A HANDBOOK OF NORMAL PENAEID SHRIMP HISTOLOGY

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TABLE OF CONTENTS

INTRODUCTION

The world-wide culturing of marine shrimp, primarily of the family Penaeidae, has reached an all time high production level. The cultured production of marine and freshwater shrimp (a majority being marine penaeid shrimp) in 1986 has been estimated at approximately 310,000 metric tons, with an estimated increase to 475,000 metric tons by 1990 (Weidner, 1987).

The culture of marine shrimp differs little from the husbandry of any other animal species, with respect to the role of disease diagnosis and prevention and its relationship to the evolution and advancement of the industry. As the industry and markets develop and expand, there are obvious monetary incentives to increase production rates at individual facilities and within the industry, as a unit. The achievement of increased production rates dictates that either stocking rates and/or overall survival be increased commensurately. The increase in either of these system parameters is intimately linked to the incidence of diseases.

The shrimp culture industry is in its infancy, relative to, for example, the poultry industry. Due in part to this fact, the incentives for scientific support have been somewhat lacking. Without such support, techniques of disease diagnosis and prevention will likewise remain in their infancy. The significant physiological differences between shrimp (an invertebrate) and the vast majority of other cultured animals (vertebrates), in turn, has restricted advances in shrimp disease diagnosis; advanced techniques and methodology used on other species have normally not been readily applied to shrimp work. Hence, the primary and most important means of disease diagnosis is by microscopy, either light or electron.

Light and electron microscopy, though useful (without these techniques the advances made to date would have been impossible), have proved to be not only labor intensive and relatively imprecise, but slow. The speed of disease determination has been further hampered by the absence of a single publication depicting the complete normal histology of penaeid shrimp. Shrimp researchers have had to depend on dated text of comparative Decapod histology (Cuenot, 1893), text of other Decapod crustaceans (Pearson, 1908; Johnson, 1980) and publications scattered throughout the extensive scientific literature.

The bulk of disease work has, in the past, been conducted by research scientists. With the dramatic increase in the culture of shrimp and the associated diseases linked to intensification, there has been a proportionate increase in the number of applied scientists; those intimately associated with diseases at the side of a commercial pond or raceway. The greater the number of people looking for diseases, the greater will be the number of observations of diseased shrimp. And in this case, the observations can impact directly and immediately on the production from that or other facilities.

The primary purpose of this handbook is to fill a void in the literature; a comprehensive handbook covering the histology of non-diseased penaeid shrimp, from which to define and identify diseased shrimp. A significant number of penaeid shrimp diseases have been newly reported within the past ten years (Lightner, 1988), many of which are of more than academic interest. It is imperative that those most in need of this information are not only aware that these diseases exist, but are capable of recognizing them in an accurate and timely fashion. This text was designed to accomplish that, and as a consequence, provide aid to both research and applied scientists, many of whom can significantly influence the fate of the industry.

TECHNIQUES

The micrographs in this handbook were produced primarily from specimens of cultured *Penaeus stylirostris*. Specimens were almost exclusively obtained from the University of Arizona's marine culture research facilities in Sonora, Mexico and Oahu, Hawaii. Additional specimens of *P. stylirostris*, and other species, were obtained from various private and government facilities, and in particular from Marine Culture Enterprises' commercial facility on Oahu, Hawaii.

The techniques of specimen fixation, though simple in nature, are of the utmost importance in the preparation of meaningful microscopic slides. Inadequate or improper fixation, if not recognized as such, can often lead to misinterpretation of the sectioned material. The relatively impervious chitinous exoskeleton of shrimp does not allow for adequate fixative penetration by simple immersion. Hence, it is imperative that immersion within a fixative be immediately preceded by injection of the fixative into vital areas.

The timing of fixation is of equal importance. Specimens should be fixed immediately following removal from the water, i.e. they should not be removed from the water and carried in an empty bucket to the place where they are to be fixed. They should instead be placed in a bucket, or similar utensil, with an adequate amount of water and then carried to the site of fixation or fixed on site. Additional care should be exercised to limit the amount of handling stress that each specimen is subjected to prior to fixation. Stress mediated histopathology, due to excessive handling, could be misinterpreted as being the state of the animal in its normal environment.

Various fixatives have been used for the preservation of shrimp and other crustaceans with varying success. Among those used are Helly's (Luna, 1968), Bouin's (Luna, 1968), 10% neutral buffered formalin (Luna, 1968) and Davidson's AFA (Humason, 1972). Our experience has shown Davidson's AFA to be the best general purpose fixative for penaeid shrimp when intended for light microscopic observations.

More precisely, the methods for specimen preparation are as follows:

Collection

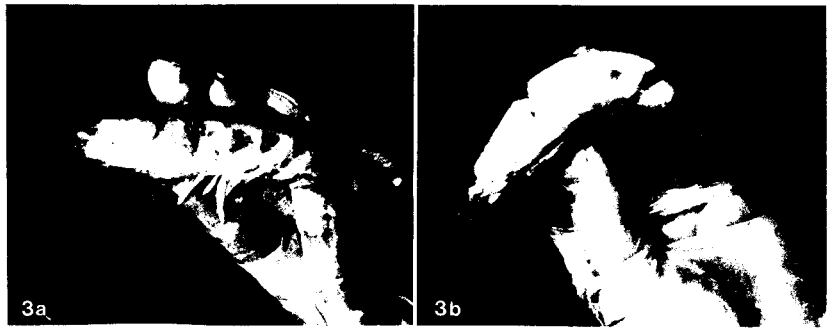
1) Collect shrimp by whatever means are available with a minimum of handling stress. For the study of presumably diseased shrimp, select those which are moribund, discolored, displaying abnormal behavior, or otherwise abnormal, except in the case of intentional random sampling for estimation of disease prevalence. Shrimp sampled for normal histology should not be abnormal in appearance nor behavior. Do not collect shrimp that are dead for any sample, unless it can be positively determined that they have died within the last few minutes. If recently dead shrimp must be sampled, be sure to make note of this condition and estimate the time since their death.
2) Transport the shrimp to the laboratory via a water filled utensil. Supply adequate aeration to the container if they are to be left for a short period of time before actual fixation.

Fixation or Preservation

1) Have ready an adequate supply of fixative; a rule of thumb is that a minimum of approximately 10 X their volume of fixative should be used for each specimen (eg. a shrimp of 10 ml volume would require 100 ml of fixative).
2) Davidson's fixative should be made as such:
   a) 330 ml 95% ethyl alcohol
   b) 220 ml 100% formalin (saturated aqueous solution of formaldehyde gas, 37–39% solution).
   c) 115 ml glacial acetic acid
   d) 335 ml tap water (preferably distilled if available)
   e) store at room temperature
3) Inject fixative (0.1 to 10 ml depending on size of shrimp), via needle and syringe (needle gauge dependant upon shrimp size; small shrimp, small needle) into the living shrimp. The site of injection should be laterally in the hepatopancreas proper (Figure 1a), in the region anterior to the hepatopancreas (Figure 1b), in the posterior abdominal region (Figure 1c) and in the anterior abdominal region (Figure 1d). Precautions should be taken to avoid skin and eye contact with the fixative. The fixative should be divided between the different regions, with the cephalothoracic region, specifically the hepatopancreas, receiving a larger share than the abdominal region. A good rule of thumb: "inject an equivalent of 5–10% of the shrimp's body weight; all signs of life should cease".

4) Immediately following injection, slit the cuticle, with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, **paying particular attention not to cut deeply into the underlying tissue.** The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral (Figure 2).

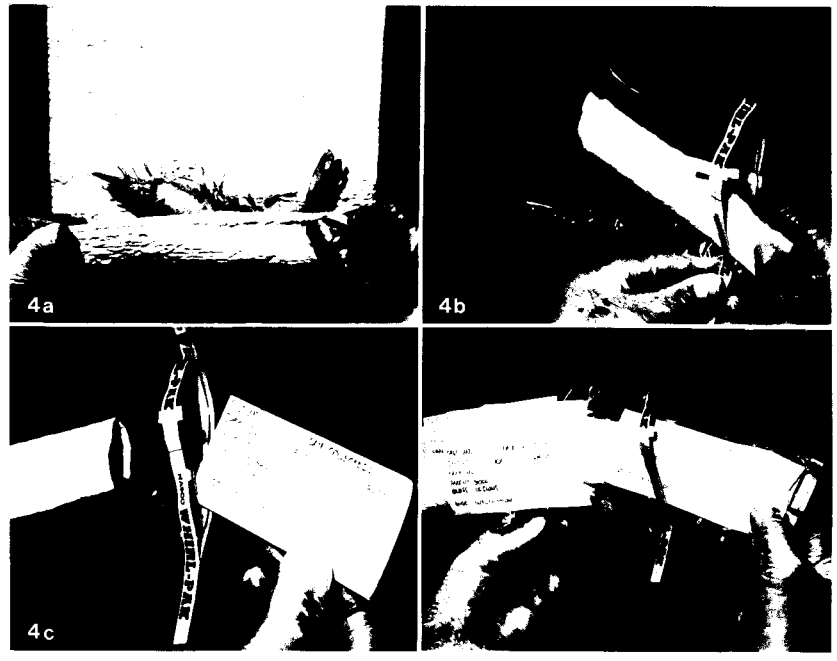
5) Shrimp larger than 12 grams, should then be transversely slit once at the abdomen/cephalothorax junction (Figure 3a) or again mid-abdominally (Figure 3b).
6) Following injection, incisions and bisection/trisection, immerse the specimen in the remainder of the fixative.



7) Allow the shrimp to remain in the fixative at room temperature for 24 to 72 hr depending on the size of shrimp (larger shrimp for longer).
8) Following proper fixation, the specimens should be transferred to 50% ethyl alcohol, where it can be stored for an indefinite period.
9) Record a complete history of the specimen at the time of collection: gross observations on the condition of the shrimp, species, age, weight, source (pond, tank or raceway identifying number), source of parent stock, and any other pertinent historical information that may at a later time provide clues to the source and cause of the problem. Use **soft-lead pencil** on paper (plastic paper if possible).

