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A Comparative Study of
Functional Morphology
of the Male Reproductive
Systems in the Astacidea
with Emphasis on the
Freshwater Crayfishes
(Crustacea: Decapoda)

*Horton H. Hobbs Jr.,
Margaret C. Harvey, and
Horton H. Hobbs III*

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ABSTRACT

Hobbs Jr., Horton H., Harvey, Margaret C., and Hobbs III, Horton H. *A Comparative Study of Functional Morphology of the Male Reproductive Systems in the Astacidea (Crustacea: Decapoda) with Emphasis on the Freshwater Crayfishes*. Smithsonian Contributions to Zoology, Number 624, 76 pages, 27 figures, 2007. — This study of the functional morphology of the male reproductive system in astacidean crustaceans has allowed for comparisons of representatives of the superfamilies Parastacoidea, Enoplometopoidea, Nephropoidea, and the Astacoidea with a focus on the crayfishes. Tissues from the testes and (to the extent possible) vasa deferentia were prepared for light and scanning electron microscopy and specimens of the following families were used: Parastacidae – *Parastacoides tasmanicus tasmanicus*, *Astacopsis franklinii*, *Parastacus nicoleti*; Enoplometopidae – *Enoplometopus occidentalis*; Nephropidae – *Homarus americanus*; Astacidae – *Pacifastacus leniusculus trowbridgii*; Cambaridae – *Cambaroides japonicus*, *Cambaroides similis*, *Cambarus (Puncticambarus) acuminatus*, *C. (Hiaticambarus) longulus*, *Procambarus (Ortmannicus) fallax*, *P. (O.) zonangulus*, *P. (Scapulicambarus) paeninsularis*, and *Orconectes (Procericambarus) rusticus*.

The single organ testis is “H-shaped” in members of the Parastacoidea, Enoplometopoidea, and Nephropoidea and consists of a pair of longitudinal lobes, each composed of an anterior and posterior lobule joined by a transverse commissure or bridge. The derived “Y-shaped” pattern of the testis of the Astacoidea is trilobed and consists of a pair of anterior lobules and a median posterior lobule that in most adult Cambaridae are joined by a trifurcate, constricted stalk, a structure that is lacking in the Cambaroidinae and Astacidae.

The sac-like acini lie in the axes of the testicular lobules and produce spermatozoa. As spermatogenesis proceeds, each acinus becomes larger and, with spermiogenesis and the expulsion of spermatozoa into the collecting ducts, undergoes one or two of three fates: (1) acinus regeneration occurs and another cycle of sperm production ensues (adopted exclusively by the Astacidae and Cambaroidinae); (2) secondary acini develop in the wall of existing acini, converting the primary acinus into a passageway to the collecting tubules; or (3) the acinus degenerates and new acini arise from collecting tubules (employed only by the Cambarinae and Cambarellinae). The first and second fates have been adopted by the Parastacoidea, the Enoplometopoidea, and the Nephropoidea. In the Cambarinae and Cambarellinae, the germinal cells are recognizable only in the acinar buds from the collecting tubules and when they assume the role of spermatogonia; they are not evident along the lengths of the tubules nor are they present within an acinus after the onset of spermatogenesis. In all other astacideans examined, the germinal cells seem always to be present in the collecting tubules. Additionally, they appear in the walls of acini by the time the spermatogenic elements are being converted to spermatids, frequently forming clusters, the primordia of secondary acini in the Parastacoidea, the Nephropoidea, and occasionally the Enoplometopoidea. Germinal cells may be disposed in a partial layer or scattered within the walls of an acinus and constitute the initial spermatogonia of a new cycle of sperm production. This is what occurs in the acini of the Astacidae, Cambaroidinae, Parastacoidea, Nephropoidea, and Enoplometopoidea.

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Preface

At the time of his death on 22 March 1994, Horton H. Hobbs, Jr. was working on this manuscript, an endeavor that had periodically occupied his efforts over a 40-year period and had dominated his work during the last few years of his life. Although most of the manuscript was completed, some work remained, particularly with reference to the figures. Margaret Harvey became involved as a doctoral student at the suggestion of C. W. Hart Jr., during a presentation at the Crustacean Society's summer 1995 meeting held at the Harbour Branch Oceanographic Institute, in Fort Pierce, Florida. We have attempted to present the manuscript in its original form and have modified it only for purposes of clarification and for figure inclusions. An appendix is added with references to some of the more recent literature.

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A Comparative Study of Functional Morphology of the Male Reproductive Systems in the Astacidea with Emphasis on the Freshwater Crayfishes

(Crustacea: Decapoda)

INTRODUCTION

This study is an outgrowth of an endeavor to determine whether or not the basic structure of the testis and the processes involved in the production of spermatozoa in the Cambaridae and Parastacidae are similar. Discovering that marked differences do indeed occur, curiosity led me to attempt to obtain what information exists pertaining to the testes in members of other families and subfamilies of the infraorder Astacidea and to broaden the scope of effort to include the male gonoducts. Surprisingly, the group for which the most complete information appears to be available is the Enoplometopoidea (Matthews, 1954; Haley, 1984). In contrast, despite the number of studies that have been made on the reproductive systems of members of the family Astacidae and the superfamily Nephropoidea, little data of the kinds needed for the comparative study undertaken were found. Too, unfortunately, there is an almost total lack of information on the functional anatomy of the male reproductive systems of members of the subfamily Cambaroidinae. While this effort has produced limited data on each of the three groups just mentioned, at least some comparisons of the male reproductive systems in representatives of the superfamilies Parastacoidea, Nephropoidea, Enoplometopoidea, and Astacoidea are now possible and are presented here. Emphasis must be made, however, that the primary concern for this study has been with the crayfishes.

In the presentation of findings, the following classification of the decapods that are included in this study has been adopted:

Order Decapoda

Infraorder Astacidea

Superfamily Parastacoidea

Family Parastacidae

Genera mentioned: *Astacopsis*, *Cherax*, *Euastacus*, *Parastacoides*, *Parastacus*, and *Samastacus*

Superfamily Enoplometopoidea

Family Enoplometopidae

Genus mentioned: *Enoplometopus*

Superfamily Nephropoidea

Family Nephropidae

Genus mentioned: *Nephrops*

Family Homaridae

Genus mentioned: *Homarus*

Superfamily Astacoidea

Family Astacidae

Genera mentioned: *Astacus*, *Austropotamobius*, and *Pacifastacus*

Family Cambaridae

Subfamily Cambaroidinae

Genus mentioned: *Cambaroides*

Subfamily Cambarinae

Genera mentioned: *Cambarus*, *Fallicambarus*, *Orconectes*, and *Procambarus*

Subfamily Cambarellinae

Genus mentioned: *Cambarellus*

There is a tremendous discrepancy in the quantity of information available pertaining to the male reproductive systems of members of the several taxa treated. As will become evident in the summaries of the literature pertaining to members of each of the groups investigated, much more information exists for the Cambaridae and Enoplometopidae than for the other families. The present study has resulted in a better understanding of the male reproductive system of the Parastacidae and Astacidae, and of some facets of the system in the Nephropidae (*Homarus*).

Because what is believed to be the most generalized condition of the male reproductive system (gonads and gonoducts) among the crayfish species investigated occurs in the Parastacidae, a description of these organs in *Parastacoides tasmanicus* (Erichson, 1846) is presented first and is followed by comparisons with other parastacids. Little information on this organ system in members of the family was available previously and, for that reason, what was known—together with new information on other members of the family—is summarized following the description. The enoplometopid system is reviewed and that of the homarid system compared with corresponding elements of the parastacids. The gonads and gonoducts of the Astacidae and Cambaridae are presented in more detail.

The descriptions of the male reproductive system in crayfishes that follow are based largely on the testes and sperm ducts of individuals collected a short time prior to and during a breeding season. In the Astacoidea living in the northern temperate zone the best panorama of testicular activity may be observed in individuals collected from August to November (Word and Hobbs, 1958; Wielgus-Serafinska, 1976). In the cambarids investigated this corresponds to the few weeks

before and after the molt from second to first form. To clarify the application of “‘first’ and ‘second’ form” to cambarid males the following is quoted from Hobbs (1981:9):

Among those [members of the Cambaridae] that have an annual reproductive cycle, the breeding (“Form I,” or “first form”) males of the population, at the end of their first season, molt and are transformed to essentially a juvenile morphology (“Form II,” or “second form”) that is retained until the advent of the next breeding season when the second semiannual molt returns them to the adult form (Form I). Thus between each breeding season there is a regression to the quasi-juvenile (Form II) stage, which may have a duration of three to perhaps as long as six months. For those species that have a seasonal reproductive cycle, the entire male population may be in the juvenile or quasi-juvenile (Form II) stage throughout most of the summer months.

In southern temperate, subtropical, and tropical zones of the northern hemisphere, seasonal differences are not so obvious (Black, 1966; Payne, 1968; Albaugh, 1973), for breeding (first form) and nonbreeding (second form) males occur throughout the year in at least some populations of a number of species (see also Hobbs, 1942, 1981). In the latter part and at the close of a mating season, almost all of the sperm-producing units may be in degenerating stages, exhibit early stages of regeneration, or are adding new units that are replacing those that have deteriorated.

In the much more limited knowledge of the testicular activity of the southern temperate Parastacoidea, apparently the best panorama of testicular activity is evident from February to April. In the Tasmanian species treated herein, this period occurs in the late summer and fall (Hamr, 1990); in their study of *Parastacus brasiliensis* (von Martens, 1869) Fontoura and Buckup (1989:912) found that “the reproductive period [?= the time during which the females are ovigerous] goes from September to February.” This suggests that the testes of the males were probably most active in the previous late summer and fall. The testis of *Samastacus spinifrons* (Philippi, 1882) was reported to be largest in February, March, and July by Bocic, Rudolph, and Lopez et al. (1988). On the basis of these observations the inference may be made that in the population studied there exist two periods of sperm production per year with corresponding peaks of testicular activity: one in the late summer and fall, and the other

in late fall and winter. In specimens of *Parastacoides tasmanicus* and *Parastacus nicoleti* (Philippi, 1882) collected in October and August, respectively, almost all of the acini in the testes of the former contained spermatogonia, and those of the latter were in various stages of degeneration, whereas specimens of the former collected in March provided a complete series of stages in the production and expulsion of spermatozoa from the testis.

The cyclic activity of the male reproductive system in the marine Enoplometopoidea and Nephropoidea appears to be affected less by annual seasons, and occurring in waves (in *Enoplometopus occidentalis* (Randall, 1840)) along the axes of the testis (Haley, 1984), most events in the formation and transportation of spermatozoa are evident throughout the year (Farmer, 1974). In the single specimen from the current study the testicular units contain only spermatogonia, spermatids, and spermatozoa, suggesting that it was preserved during a mating season.

There is so little variation in the basic histology of the sperm ducts of the crayfishes that the common features are presented here and treated only briefly in the individual descriptions. The duct consists basically of an epithelial lining in which the nuclei are situated adjacent to the basement membrane. This is surrounded by a layer of connective tissue that radiates to become enmeshed with a layer of striated muscle fibers extending longitudinally, and a more superficial encircling layer. (Sometimes these muscle layers are indistinct and the fibers are obliquely disposed and overlap.) Variations in the appearance of these layers are determined in part by the diameter of the lumen of the duct, which varies with the sexual cycle. As might be anticipated, the muscular layer is thickest when the lumen of the duct is devoid of spermatozoa or a spermatophore and thinnest entally when the spermatozoa are most abundant and more ectally when the spermatophore is completely formed. Even the muscular wall of the ejaculatory duct may appear exceedingly thin when encompassing a spermatophore. With the approach of the breeding season the acidophilic epithelial layer consists of tall columnar cells with the basal nuclei slender, compressed, and sometimes extending half the length of the cell. As the cells contribute their exudate to the formation of the spermatophore and the lumen of the duct increases, the cells become shorter, often cuboidal, and the nuclei become ovate to subspherical. At the close of the breeding season, frequently the free borders of the epithelial layer have broken down in secretion, sometimes parts of the layer virtually disintegrating.

In this study no outer epithelial coat encasing any part of the sperm duct in any of the materials examined has been identified.

MATERIALS AND METHODS

Materials

Specimens of *Parastacoides t. tasmanicus* were collected in the Needles Mountain, Southwest National Park and environs, Tasmania, on 22 October 1987 and 8 March 1988, and those of *Astacopsis franklinii* (Gray, 1845) from Guy Fawkes Rivulet in Hobart, Tasmania on 8 March 1988. Materials of *Parastacus nicoleti* were collected in the vicinity of Valdivia, Chile. The single specimen of *Enoplometopus occidentalis* from Hawaii, lacking other data, was in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Specimens of *Homarus americanus* Milne Edwards, 1837 (a) were obtained in local markets on 8 January 1988 and December 1991. The material of *Pacifastacus leniusculus trowbridgii* (Stimpson, 1857) was taken from the Willamette River near Eugene, Oregon, on 8 November 1988. Specimens of *Cambaroides japonicus* (de Haan, 1842) were collected from Zenibako, near Sapporo, Hokkaido, Japan, on 17 April and 11 October 1989. Representatives of *Cambarus (Puncticambarus) acuminatus* Faxon, 1884 and *C. (Hiaticambarus) longulus* Girard, 1852 were collected in the vicinity of Charlottesville, Virginia, throughout the year in the 1950s, and those of *Procambarus (Ortmanicus) fallax* (Hagen, 1870) and *P. (Scapulicambarus) paeninsulanus* (Faxon, 1914) in the vicinity of Gainesville, Florida, by Horton H. Hobbs, Jr. in the 1940s. Specimens of *Orconectes (Procericambarus) rusticus* (Girard, 1852) were obtained from Clark County, Ohio, in 1993. [Harvey and Hobbs III were unable to determine the locations and dates collected for *Cambaroides similis* (Koelbel, 1892), *Cambarus bartonii cavatus* Hay 1902, and *Procambarus zonangulus* Hobbs and Hobbs 1990].

Preparations for Scanning Electron Microscopy

Entire or pieces of testes were fixed in Bouin's Fluid for varying lengths of time (6 hours to several days) and stored in 70% ethyl alcohol. Hydration followed at 30-minute intervals in 50% and 30% alcohol, and distilled water where, after washing, the specimens were left for one hour. They were then transferred to 1.0% osmium tetroxide for two hours (two changes) and washed in distilled water for 30 minutes. After being transferred to 50% alcohol for 30 minutes and 70% alcohol overnight, the specimens were clipped in small pieces and immersed in DMP (2,2-dimethoxypropane) for three 15-minute changes, then in absolute alcohol for two 30-minute changes, followed by

critical point drying for 30 minutes, and left in a desiccator overnight before mounting on stubbs. Photographs were made using a Cambridge stereoscope or Hitachi SEM at 10kv.

In preparations of the "clusor," which is located within the coxa and phallic papilla, the fifth pereopod was removed at the base of the coxa and the podomeres distal to the coxa dismembered from the latter. Working under a stereoscopic microscope, at least half of the coxa was trimmed from that bearing the phallic papilla. The latter half was placed in lactic acid with four or five drops of Chlorazol Black E and placed in an oven at a temperature of 150°C for approximately 45 minutes, thereby clearing the fractured podomere of all noncuticular elements. Dehydration and examination followed, as noted above.

