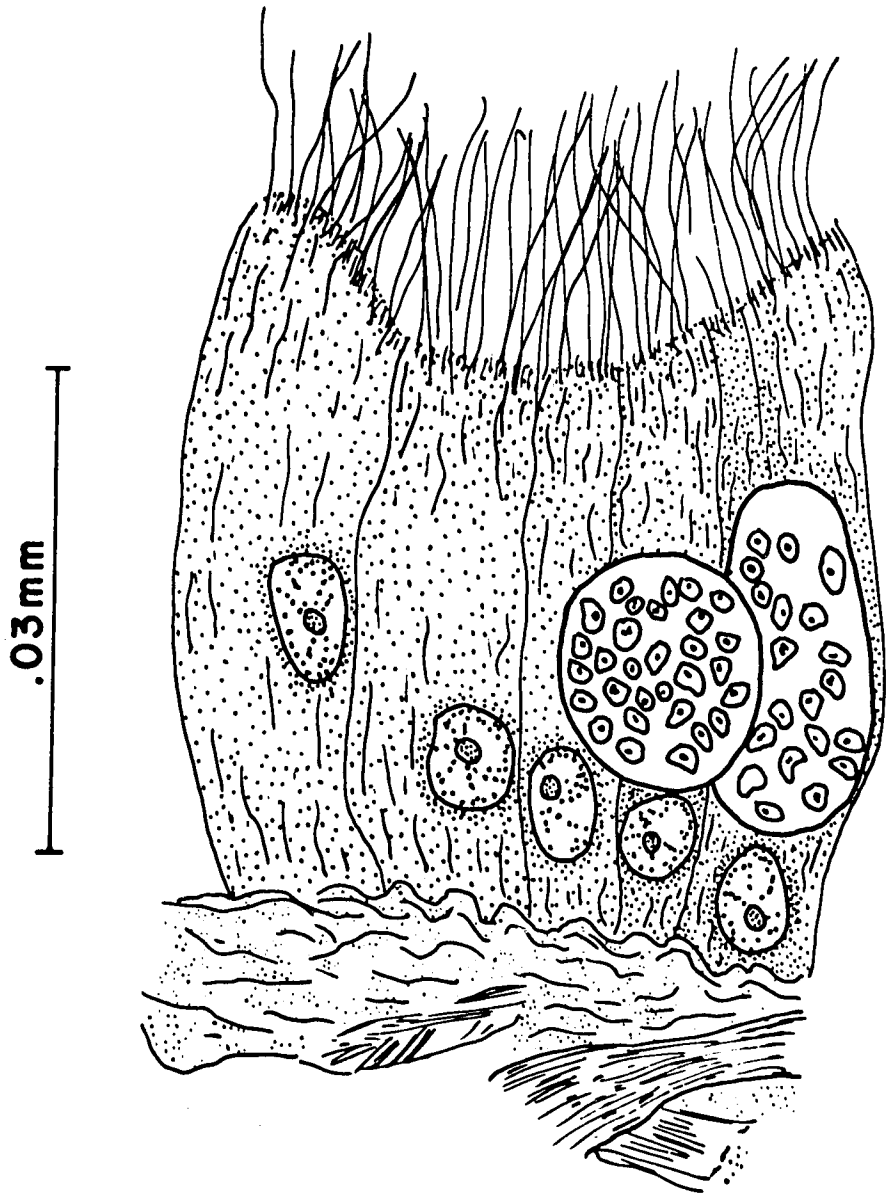

Figure 39

Figure 40. Photomicrograph of a section through the ciliated columnar epithelium of the cecum of A. pilosa showing the intercellular cysts of an unidentified sporozoan parasite. Fixed in Bouin-DuBoscq, stained with Gomori's Trichrome. (x 800)

Figure 41. Longitudinal section of the intestine of A. pilosa showing a dark staining wandering cell in the lumen. Fixed in Bouin-DuBoscq, stained with Masson's Trichrome. (x 200)