Transportation or Shipment for Processing

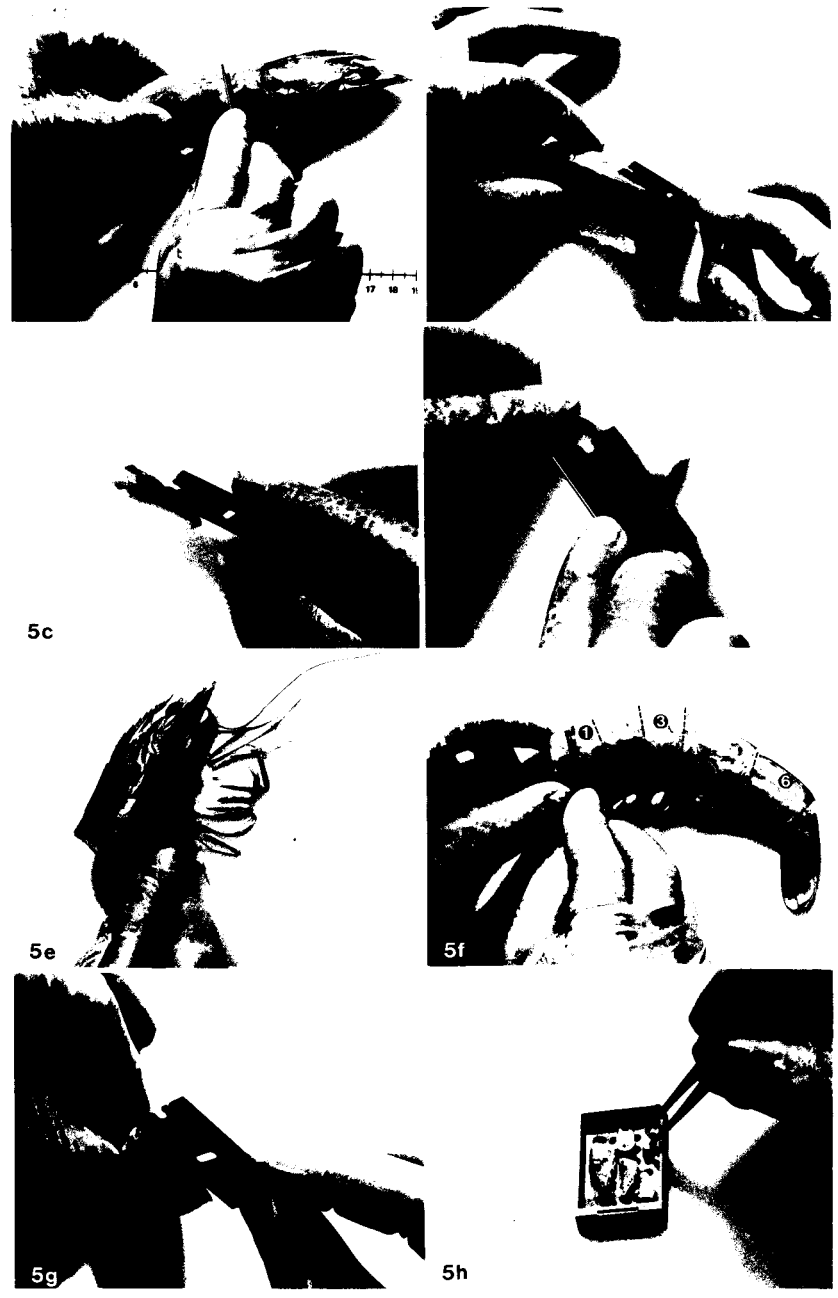
1) remove the specimens from the 50% ethyl alcohol.
2) wrap with paper towels to completely cover (Figure 4a).
3) place towel-wrapped specimen in a sealable plastic bag and saturate with 50% ethyl alcohol (Figure 4b).
4) include the history, as recorded above, with the shipment (Figure 4c).
5) place bag within a second sealable bag.
6) multiple small sealable bags can again be placed within a large sealable bag (Figure 4d).



Preparation for Embedding

Following a prescribed set of procedures to prepare the specimen for histological sectioning is not essential, but helpful for proper visualization of organ systems, especially if prepared slides are to be compared to those in this manual. Therefore, the following procedures and accompanying figures are provided to assist in establishing a standardized method, which we refer to as a **"gut-gill panorama"** procedure (the procedures pertain to shrimp larger than approximately 5.0 cm in total length, unless stated otherwise):

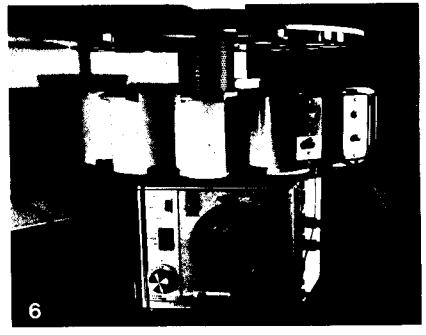
1) remove preserved shrimp from 50% ethyl alcohol and place on a cutting surface (wood, plastic, paraffin).
2) utilizing a single edge razor blade or scalpel, bisect shrimp transversely (for shrimp greater in length than 3.0 cm) at the junction of the cephalothorax and abdomen (Figure 5a).
3) longitudinally bisect the cephalothorax just lateral of the mid-line (Figure 5b) [or, if possible, the whole specimen for shrimp less than 3.0 cm in length (Figure 5c)].
4) from the half of the cephalothorax without the mid-line, remove, with a diagonal cut starting at the distal surface, the branchiostegal region containing the gills (Figure 5d).
5) remove the distal 80% of head appendages if these are not to be studied or if the appendages would get in the way during embedding (Figure 5e).
6) utilizing a razor blade, separate abdominal segments #1, 3 and 6 from the remainder of the abdomen, remove distal ends of the uropods (Figure 5f).
7) longitudinally bisect the 6th abdominal segment, as in the manner of the cephalothorax (Figure 5g).
8) depending on the size of shrimp, the available tissue blocks and the size of the anticipated embedding mold, place either all or any number of the following tissue blocks into histological embedding cassettes (Figure 5h):
   - half of complete shrimp with mid-line, cut-side down (less than 3 cm).
   - half of cephalothorax with mid-line, cut-side down.
   - branchiostegal region, cut side up.
   - other half of complete shrimp, or cephalothoracic region from this half, (without the mid-line), cut-side up (less than 3 cm).
   - other half of the cephalothorax (without the mid-line) placed with the cut-side up.
   - transverse block(s) of abdominal segments #1 and/or #3.
   - longitudinal block of 6th abdominal segment (with mid-line) placed with cut-side down.
9) tissue blocks should not exceed 1/4" in thickness for the thinnest dimension.
10) if particular organ systems or regions are required, adjust the hand sectioning into blocks to correspond to those areas.



Paraffin Embedding

The paraffin embedding of shrimp tissues differ little from that of vertebrate tissues, hence general information concerning embedding procedures can be obtained in a manual of standard histological techniques. The following is thus meant to be illustrative of general procedures and equipment as used in our laboratory and to point out those techniques which may be unique or helpful when working with shrimp tissue.

1) The desired tissues, enclosed within embedding cassettes, can be processed in an automatic tissue processor, such as that illustrated below (Figure 6).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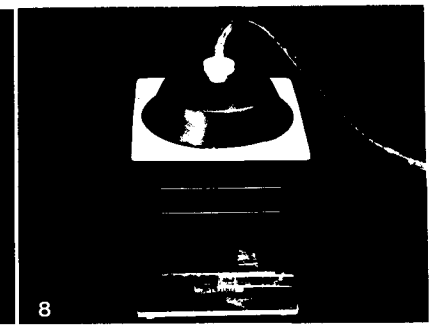
2) Shrimp tissues are routinely processed at our laboratory in the following solutions (in the order they are presented): a) 70% ethyl alcohol (EtOH)—two separate 1 hr baths, b) 80% EtOH—two separate 1 hr baths, c) 95% EtOH—two separate 1 hr baths, d) 100% EtOH—two separate 1 hr baths, e) clearing agent [xylene, Hemo De (Fischer Products), etc.]—two separate 1 hr baths, f) paraffin [eg. Paraplast X-TRA (Monoject Scientific)]—two separate 1 hr baths.
3) Tissues successfully embedded, as accomplished above, are then placed in embedding molds to form blocks ready to be sectioned. The blocking process is accomplished with an embedding center (Figure 7) and vacuum infiltrator (Figure 8) as illustrated below. An embedding center is not essential; cold trays and melted paraffin can be used, although vacuum infiltration for approximately 20 min. prior to blocking will eliminate partially infiltrated tissue.

Sectioning

Like that of tissue embedding, sectioning of shrimp tissue varies little from routine vertebrate sectioning. Essential equipment in-


7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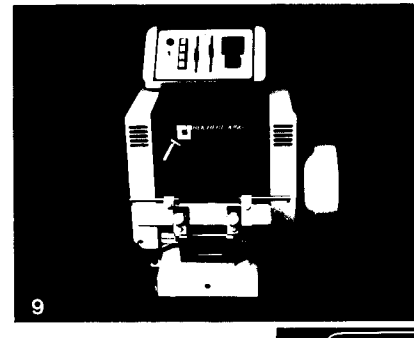
cludes a microtome (Figure 9) and a water bath (Figure 10). An automatic microtome knife sharpener (Figure 11) is required unless disposable knives are to be used. A manual of histology techniques should be consulted for routine procedures.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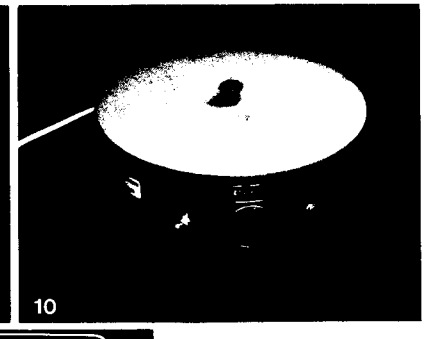
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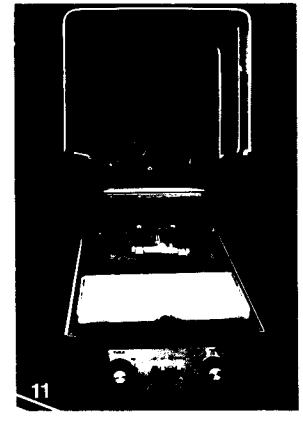
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A few procedures found to be useful in sectioning shrimp tissue:

1) the block containing the mid-line should be used when attempting to section the stomach, midgut or hindgut in the longitudinal plane. It should be face-trimmed toward the midline until the gut can be seen macroscopically, at which time sections can then be saved.

2) small or otherwise difficult sections may at times tend not to spread in the water bath. To alleviate this, place the ribbon of sections on a slide which has 50% ethanol on its surface. Immerse the slide and ribbon in the water bath and the ribbon should float off and spread.

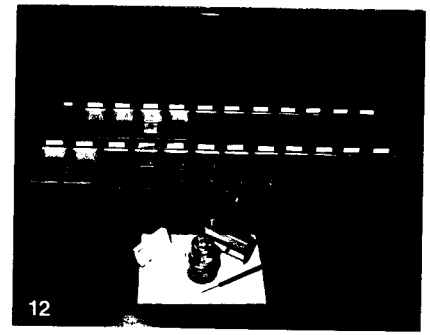
Staining

Routine histological preparations typically employ a standard stain; shrimp histology does not differ from the norm. The majority of slides used for the preparation of micrographs in this manual were stained with modified Mayer's hematoxylin and Phloxine/eosin (Sheehan and Hrapchak, 1980) stain (H&E). They are prepared with our routine lab procedures, as outlined below, in a staining module, as illustrated (Figure 12).

Staining Sequence, Solutions and Times

1) Hemo De - 5 min.
2) Hemo De - 5 min
3) 100% EtOH - 10 dips
4) 100% EtOH - 10 dips
5) 95% EtOH - 10 dips
6) 95% EtOH - 10 dips
7) 80% EtOH - 10 dips
8) 80% EtOH - 10 dips
9) 50% EtOH - 10 dips
10) distilled water - 6 rinses*
11) hematoxylin - 4-6 min.
12) running tap water - 4-6 min.
13) Phloxin/eosin - 2 min.
14) 95% EtOH - 10 dips
15) 95% EtOH - 10 dips
16) 100% EtOH - 10 dips
17) 100% EtOH - 10 dips
18) Hemo De - 10 dips
19) Hemo De - 10 dips
20) Hemo De - 10 dips
21) Hemo De - 10 dips

* - change water for each rinse.