Preparations for Light Microscopy

To the extent possible, the testes were removed with much of the vasa deferentia intact and placed in a small Stender dish with van Harreveld's perfusion fluid (van Harreveld, 1936). There they were oriented much as they were in the crayfish and after the perfusion fluid was removed the specimens were gently flooded with one of the following fixatives: Bouin's, Sanfelice's, Carnoy's, or A.F.A. Dehydration was accomplished in the usual ethyl alcohol series after which the specimens were cleared either in xylene, toluene, or chloroform, followed by infiltration with paraffin. In embedding, most of the specimens were positioned for frontal and cross sections. Serial sections were prepared at 4 to 20 μm using a rotary microtome. The stains employed were Heidenhain's iron, Weigert's iron, Delafield's and Harris' hematoxylin counterstained with eosin, fast green, orange G, or Biebrich scarlet. Other preparations were treated with Mallory's or Masson's Trichrome stains, and a few with periodic acid-Schiff stain.

To soften the coxae of the fifth pereopod for sectioning after fixation, the podomere was transferred to several changes (24 hours each, the number depending on the degree of calcification of the cuticle) of 2.0% HCl, after which the procedure outlined above for dehydration and slide preparation was followed.

The specimens of the testes of *Enoplometopus occidentalis*, *Cambaroides similis*, and a few others were obtained from previously preserved animals and post-fixed in Bouin's fixative.

Preparation of Illustrations

Photographs were made using a Zeiss Universal Microscope equipped with Nomarski Optics and an NPS-5

Polaroid camera. Drawings were prepared with the aid of camera lucidas accompanying Wild compound and stereoscopic microscopes.

TERMINOLOGY

One of the decisions that had to be reached in presenting this comparative study was how best to designate the male gonads. Were comparisons not involved, no difficulty should arise in referring to that of the lobsters and parastacids as paired testes, and that of the Astacoidea (astacids and cambarids), since they are not paired, as a testis. In earlier drafts of the manuscript, however, awkward situations arose in attempting to use both the singular and plural designations. Although in this concept of the evolution of the testes, the nonpaired condition existing in members of the Astacoidea represents the derived condition, and thus I designate herein the gonad of male astacideans and their allies a *testis*. Among other advantages in doing so, this permits the use of "H-shaped" in describing the parastacoidean, enoplometopoidean, and nephropoidean testis and "Y-shaped" in designating that of the astacoideans (see Figs. 1, 27). The H-shaped testis is considered to consist of a pair of *longitudinal* lobes, each composed of an anterior and posterior lobule, joined by a *transverse commissure* or *bridge*. The Y-shaped, or trilobed, testis consists of a pair of *anterior lobules* and a median *posterior lobule*, which in the majority of the adult Cambaridae are joined by a trifurcate, constricted *stalk*, a feature lacking in the Cambaroidinae and Astacidae.

In selecting a general terminology for designating the elements of the variously modified male genital ducts, one must attempt to take into consideration the diverse conditions ranging from the apparent simplest arrangement found in members of the genus *Parastacus* to that of the highly specialized segments existing in the Nephropoidea. Johnson (1960) is followed in referring to each of the paired ducts coursing from the primary collecting duct/s of the testis to the phallic papilla on the coxa of the fifth pereopod as a *sperm* duct. The duct consists of a proximal *vas deferens* (composed of one or more segments), followed by a highly muscular *ejaculatory duct*, and most distally a cuticle-lined *terminus* located within the phallic papilla and coxa, which in the Astacoidea and in some Parastacoidea is modified into a complex trough-like apparatus here designated the *clusor*. The lumen of the terminus, or clusor, communicates with the exterior through the very short *genital atrium*, which in some crayfishes appears to be at least partly eversible, opening on the phallic papilla. In the Astacoidea, Enoplometopoidea,

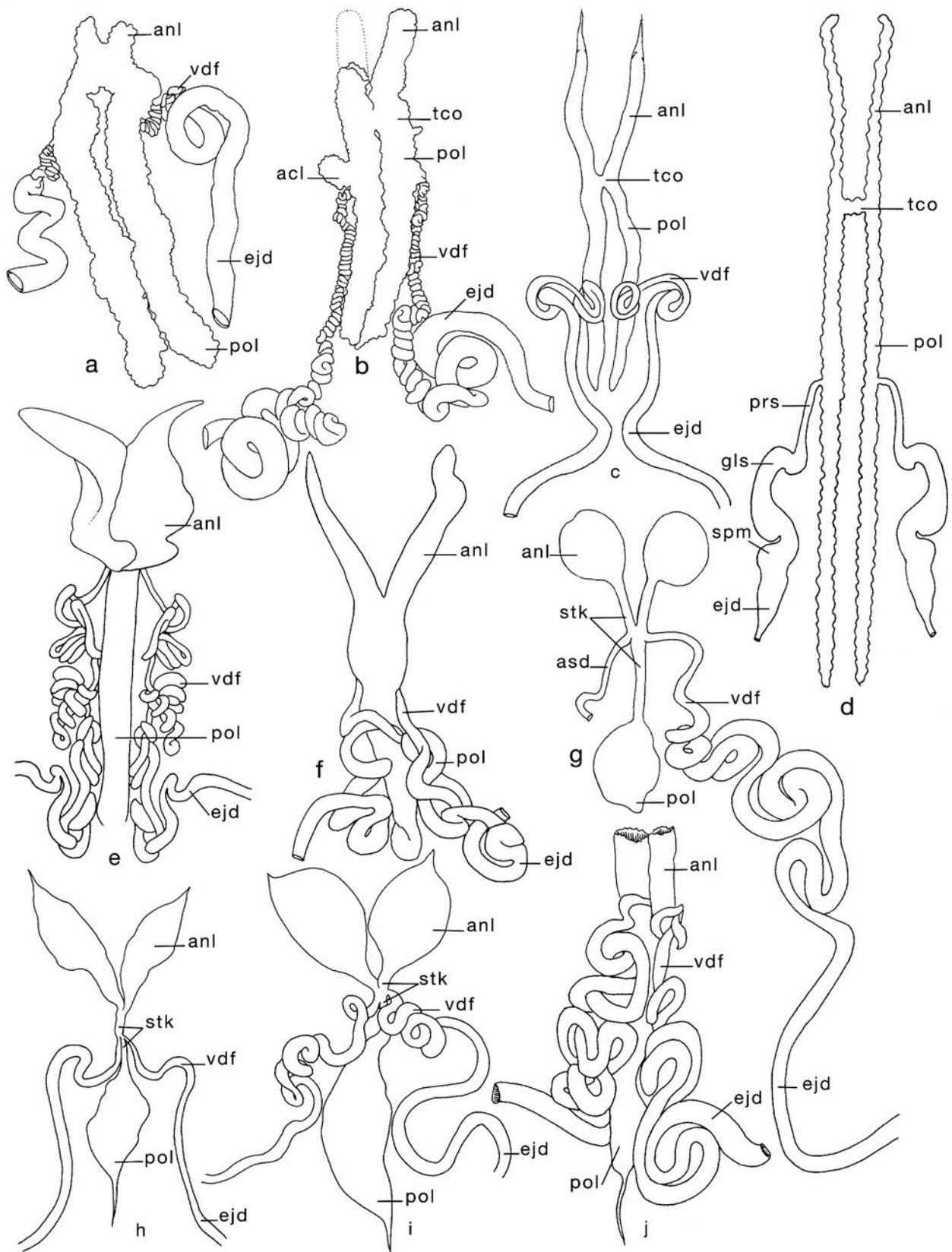


FIGURE 1. Semi-diagrammatic representations of gross structure of testis and sperm ducts, in dorsal aspect, of: a, *Parastacoides tasmanicus*; b, *Astacopsis franklinii*; c, *Enoplometopus occidentalis*; d, *Homarus americanus*; e, *Pacifastacus leniusculus trowbridgii*; f, *Cambaroides japonicus*; g, *Procambarus zonangulus*; h, *Orconectes rusticus*; i, *Cambarus bartonii cavatus* Hay, 1902; j, *Cambaroides similis*.

and Nephropoidea the papillae are membranous and are borne on the mesial or mesioventral surfaces of the coxae. In the Parastacoidea they are decidedly more variable in constitution and are situated on the ventral or mesioventral surfaces of the coxae. In the descriptions that follow an attempt has been made to equate these terms with previously employed designations.

In choosing *acinus* to designate the primary sperm-producing unit of the testis over “cyst,” “follicle,” “vesicle,” or “seminiferous tubule” (the latter quite inappropriate), I have used the term adopted by Huxley (1880). As for the two types of cells of the acinus most frequently referred to herein, *generative cells* and *accessory cells* (the latter proposed by Haley, 1984) seem preferable over, among others, “germinal epithelium” for the former and “Sertoli cells,” “sustentacular cells,” “nurse cells,” or “otheocytes” for the latter. Acini that are joined individually to a protubule, collecting tubule, or primary collecting duct are here designated *primary acini*, frequently referred to simply as *acini*. Those that join the collecting tubule or the primary collecting ducts through a broad common sinus (derived from preexisting acini) are referred to as *secondary acini*. Primary acini occur in the testes of all Astacidea; secondary acini seem to be limited to the Eno-plometopoidea, Nephropoidea, and Parastacoidea.

As for the designations applied to the ducts within and leading from the testis, the following have been adopted: coursing in or about the axes of the testicular lobules are the *primary collecting ducts*, which, corresponding to the disposition of the lobules, also exhibit a basically H- or Y-shaped pattern. (The transverse duct joining the primary collecting ducts of the H-shaped pattern may or may not be present.) Radiating from the primary collecting ducts are the unbranched or branching *collecting tubules*, which are affixed directly or via a *protubule* (short pedicel in which the lumen has not yet appeared) to the acini. When Johnson (1960:238) proposed the term *shaper* to designate the subterminal, trough-like, cuticular contraction in the sperm duct of *Cambarus longulus*, he was under the impression “that the shaper is functional in transforming the tube-like spermatophore, as found in the ejaculatory duct, into a thin crescent-shaped ribbon and thereby facilitating its movement through the groove of the first pleopod.” While perhaps this is one of its functions, in view of the presence of this apparatus in decapods in which the spermatophore is definitely not ribbonlike (e.g., some penaeid shrimps; see Johnson, 1964) and/or does not traverse such a groove in the first pleopod of the Nephropoidea and in at least some Parastacoidea, the primary function might be otherwise. It is suggested that

this highly modified structure near the terminus of the sperm duct was perhaps an early astacuran invention that serves primarily in the same capacity as would a sphincter in opening and closing the lumen of the sperm duct. In view of this more probable primary function and, inasmuch as the term *shaper* has not become entrenched in the literature [to my knowledge having appeared in only one published article, (Johnson, 1960)], the term *clusor* (Latin for “closer”) is suggested and employed as a more appropriate designation.

Located within the coxa and phallic papilla, and connected through a series of fine ducts to the lumen of the clusor and sometimes to the basal part of the genital atrium, are clusters of glands, resembling tegumental glands that are referred to as *terminus glands* because of their being limited to the terminus segment of the sperm duct. There is evidence that their presence and absence is associated in some manner with the seasonal production of spermatophores (Hobbs, unpublished data).

The term *spermatophore* is used herein not only to designate the sperm packets that are discharged from the phallic papilla and applied to some part of the body or placed within the seminal receptacle (*annulus ventralis*) of the female, but also to the enveloped sperm mass within the sperm duct.

PARASTACOIDEA

The H-shaped testis of *Parastacoides t. tasmanicus* (Figs. 1a, 3a) consists of a pair of subcylindrical, longitudinal lobes joined near their anterior ends by a short transverse bridge. The anterior lobules, flanking the pyloric region of the stomach, extend anterodorsally appressed against the anteriorly elevated parts of the hepatopancreas, and the longer posterior lobules lie horizontally on the posterior lobes of the latter and invade the anterior part of the abdomen. The superficial, delicate, squamous epithelium and underlying loosely adhering connective tissue layer bulge in irregular, rounded, knoblike prominences (Fig. 2a,b) reflecting the underlying compound structure of the organ. Extending posterolaterally from the longitudinal lobes are the paired vasa deferentia that exit the testis laterally a short distance posterior to the transverse bridge. The segment closest to the testis has a narrow diameter and courses posteriorly along the posterior testicular lobules in tight coils; in the next segment, which begins to turn laterally, the increased diameter of the duct is reflected in the size of the coils. With a gradual further increase in diameter, the coiled segment joins the ejaculatory duct, which

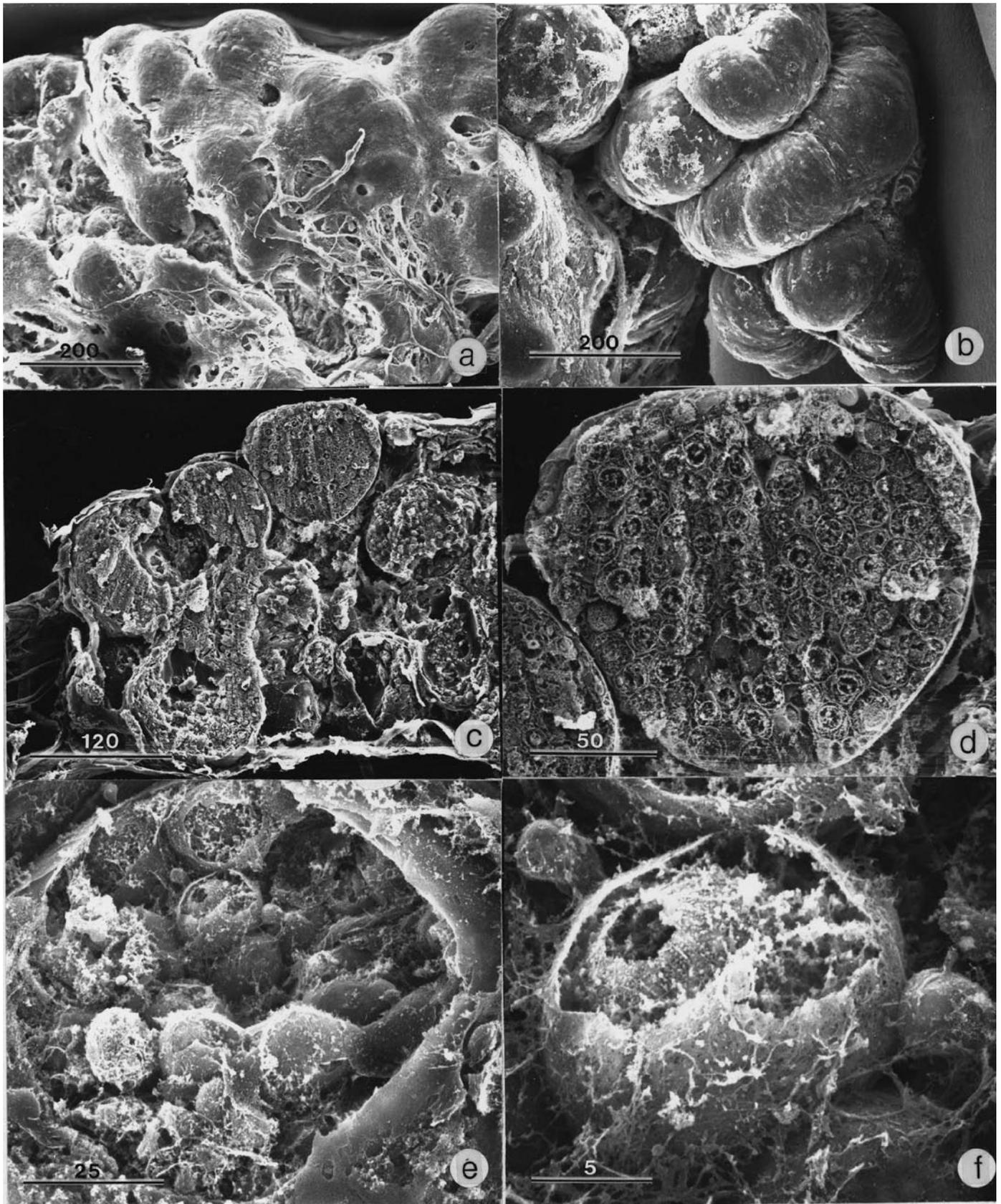


FIGURE 2. Scanning electron micrographs (μm) of the testis of *Parastacoides tasmanicus*: a, compound and simple acini ensheathed in connective tissue-epithelial coat; b, compound acini similarly covered; c, fractured compound and simple acini with spermatids and spermatozoa; d, part of same enlarged; e, fractured acinus with encapsulated (?) spermatozoa; f, enlargement of part of "e." Numbers on scale bars represent microns.

very loosely coiled proximally becomes almost straight in coursing ventrally and posterolaterally toward the phallic papilla on the ventromesial surface of the coxa of the fifth pereopod. At the base of and extending into the papilla, the duct joins the terminus that opens to the exterior through the short, apparently eversible, genital atrium.