Figure 40

Figure 41

Figure 42. Section through the anal opening of the digestive tract of A. pilosa. Fixed in Baker's Formalin, stained with Hematoxylin and Eosin. (x 600)

Figure 43. Wandering cell in the intestine of A. pilosa displaying the characteristic bean-shaped nucleus and vacuolated cytoplasm. Fixed in Baker's Formalin, stained with Hematoxylin and Fast Green. (x 900)

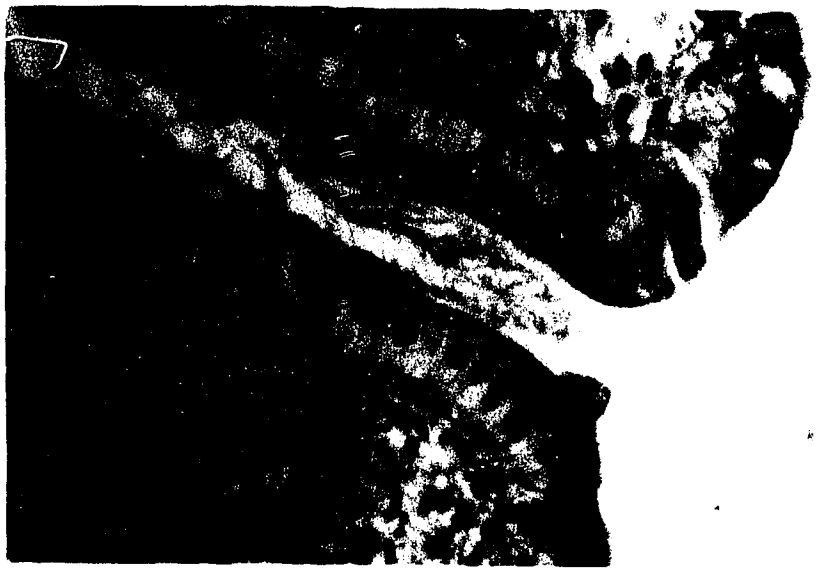

Figure 42

Figure 43

Figure 44. Portion of a longitudinal section through the intestine of A. pilosa. Camera lucida drawing from Bouin's fixed material. G.C., gland cell; E.C., epithelial cell; BA.M., basement membrane; AM., amoebocyte.

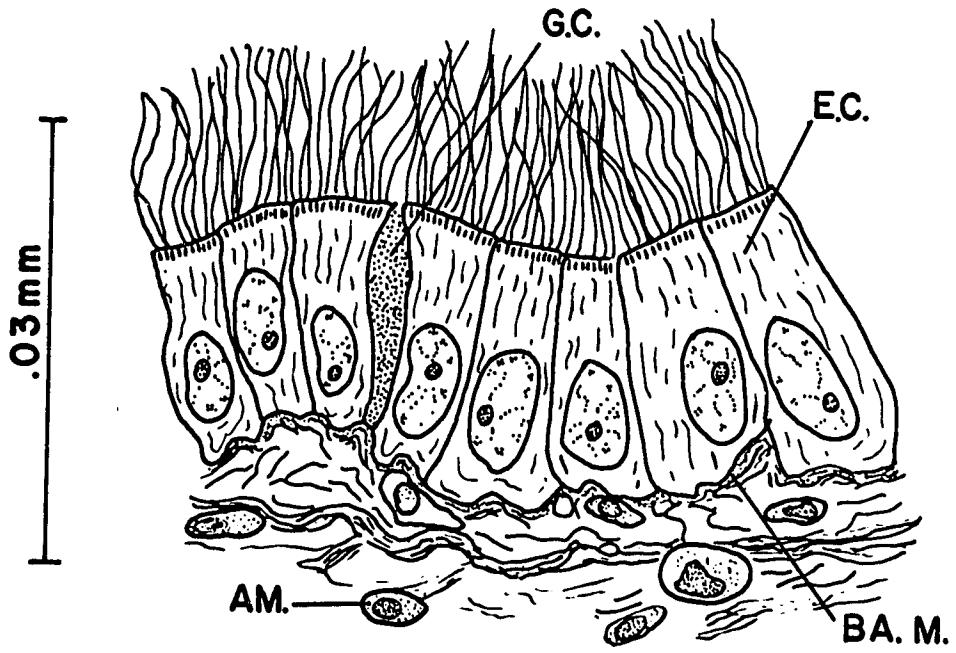

Figure 44

Figure 45. Section of the epithelium of the posterior part of the intestine at the anal opening. Camera lucida drawing from Baker's Formalin fixed material.