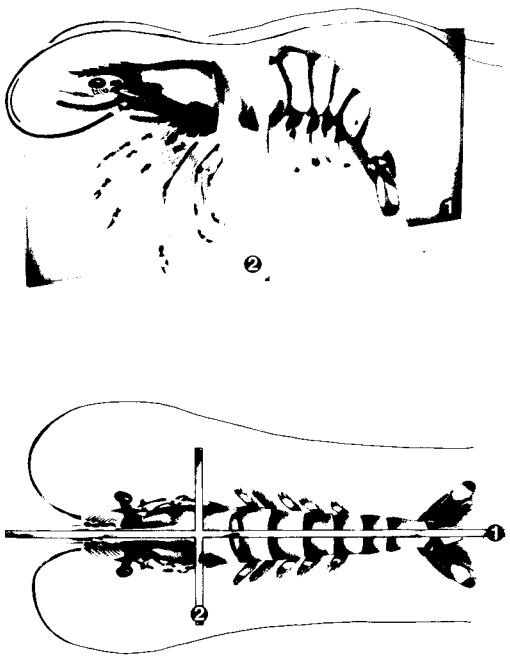


Stains, other than H&E, have been used in the production of this manual to demonstrate the presence of particular tissue types. As is often the case, black and white micrographs may not adequately distinguish the particular tissue from other surrounding tissues. We have attempted to add to the value of the text by describing the exact colors noted.

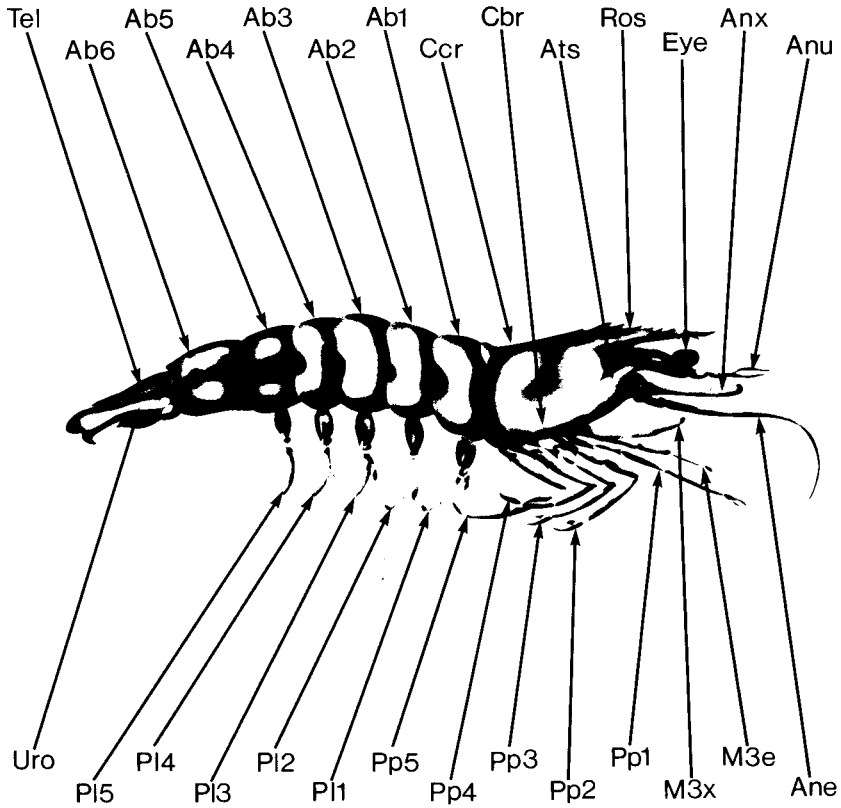
TEXT AND CAPTION NOMENCLATURE

A conscious effort was made to be consistent in the presentation of micrographs throughout the manual. Although we feel that we have achieved that end, in the process we may not have been consonant with the total wealth of information from which much of this manual was drawn. To assist the end user in the clarification of such matters as nomenclature, the Bibliography Section is divided into subsections corresponding to anatomical sections. The bibliographic subsections include an extensive list of literature (that used in defining nomenclature for this book and as general reference) addressing either penaeid histology or, when the information is lacking for penaeids, histology of related decapods or crustaceans.

Routine sectioning of specimens is normally accomplished in one of two planes: 1) the longitudinal or sagittal plane; from the dorsal to the ventral surface through the long axis, and 2) the transverse or cross section; from the dorsal to the ventral surface through the short axis. These planes of sectioning are pictorially represented and numbered below, in both a perspective and top view. Reference to these planes of section within the micrograph captions and the text is as depicted in the figures below.



The gross anatomy of penaeid shrimp has been well described in the existing literature. The following drawing has been provided as a quick reference to assist in the understanding of penaeid shrimp gross terminology, as used throughout this manual.



Morphological nomenclature and histological points of interest have been consistently labelled with three (3) character abbreviations to eliminate overlapping of abbreviations. The abbreviations used in the figure above and throughout this manual are as follows:

ABBREVIATIONS USED IN TEXT AND CAPTIONS

A — toward the anterior region of the shrimp
Ab1 — 1st abdominal segment
Ab2 — 2nd abdominal segment
Ab3 — 3rd abdominal segment
Ab4 — 4th abdominal segment
Ab5 — 5th abdominal segment
Ab6 — 6th abdominal segment
Afc — afferent vessel, central axis
Afp — afferent vessel of primary filament
Afs — afferent vessel of secondary filament
Agb — antennal gland bladder
Agn — antennal gland labyrinth, non-secreting
Ags — antennal gland labyrinth, secreting
Agt — antennal gland tubule
Ala — alary muscle
Ama — anterior mandibular adductor muscle
Amc — cecum, anterior midgut
Ana — anus
Ane — antenna, endopodite
Ann — antennal nerve
Ans — antennal scale
Anu — antennule
Anx — antenna, exopodite
Aoa — aorta, anterior
Aop — aorta, posterior
Aos — artery, segmental
Api — apical end
Arf — artifact of fixation
Arh — artery, hepatic
Ars — artery, sternal
Art — arterial vessel
Ats — antennal spine
Aux — auxiliary channel
Axn — axons
Bac — bacteria or bacterial flocculent
Bas — basement membrane
Bcl — basal cells
Bel — organ of Bellonci with onion bodies
Bnc — binucleated cell
Brb — brush border or microvilli
Cam — carapace adductor muscle
Cap — capillary
Cbr — cephalothorax, brachiostegal region
Ccn — cone cell, nuclei
Ccr — cephalothorax, cardiac region
Ceb — cell boundaries
Cen — central axis of gill
Cep — cuticular epidermis or epithelium
Cir — circumesophageal connective
Cnf — connective tissue, fibrous
Cns — connective tissue, spongy
Coc — central ovarian cavity
Con — cone cells
Cor — cortical glia
Crc — crystalline cones
Cre — cell rind, medulla externa
Crf — collagen and reticulin fibers
Cri — cell rind, medulla interna
Crn — cell rind nuclei, lamina ganglionaris
Crt — crystalline tracts
Cu1 — epicuticle sublayer
Cu2 — exocuticle sublayer
Cu3 — endocuticle sublayer
Cu4 — membranous cuticle sublayer
Cut — cuticle
Cxn — cuticle sublayers, trichrome blue
Cyt — cytoplasm
D* — toward the dorsal region of the shrimp
Dip — distal pigment granules
Dis — distal direction
Ecc — epicorneagenous cell, cytoplasm
Ecn — epicorneagenous cell, nuclei
Eec — epithelium, anterior midgut cecum
Efc — efferent vessel, central axis
Efp — efferent vessel of primary filament
Efs — efferent vessel of secondary filament
Ela — elastin fibers
Emc — epithelium, posterior midgut cecum
End — endothelia
Epc — epicardial cell, cytoplasm
Epf — epithelium, foregut
Eph — epithelium, hindgut
Epi — epicardium
Epl — epithelium, labyrinth
Epm — epithelium, midgut
Epn — epicardial cell, nuclei

Epp — epipodite or mastigobranchia
Eps — epithelia, squamous
Ept — epithelia
Esm — esophageal musculature
Eso — esophagus
Eye — compound eye
Faz — fasciculated zone
Fcl — follicle cell
Fld — longitudinal vas deferens fold
Fms — food, sufficiently masticated
Fsm — sensory/motor fibers & interneurons
Gaa — ganglia, antennal
Gal — ganglia, labral or tritocerebral
Gan — ganglia, cell body and nucleus
Gas — ganglia, segmental
Gat — ganglia, antennule neuropile
Gic — giant cells
Glc — glial cells
Glo — globuli cells
Gnf — giant nerve fiber
Gss — gastric sieve
Han — organ of Hanström
Hce — hemocyte, crossing endothelium
Hec — hemocoel
Hel — hematopoietic organ, tubule
Hem — circulating hemocytes
Heo — hematopoietic organ
Hep — hepatopancreas
Hey — hemocytes, young, non-circulating
Hgf — hindgut folds
Hpb — hepatopancreatic B-cell
Hpc — hepatopancreatocytes
Hpd — hepatopancreas, primary duct
Hpe — hepatopancreatic E-cell
Hpf — hepatopancreatic F-cell
Hpr — hepatopancreatic R-cell
Hrt — heart
Hsd — hepatopancreas, secondary duct
Hta — hepatopancreatic tubule, apical
Htm — hepatopancreatic tubule, medial
Htp — hepatopancreatic tubule, proximal
Ils — inter-lobular space
Inc — inclusion body
Int — intima
Iss — interstitial sinuses
Jun — junction of midgut and hindgut or stomach
Lab — labrum
Lac — lacunae or hemal space
Lam — lamina ganglionaris
Lap — lappets
Lcc — labyrinth-coelomosac complex
Lcg — lower cardiac groove
Lco — lumen, coelomosac
Lig — ligament, suspensory
Lpp — labral posterior process
Lsg — longitudinal inter-setal grooves
Luc — lumen, cecal
Lul — lumen, labyrinth
Lum — lumen
Lvp — primary vas deferens lumen
Lvs — secondary vas deferens lumen
Lym — lymphoid organ
M1c — 1st maxilliped, coxopodite exites
M1e — 1st maxilliped, exopodite
M2s — 2nd maxilla, scaphognathite
M3m — 3rd maxilliped, meropodite & ischiopodite
Maf — 1st maxilla
Man — mandible
Mao — mandibular organ
Map — mouth appendages
Mas — 2nd maxilla
Mdp — mandibular palp
Mee — medulla externa
Mef — myoepithelial fibers
Mei — medulla interna
Met — medulla terminalis
Mfn — myoepithelial fiber nucleus
Mhj — midgut-hepatopancreas junction
Mid — midgut
Mit — mitotic figure
Mlt — muscle, lateral anterior thoracic
Mpf — maxilliped, first
Mps — maxilliped, second
Mpt — maxilliped, third
Mse — 2nd maxilliped, exopodite
Msf — muscularis frontalis
Msl — muscle, longitudinal or longitudinal section
Msx — muscle, circular or transverse/cross section
Msz — muscle, circular and longitudinal
Mus — muscle, striated