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. Meandering about the axes of the two longitudinal lobules and joining in the transverse bridge (Fig. 3a) are the long primary collecting ducts. From along their entire lengths radiate the branching collecting tubules that lead to one or several individual or compound acini, which are saclike, sperm-producing units.

The primary collecting ducts are lined by a simple epithelium. Along much of their length the epithelium forms a very thin squamous layer (Fig. 4c), but in some areas it becomes cuboidal and, as it approaches the collecting tubules, sometimes even columnar. In many areas the free borders of the cells are frayed as though disintegrating in secretion. The oval nuclei of these epithelial cells (Fig. 5g) are comparatively small ($8.2\text{--}9.2 \times 3.8\text{--}6.4 \mu\text{m}$), each containing a number of darkly staining elements, one of which appears always to be larger than the others. In segments in which the duct is lined with cuboidal cells, often scattered or small clusters of cells with large subspherical nuclei (diameter approximately $10.5 \mu\text{m}$) and well-defined limiting membranes may be observed wedged between the bases of the epithelial cells (Fig. 5a). These cells resemble in every respect the spermatogonia that make up the dominant element of newly organized acini (see below). Although here they are designated *generative cells* because of their position lying where a functional acinus may or may not develop, insofar as can be determined, at least some of them differ from spermatogonia only temporally, indeed contributing their progeny to the spermatogonial cluster making up the cores of the young acini. That some, and potentially all, of these are precursors of spermatogonia seems certain. Superficial to the basal lamella are obliquely dispersed striated muscle fibers embedded in a connective tissue layer. As the primary collecting duct approaches its junction with the vas deferens, the epithelial lining changes swiftly from squamous through cuboidal to tall columnar cells. A striking feature of the primary collecting ducts that has not been observed in the collecting tubules, or anywhere within the testicular ducts in cambarids, are the very narrow ($4\text{--}7 \mu\text{m}$) to rather broad ($40\text{--}50 \mu\text{m}$), bare, two-layered acidophilic ligaments and membranes (Fig. 4a) that stretch across the lumen, but no-

where do they completely block it. The ends of some of these ligaments and membranes seem to be anchored in the superficial connective tissue layer of the ducts. Some, however, appear to have been secreted by, and to be attached to, the epithelial lining. Except for an occasional nodelike swelling, the composition, which is acidophilic, appears to be homogeneous and without associated nuclei. Spermatozoa are observed frequently to adhere to them in comparatively large numbers; there is no clear evidence as to their function, although one is tempted to suggest that in some manner they might aid in moving the spermatozoa toward the vasa deferentia.

The collecting tubules branching from the primary collecting ducts have precisely the composition of the latter. These tubules lead to branched tubules, the epithelial lining of which is uniformly cuboidal with the small nuclei situated basally, and with the cytoplasm toward the free border highly vacuolate.

ACINI. The swollen, blind, proximal ends of the collecting tubules are the acini (Fig. 4). They are devoted to the production of spermatozoa and part of the seminal fluid in which the sperm cells exit the testis. They may be simple (primary acini) or compound (secondary acini) sacs and have had their origin either in the wall of one of the primary collecting ducts, that of a collecting tubule, or that of an active or spent acinus. Their beginning as simple acini is signaled by a local aggregation of generative cells lying at the base of the epithelial lining of a duct or tubule (Fig. 5g). (Whether the aggregation results from cell division alone or also involves active or passive migration of generative cells is not known, but mitotic divisions have been noted in the very small clusters, cells which are now clearly spermatogonia [Fig. 5c].) As these spermatogonia, with oval to subspherical nuclei (9.2×12.4 or about $10 \mu\text{m}$ in diameter), continue to divide, each cluster increases in size, causing a distinct bulge in the outer contour of the tubule (Figs. 5b, 6b).

Concomitantly, the cuboidal epithelium abutting the lumen is apparently stretched so that it forms a thin squamous epithelial membrane lying between the dividing spermatogonia and the lumen of the tubule (Fig. 6c). Sometimes, and perhaps even frequently, the membrane becomes ruptured and the spermatogonia immediately underlying it disintegrate, contributing to the acidophilic content of the adjacent lumen. There is some evidence that the membrane can be repaired, but, if not, apparently all of the spermatogonia that were shielded by it may suffer a similar fate. Usually the membrane remains intact and a layer of accessory cells (with darkly staining nuclei about $7 \times 8.5 \mu\text{m}$ in diameter) develops on the superficial side of the cluster, resulting in the latter being completely enclosed

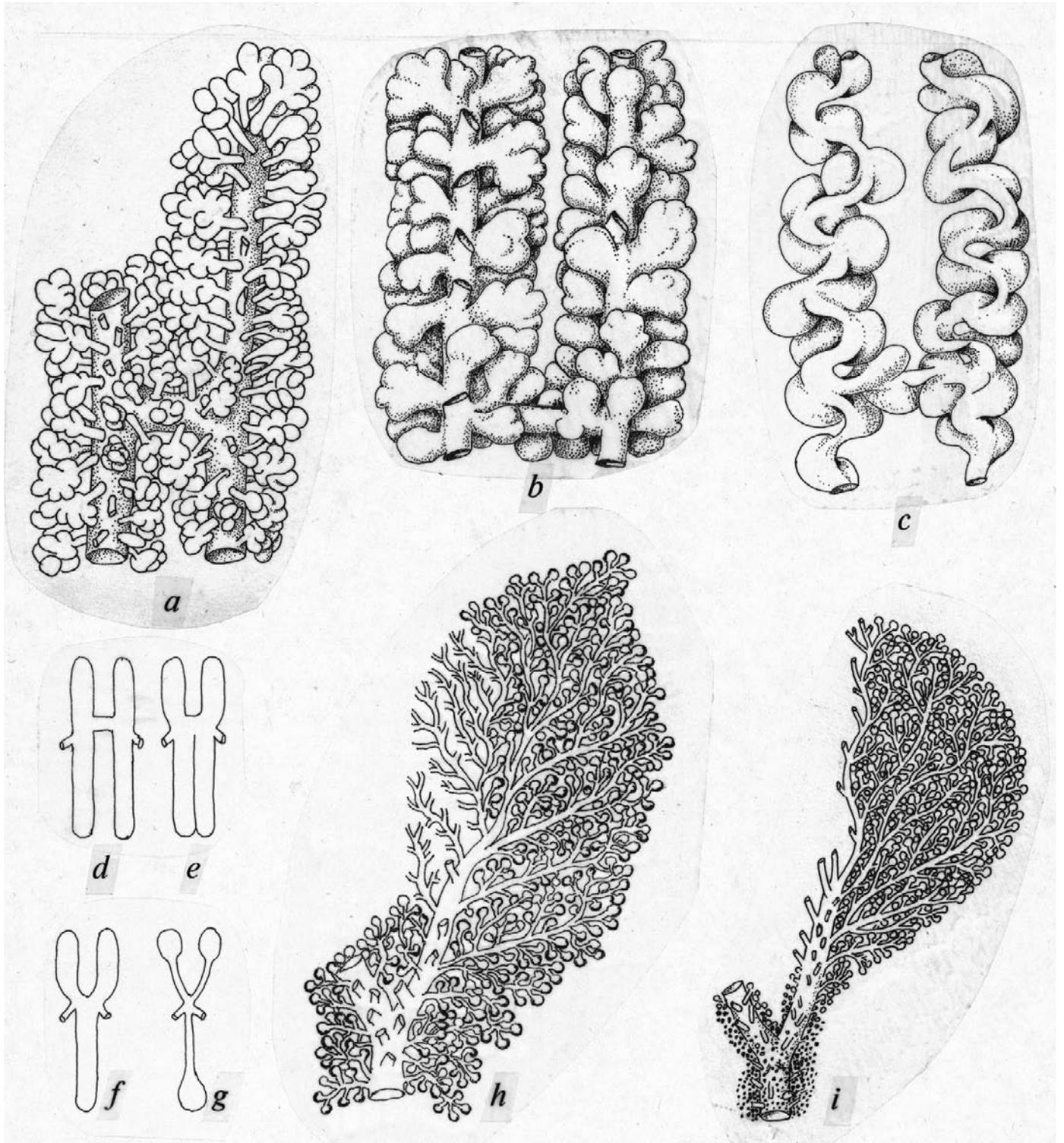


FIGURE 3. Diagrammatic representation of part of testis showing relationship of the acini to primary collecting ducts: a, H-shaped testis of Parastacoidea; b, H-shaped testis of Nephropoidea; c, H-shaped testis of Enoplometopoidea; d-g, diagrammatic representation of possible origin of Y- from H-shaped testis suggested by Suko (1955); h, Y-shaped testis of Astacidae and Cambaroidinae; i, Y-shaped testis of Cambaridae except Cambaroidinae.

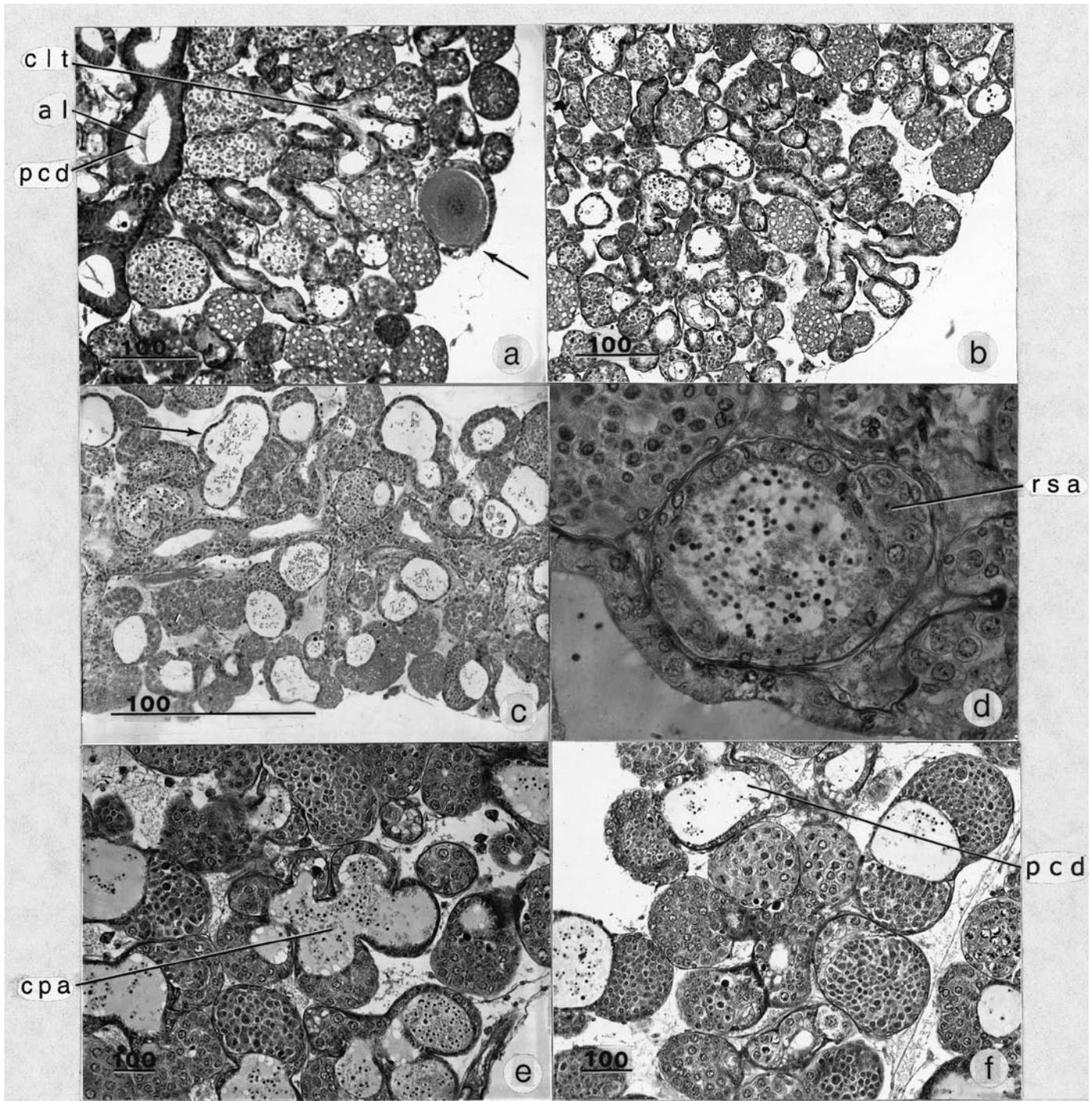


FIGURE 4. Testis of *Astacopsis franklinii* (a, b) and *Parastacoides tasmanicus* (c-f) (μm): a, simple acini, collecting tubules, primary collecting duct, and oocyte (arrow); b, simple acini and collecting tubules; c, section of testis through primary collecting duct and associated acini; d, simple acinus, having produced a generation of spermatozoa, regenerating in preparation for production of another; e, f, simple and compound acini in various stages of spermatogenesis, others in degeneration and regeneration.