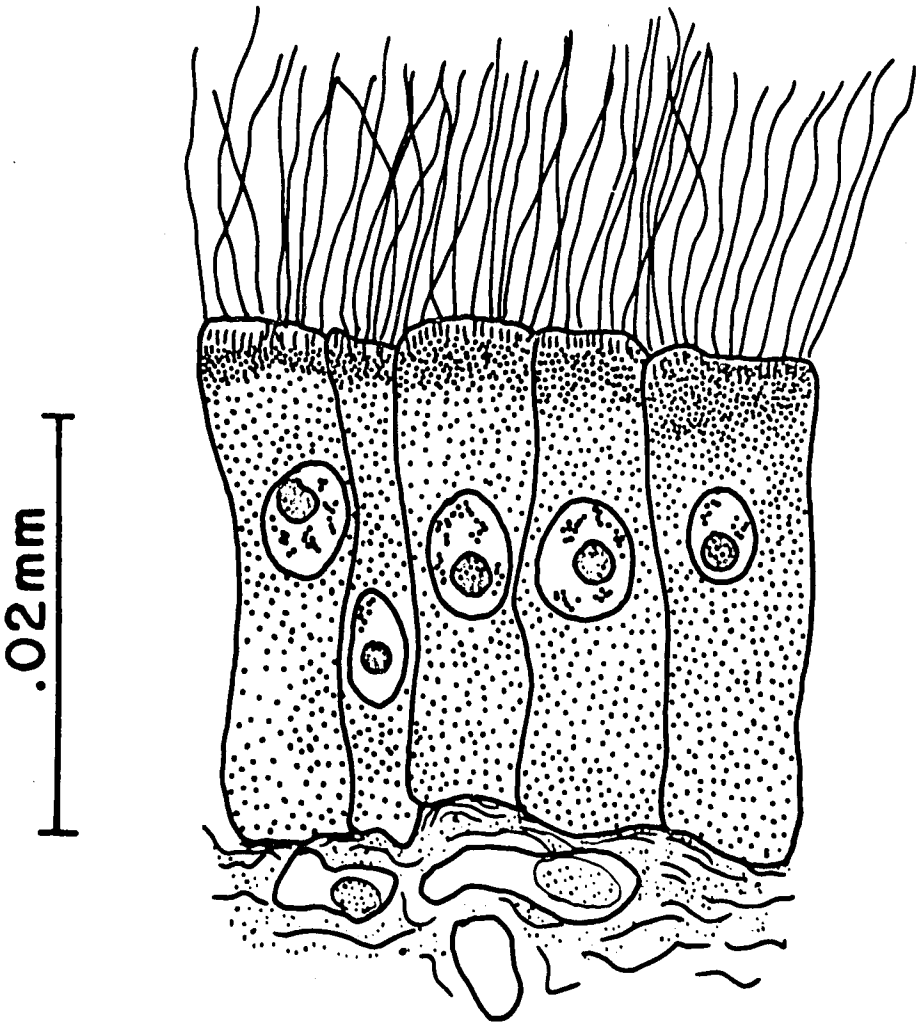

Figure 45

SECTION VIII

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SECTION IX

APPENDIX

FORMULARY OF STAINS AND PROCEDURES

Baker's Formalin (Humason, 1962, p. 14)

Formalin	10.0 ml.
Calcium chloride (anhydrous) 10% aqueous solution (10 gm./100 ml. water)	10.0 ml.
Distilled water	80.0 ml.