Myo — myocardium
Ncl — nurse cell
Nep — nephrocyte
Neu — neurilemma
Ngf — nervous and glial fibers, lamina ganglionaris
Ngh — nervous/glial fibers, lam. gang., horz. to distal border
Ngv — nervous/glial fibers, lam. gang., vert. to distal border
Nlg — neuropil of lamina ganglionaris
Nme — neuropil, medulla externa
Nra — nerve, antennal
Nrp — nerve, periopod or pleopod
Nrv — nerve tract
Nsc — neurosecretory cells
Nuc — nucleus
Nuu — nucleolus
Onb — onion bodies
Onc — optic nerve cord
Ooc — oocytes, previtellogenic
Oog — oogonia
Opp — optic peduncle
Ost — ostium
Ov1 — ovum, maturing, postvitellogenic
Ov2 — ovum, mature, postvitellogenic
Ova — ovaries
Ovd — oviduct
P — toward the posterior region of the shrimp
Par — paragnath
Pbc — pre-branchial chamber
Per — pericardium
Pid — pillar process, distal portion
Pil — pillar process epithelia
Pl1 — 1st pleopod
Pl2 — 2nd pleopod
Pl3 — 3rd pleopod
Pl4 — 4th pleopod
Pl5 — 5th pleopod
Ple — pleopod
Pmc — cecum, posterior midgut
Pod — podocytes or coelomosac epithelium
Pp1 — 1st pereiopod
Pp2 — 2nd pereiopod
Pp3 — 3rd pereiopod
Pp4 — 4th pereiopod
Pp5 — 5th pereiopod
Pri — primary gill filament
Prm — peritrophic membrane
Pro — primary optical nerve fibers
Prp — pereiopod
Raz — reabsorption zone
Rcb — retinular cell bodies with pigment
Res — reserve cells
Ret — retinular cell, nuclei
Rha — rhabdoms and retinular cell bodies
Ros — rostrum
Rpb — rod-like peripheral bodies
Rpd — retinular proximal band, distal
Rpp — retinular proximal band, proximal
Sac — stomach, anterior chamber
Sat — satellite cell
Sec — secondary gill filament
Sep — septum
Ser — serosa
Sga — subgastric artery
Shj — stomach-hepatopancreas junction
Sig — sinus gland
Sin — sinus, hemolymph
Slt — supralateral teeth
Smc — stromal matrix cells
Sp0 — spermatogonia
Sp1 — primary spermatocyte
Sp2 — secondary spermatocyte
Sp3 — spermatid
Sp4 — spermatozoa
Spc — stomach, posterior chamber
Spd — stomach, posterior, dorsal subchamber
Spk — spermatozoa, spike
Spv — stomach, posterior, ventral subchamber
Sta — eye stalk
Sup — supraesophageal ganglion
Tad — terminal ampoule duct
Tam — terminal ampoule
Tec — aluminal testicular cords
Teg — tegmental gland
Tel — telson
Ten — tendon or tendon-like
Tes — testes
Twz — terminal web region
Ucg — upper cardiac groove
Uro — uropods
Vac — vacuole

Val — valve or valve cusps
Vap — vacuolated apical complex
Vnc — ventral nerve cord
Vsd — vas deferens
Wal — vessel or organ wall
Ygb — yolk globules
Yor — Y organ
Zop — zone of proliferation
* — an attempt was made, whenever possible and appropriate, to orient the micrographs such that dorsal is toward the top of the page.

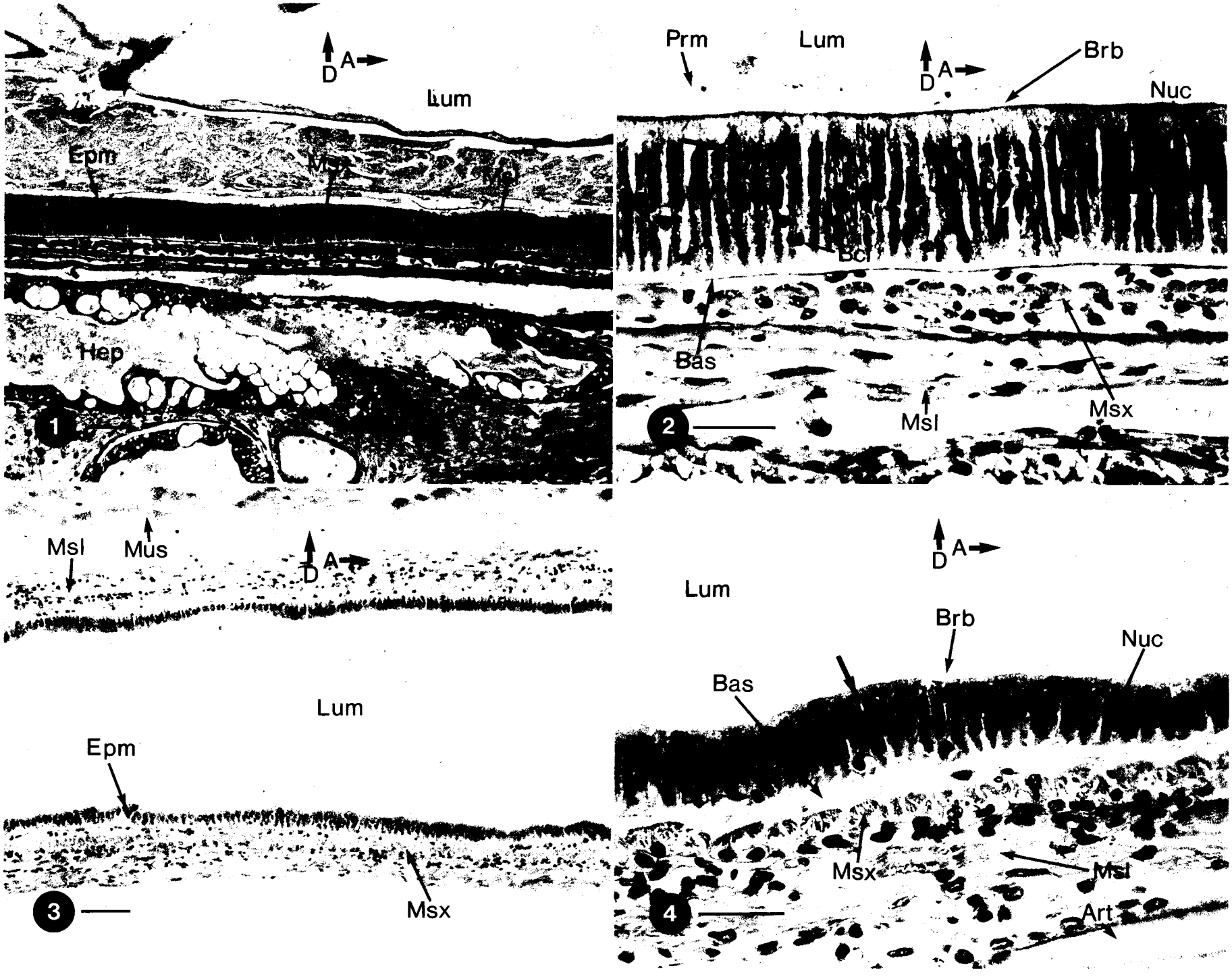
MIDGUT

FIGURE 1 Overall longitudinal view of the anterior region of the midgut, just posterior to its union with the stomach. The mucosal epithelium (Epm) is composed of simple columnar cells. These are supported by a basement membrane (not visible in this preparation), a layer of circular muscles (Msx) and finally by a layer of longitudinal muscles (Msl). The midgut, in this region, passes between the dorsal and ventral lobes of the hepatopancreas (Hep). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 100 μm.

FIGURE 2 Enlarged view of the anterior region of the midgut epithelium. The cells are simple columnar, with medial nuclei (Nuc) and prominent nucleoli (Nuu). The surface adjacent to the lumen (Lum) has a brush or microvillous border (Brb). Distal to the nuclei is a dense region (bold arrow) of cellular organelles, presumably mitochondria, secretory granules and golgi bodies. The epithelial cells are supported by the basement membrane (Bas), circular muscle (Msx) and longitudinal muscle (Msl). Proximal to the medial nuclei are often found basal cells (Bcl). In this preparation, what appears to be the peritrophic membrane (Prm) is seen surrounding the chymous mass within the midgut lumen. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.

FIGURE 3 Overall longitudinal view of central region of the midgut. The morphology is quite similar to that of the anterior region of the midgut: simple columnar epithelium (Epm), subtended by both circular (Msx) and longitudinal muscle (Msl) layers. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 100 μm.

FIGURE 4 Enlarged view of the central region of the midgut epithelium. The cells are still simple columnar, but slightly shorter than those in the anterior region. A brush border (Brb) is evident, as is a slightly reduced dense region of mitochondria, secretory granules and golgi bodies (bold arrows). A thin basement membrane (Bas), layers of circular muscle (Msx), and longitudinal muscle (Msl) are noted. Note the basal cells (Bcl). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.


D
A
Lum
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Msl
Hep
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Msl
Mus
D
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Brb
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D
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Lum
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Bas
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Msx
Msl
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4

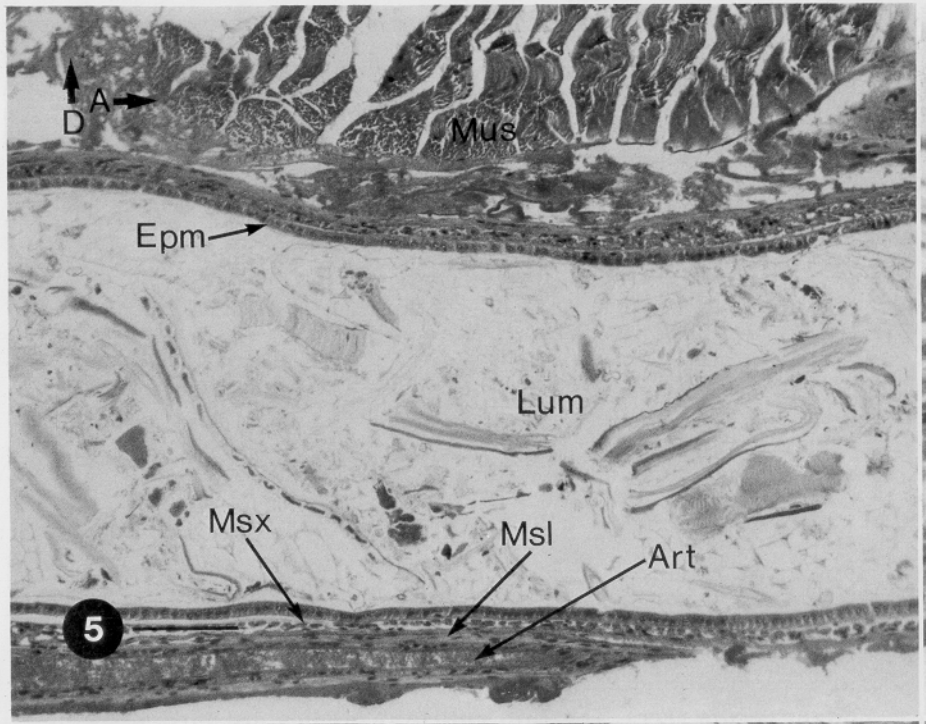
MIDGUT

FIGURE 5 Overall longitudinal view of the posterior region of the midgut. The lumen (Lum) is filled with chyme or partially digested food. The same basic sequence is noted here as had been pointed out for the other two regions of the midgut; an epithelial layer (Epm) supported basally by a circular muscle layer (Msx) and a longitudinal muscle layer (Msl). Typically the midgut is surrounded by abdominal muscle (Mus) and is seen here lying above an arterial vessel (Art). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 100 μm.

FIGURE 6 Enlarged view of the epithelium (Epm) and associated tissues of the posterior midgut region. In this region the morphology is nearly identical to that of the two anterior regions; only here the epithelium ranges from nearly squamous (or low cuboidal) to cuboidal, as opposed to columnar. The cell surface facing the lumen (Lum) is lined with a brush border or microvilli (Brb). Nuclei (Nuc) are medially located. The densely staining region of mitochondria, secretory vacuoles and golgi bodies is reduced to a non-detectable level. The epithelial cells are supported by a thin basement membrane (Bas), a layer of circular muscle (Msx) and a layer of longitudinal muscle (Msl). In this preparation, the longitudinal muscle layer contains an arterial hemolymph vessel (Art). Profiles of the peritrophic membrane (Prm) are noted. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.