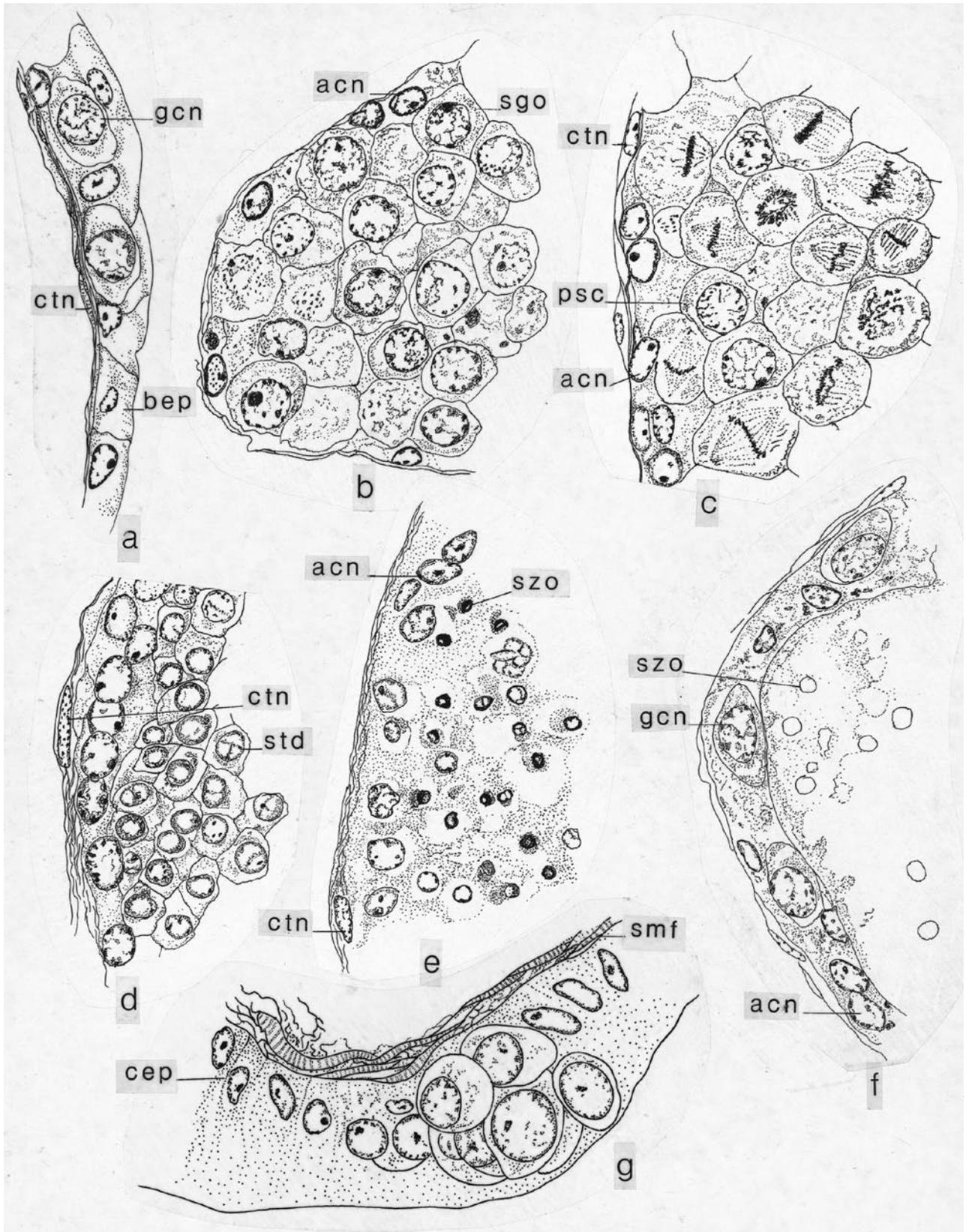


FIGURE 5. Elements of testis of *Parastacoides tasmanicus*: a, wall of primary collecting duct; b, part of acinus with spermatogonia; c, part of acinus with primary spermatocytes; d, part of acinus with spermatids; e, part of acinus with spermatids and spermatozoa; f, part of acinus with spermatozoa; g, wall of acinus containing spermatozoa (not shown) in which rudiment of secondary acinus present.

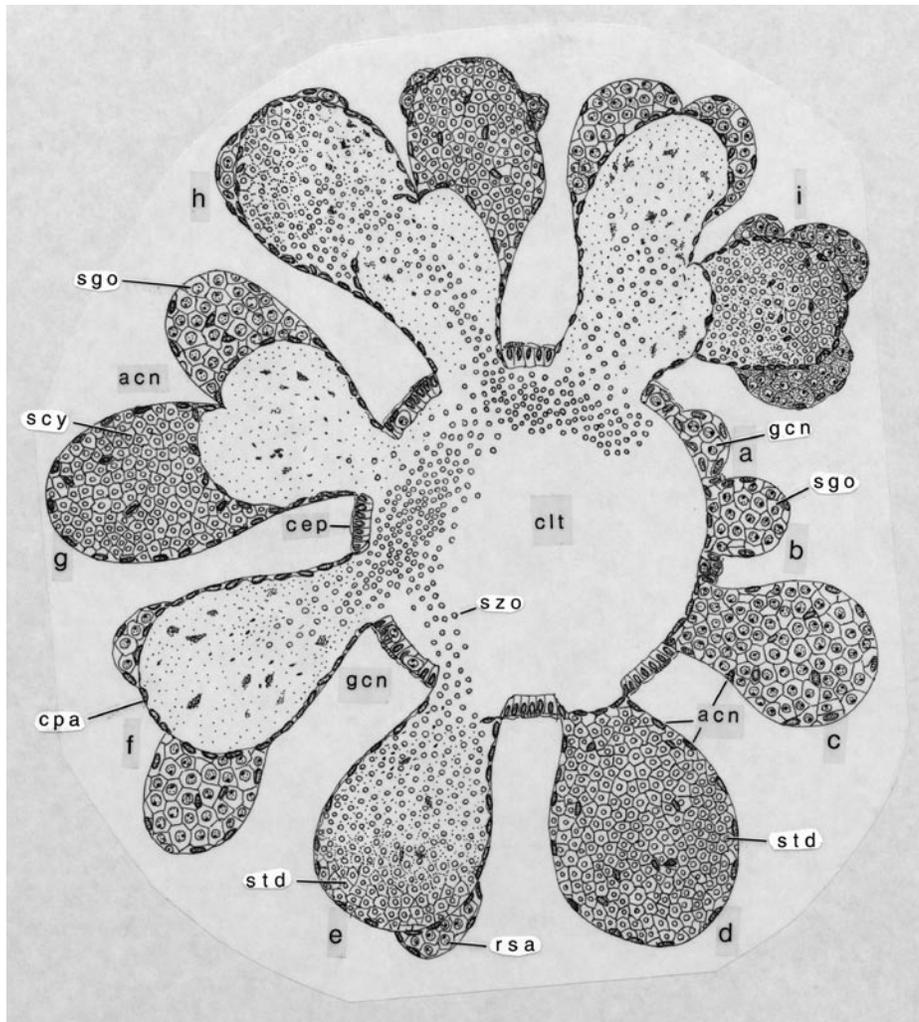


FIGURE 6. Schematic diagram of life cycle of acinus in Parastacoidea, Enoplometopoidea, and Nephropoidea: a, accumulation of generative cells on wall of collecting tubule or primary collecting duct; b, young acinus; c, with spermatogonia; d, with spermatids; e, with spermatids, spermatozoa (being released into collecting tubule), and developing secondary acinus; f, breakdown of primary acinus and discharge of spermatozoa, rudimentary and clearly defined secondary acini developing in wall of spent primary acinus; g, secondary acini, larger one with spermatocytes and smaller with spermatogonia; h, breakdown of protective membrane in secondary acinus containing spermatozoa, another with spermatids, and both with rudimentary acini; i, complete breakdown of one secondary acinus discharging contents into collecting tubule, other with spermatids transforming to spermatozoa, both secondary acini with young acini developing on their walls. (Primary acini may regenerate, producing additional generations of spermatogonia instead of giving rise to secondary acini; this is more typical of *Enoplometopus*).

in a thin-walled coat. Rarely are accessory cells seen in such numbers occurring other than superficial to the germinal elements of the acinus, although cells with similar nuclei are scattered among the developing spermatocytes, spermatids, and newly transformed spermatozoa. The cell boundaries of this accessory layer are not clearly defined in these preparations, and the nuclei associated with it could well be those of a syncytium resulting from the coalescence of accessory cells.

Although mitotic figures appear among the neighboring multiplying spermatogonia, there is no suggestion of nuclear reorganization in the increasing number of nuclei of the accessory elements (cells or syncytia?). In contrast to the lack of synchrony in the mitotic divisions of the spermatogonia, when the first meiotic division occurs, many of the cells within an acinus exhibit spindles that are at the same or approximate stage (Fig. 5c). (As will be seen, unlike observations on the cambarid testis, there is

little evidence in the parastacid material examined that coincident activity exists in many nearby acini. Seldom were even two adjacent acini observed exhibiting similar metaphase, anaphase, or telophase stages.) The second meiotic division of the spermatocytes and their subsequent transformation to spermatids and spermatozoa within a single acinus proceed synchronously (Figs. 5d–f; 6c–e).

With the completion of spermiogenesis, the spermatozoa are suspended in a massive syncytium that apparently rapidly degenerates, the content of the acinus coming to appear no different from that present in the collecting tubule leading from the acinus. Nuclear fragments together with both basophilic and acidophilic particles and often nuclei that are characteristic of accessory cells are scattered among the spermatozoa in an acidophilic matrix (Fig. 5e). With the rupture of the squamous epithelial layer that had separated the content of the acinus from that of the lumen of the collecting tubule, the material in the acinus is added to that already present in the tubule. As for the wall of the acinus, nothing appears to remain of the (syncytial?) coat that surrounded those cells that were undergoing spermatogenesis. In some areas, however, what appears to be a new squamous layer comes to lie next to the lumen, separated from the connective tissue coat by a basal lamella. In some such areas generative cells appear and cluster long before the remaining content of the acinus had been shed (Fig. 6e–g). Even before the spermatids are converted to spermatozoa, formations of new secondary acini are frequently initiated. Muscle fibers, which appear at the efferent end of the acinus at about the same time that the spermatocytes become spermatids, extend proximally over one-third to one-half of the acinus. Nuclear elements have not been associated with these striated muscle fibers. Perhaps some of those that have been interpreted as nuclei of the connective tissue are actually components of the muscular elements. It should be understood also that in many, if not most acini, degenerating cells may be observed during all of the stages of sperm production.

The secondary acini, which develop in the walls of preexisting acini, may be recognized readily as units of compound, individually stalkless acini (Figs. 4f, 6h), rather than being simple sac-like lobules. Secondary acini continue to “bud” from preexisting ones so that as many as eight or ten may open through an expansive sinus into a collecting duct (Figs. 4f, 6i). (Sperm production in these acini does not differ from that in primary acini.) As in the testes of members of the Enoplometopodea and Nephropodea, but differing from those of the Astacoidea, acini exhibiting different stages of spermatogenesis and spermiogenesis

are juxtaposed along the length of the testicular lobules, and acini in early formative stages are intimately associated with those that are spent (Fig. 4e,f).

MICROANATOMY OF THE SPERM DUCT

Three sections are recognized in the paired sperm ducts of *Parastacoides t. tasmanicus* (Fig. 7a–f): vas deferens, ejaculatory duct, and terminus.

VAS DEFERENS: TESTICULAR SEGMENT. The short, entalmost portion of each vas deferens is embedded within the anterior part of the posterior testicular lobule. It consists of a columnar epithelial lining resting on a basement membrane, which, in turn, is enveloped by a connective tissue coat in which predominantly circular to obliquely oriented muscle fibers are interwoven. Except for the muscular element being more prominent, this part of the vas deferens does not differ strikingly from segments of the primary collecting duct that are lined with a glandular, columnar epithelium.

The content of the lumen of this proximal-most segment of the vas deferens consists of secretions from the epithelial linings of the collecting tubules and primary collecting ducts as well as breakdown products of the nuclei and cytoplasm of the accessory cells (syncytia) and cytoplasmic products of the spermatids which were not incorporated in the final sculpturing of the spermatozoa; within this fluid matrix are suspended the spermatozoa.

COILED SEGMENTS. These segments correspond to the “proximal and middle vas deferens” of Talbot and Beach (1989). The sperm duct, enveloped in a squamous epithelium, emerges from the testis in a series of loops and coils (Fig. 1a), but the histological features do not differ in any conspicuous aspect from those of the testicular one. The diameter of the lumen in the proximal part is little greater than that of the latter, but it increases gradually more distally. The muscle fibers are still rather sparse and seem to be dispersed in a single oblique layer. Within the coiled mass, however, extrinsic muscle fibers are present in the connective tissue, binding one section of the coil to another. In many areas, the free borders of the basically basophilic epithelial lining are disrupted in secretion, and, in some of the coils, as much as half of the circumference of the lining has completely disintegrated, exposing the basement membrane to the content of the lumen (Fig. 7a–d).

It is in this segment of the vas deferens that the content of the lumen becomes surrounded by a thin homogeneous, highly refractile, acidophilic layer corresponding to that identified by Beach and Talbot (1987, figs. 17, 18)

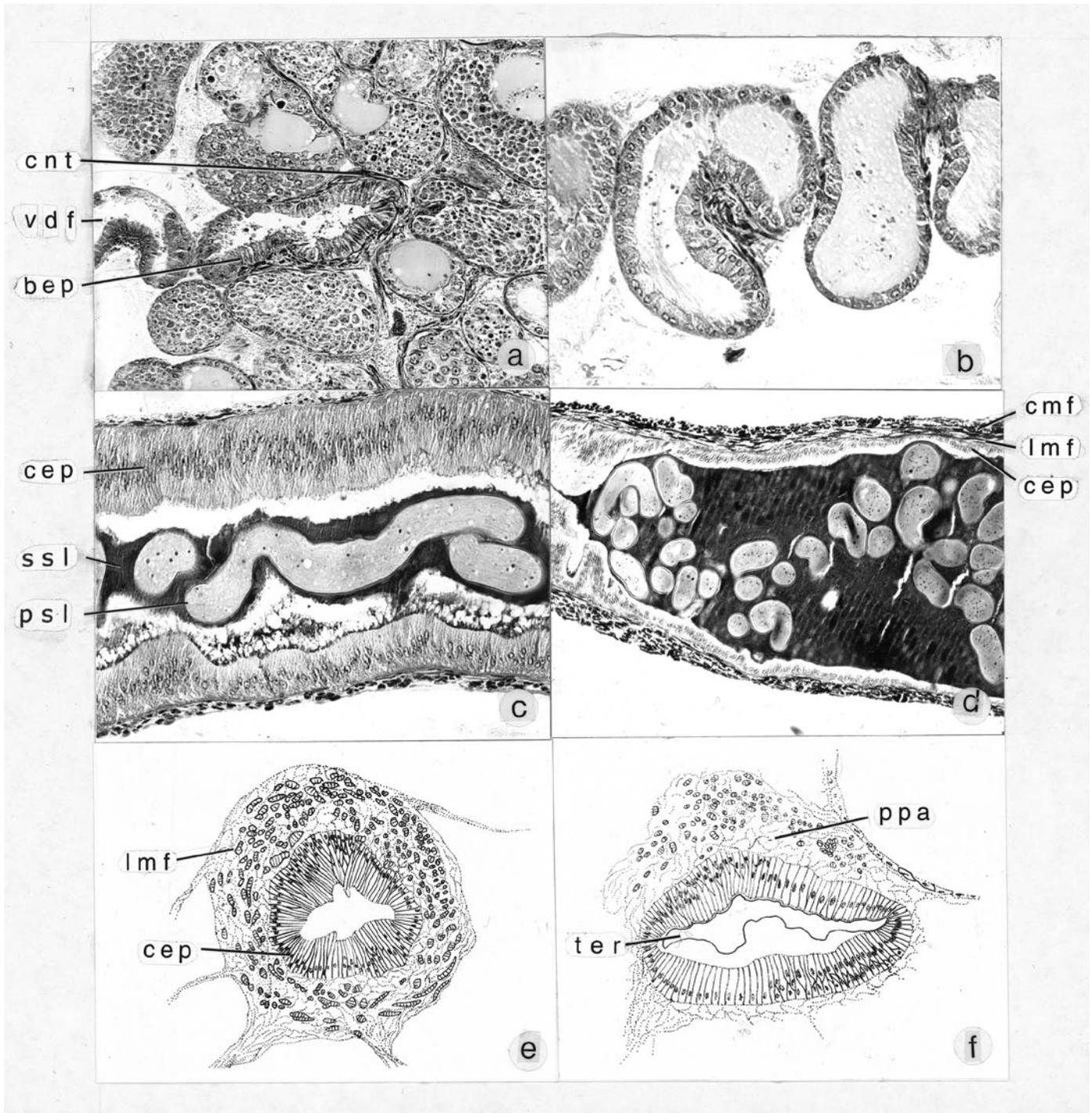


FIGURE 7. Sperm duct of *Parastacoides tasmanicus*: a, part of testicular segment of vas deferens; b, cross section of ental part of coiled segment of vas deferens; c, longitudinal section of ectal part of coiled segment of vas deferens; d, longitudinal section of ejaculatory duct with spermato-
phore; e, cross section of ectal part of ejaculatory duct; f, cross section of terminus in phallic papilla, clusor lacking.

as the *primary spermatophore layer* in the spermatophores of *Cherax albidus* Clark, 1936, and *C. tenuimanus* (Smith, 1912). At this level, the developing spermatophore consists of a refractile tube filled with a weakly acidophilic matrix in which are suspended widely spaced spermatozoa and finely divided acidophilic and basophilic particles. As the spermatophore is moved ectally along the coiled segment, an additional, strongly acidophilic secretion is added to the content of the lumen by the lining epithelium, and with the increase in the diameter of the cavity (and concomitant loosening of the coils), the tube is displaced from its central position in the duct and thrown into irregular loops, so that in sectioned preparations it appears as many small, irregular (in shape and size), sausage-like bodies embedded in the granular *secondary spermatophore layer* (Fig. 7b–d). Following the loosely coiled segment, the vas deferens straightens but histologically does not appear to differ from the distal part of the coiled segment.