Bouin-DuBoscq (Humason, 1962, p. 13)

80% ethyl alcohol	150.0 ml.
Formalin	60.0 ml.
Glacial acetic acid	15.0 ml.
Picric acid crystals	1.0 gm.

Bouin's Fixative (Humason, 1962, p. 13)

Picric acid, saturated aqueous	75.0 ml.
Formalin	25.0 ml.
Glacial acetic acid	5.0 ml.

Carnoy's Fixative (Humason, 1962, p. 16)

Glacial acetic acid	20.0 ml.
Absolute ethyl alcohol	60.0 ml.

Champy Fixative (Humason, 1962, p. 17)

Potassium dichromate, 3% aqueous (3 gm./100 ml. water)	7.0 ml.
Chromic acid, 1% aqueous (1 gm./100 ml. water)	7.0 ml.
Osmic acid, 2% aqueous (2 gm./100 ml. water)	4.0 ml.

Freeze-Substitution (Barka and Anderson, 1963, p. 18)

Osmium tetroxide	1.0 gm.
Acetone	100.0 ml.

Procedure:

1. Quench small tissue block in isopentane with liquid nitrogen.
2. Transfer to precooled dehydrant at -70°C . . . 6 days
3. Bring tissue to 0°C and wash in pure solvent (acetone) . . . 18 hours

Gendre's Fluid (Barka and Anderson, 1963, p. 414)

95% ethyl alcohol, saturated with picric acid	80.0 ml.
Formalin	15.0 ml.
Glacial acetic acid	5.0 ml.

Helly's Fixative (Davenport, 1960, p. 168)

Potassium dichromate	2.5 gm.
Mercuric chloride	5.0 gm.
Water	100.0 ml.
Formalin (add just before use)	5.0 ml.

Neutral Buffered Formaldehyde Solution (Barka and Anderson, 1963, p. 415)

40% formaldehyde solution	100.0 ml.
Water	900.0 ml.
Acid sodium phosphate monohydrate ($NaH_2PO_4H_2O$)	4.0 gm.
Anhydrous disodium phosphate (Na_2HPO_4)	6.5 gm.

Palade's Fixative (Barka and Anderson, 1963, p. 415)

Veronal-acetate buffer (14.7 gm. sodium veronal, 9.7 gm. sodium acetate, distilled water to make 500 ml.)	5.0 ml.
0.1 N HCl	5.0 ml.
Distilled water	2.5 ml.
2% osmium tetroxide solution	12.5 ml.

Perenyi's Fixative (Humason, 1962, p. 20)

Chromic acid, 1% aqueous (1 gm./100 ml. water)	15.0 ml.
Nitric acid, 10% aqueous (10 ml./90 ml. water)	40.0 ml.
95% ethyl alcohol	30.0 ml.
Distilled water	15.0 ml.

Susa's Fluid (Barka and Anderson, 1963, p. 415)

Distilled water	80.0 ml.
$HgCl_2$	4.5 gm.
NaCl	0.5 gm.
Formaldehyde solution (40%)	20.0 ml.
Glacial acetic acid	4.0 ml.
Trichloroacetic acid	2.0 gm.

Heidenhain's Iron Hematoxylin (Humason, 1962, p. 134)

Solution A:

Ferric alum, $Fe_2(SO_4)_3(NH_4)_2SO_4.24H_2O$	4.0 gm.
Distilled water	100.0 ml.

Solution B (stock):

Hematoxylin	10.0 gm.
95% ethyl alcohol	100.0 ml.

Procedure:

1. Xylol to water
2. Mordant in 4% iron alum 30 minutes
3. Wash in running water 5 minutes
4. Stain in hematoxylin (5 ml. Sol. B in 100.0 ml. distilled water) 30 minutes
5. Wash in running water 5 minutes
6. Destain in 2% aqueous iron alum
7. Wash in running water 30 minutes
8. Counterstain in Orange G. (saturated solution in 95% ethyl alcohol) 2 minutes
9. Dehydrate, clear and mount.