FIGURE 7 Overall cross-sectional view of the central midgut region. The midgut lies ventral to the posterior aorta (Aop) or dorsal abdominal artery and ovarian lobes (Ova). The midgut, posterior aorta and ovarian lobes lie within the hemocoel, which is completely surrounded by abdominal muscle (Mus). As noted previously, the mucosal epithelium (Epm) is surrounded by layers of both circular and longitudinal muscle (Msz), which in turn are surrounded by a layer of fibrous connective tissue or serosa (Ser). Transverse 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 100 μm.

FIGURE 8 Enlarged cross-sectional view of the epithelium (Epm) and associated tissues in the central midgut region. The epithelial cells, as previously noted in longitudinal sections, have a brush border (Brb) in contact with the lumen (Lum), and the nuclei (Nuc) are approximately medial in location. The basement membrane (Bas) here is thick and undulating. Very small bundles of longitudinal muscle (Msl), which had not been previously noted in this text, lie between the basement membrane and the large bundle of circular striated muscle (Msx). Distal to these are the large longitudinal muscle bundles. Transverse 4–5 μm paraffin section, Masson's trichrome stain, Davidson's fixative, bar length = 20 μm.


D
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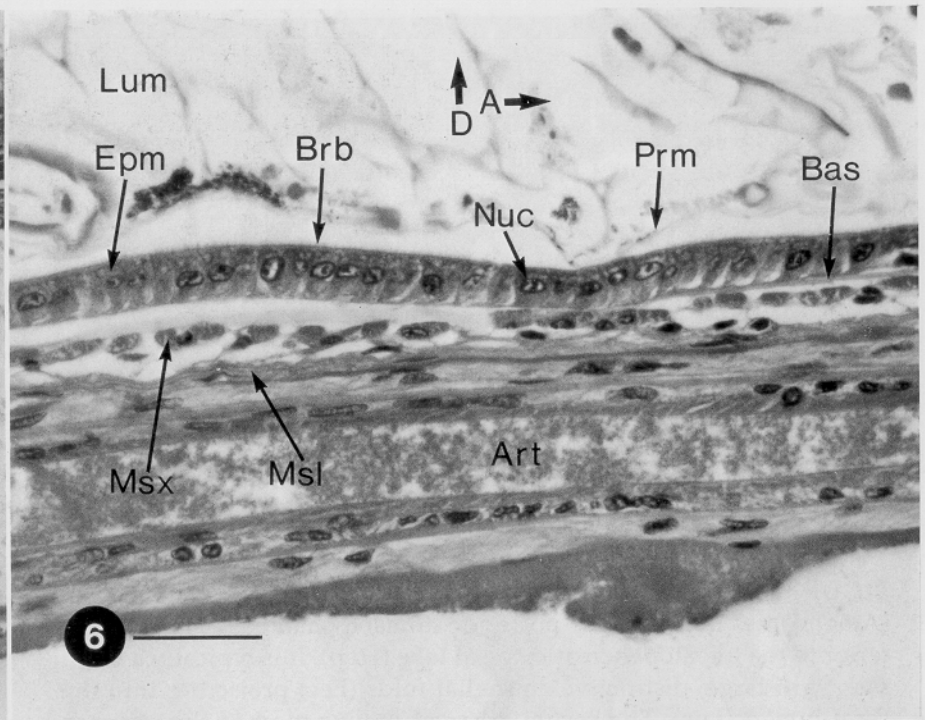
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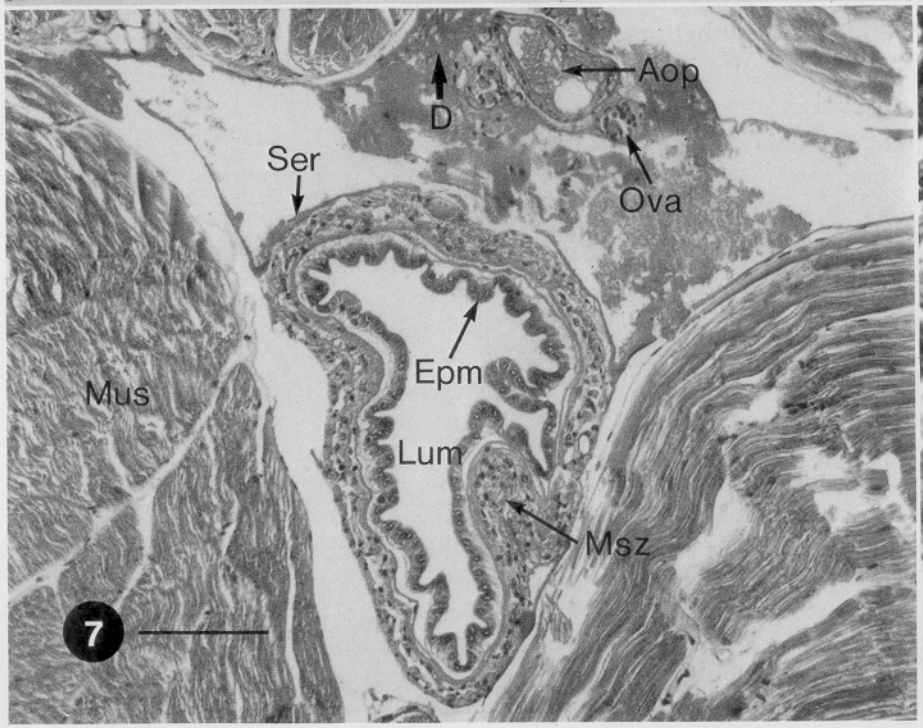
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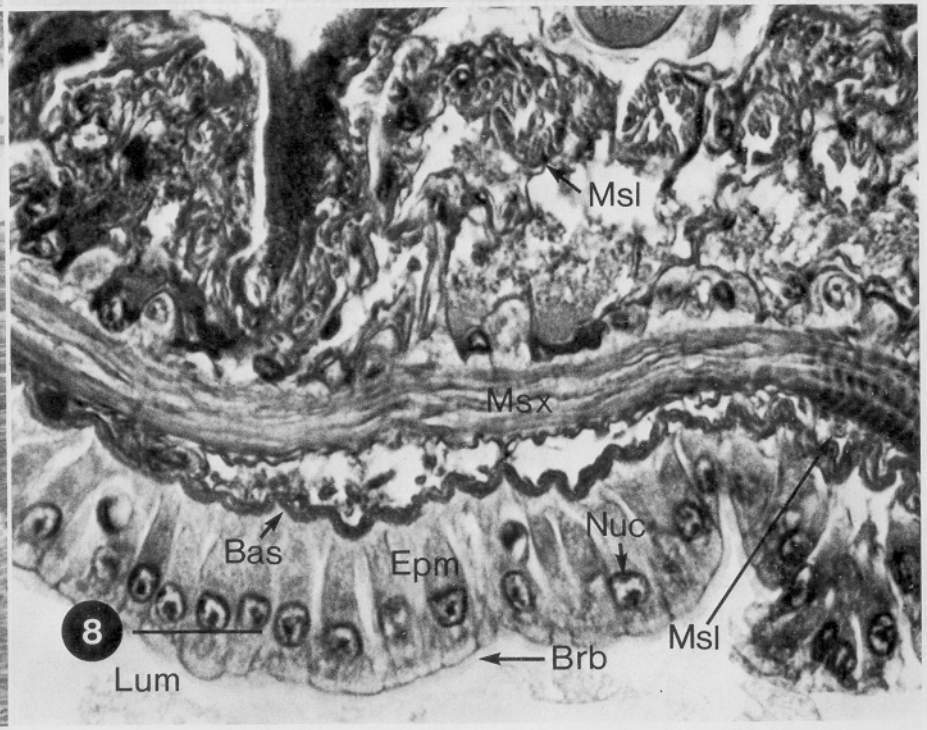
Msl
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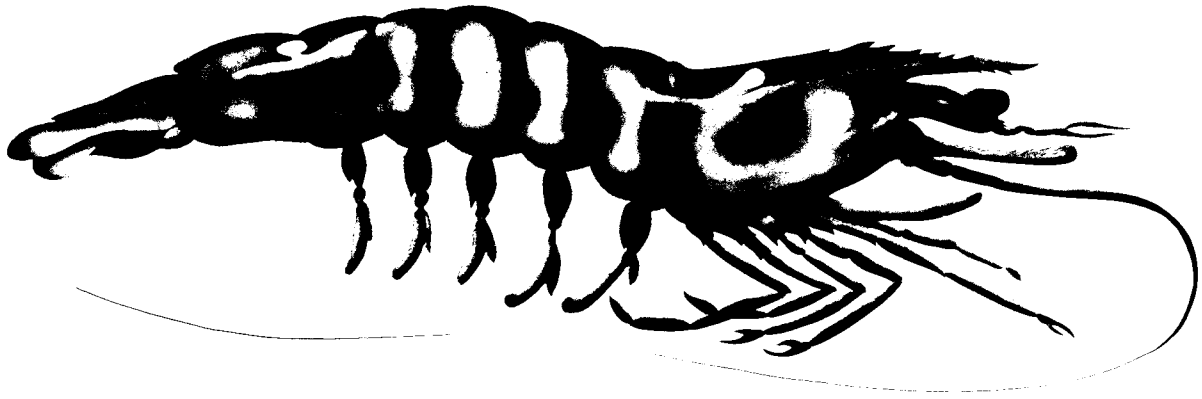