EJACULATORY DUCT. This section of the sperm duct (Fig. 7d), which corresponds to the “distal vas deferens” of Talbot and Beach (1989), is readily recognizable by the very thick coat of striated muscle fibers, which is embedded in connective tissue. The muscle fibers are, for the most part, arranged in superficial circular and deeper longitudinal layers, although in some areas they appear to be disposed obliquely and interwoven in a basket-weave pattern. The lining of the duct is composed of a basophilic, cuboidal to tall columnar, glandular epithelium. Within the coxa, more of the muscle fibers become arranged obliquely, and approaching the junction with the cuticular terminus, the circular and longitudinal layers are hardly recognizable. Nearing the phallic papilla, strands of connective tissue extend from the duct to the wall of the coxa, passing through the haemocoelic spaces between the skeletal muscles. A short distance ental to the junction, the muscle layer on one side of the duct, consisting principally of longitudinal fibers, becomes conspicuously much thicker than elsewhere around the circumference and, continuing toward the junction, except for the thickened mass, the muscle coat becomes progressively more reduced. The epithelial layer of the ejaculatory duct within the coxa is columnar and does not appear to differ from the more ental area (Fig. 7e). In the materials examined, there is no spermatophore within the lumen, and in some areas the epithelial layer appears to be strongly irregular in height (probably resulting from the contraction of the lumen); for a short distance there exists a somewhat conspicuous fold accompanied by an intrusion of the underlying connective tissue, in cross section presenting a “typhlosole-like” appearance, but this persists for only a short distance and,

at either end, the apparent folds are irregular and are not accompanied by connective tissue invasions.

In the specimens examined, the developing spermatophore fills the lumen of the ejaculatory duct above the level of the coxa where it receives additional elements of the secondary spermatophore layer, a strongly acidophilic mass in which are suspended subspherical basophilic granules. Ectally, within the coxa, the lumen of the ejaculatory contains a very delicate, almost hyalin membrane obviously secreted by the underlying epithelium. That this secreted material might, with the passage of the spermatophore through this segment of the duct, constitute a lubricant or perhaps the most superficial part of the secondary coat seems possible. As the duct reaches the phallic papilla, it joins the cuticle-lined terminus.

TERMINUS. Fixation of the material on which this description is based is rather poor, shrinkage causing the tissue to pull free of the cuticular elements (the coxal wall and the lining of the terminus). Also, the spacious segment of the haemocoel within the phallic papilla is much compressed. Even so, major features are readily discernible. At the base of the phallic papilla the ejaculatory duct joins the terminus, which is lined by a uniformly thin, unadorned, acidophilic cuticle underlain by a cuboidal to subcolumnar, basophilic epithelium. The latter is surrounded by a loose connective tissue layer devoid of muscle fibers, except proximally along one side of the duct. This band of fibers is an extension of the asymmetrical muscular thickening mentioned above in the description of the ectal part of the ejaculatory duct. Extensions of the connective tissue that binds these fibers together are inserted on the wall of the papilla along the proximal part of its overhanging side.

On the basis of shape of the terminus in these preparations, it is likely that when not traversed by a spermatophore it is elongate oval in cross section with the long sides of the tubular cuticular lining closely applied to one another. With the contraction of the muscle extending from the ectal part of the ejaculatory duct and along the corresponding long wall of the terminus, the shape of the lumen of the latter would be converted from a slit to a more broadly oval space. Moreover, if the more apical part of the terminus, which in *P. tasmanicus* is inseparable from the terminal genital atrium, is eversible, this muscle would serve to retrieve the everted part of the terminus.

The terminus in this crayfish lacks the clusor that is typical of those of the members of the Nephropoidea, Astacidae, Cambaridae, and at least two of the South American parastacids: *Parastacus nicoleti* and *Samastacus spinifrons* (Philippi 1882).

SPERMATOPHORE AND SPERMATOZOA. The definitive structure of the spermatophore consists of a convoluted cylinder with a retractile wall (primary spermatophore layer deposited in the coiled segment) containing widely spaced spermatozoa suspended in a matrix derived from the testis and the testicular and proximal part of the coiled segments of the vas deferens. This cylinder is embedded in a thick secondary spermatophore layer that is added in the loosely coiled segment of the vas deferens and ejaculatory duct. The spermatophore appears not to differ from that described and illustrated for members of the genus *Cherax* by Beach and Talbot (1987, figs. 17, 18).

The subcircular, weakly biconvex spermatozoa are 3–3.5 μm in diameter, lack rays that are characteristic of those of other Astacidea, and are not individually encapsulated as are those of astacids and cambarids. They seem to resemble the spermatozoa of the two species of *Cherax* studied by Beach and Talbot (1987) and are much more like those of *Panulirus* reported by Matthews (1951) than those of astacideans.

Notes on the Testis and Vas Deferens of *Astacopsis franklinii*

With few exceptions, the gross (Fig. 1b) and microstructure and functioning of the testis of *Astacopsis franklinii* closely resemble those of *P. tasmanicus*. As pointed out by Hamr (1990:31), “the posterior lobes of the testes are also less distinct from each other in *Astacopsis* and do not extend past the first coils of the middle section of the vas deferens.” In adults of the “Eastern form” of the species (= *A. franklinii* (Gray 1845)) s.s. (see Hamr, 1992), he found “a small additional lobe (fig. 1b, acl) of the testis which emerges from the left posterior lobe just above the origin of the vas deferens” (op. cit., p. 32). Such a lobe was not observed in the “Western form” (= *A. tricornis* Clark, 1936) or in *Astacopsis gouldi* (Clark, 1936). As for the vasa deferentia, the coiled segment is comparatively much longer in *A. franklinii*, s.s., than it is in *P. tasmanicus*. Turning to the microscopical anatomy of the testis, the development of secondary acini in material examined is less conspicuous, however, for most of the newly appearing acini seem to have arisen as buds from the collecting tubules (Fig. 4b). Too, within those acini in which metaphase plates are present, there are cells with tripolar spindles (like Fig. 14d), a feature not observed in *P. tasmanicus* but occurring often in many Astacura and found in this study to occur frequently in *Homarus americanus*. Whereas in none of the preparations of the testes of *P. tasmanicus* were oocytes observed, several with diameters

ranging from 210 to 595 μm were found in the testis of *A. franklinii* (Fig. 4a, arrow).

Insofar as can be determined, the form of the spermatophore of the Parastacidae (observed in representatives of three genera of Australian crayfishes this should stand, *Astacopsis*, *Cherax*, and *Parastacoides*) is unique among the Astacura but is markedly similar to that of at least some members (Scyllaridea) of the Palinura. The similarity of the spermatophores of the two species of *Cherax* investigated by Beach and Talbot (1987) and Talbot and Beach (1989) to those of *Panulirus* was pointed out by them in the latter publication.

Notes on the Male Reproductive System of Other Parastacidae

The earliest recorded observations on the reproductive system of the Parastacidae was that of von Martens (1870), who discussed the abnormal presence of gonopores on the third and fifth pereopods in a specimen of *Cherax preissii* Erichson, 1846. In it, he found vasa deferentia but neither ovary nor oviduct could be located. He also pointed out that the males of *Parastacus pilimanus* (von Martens, 1869) and *P. brasiliensis* (von Martens, 1869) had gonopores on the third and fifth pereopods.

Von Ihéring (1893) noted that in *Parastacus*, genital openings occur on the coxae of the third and fifth pereopods. He briefly described the “gland genitale” of an unidentified member of the genus as a large gland located in the posterior part of the cephalothorax. It consisted of a number of lobes, flattened on their faces of contact, and having diameters of about 2 mm. It was described as being situated under the heart but above the intestine, unpaired, and possessing a rather short posterior lobe. Posteriorly the paired, short gonoducts led to the bases of the coxae of the fifth pereopods where they terminated in the genital aperture situated on a small cone. Uncertainty existed as to whether or not a very delicate, more anteriorly situated conduit led to the coxae of the third pereopod.

Faxon (1898) reported gonopores on the coxae of the third and fifth pereopods in *Parastacus saffordi* Faxon, 1898; *P. defossus* Faxon, 1898; and *P. hassleri* Faxon, 1898 (= *P. pugnax* (Poepfig, 1835)), and the absence of that on the third in males of *P. agassizii* Faxon, 1898 (= *Samastacus spinifrons* (Philippi, 1882)). He made no comments concerning the testis or vas deferens.

Lonnberg (1898), studying specimens of *Parastacus hassleri*, found that while gonoducts led to the coxae of both the third and fifth pereopods, actual apertures were lacking on the third in males and on the fifth in females. His

description of the gonads included an important account of their anatomy in which he stated that they were “composed of two portions of nearly the same size and placed in a straight angle to each other, namely one anterior [sic] vertical, and one posterior horizontal, which latter is longer in the most developed glands. The former is laterally, the latter ventrally imbedded in the liver” (p. 346). He recognized a possible connection between the paired ovarian elements, but no mention was made of a transverse bridge in the testis. Of particular interest was his confirming the observations of von Ihéring (1893) that the vasa deferentia as compared to those of the “common crayfish” are “very much shortened . . . and do not make any convolutions.” Most of Lonnberg’s observations were corroborated by Runnstrom (1925), who, primarily interested in hermaphroditism in decapod crustaceans, described and illustrated the male and female reproductive systems in *Parastacus hassleri*. He found a transverse bridge joining the paired lobes (testes), and in a series of sections of the testis he found unmistakable oocytes at the bend of the organ and near the oviducts. His description of the vasa deferentia leaving the testis as being slender and straight and becoming thicker and weakly curved more ventrally suggests the possibility that these ducts in the American parastacids are strikingly different from those of the Australian species that have been investigated in being shorter, less coiled, and, in *P. pugnax* at least, exiting the testis much posterior to the transverse bridge.

In a study of the genital pores of 118 specimens in a population of *Parastacus nicoleti*, Rudolph (1990) found 3.4% with male and female gonopores, 44.9% with male and traces of female apertures, 7.6% with only female gonopores, 34.7% with only traces of female gonopores, and 9.3% with a single male gonopore and paired traces of female gonopores (these percentages are from his table 2). He suggested the possibility of hermaphroditism occurring in this crayfish with inversion being protandric.

The only other studies of the testis of parastacids of which I am aware are those of Turvey (1980) and Hamr (1990). The former described the H-shaped contour of the testis of *Euastacus spinifer* Heller (1865) and noted that in some males a second transverse bridge was present near midlength of the testis. The sperm duct is apparently similar to that noted in *Cherax* by Talbot and Beach (1989) and *Parastacoides* and *Astacopsis* observed in this study. Those parts of Hamr’s (1990) study devoted to the male reproductive systems of *Parastacoides tasmanicus*, *Astacopsis franklinii*, and *Astacopsis gouldi* were largely concerned with the reproductive cycle in representatives of the last two mentioned genera, emphasizing gross changes

in the testis and vas deferens of *Astacopsis franklinii* and *Astacopsis gouldi* throughout the year. Of special interest was his statement that “Spermatozoa contained within the tubes [vasa deferentia] are round, conspicuously nucleated and lacking the rays characteristic of the sperm of other Astacidea” (Hamr, 1990:32).

The Testis and Sperm Ducts of Parastacus nicoleti

Gross features of the testis resemble those of the Australian parastacids, and the basic internal features are much like those described above for *Parastacoides tasmanicus* and *Astacopsis franklinii*. In the preponderance of primary acini and the occurrence of oocytes within one or more of the testicular lobes—in one of the specimens examined, one of the anterior lobules of the testis is packed with oocytes—it shares more in common with *A. franklinii* (Figs. 4a, 8a, arrow). The area of the testis in the vicinity of the exodus of the sperm duct differs from both in the increased height of the columnar epithelial lining and the heavier muscular coat of the primary collecting ducts (Fig. 8b).

The most noteworthy difference noted between the male gonad and gonoducts of the Australian and South American parastacids is the apparent lack of the coiled segments of the vasa deferentia (as defined herein) in the South American species. The sperm duct in *P. nicoleti* extends directly posterolaterally and ventrally from the testis, lacking coils or loops, and the muscular coat is much more strongly developed than in the Australian species studied. Even the testicular segment of the duct is strongly muscular. One is tempted to suggest that in *P. nicoleti* almost all the vas deferens segment of the sperm duct is wanting, perhaps in its evolution having progressed one step beyond the condition existing in the Enoplometopoidea (see below). Not only were the coiled sectors of the vasa deferentia withdrawn into the testis, but they had been reduced to such an extent that they were no longer recognizable as such (Fig. 8c).

ENOPLOMETOPOIDEA

The studies of Matthews (1954) and Haley (1984, 1986) on the male reproductive system of *Enoplometopus occidentalis* provide the most complete accounts of the entire system existing for any member of the Astacura. Presented here is a resumé of their work, bolstered by information gleaned from poorly preserved material of a single specimen available, and a comparison of the system with that of the crayfishes and lobsters examined.