Ehrlich's Hematoxylin and Eosin

Solution A:

Hematoxylin	2.0 gm.
Ammonia alum, $Al_2(SO_4)_3(NH_4)_2SO_4.24\ H_2O$	3.0 gm.
Alcohol, methyl or ethyl	100.0 ml.
Glycerol	100.0 ml.
Distilled water	100.0 ml.

Solution B:

1% eosin Y in 95% ethyl alcohol	10.0 ml.
95% alcohol	45.0 ml.

Procedure:

1. Xylol to water
2. Stain in a 1:9 dilution (1 part Solution A: 9 parts distilled water) 30 minutes
3. Wash in running water 30 minutes
4. Counterstain in eosin
5. Dehydrate, clear and mount

Safranin O and Fast Green (Lillie, 1948, p. 148)

Procedure:

1. Xylol to water
2. 0.5% aqueous Fast Green
3. 1% acetic acid 1 minute
4. 0.1% Safranin O 45 seconds
5. Dip in two changes of acetone for one minute each
6. Dip in equal mixture of acetone and xylol one minute
7. Clear in xylol and mount

Kernechtrot Nuclear Stain (Gurr, 1963, p. 102)

Solution:

Nuclear fast red	0.1 gm.
Aluminium sulphate	5.0 gm.
Water	100.0 ml.

Procedure:

1. Xylol to water
2. Stain in Kernechtrot stain for 5 minutes
3. Rinse in distilled water 1 minute
4. Counterstain in Fast Green (0.5% in 95% alcohol) 2 minutes
5. Dehydrate, clear and mount.

Gormori's Trichrome (Bullock, Personal communication, 1964)

Solution:

Chromotrope 2R	0.6 gm.
Fast Green	0.3 gm.
Phosphotungstic Acid	0.7 gm.
Glacial Acetic Acid	1.0 ml.
Distilled water	100.0 ml.

Procedure:

1. Xylol to 70% ethanol
2. 70% ethanol, 2 changes, 5 minutes each
3. Stain in trichrome 10 minutes
4. Rinse in 1% acetic acid in 90% ethanol until excess stain no longer flows from slide - 15 seconds
5. Dip twice in 100% ethanol
6. Dehydrate in second change of 100% ethanol 30 seconds
7. Clear in xylol and mount

Masson's Trichrome (Humason, 1962, p. 152)

Solution A:

Acid fuchsin, 1% aqueous	10.0 ml.
Ponceau dxylidine, 1% aqueous	90.0 ml.
Glacial acetic acid	1.0 ml.

Solution B:

Phosphomolybdic acid, 1% aqueous	50.0 ml.
Phosphotungstic acid, 1% aqueous	50.0 ml.

Solution C:

Fast Green	2.5 gm.
Distilled water	100.0 ml.
Glacial acetic acid	2.5 ml.

Procedure:

1. Xylol to water
2. Mordant with iron alum (4.0 gm. ferric ammonium sulfate in 100 ml. distilled water) 30 minutes
3. Wash in running water 5 minutes
4. Stain in hematoxylin (Heidenhain iron hematoxylin) 30 minutes
5. Wash in running water 5 minutes
6. Differentiate in 2% iron alum
7. Wash in running water 20 minutes
8. Stain in Solution A 5 minutes
9. Rinse in distilled water
10. Treat with Solution B 10 minutes
11. Stain in Solution C 5 minutes
12. Differentiate in 1% acetic acid 2 minutes
13. Dehydrate quickly in 70% and 95% ethyl alcohol
14. Dehydrate in absolute alcohol, 2 changes: 3 minutes each
15. Clear and mount

Mallory's Phosphotungstic Acid Hematoxylin (Lillie, 1948, p. 62)

Solution:

Hematoxylin	0.5 gm.
Phosphotungstic acid	10.0 gm.
Distilled water	500.0 ml.