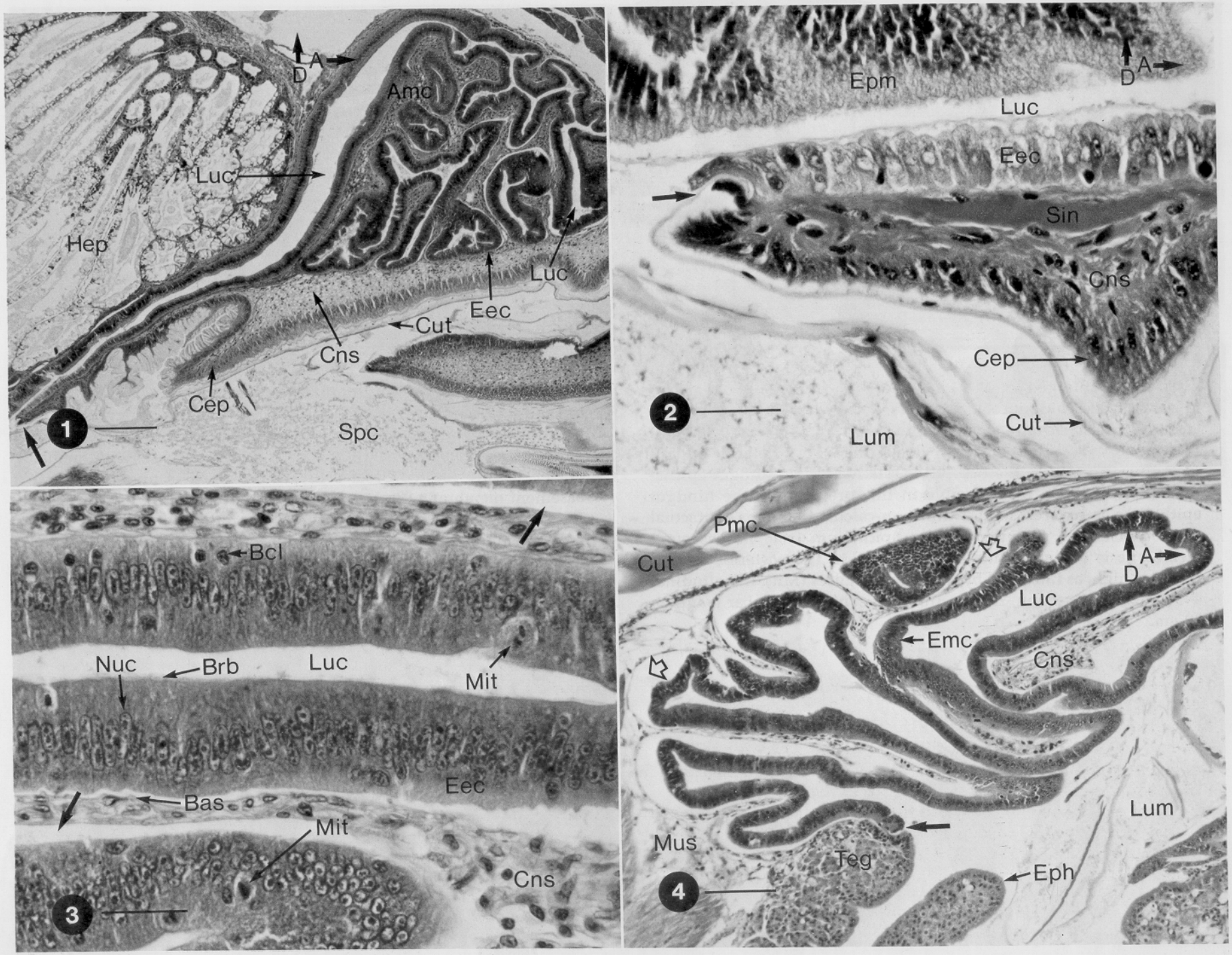
MIDGUT CECA

FIGURE 1 Overall longitudinal view of anterior midgut cecum (Amc). It lies dorsal to the posterior stomach chamber (Spc) and anterior to the hepatopancreatic dorsal lobe (Hep). It is typically a blind sack with large, distinctive epithelial folds (Eec) projecting into the cecal lumen (Luc). The cecum arises at the junction of the stomach and the midgut proper. Embryologically, the cecum is a part of the midgut, thus the midgut begins at the junction (bold arrow) of the cecum and stomach. The stomach epithelium (Cep) lies basal to a thin layer of non-calcified cuticle (Cut), unlike the anterior midgut cecum or midgut proper. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 200 μm.

FIGURE 2 Expanded view of anterior midgut cecum/stomach epithelial junction (bold arrow). The epithelial layers, on either side of the junction, are quite distinctive. The stomach epithelium (Cep), in this view, is atypically basophilic relative to those of the anterior midgut cecum (Eec). The thin cuticle (Cut) lies just medial to the stomach epithelium (Cep) and ends at the junction. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.

FIGURE 3 Enlarged view of anterior midgut cecum and its associated epithelial lining (Eec). The lumen (Luc) is bounded by simple columnar cells. The nuclei (Nuc) are situated midway between the basement membrane (Bas) and the luminal surface. Medial to the nuclei are basal cells (Bcl), often with condensed, basophilic nuclei. The midgut cecum generally has a high mitotic index, as noted by the number of mitotic figures (Mit) interspersed throughout the epithelial layer. The bold arrows point to areas where there has been artifactual separation of the epithelium from the basement membrane. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.

FIGURE 4 Overall longitudinal view of the posterior midgut cecum (Pmc). This organ system has been erroneously referred to as the hindgut cecum, but embryologically it arises from the midgut. It is similar to the anterior midgut cecum, except in its shorter connection to the midgut lumen (Lum). It likewise has lobular projections of the epithelium (Emc) into its lumen (Luc). It lies just beneath the main body cuticle (Cut) and its epithelium connects directly to the hindgut epithelium (Eph). Tegmental glands (Teg) are noted within the hindgut folds near the junction (bold arrow) of the hindgut and the posterior midgut cecum. Artifactual separation of the epithelium from the basement membrane is noted again (hollow arrows). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 100 μm.


D
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Bas
Mit
Cns
3
Pmc
Cut
D
A
Luc
Emc
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Teg
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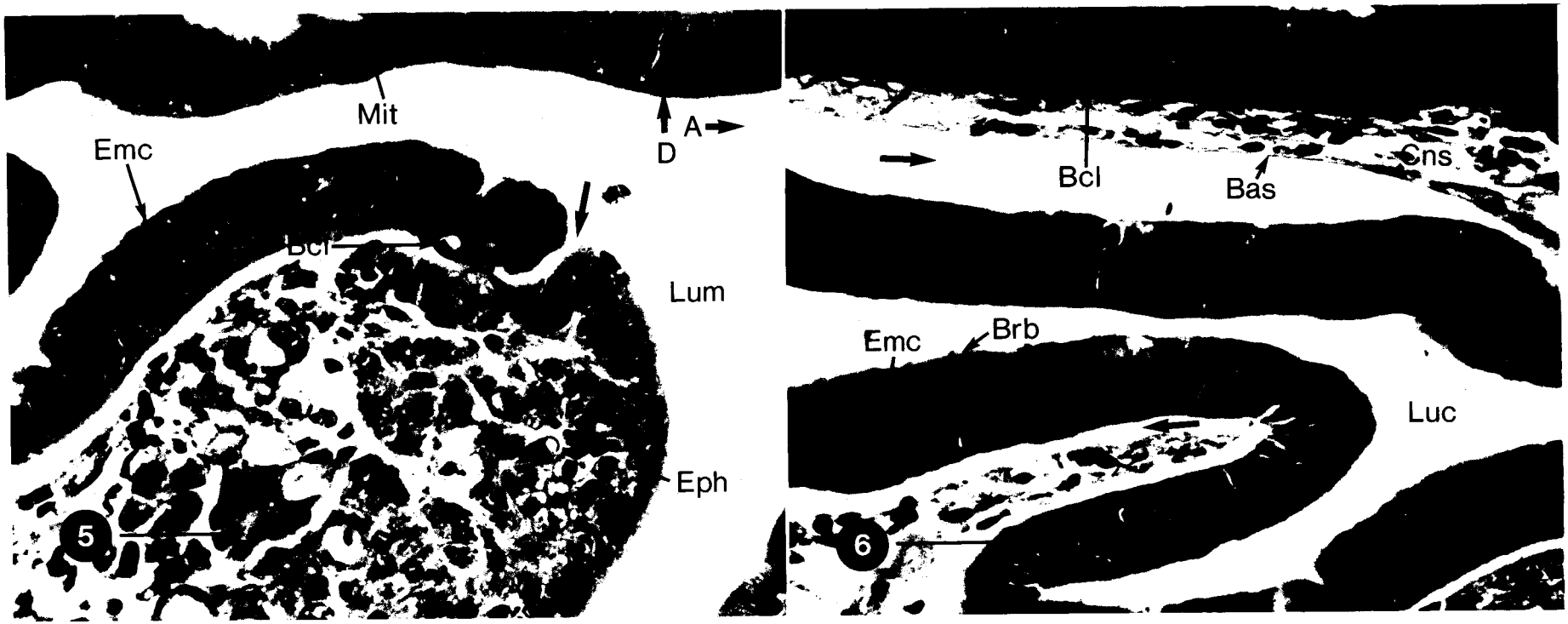
4

MIDGUT CECA

FIGURE 5 Enlarged longitudinal view of the junction (bold arrow) of the posterior midgut cecum and the hindgut. As in the case of the anterior midgut cecum - stomach, the union of the posterior midgut cecum and the hindgut is clearly demarcated. The posterior midgut epithelium (Emc) is quite basophilic relative to the hindgut epithelium (Eph). Although not visible in this preparation, the hindgut epithelium is covered with a thin non-calcified cuticle. Artifactual separation of the posterior midgut epithelium from its basement membrane is evident. Mitotic activity within the posterior midgut cecum is high, as indicated by the mitotic figures (Mit). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.

FIGURE 6 Expanded longitudinal view of the posterior midgut cecum and its associated epithelial layer (Emc). Its morphology is nearly identical to that of the anterior midgut cecum: a microvillous brush border, simple (though slightly shorter) columnar cells with medial nuclei, basally located basal cells, all of which is underlain by a basement membrane (Bas) and connective tissue (Cns). It likewise is seen to have artifactual separation of the epithelium from the basement membrane. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 30 μm.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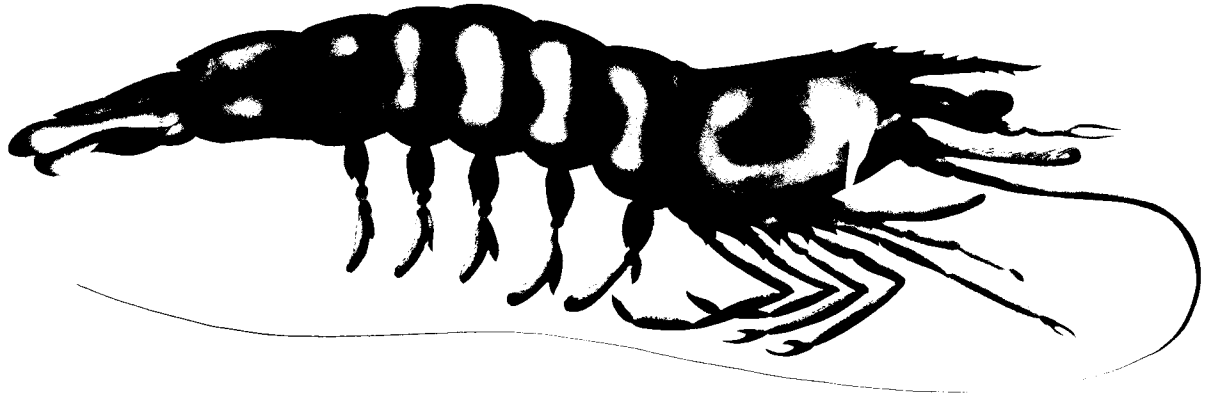