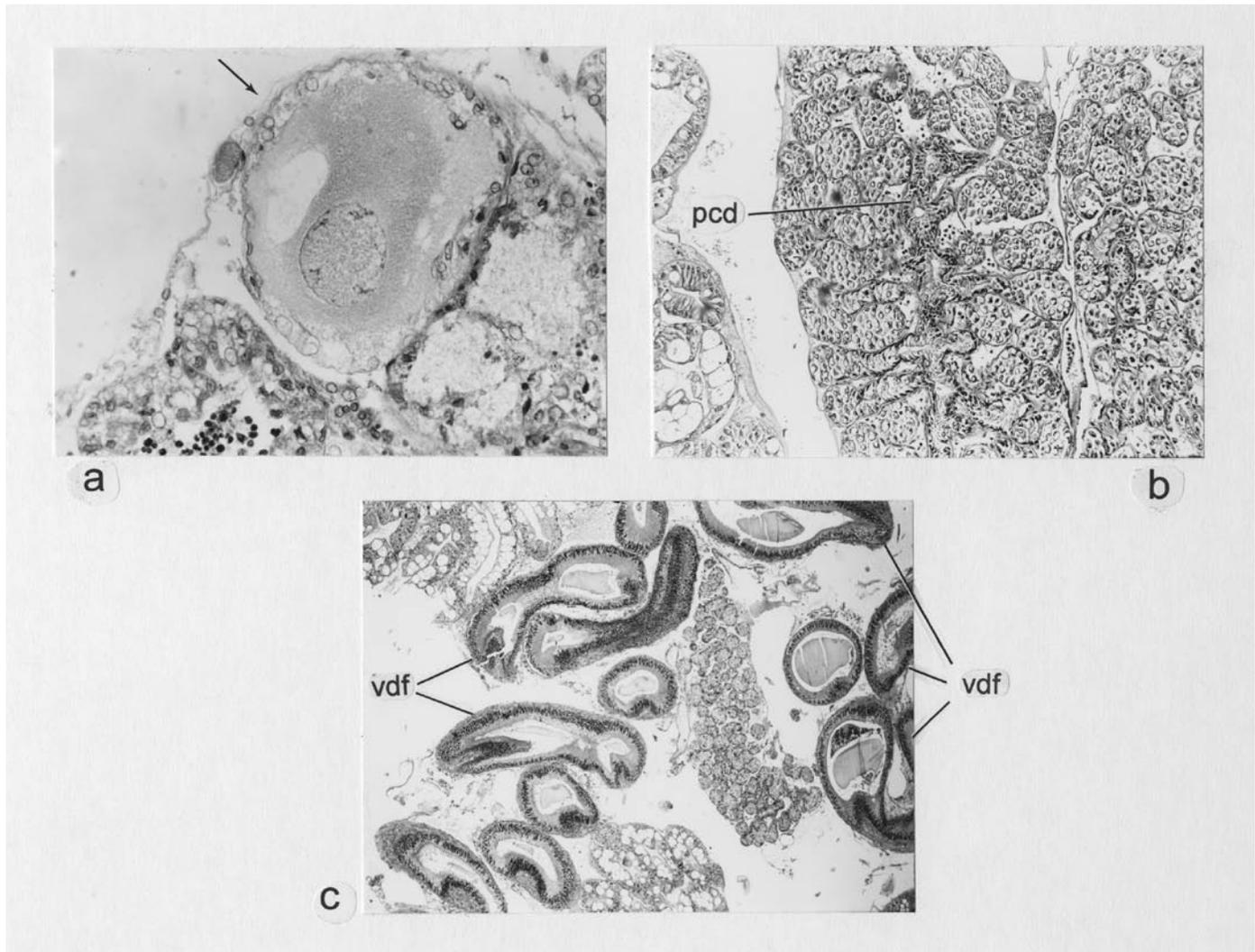


FIGURE 8. Testis and sperm duct of *Parastacus nicoleti*: a, cross section through anterior lobule containing oocyte; b, primary collecting duct; c, portion of testicular segment of vas deferens.

The basic macroanatomy of the testis (Fig. 1c) differs in no conspicuous way from that of the Nephropoidea, being H-shaped with the slender lateral lobes joined by a transverse bridge far anterior to the exodus of the sperm duct. The latter, however, is strikingly different from that of *Homarus americanus*, longer and lacking the sharply demarcated regions. It is more like that of some of the cambarids, the vas deferens exhibiting a few loose coils proximally before straightening and merging with the almost straight ejaculatory duct ("descending vas deferens" of Haley), which is comparatively larger in diameter than that of the cambarids.

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. Each lobule of the testis consists of a highly convoluted collecting tubule bearing lobulate acini, which are so broadly affixed to the tubule that, as Haley pointed out, they form much of the wall of the tubule. Scarcely anywhere along most of the length of the latter are intervals of more than a few microns uninterrupted by the broad mouth of an acinus.

The wall of the collecting tubule consists of a cuboidal epithelium with small, spherical, darkly staining nuclei. In material examined in this study, this layer rests on a base-

ment membrane adjacent to which is a narrow layer of what appears to be a fibrous connective tissue with compressed nuclei that are also strongly chromatophilic. [The most superficial layer was perhaps correctly interpreted by Haley (1984) as being a squamous epithelium, but in the material I was unable to distinguish it.] Unlike the collecting tubules of the lobsters and the crayfishes, there are few, if any, muscle fibers in the connective tissue layer. Thus, what force is at work in propelling the products of the acini along the tubules to the vas deferens is not at all clear. Haley (1984) was of the opinion that the scattered cells he tentatively identified as muscular elements in the wall of the collecting tubule were unlikely to provide the main locomotive force, considering any contribution by them to be secondary to “secretion pressure” in the acini. According to him, the more distal part of the collecting tubule, which is situated in the midsection of the testis, is devoid of acini and is histologically like that just described (Haley, 1984, fig. 11). In the current preparation there is a tubule or duct (Fig. 9a), the wall of which does not differ from that of an acinus in which the germinal elements are in the spermatogonial stage (see below); this duct resembles the “collecting tubule” illustrated by Matthews (1954, fig. 4).

ACINI. The acini, which occur as bulges in the walls of the collecting tubules are, as noted above, in

broad communication with the latter (Fig. 3c). As in other astacidians, the walls of those acini that abut the limiting membrane of the testis consist of a superficial layer of connective tissue surrounding a basement membrane, internal to which are generative cells and/or their progeny (spermatogonia, spermatocytes, spermatids, and spermatozoa), along with accessory cells, the antecedents of which are also likely generative cells. As in the testis of the Nephropoidea, the interior elements of each acinus undergo a cyclic change, so that initially the generative cells give rise to a mass of spermatogonia surrounded and intruded by accessory cells. The usual spermatogenic stages follow, giving rise to spermatids that, in transforming to spermatozoa, lose their cell boundaries and become suspended in a syncytium, bounded by a layer of accessory cells. Insofar as I am able to determine, the cell membranes of those accessory cells that were dispersed among the developing spermatogonia disappear, leaving their prominent, darkly staining nuclei in the syncytial mass that encompasses the spermatozoa. With the rupture of the accessory cell membrane facing the lumen of the collecting tubule, the syncytial content of the acinus, including the spermatozoa and disintegrating accessory cell nuclei, is discharged into the tubule. In the meantime, generative cells lying adjacent to the basement membrane have begun a new cycle of sperm production. Compound (secondary) acini that are



FIGURE 9. Sperm ducts of *Enoplometopus occidentalis* (a) and *Pacifastacus leniusculus trowbridgii* (b–c): a, sperm clusters in poorly fixed primary collecting duct; b, longitudinal section of coiled section of sperm duct lacking primary spermatophore sheath; c, longitudinal section of more distal part of coiled section of sperm duct with primary spermatophore sheath.

so common in the testis of *Homarus* appear infrequently in that of *Enoplometopus*, but one is clearly depicted in Haley's figure 5 (1984). Nevertheless, the cyclic production of spermatozoa appears to be markedly similar in the two. Haley found that clusters of acini tend to be at similar stages in the spermatogenic cycle and was of the opinion that spermatogenic activity occurs in waves along the testis.

MICROANATOMY OF THE SPERM DUCT

Following this interpretation of the primitive gross morphology of the sperm duct, it is suggested that the distal acinus-free part of the collecting tubule described by Haley (1984) corresponds to at least the proximal part of the coiled segment of the vas deferens of the parastacid and spiny lobster sperm duct, which has been enveloped into the testicular mass. Haley (1984) found this segment to be no different in structure from that of the acinus-bearing collecting tubule. That part of the vas deferens emerging from the testicular mass is lined by a tall, glandular, basophilic, columnar epithelium with darkly staining nuclei resting on a basement membrane which is surrounded by a connective tissue coat and striated muscle fibers that are asymmetrically situated, and denser (according to Haley) in two longitudinal bands than elsewhere around the circumference. More distally, as the vas deferens merges with the ejaculatory duct, the muscular layer becomes more uniformly dispersed, thicker, and clearly stratified into well-defined inner longitudinal and outer circular layers. If a spermatophore is not present in the distal part of the ejaculatory duct, the longitudinal folds projecting into the lumen are more numerous. Joining the lumen in the distal part of the duct is a simple tubular gland, the body of which is embedded in the muscular layer. Histologically, the epithelial lining of the gland does not appear to differ from that lining the ejaculatory duct. As the latter passes through the coxa, the muscular layers become indistinguishable; most, if not all, of the fibers becoming longitudinally or obliquely directed. Moreover, the muscular coat becomes much thicker on one side (that corresponding to the concave side of the clusor as described immediately below) than on the other. No extrinsic muscle extending from the coxal wall to the distal part of the ejaculatory duct was recognized in this study.

TERMINUS. The terminus (Fig. 10c) is much like that of the other astacurans studied except *Parastacoides tasmanicus* (Fig. 10a). As in *Homarus americanus*, the clusor is much larger in proportion to the diameter of the coxa than in any of the crayfishes, spanning more

than three-fourths the lumen of the coxa. Unlike the clusor in *Pacifastacus l. trowbridgii* (Fig. 19b), longitudinally or obliquely oriented muscle fibers are distributed for more than half the proximal length of the clusor, and the band on the concave side is heavier and extends farther distally than does the coat on the convex side (Fig. 19d,g), in this respect resembling members of the Cambaridae. Along its length, fibers are bound to the dense connective tissue layer, through which they are anchored in the cuticle by tonofibrils crossing the epithelial lining (Fig. 19a,i). In the specimen examined in this study, there is no indication of terminus glands; furthermore, the distal end of the clusor reaches the exterior, not terminating in a recognizable genital atrium. It is entirely possible that the apparent absence of the latter results from the protracted state of the clusor; on the assumption that it may be retractable, then the atrium might well become evident.

SPERMATOPHORE AND SPERMATOZOA. The sperm mass passing from the collecting tubules to the vas deferens consists of clusters of spermatozoa embedded in a fluid matrix secreted by the epithelial lining of the collecting tubule (Fig. 9a). According to Matthews (1954), each cluster is the product of a single acinus; this includes the spermatozoa that are enveloped by the remains of the syncytial cytoplasmic mass and breakdown products of the previously accompanying accessory cells. As the mass passes along the proximal part of the coiled segment it becomes shaped into a continuous cylindrical core, around which is added the primary spermatophore layer and, before reaching midlength of the coiled segment, much of the secondary spermatophore layer (= "outer bounding layer" of Haley, 1984) is added. The asymmetrical shaping of the spermatophore is correlated with the morphology of the coiled segment of the vas deferens. A longitudinal epithelial fold along the proximal part of the coil is flanked by two longitudinal muscular thickenings that lie superficial to invaginations of the deeper epithelial layer. These two invaginations cause a narrowing of the lumen between them, transforming the shape of the cross section of the latter from cylindrical to bell shaped. The spermatophore contained therein fills the lumen. Since the primary spermatophore layer is of uniform thickness around the spermatozoa-bearing core, the differential thickness of the spermatophore wall results from that of the secondary spermatophore layer, which is thin in the constricted area termed the "foot" by Haley, but thickens gradually toward the "rim of the bell" and there becoming massive, forming what Haley termed the "cap." Thus the asymmetry of the spermatophore results from the morphology of the coiled segment of the vas deferens and from the disposition of the



FIGURE 10. Scanning electron micrographs of terminus at base of and within phallic papilla in: a, *Parastacoides tasmanicus*; b, *Parastacus nicoleti*; c, *Enoplometopus occidentalis*; d, *Homarus americanus*; e, *Pacifastacus leniusculus trowbridgii*; f, *Procambarus zonangulus*; g, *Orconectes rusticus*; h, *Cambarus acuminatus*.

secreted components of the secondary layer by the bounding glandular epithelial layer.

The spermatozoa consist of a lens-shaped body with a concave nucleus extending into the bases of three radial arms (Haley, 1986).

NEPHROPOIDEA

Much of this comparatively brief account of the gross anatomy of the testis and the structure and functioning of the vas deferens, spermatophore, and spermatozoa in the Nephropoidea is based on the following: Herrmann (1890), Gerstaecker (1895), Herrick (1895, 1909), Gerstaecker and Ortmann (1901), Wollebaek (1909), Worley (1939), Johnson (1964), Chevallier (1965), Pochon-Masson (1965a, b; 1968), Farmer (1974), Phillips et al. (1980), Talbot and Chanmanon (1980a, b), Aiken and Waddy (1980), Kooda-Cisco and Talbot (1982, 1984, 1986), Talbot et al. (1983).

The basic structure of the testis differs little from that of the Australian crayfishes mentioned above. The organ is apparently usually H-shaped, or the transverse bridge joining the two lobes may be lacking in the American lobster (Herrick, 1909). In the two specimens of *Homarus americanus* studied herein, the transverse bridge was present (Fig. 1d). The most conspicuous differences in the gross structure of the reproductive system as compared with those of *Parastacoides* and *Astacopsis* are the more massive bulges in the testicular walls (Fig. 11a) and the form of the sperm duct (Fig. 12). The latter is proportionately much shorter and consists of a comparatively narrow, short "proximal segment" followed by a prominent, enlarged, sinuous "glandular segment" that is joined to the "ejaculatory duct" by a very short sector designated by several of the authors cited above as the "sphincter muscle." Aiken and Waddy (1980) remarked on the apparent lack of a description of the testis of *Homarus* that might permit a comparison with that of *Panulirus penicillatus* (Olivier, 1791) by Matthews (1951). The following observations are offered based on this study of the testis of *Homarus americanus*.

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. Although the testis of the American lobster is a much more compactly structured organ, and sectioned material appears at first glance to be strikingly dissimilar to that of the parastacids, the basic patterns of structure and apparent functioning of the homarid testis agree well with those described for the three

parastacids examined in this study. Being more compact, the collecting tubules radiating from the primary collecting ducts are proportionately shorter and, for the most part, have a greater diameter. In these respects they are more like those of *Parastacoides* than like those in *Astacopsis*. The ducts and tubules (Fig. 12b,c) are lined by a glandular epithelium that ranges from cuboidal to columnar cells, the free borders of which are often frayed and continuous with the adhering acidophilic secretion. This epithelial layer, resting on a basal lamella (basement membrane), is surrounded by a connective tissue layer in which are embedded obliquely disposed striated muscle fibers.

ACINI. Like the ducts and tubules, the acini are also more robust than in the parastacids (Figs. 3b, 11, 13, 14). As in the latter, they may originate from the collecting tubules (and no doubt, at least in younger individuals, from the primary collecting ducts) or from active or spent acini. Too, there is ample evidence that the same acinus may produce more than one, probably several, "generations" of spermatozoa. The same cell types observed in the acini of parastacids are present in those of *H. americanus* and their relative abundances depend on the stage in the life cycle of the acinus.