Procedure:

1. Xylol to water
2. 0.5% sodium thiosulfate 5 minutes
3. Wash in tap water
4. 0.25% potassium permanganate 5 minutes

5. Wash in tap water
6. 5% oxalic acid 5 minutes
7. Wash in running water 2 minutes
8. Stain in phosphotungstic acid hematoxylin overnight (12-24 hours)
9. Dehydrate rapidly through 95% and absolute ethyl alcohol
10. Clear and mount

Thionin (Lillie, p. 148)

Procedure:

1. Xylol to water
2. Stain in thionin (0.5 gm. thionin in 500.0 ml. distilled water) for 30-60 seconds
3. Dehydrate in two changes of acetone for 1 minute each
4. Dip in an equal mixture of acetone and xylol 1 minute
5. Clear in xylol and mount

Buffered Azure Eosinate (Lillie, p. 83)

Solution A: Buffers (Lillie, p. 263)

pH	M/10 Citric Acid	M/5 disodium phosphate
2.5	20.0 ml.	0.0 ml.
3.05	16.0 ml.	4.0 ml.
4.1	12.0 ml.	8.0 ml.
4.95	10.0 ml.	10.0 ml.
6.0	8.0 ml.	12.0 ml.
8.3	0.0 ml.	20.0 ml.

Solution B:

1. Dissolve 10.0 gm. Azure C in 600.0 ml. distilled water
2. Dissolve 8.0 bm. of Eosin Y in 100.0 ml. distilled water
3. Mix 1. and 2. and filter out resultant precipitate
4. Wash precipitate with 2 washes of 50.0 cc. distilled water and 2 washes of 25.0 cc. alcohol
5. Dry at 40°C
6. Dissolve 1.0 gm. in 50.0 cc. glycerol and 50.0 cc. methanol

Procedure:

1. Xylol to water
2. Stain 1 hour in buffered azure eosinate solution containing 0.5 cc. solution B, 5.0 cc. acetone, 2.0 cc. of buffer (Solution A) and distilled water to make 40.0 cc.
3. Rinse in distilled water
4. Two changes of acetone and 1 change acetone and xylol (equal parts)
5. Clear in xylol and mount.

Periodic Acid Schiff (Barka and Anderson, 1963, p. 73)

Solution A:

1. Dissolve 1.0 gm. basic fuchsin in 200.0 ml. boiling distilled water. Cool to 52°C., filter, and add 20.0 ml. N HCl to the filtrate
2. Cool to 25°C and add 1.0 gm. sodium metabisulfite ($Na_2S_2O_5$)
3. After solution has remained for 16 to 24 hours in the dark, add 2.0 gm. activated charcoal, shake, and filter into a dark bottle.

Solution B:

1. Bisulfite solution consists of 10.0 ml. N HCl and 10.0 ml. of 10% sodium bisulfite ($Na_2S_2O_5$) and 200.0 ml. distilled water.

Procedure:

1. Xylol to water
2. Oxidize section S with 0.5% aqueous periodic acid for 10 minutes
3. Wash in tap water 5 minutes
4. Schiff reagent (Solution A) for 30 minutes
5. Rinse in 3 changes of freshly prepared bisulfite solution (Solution B) for 2 minutes each
6. Wash in tap water 2 minutes
7. Dehydrate and mount

Acetylation:

1. Place sections for 6 hours at 58°C in a 2:3 mixture of acetic anhydride: pyridine
2. Rinse in ethanol for 2 minutes and briefly in distilled water
3. The acetylated sections are then subjected to the PAS reaction together with a nonacetylated control section

Best's Carmine (Gormori, 1958, p. 67)

Solution:

Carmine	2.0 gm.
Potassium carbonate	1.0 gm.
Potassium chloride	5.0 gm.
Distilled water	60.0 ml.