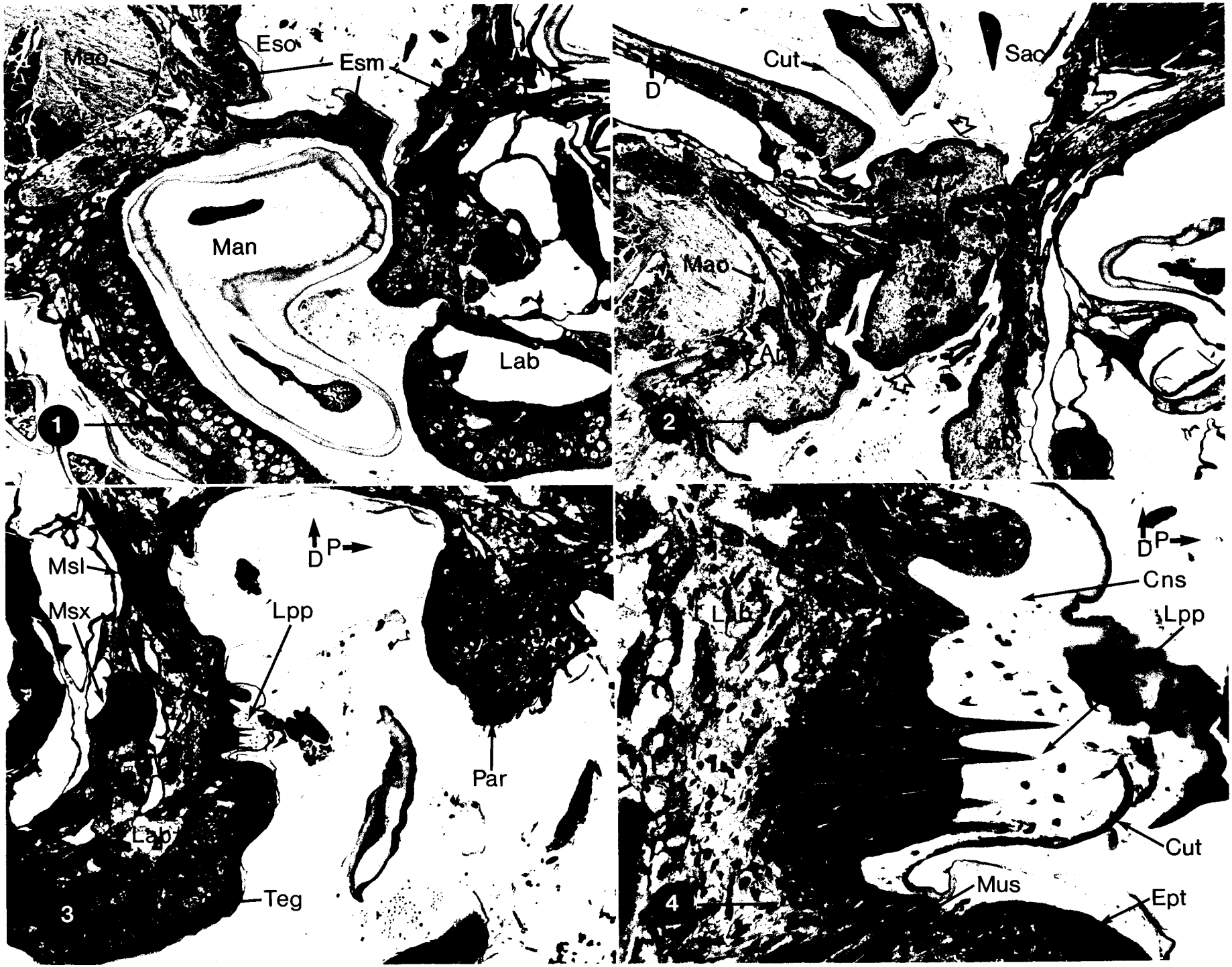
ORAL REGION

FIGURE 1 Overall longitudinal view of oral region including, mouth, surrounding appendages and esophagus. The most anterior appendage is the labrum (Lab), a thinly chitinized, fleshy, non-paired, large, protuberance that extends posteriorly over the buccal opening. Just posterior to the labrum (sectioned through its apical end) is one of the heavily chitinized, paired mandibles (Man), each of which has an incisor and molar surface, used for tearing and grinding of food items. Posterior to the mandibles are the paired paragnatha (Par). This preparation is slightly lateral to a mid-sagittal section and thus the esophagus (Eso) appears to be occluded. Within the wall of the esophagus are esophageal muscles (Esm). A major nerve tract (Nrv) is noted just dorsal to the mandible, as is the mandibular organ (Mao). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 400 μm.

FIGURE 2 An additional overall longitudinal view of the oral region. This section is likewise just lateral to a mid-sagittal. The extent of the esophageal musculature (Esm) can be seen. The esophagus enters the stomach slightly posterior to the anterior-most region of the anterior chamber (Sac). The stomach is lined with a thin layer of non-calcified cuticle (Cut). The esophagus extends approximately from arrow to arrow. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 400 μm.

FIGURE 3 Overall longitudinal view of appendages surrounding the mouth. Of particular note is the structure referred to by Young (1959) as the labral posterior feeding process (Lpp). The labrum (Lab) and the paragnatha (Par) both contain numerous specialized tegmental glands (Teg). The labrum contains large muscle bundles in both longitudinal (Msl) and cross section (Msx). Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 200 μm.

FIGURE 4 Expanded view of the labral posterior feeding process (Lpp). The fibrous extensions, which presumably extend beyond the cuticle (Cut), are surrounded by a cellular, spongy connective tissue (Cns). The insertion of muscles bundles (Mus) near it may substantiate its function, as suggested by its name. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 50 μm.


Mao
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Esm
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1
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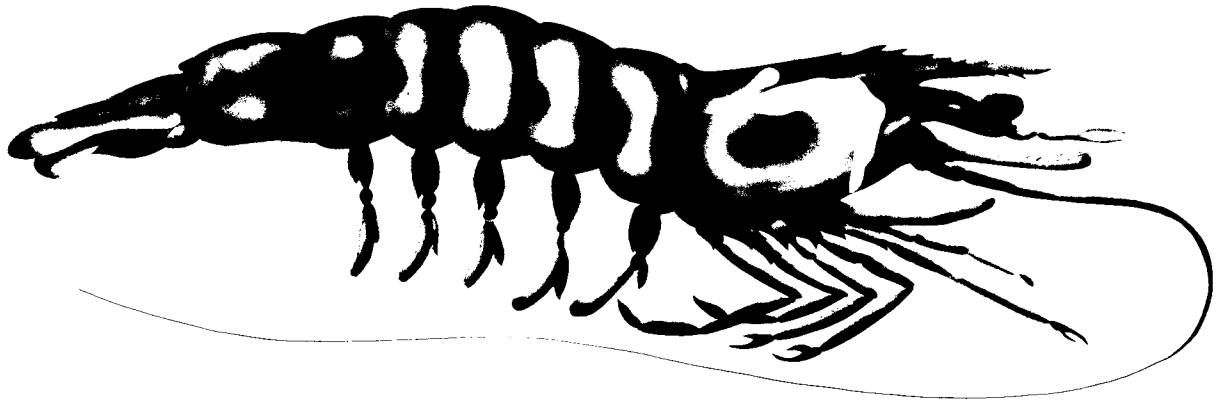

STOMACH

FIGURE 1 Composite longitudinal orientation view of the complete stomach from a less than 2.0 gram juvenile. The stomach begins anteriorly at the ventral esophagus (Eso) and extends posteriorly to the approximate mid-point (anterior-posterior) of the hepatopancreas (Hep). The stomach is distinctly divided into an anterior (Sac) and posterior chamber (Spc), the latter divided again into a dorsal and ventral subchamber. Other terms have been commonly used in the literature: "cardiac stomach" or "anterior proventriculus" for the anterior chamber and "pyloric stomach" (which is actually closer to the heart and pericardial chamber than the "cardiac stomach") or "posterior proventriculus" for the posterior chamber. Both regions of the stomach are lined with a layer of cuticle, normally non-calcified except for individual tooth-like projections or grinding surfaces (ossicles). Dorsal to the anterior chamber (Sac) is a thin layer of muscle (Msl), several nodules of hematopoietic tissue (Heo) and the exoskeletal cuticle (Cut). On occasions, antennal gland tubules are noted in this area. The inner wall of the anterior chamber is often seen to have highly exaggerated folds projecting into the lumen (Lum); these are usually associated with the absence of ingested food. Consumed food items, are reportedly masticated, even to the point of trituration, within this region; leading to it often being called a "gastric mill". Ventral to the anterior chamber is muscle (Msl) and a significant region of antennal gland tubules (Agt). The posterior chamber comprises a dorsal and ventral subchamber, with the gastric sieve (Gss) being the major component of the latter. Its intimate association with the primary hepatopancreatic ducts (Hpd) alludes to its function as a screening structure for masticated food delivery to the hepatopancreas proper. The gastric sieve is a complex structure of cuticular setae and grooves (see Figure 2). The region dorsal to the posterior chamber contains the anterior midgut cecum (Amc) and a nodule of hematopoietic tissue (Heo). Dorso-posterior to the posterior chamber is the dorsal lobe of the hepatopancreas (Hep), while the ventral lobe of the hepatopancreas borders nearly the complete ventral margin of the posterior chamber. The imposing ventral structure of the ventral subchamber is referred to as the inferior ampullary ossicle (Iao); often in longitudinal section its tissue (and that of other similar ossicles) will artifactually separate into single-cell layers, making interpretation of function, based on structure, difficult. The posterior extremity of the stomach ends ventrally at the anterior lip of the primary hepatopancreatic duct (Hpd) (bold arrow) and dorsally at the anterior midgut cecum (Amc) main duct anterior lip (hollow arrow). The cuticular stomach lining likewise terminates at these respective points. Line A-B defines the approximate plane of section of Figure 2. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 20 μm.


D
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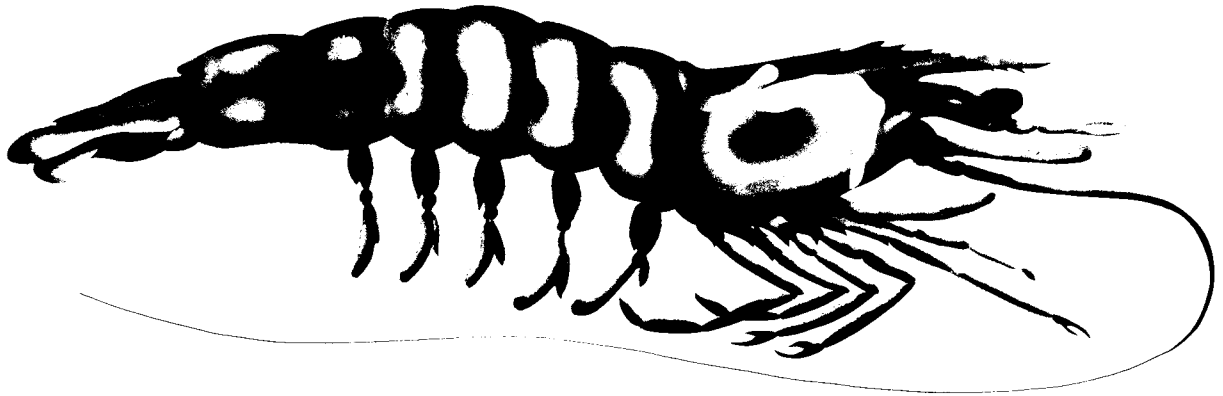