In its earliest stage, the young acinus appears as a bulge (Figs. 6a,b; 11a) in the wall of a collecting tubule, consisting of a cluster of generative cells bounded superficially by a layer of connective tissue and, at least initially, on the deep side by the epithelial lining of the tubule. These generative cells undergo a series of mitotic divisions resulting in a mass of cells, most, or perhaps all of which are spermatogonia; likely some of those adjacent to the connective tissue and fewer among the spermatogonia are modified (?), acquiring smaller, more darkly staining nuclei. These are the accessory cells (Fig. 11b), the forerunners of which have not been definitely identified. As the spermatogonia within an acinus (Figs. 6c-e; 11c) are subsynchronously transformed to primary and secondary spermatocytes and spermatids, the volume of the acinus increases, and by the time spermiogenesis is initiated in the spermatids, the nuclei of the accessory cells flanking the connective tissue coat and those scattered among the spermatids are clearly distinct (Figs. 6d, 11d). In the meantime, a few generative cells have appeared between the connective tissue coat and the superficial accessory cell layer (Figs. 6e,i; 11d). Accompanying the conversion of spermatids to spermatozoa, the new generation of generative cells multiply and, pressing upon the adjacent deep layer of accessory cells, aid in if not largely effecting a concentration of the spermatozoa and displacing them and the original superficial accessory cell layer to the center of the acinus and toward the

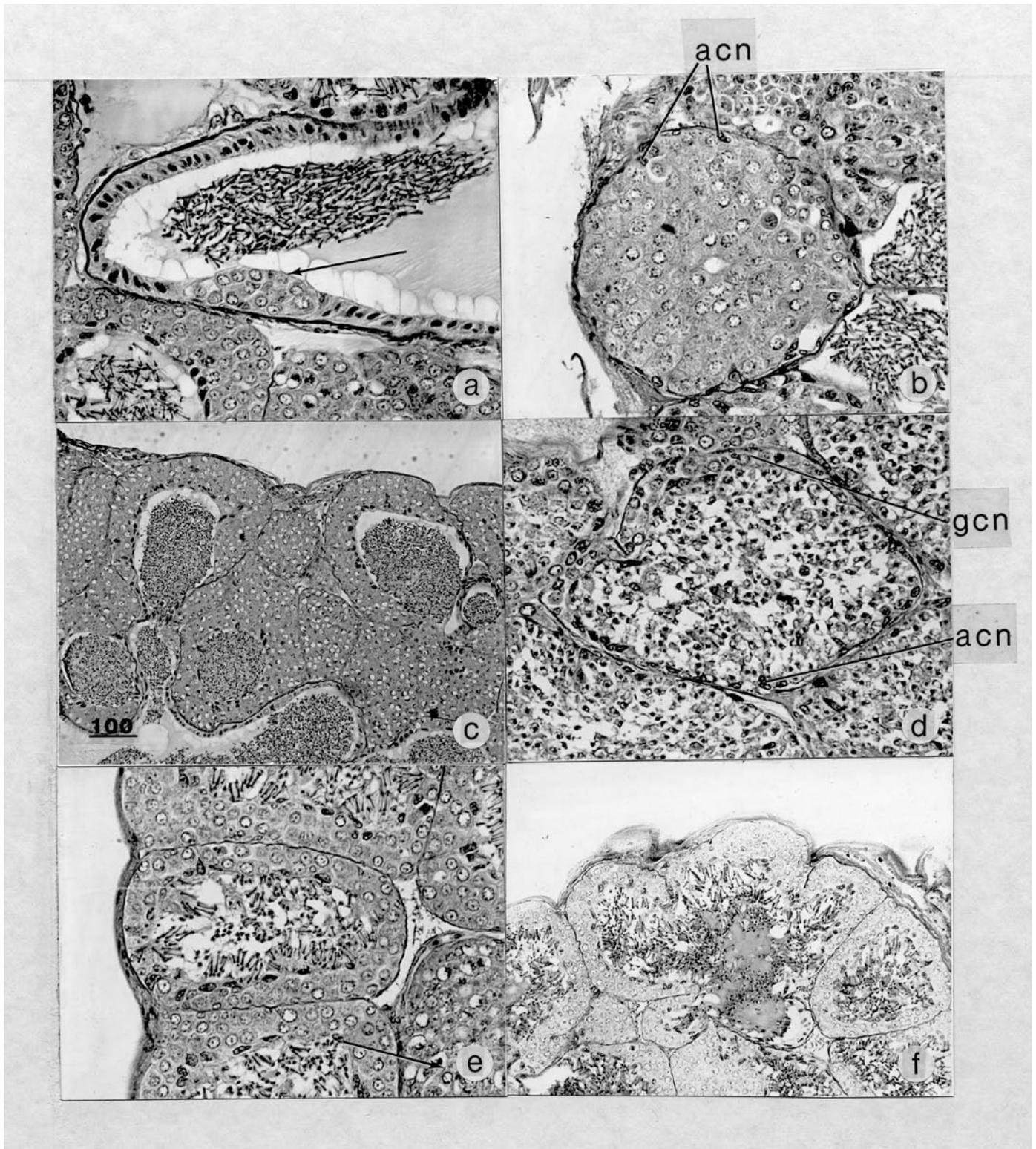


FIGURE 11. Elements of testis of *Homarus americanus*: a, young acinus, part of collecting tubule with rudimentary acinus in lower wall (arrow); b, acinus containing spermatogonia, accessory cells indicated; c, acinus containing spermatogonia (note accessory cell layer lining lumen); d, acinus containing spermatids transforming to spermatozoa, breakdown products including remnants of accessory cells (note generative cells in lower periphery of acinus), generative cell and accessory cell nuclei are indicated; e, regenerating acinus (arrow) with core of spermatozoa and breakdown products of acinus; f, tri-lobed compound (secondary), regenerating acini discharging spermatozoa flanked by simple acinus on right. (See Figure 14 for labels.)

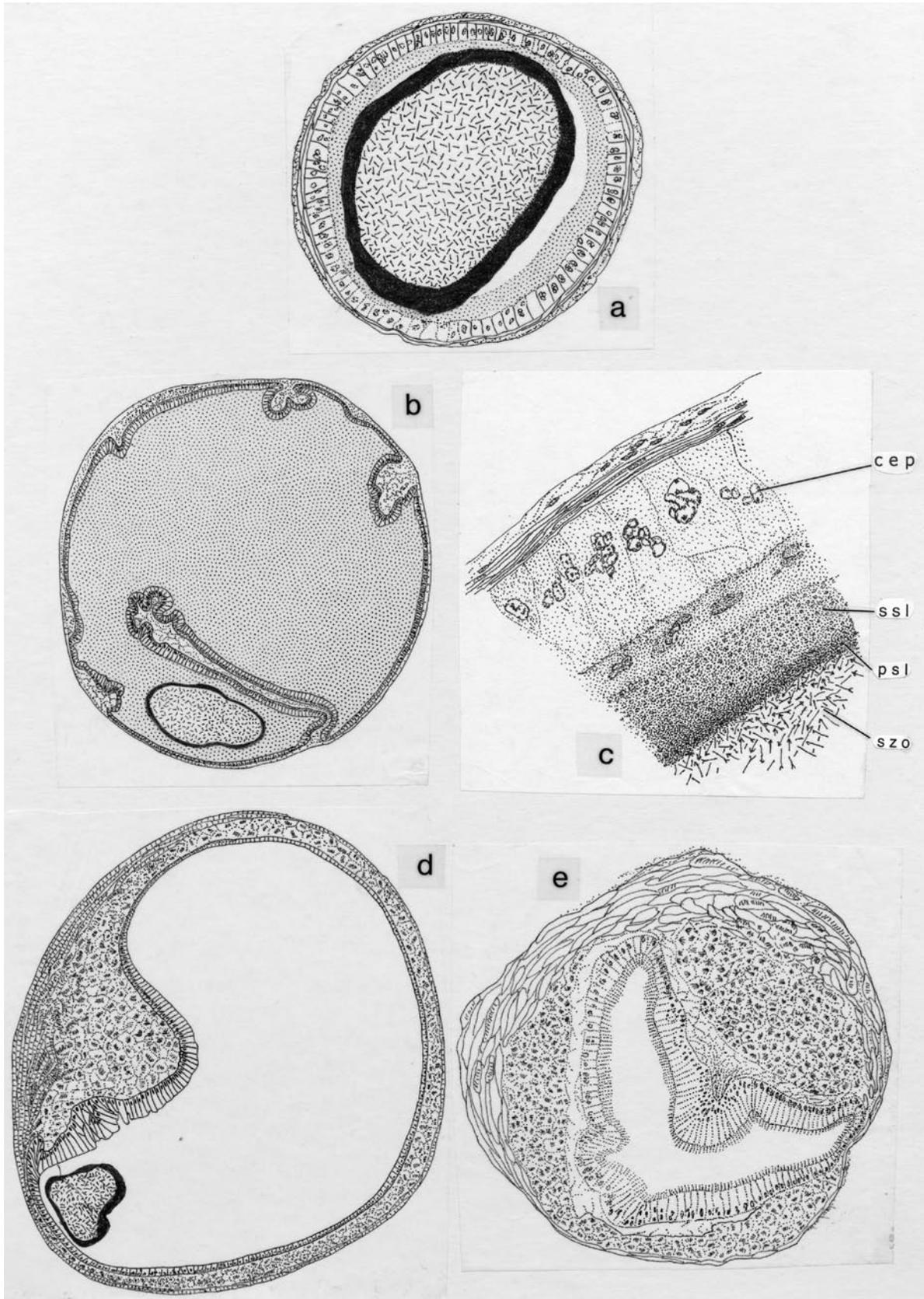


FIGURE 12. Sperm duct of *Homarus americanus*: a, cross section through proximal segment; b, cross section through glandular segment; c, enlargement of part of wall of "b"; d, cross section through sphincter segment; e, cross section through ejaculatory duct.

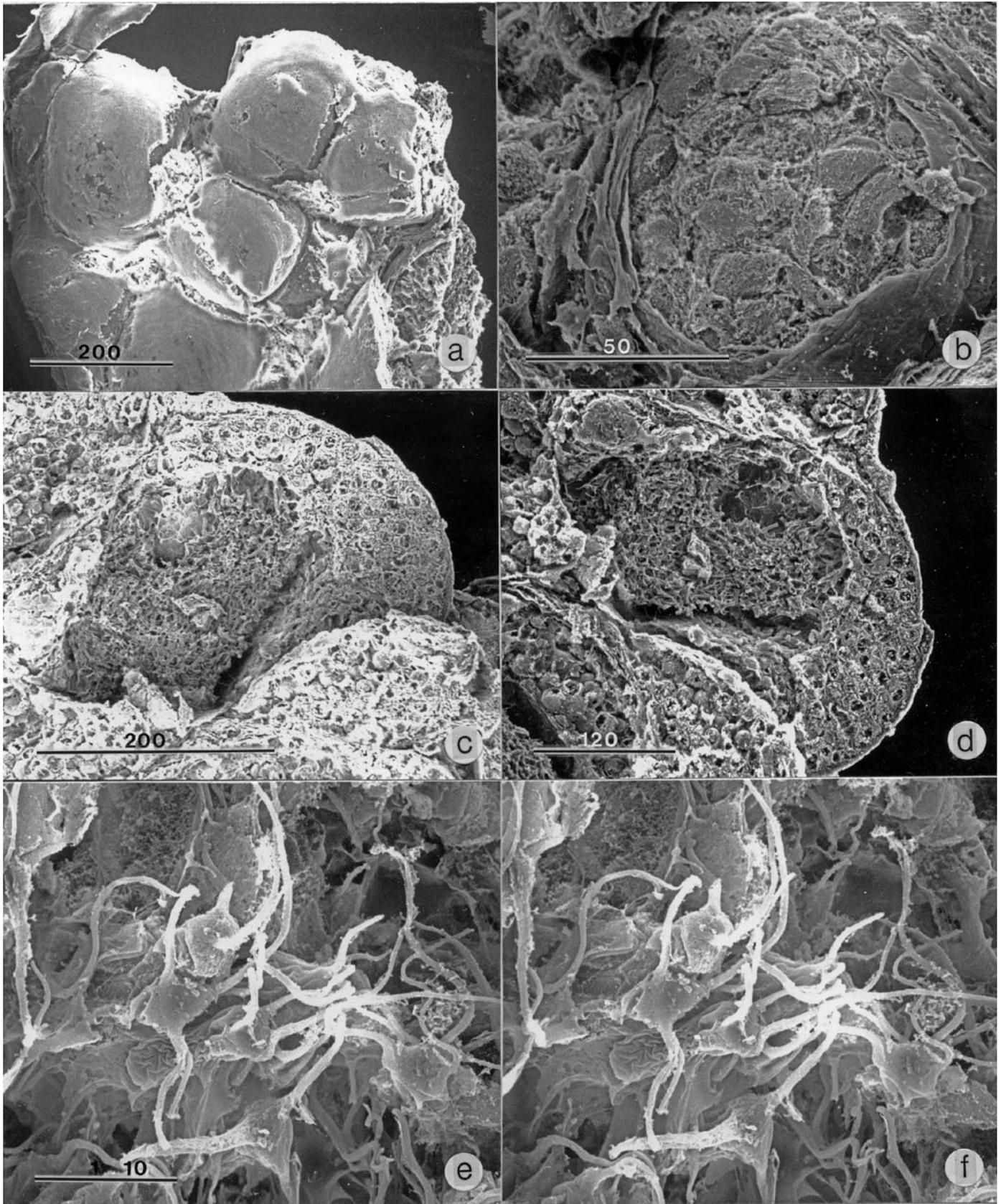


FIGURE 13. Scanning electron micrographs of the testis of *Homarus americanus* (μm): a, cluster of acini in testicular lobe; b, acinus ensheathed in connective tissue-epithelial coat; c, d, regenerating acinus with core of spermatozoa (note accessory cell layer separating spermatocytes from cluster of spermatozoa); e, f, stereoscopic view of spermatozoa within acinus (note three rays).

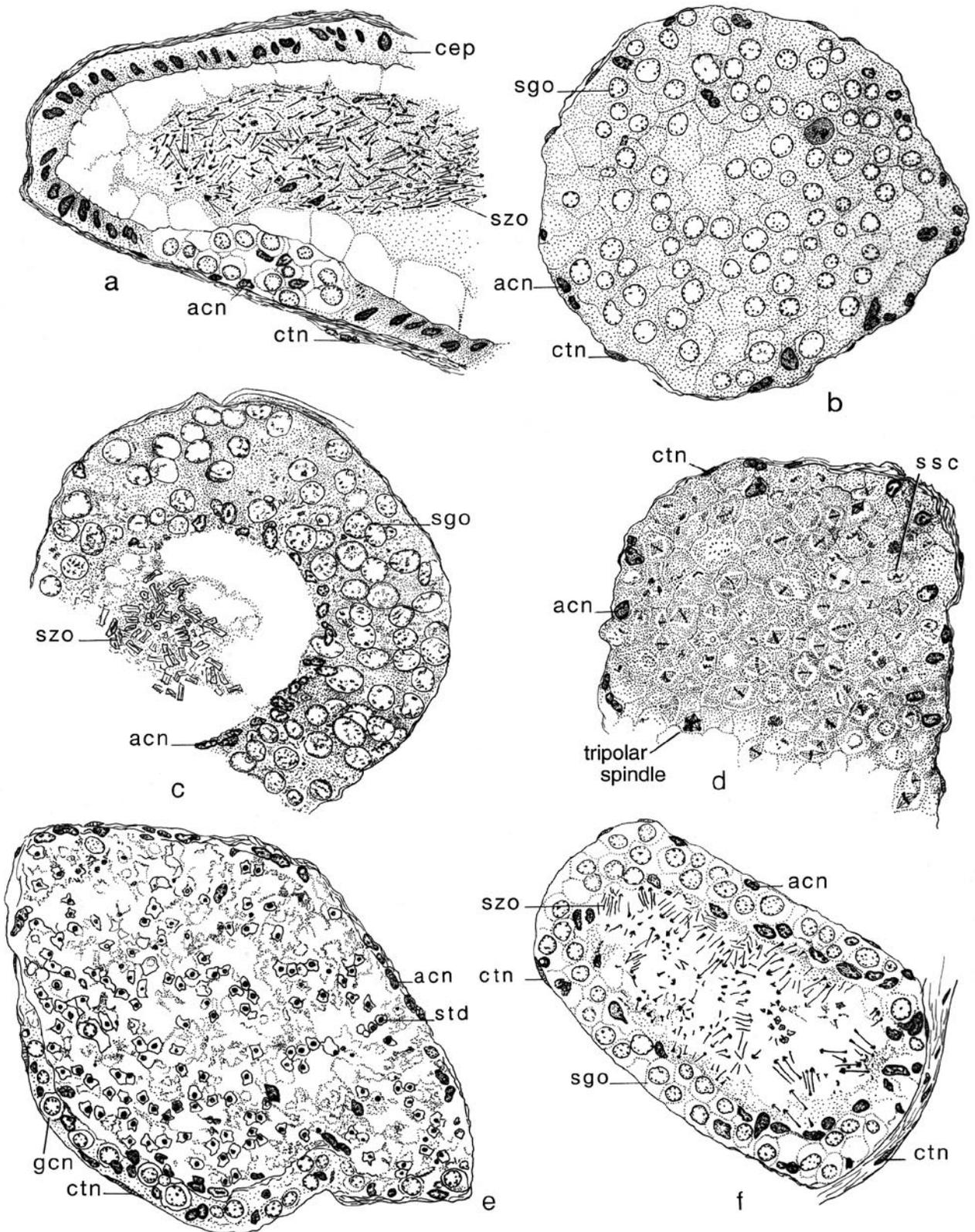


FIGURE 14. *Homarus americanus*: drawings of same elements photographed in Figure 11, except: d, spermatocytes at second meiotic division (note shared centriole by two spindles at lower right (arrow) and tripolar spindle at lower left of center (arrow)); e, acinus containing spermatids transforming to spermatozoa (note generative cells in lower wall of acinus preparing for another cycle of sperm production); f, regenerating acinus with core of spermatozoa and breakdown products of acinus, spermatogonia already present in new wall.

collecting tubule (Figs. 6e,f; 11e). The original accessory layer together with those accessory cells that were scattered among the spermatids disintegrate and, along with the discarded elements of the spermatids, travel with the compacted sperm mass into the collecting tubule. During this exodus from the acinus into the tubule, the dividing generative cells have begun a new cycle of sperm production within the spent acinus (Fig. 11e).