Simmer mixture 2 minutes, cool and add 20.0 ml. concentrated ammonia water

Procedure:

1. Xylol to water
2. Stain nuclei with hematoxylin
3. Stain 5 to 10 minutes in dilute mixture (dilute 10.0 ml. of stock with 15.0 ml. concentrated ammonia water and 15.0 ml. of 95% alcohol)
4. Differentiate in 60% ethanol to which a few drops of ammonia water are added
5. Dehydrate, clear and mount

Mayer's Mucicarmine (Gomori, 1958, p. 68)

Solution:

Carmine	1.0 gm.
Anhydrous aluminum chloride	0.5 gm.
Aluminum hydroxide	1.0 gm.
50% alcohol	100.0 ml.

Simmer gently until it turns into a deep ruby red liquid. Let stand 24 hours and filter

Procedure:

1. Stain nuclei in hematoxylin
2. Rinse slide in water
3. Stain in mucicarmine 15 minutes
4. Rinse in water
5. Dehydrate, clear and mount

Alcian Blue (Barka and Anderson, 1963, p. 78)

Procedure:

1. Xylol to water
2. Stain in 0.1% Alcian blue in 0.01 N HCl for 10 minutes
3. Rinse briefly in 3 changes of distilled water followed by a brief rinse in 0.01N HCl
4. Rinse again in distilled water
5. Use a red nuclear counterstain (Safratin O or Kernechtrot)
6. Dehydrate, clear and mount

Astra Blue (Barka and Anderson, 1963, p. 78)

Procedure:

1. Xylol to water
2. Stain with 1% Astra blue in 1% acetic acid for 10 minutes
3. Rinse in 1% acetic acid and in distilled water.
4. Stain nuclei with Safranin O or Kernechtrot
5. Dehydrate, clear and mount

Oil Red O (Barka and Anderson, 1963, p. 120)

Solution:

Stain is prepared from a filtered stock solution of isopropyl alcohol saturated with Oil Red O. Dilute 6.0 ml. of stock with 4.0 ml. distilled water. Let stand for 10 minutes and filter.

Procedure:

1. Stain frozen sections in Oil Red O Solution for 30 minutes
2. Wash in tap water
3. Stain nuclei with hematoxylin
4. Rinse in distilled water and mount in glycerin jelly

Oil Blue N (Barka and Anderson, 1963, p. 120)

Procedure:

Same as for Oil Red O, substituting Oil Blue N

Mercuric Bromphenol Blue (Mazia et al., 1953)

Solution:

Mercuric chloride	10.0 gm.
Bromphenol blue	0.1 gm.
95% ethanol	

Procedure:

1. Xylol to water
2. Treat with solution 15 minutes
3. Wash in running water 15 minutes
4. Dehydrate, clear and mount

Gomori's Alkaline Phosphatase (Bullock, personal communication, 1964)

Solution:

3% sodium glycerophosphate	8.0 ml.
2% calcium nitrate	10.0 ml.
1% sodium barbital	10.0 ml.
Distilled water	20.0 ml.

Procedure:

1. Fix in 80% ethanol and embed in paraffin
2. Xylol to water
3. Incubate at 37°C for 1 hour in solution above. Use a control slide in solution omitting sodium glycerophosphate.
4. Rinse in 2% Calcium nitrate 2 minutes
5. Treat with 2% Cobalt chloride 5 minutes
6. Distilled water, 3 washes of 2 minutes each
7. Place in dilute Ammonium sulfide (0.5% in distilled water) 5 minutes
8. Dehydrate, clear and mount

Von Kossa Stain (Barka and Anderson, 1963, p. 170)

Procedure:

1. Xylol to water
2. Place sections for 30 minutes in the dark into 1% solution of silver nitrate
3. Rinse in distilled water
4. Treat with 0.5% pyrogallic acid for 3 minutes
5. Rinse in two changes distilled water
6. Fix in 5% sodium thiosulfate solution for 5 minutes
7. Dehydrate, clear and mount

Prussian Blue Reaction (Fretter, 1937)

Procedure:

1. Fix in a mixture of equal parts of Bouin's and 5% ammonium sulphide
2. Xylol to water
3. Treat in 10% potassium ferrocyanide for 10 minutes
4. Counterstain in Orange G
5. Dehydrate, clear and mount