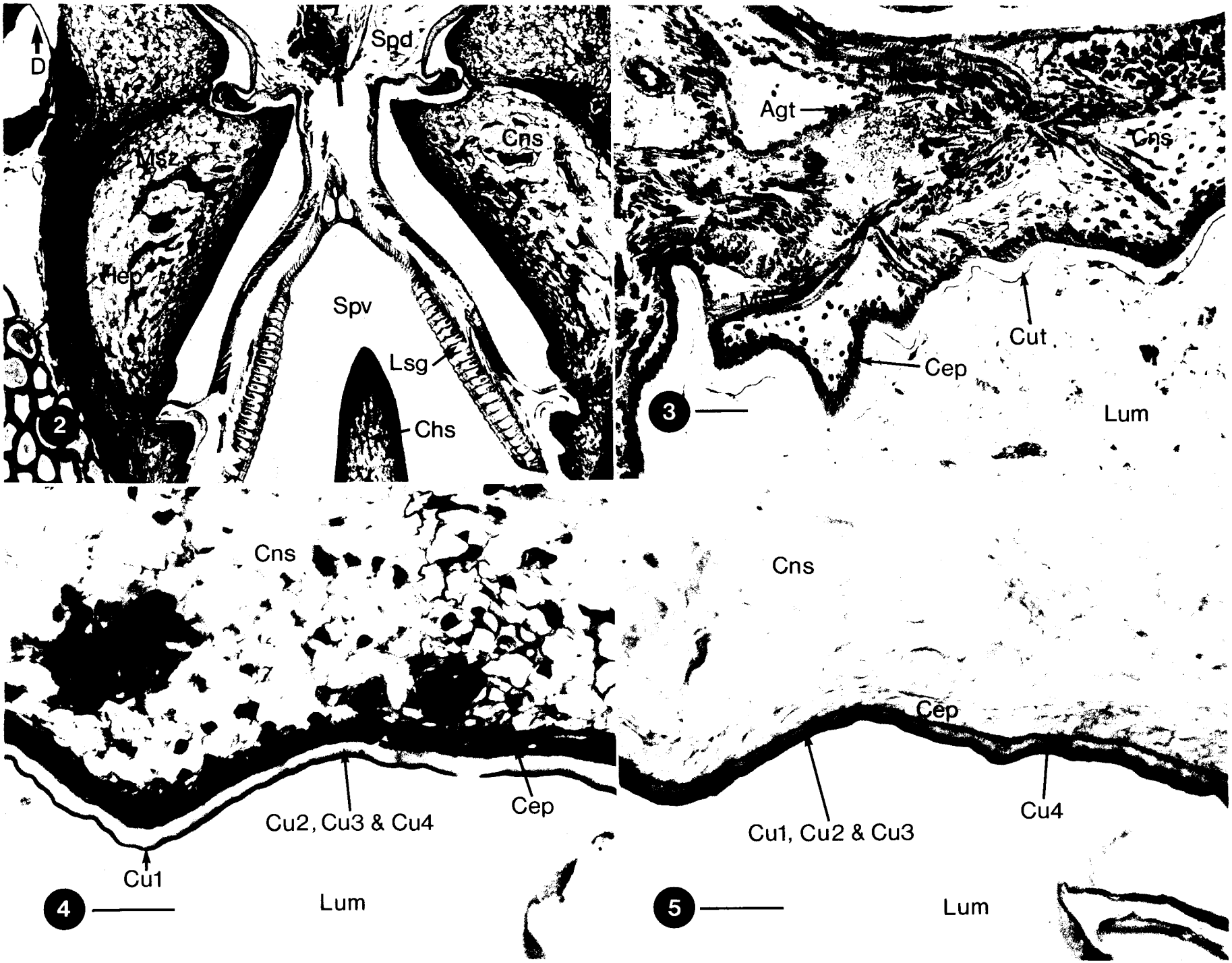
STOMACH

FIGURE 2 Cross sectional orientation view of the posterior chamber. Both the dorsal (Spd) and ventral subchambers (Spv) of the posterior chamber have been sectioned; the plane of section corresponds to that depicted as line A-B in Figure 1. The gastric sieve's cuticular structure indicates that masticated food, when forced into and through the sieve, is divided roughly in half and directed, via the longitudinal inter-setal grooves (Lsg), to each of the two hepatopancreatic primary ducts (not noted in this section). The dorsal subchamber is distended by food items (bold arrow). Thick layers of circular and longitudinal muscles (Msz) surrounds the entire stomach, with spongy connective tissue (Cns) occupying major regions. Transverse 4–5 μm paraffin section, Masson's trichrome stain, Davidson's fixative, bar length = 400 μm.

FIGURE 3 Enlarged longitudinal view of anterior chamber wall. Immediately beneath the thin non-calcified cuticle (Cut) is the simple, columnar cuticular epithelium (Cep), slightly separated from the cuticle. The stomach lumen (Lum) is filled with partially masticated food. Worthy of noting is the number and varied orientation of muscle bundles (Msl), all of which play a role in the movement of food against the triturative ossicles. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 50 μm.

FIGURE 4 Enlarged view of the anterior chamber cuticular lining. Masson's trichrome stain differentially stained the sublayers of cuticle. The outer-most (Cu1) or epicuticle layer is stained intensely red, while the second (Cu2) or exocuticle and the third (Cu3) or endocuticle are stained light blue. The innermost (Cu4) or "membranous layer" has been stained light blue like that of the two layers above it. An epithelial cell layer (Cep) subtends the cuticle. Supporting the cuticle and cuticular epithelium is a thick layer of spongy connective tissue (Cns). Longitudinal 4–5 μm paraffin section, Masson's trichrome stain, Davidson's fixative, bar length = 30 μm.

FIGURE 5 Additional enlarged view of the anterior chamber cuticular lining. PAS stain differentially stained the sublayers of cuticle distinctly from that of Masson's trichrome. The three outer layers: epicuticle, exocuticle and endocuticle (Cu1, Cu2 and Cu3, respectively) are shades of blue-purple (progressively darker toward the outer layer), while an inner layer, adjacent to the cuticular epithelium (Cep) is stained intensely red-purple. PAS stains of exoskeleton cuticle, in the antennae for instance, lack this inner most red-purple region. Perhaps the red-purple region in the stomach is the membranous layer (Cu4) and, due to a unique function, it is biochemically different from the same layer in other areas. Longitudinal 4–5 μm paraffin section, PAS stain, Davidson's fixative, bar length = 30 μm.


D
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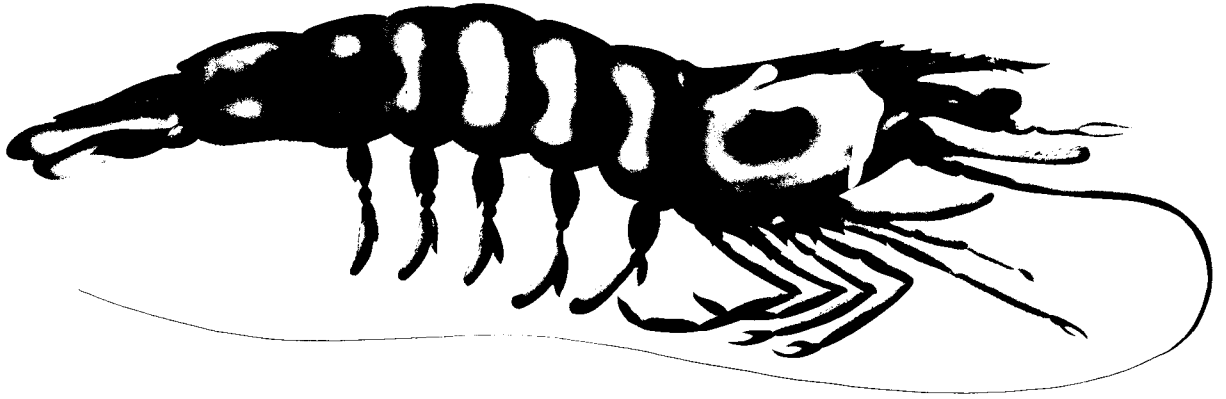
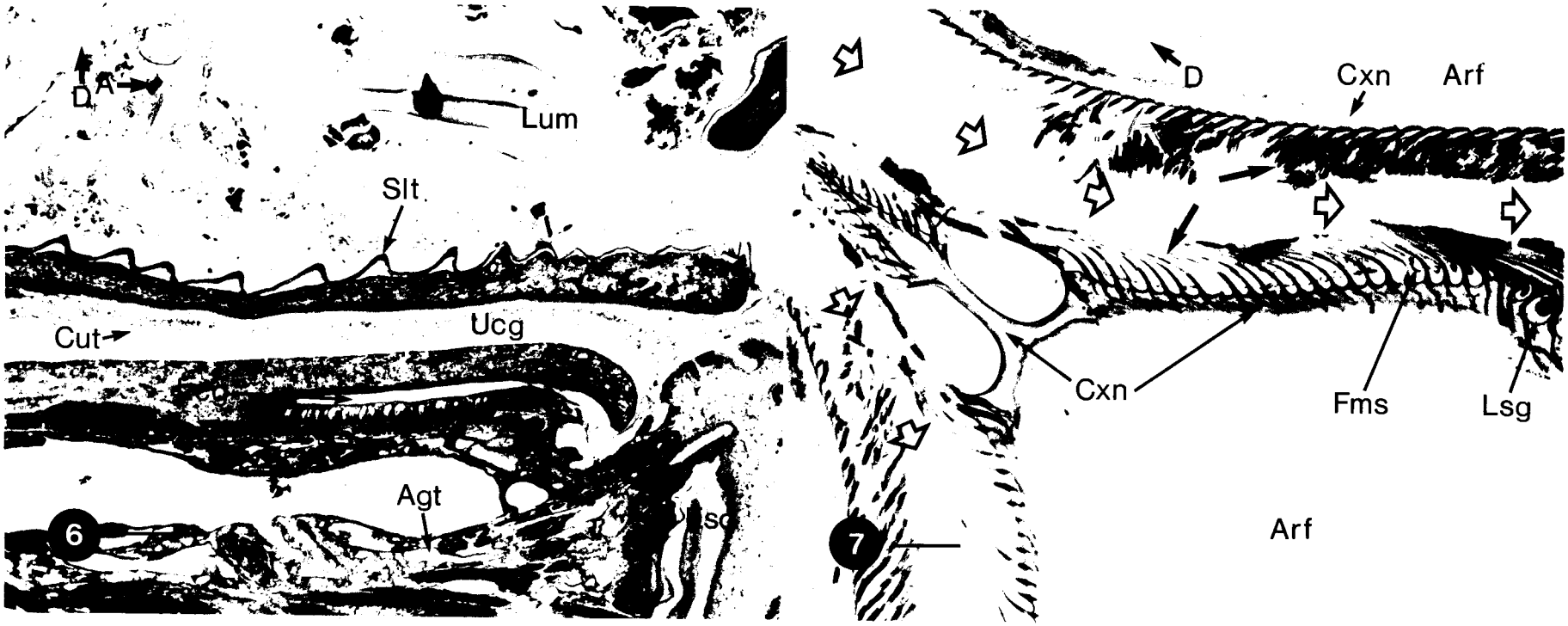


STOMACH

FIGURE 6 Enlarged view of the anterior chamber. Of particular note are the supralateral teeth (Slt) and the upper (Ucg) and lower (Lcg) "cardiac grooves". The former are functional components of the gastric mill and the latter have been proposed as pathways for movement of enzymes from the hepatopancreas to the anterior chamber. Material within the lumen (Lum) appears to be partially masticated. Longitudinal 4–5 μm paraffin section, H&E stain, Davidson's fixative, bar length = 400 μm.

FIGURE 7 Enlarged view of the gastric sieve. The setae per se (bold arrows) stain intensely red, similar to an epicuticular sublayer, while the remainder of the sieve stains (Cxn) light blue, comparable to exo- and endocuticular sublayers. As noted in Figure 2, the masticated food moves through the sieve in the directions indicated by the hollow arrows. Food that has been reduced sufficiently (Fms) in size will be allowed to pass between the setae and into the longitudinal inter-setal grooves (Lsg), via which it will pass to the hepatopancreatic primary ducts. The remainder, presumably, will by-pass the hepatopancreas and pass directly to the midgut, without further digestive action. As in most preparations of the gastric sieve region, the sieve per se has artifactually separated (Arf) from its epithelial layer, and, in this figure, the epithelium is not within view. Transverse 4–5 μm paraffin section, Masson's trichrome stain, Davidson's fixative, bar length = 50 μm.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