Apparently often, the generative cells in the walls of an acinus become situated in such a manner as to bring about one to several bulges in the walls of a primary acinus, thus giving rise to one or more secondary acini (Fig. 6e,h,i). Thus a single acinus may (1) give rise to at least two (probably several) generations of sperm without markedly altering its shape or maximum volume (when sperm are first produced) and/or (2), with the addition of secondary acini, become converted into a much larger, lobulated, sperm-producing complex (Fig. 6).

MICROANATOMY OF THE SPERM DUCT

As noted above, four sections of the sperm duct have been recognized in the lobsters (Fig. 12). Its gross structure is so different (interpreted as being the most divergent from the generalized condition observed in the *Astacura*) from what is believed to be the generalized condition, exemplified by the *Parastacoidea*, that except for the ejaculatory duct, I am reluctant to suggest homologies existing in the recognized sections in the two. Accordingly, the terminology previously applied to the sperm duct in the lobsters is adopted, adding, however, the cuticular-lined terminus (Fig. 10d).

The basic structure of the sperm duct does not differ from that of *Parastacoidea tasmanicus*, the lumen lined by a layer of glandular, columnar epithelium resting on a basement lamella that is surrounded by a loose connective tissue in which are embedded an inner longitudinally disposed layer of striated muscle fibers girded by a more superficial layer of encircling ones. It has not been possible to determine whether or not the entire duct is surrounded by a layer of squamous epithelium.

VAS DEFERENS: PROXIMAL SEGMENT. The proximalmost part of the sperm duct is lined by a tall, primarily basophilic, columnar epithelium, the units ("cells") of which are multinucleate. The height of the layer is no doubt determined in part by the bulk of the content of the lumen, but in the material examined in this study it is as great or greater than that of the combined, more superficial layers. The nuclei occur in clusters of as many as twelve, perhaps even more. The clusters and the individual nuclei are so variable in size and shape (the

nuclei spherical to spindle shaped [Fig. 12]) that a record of precise measurements would be as misleading as helpful. Surrounding the epithelial lining is a layer of fibrous connective tissue in which is embedded a thin ring of circular muscle fibers, nowhere more than five strands, closely adjoining the base of the epithelium; longitudinally disposed fibers, if present at all, are sparsely dispersed as isolated ones wedged between the epithelium and the circular fibers. A loose, fibrous connective tissue constitutes the superficial layer of the duct.

Within the proximal segment, the sperm mass issuing from the testis receives the primary spermatophore layer (Fig. 15). A homogeneous matrix supporting acidophilic subspherical globules is produced by the epithelium. These globules are produced in rings encircling the sperm mass, and as more are added, those closer to the mass coalesce, leaving minute acidophilic particles supported in the matrix.

The formation of the intermediate spermatophore layer (Kooda-Cisco and Talbot, 1982) appears also to be initiated in the proximal segment, and much of the organization of the epithelial layer seems to break down, sloughing segments of the nuclear masses along with the basophilic granular exudate. Perhaps the nuclear fragments are responsible in part for the inclusions and/or granules noted in the intermediate layer by Kooda-Cisco and Talbot.

GLANDULAR SEGMENT. The most conspicuous element of the glandular segment is a series of prominent, complex longitudinal evaginations into the lumen of that part of the wall of the duct lying deep to the layer of circular muscle (Fig. 15). One of these, here designated the "velum," is much more prominent than the others, reaching centripetally for at least half the diameter of the duct. Making parts of the invaginations (especially the basal sides of the fold) more complex are invaginations of the epithelial layer, forming shallow, simple glands that are tubular or globular (Fig. 15c). In addition, similar but smaller simple or compound circular folds are present on the non-evaginated sections of the wall (Fig. 15d). There appears to be no difference between either the structure or the staining properties of the epithelium in this segment of the sperm duct and that lining the proximal segment. The nuclear clusters are basally situated and the granular cytoplasm exudes a strongly acidophilic product incorporated in the intermediate layer of the spermatophore. Superficial to the epithelial layer, and supported by connective tissue, is a single layer of longitudinally disposed muscle fibers in which the fibers become more numerous at the bases of the evaginations, but elsewhere around the circumference may even be absent. Surrounding this partial layer is

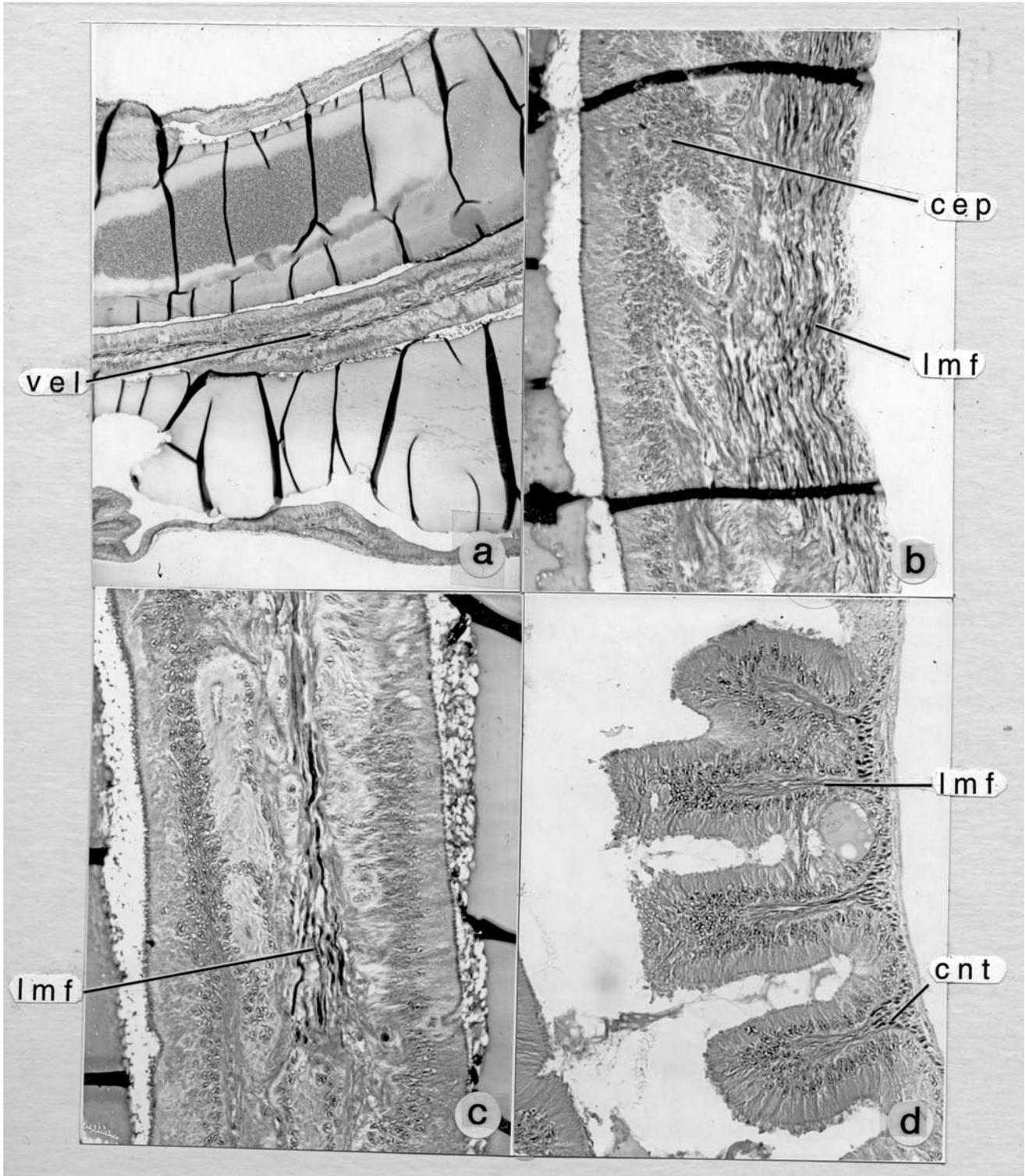


FIGURE 15. Sections through glandular segment of the sperm duct in *Homarus americanus*: a, longitudinal section showing velum dividing lumen with spermatozoa surrounded by primary spermatophore coat on left top and intermediate coat being added on both sides (black lines are artifacts caused by folding of mostly spermatophore layers); b, longitudinal section of wall with heavy layer of longitudinal muscle fibers and simple glands formed by folding of epithelial layer; c, enlarged view of velum showing connective tissue with longitudinal muscle fibers flanked by glands of units of epithelial layer; d, folds in the epithelial wall bounding extensions of connective tissue and longitudinal muscle fiber layer.

a narrow band of circular muscle fibers and connective tissue similar to that in the proximal segment. In some areas along the surface of the duct the muscle fibers seem to be exposed directly to the haemocoel.

The importance of the evaginations in the production of the intermediate layer of the spermatophore is reflected in the increased epithelial component, which produces the most massive component of the spermatophore. But the velum also appears to serve an additional function in partially partitioning the lumen so that as the developing spermatophore enters the glandular segment of the duct, it is shunted to one side, and a major part of the secretion of the segment is produced on the other. This results in the transposition of the core of spermatozoa from the center of the developing spermatophore to a more superficial position, thus preparing the developing spermatophore for further shaping in the sphincter and ejaculatory duct.

Within this segment of the sperm duct the primary spermatophore layer no longer exhibits the aligned subspherical globules noted in the proximal segment but consists of a homogeneous mass that appears to have been laid down in closely adhering columns situated perpendicular to the sperm core. These columns continue in the intermediate layer but are set off at the junction of the two coats by short, fine, refractive lines similar to others scattered through the columnar elements of the intermediate layer. Small irregular bodies and granules are scattered throughout the acidophilic intermediate layer but are more abundant on the side of the velum opposite the sperm core.

SPHINCTER MUSCLE. Replacing the several evaginations of the wall of the duct into the lumen is a single massive one that occupies between one-fourth and one-fifth the diameter of the duct, converting the shape of the lumen, and hence that of the developing spermatophore, from subcircular in section to comma shaped. The comparatively small sperm mass, now heart shaped in section, has been shunted to the tip (base) of the comma. The multinucleate epithelial lining varies from tall columnar over the evagination to subsquamous on the opposite side of the lumen. The free border of this weakly basophilic layer is produced in slender projections resembling cilia that are recognizable even in the surrounding amorphous exudate of the layer. The nuclei in this layer appear to be no different from those observed in the proximal and glandular segments of the duct. A pronounced thickening of the underlying layer of connective tissue and longitudinal muscle fibers make up the major component of the evagination. This layer gradually diminishes in thickness along the upper side of the "comma" but is decreased abruptly at the basal end; nevertheless, except in the area flanking

the sperm mass, the thickness of this layer is at least as great as the height of the adjacent epithelium. The more superficial layer, composed of connective tissue and circularly to obliquely oriented muscle fibers, varies in thickness but is proportionately heaviest on the side opposite the evagination. Only a connective tissue coat with spaces containing blood cells has been identified girding the duct. Except for an alteration in the shape of the sperm mass and the encompassing developing spermatophore, there is no conspicuous difference from that described in the glandular sector of the duct.

EJACULATORY DUCT. Typifying this segment of the ejaculatory duct is a broadly V-shaped lumen, one arm of which lies subparallel to the circumference of the duct and the other projecting into the central mass at an angle of approximately 75 degrees. This duct is lined by a tall columnar epithelium which, like that lining the other segment of the duct, is multinucleate, the nuclei occurring in clusters that for the most part are basally situated. Too, the free border is like that of the sphincter segment bearing, or produced in, cilia-like elements, which, despite their rather regular dispersal, appear to be nothing more than the fused surfaces of globules being secreted by the epithelium. As elsewhere in the duct, there is an underlying layer of connective tissue separating the epithelial layer from the bundles of longitudinal muscle fibers. These bundles, although forming a continuous ring on the superficial flank of the epithelial layer, are concentrated in three prominent masses: the largest in the base of the "V," a slightly smaller one adjacent to the arm of the "V" projecting toward the central mass, and the least thick adjacent to the arm lying subparallel to the surface. The positions of the larger two masses of longitudinal fibers are reflected in the eccentric position of the epithelial layer and lumen as well as in the shape of the lumen itself. On the superficial flank of the two larger longitudinal muscle masses there is a thick layer of circular to oblique muscle fibers, whereas that adjacent to the smallest mass is decidedly reduced. Near the tip of the arm subparallel to the outer contour of the duct, the epithelial lining so closely approaches the surface that there is little room for muscle fibers and the few that are present belong to the outermost layer of circular-oblique fibers. Here, as elsewhere along the length of the sperm duct, I have been unable to observe a epithelial layer superficial to the loose connective tissue layer.

Unfortunately in the material examined during this study, there was no spermatophore in the lumen of the ejaculatory duct. Only a frothy, acidophilic exudate from the epithelium was present. It is perhaps likely that the third coat termed the "secondary spermatophore layer" or

“outer bounding layer” recognized by Kooda-Cisco and Talbot (1982) is the extruded spermatophore.

SPERMATOPHORE AND SPERMATOZOA. The spermatophore and spermatozoa of the Nephropoidea are markedly different from those of the parastacids. Those of *Homarus americanus* were studied by Herrick (1895, 1909), Talbot and Chanmanon (1978), and Kooda-Cisco and Talbot (1980, 1982, 1984, 1986). Hermann (1890), Gerstaecker and Ortmann (1901), Wollebaek (1909), and Pochon-Masson (1965a, b) investigated the sperm of *H. vulgaris* Milne Edwards, 1837, and the spermatophore of *H. gammarus* (Linnaeus, 1758) was detailed by Talbot et al. (1983). The spermatophore of *Nephrops norvegicus* (Linnaeus, 1758) was described and depicted by Farmer (1974), and the sperm by Chevallier and Maillet (1965a, b).

In the preparation of the vas deferens of *H. americanus*, the spermatophore consists of a central core of tightly packed sperm enclosed in a primary spermatophore layer (staining tannish orange in Mallory's Trichrome), which is approximately one-sixth as wide as the core of spermatozoa, and a secondary spermatophore (or “outer bounding”) layer (staining blue in Mallory's Trichrome) highly variable in width (often asymmetrically so), ranging from less than that of the primary spermatophore layer to slightly greater than the width of the central core of spermatozoa (Fig. 15). More details of its structure may be found in Kooda-Cisco and Talbot (1982) who, employing transmission electron microscopy, recognized an “intermediate layer” between the primary and secondary layers of the spermatophore.

Spermatozoa: All accounts of the spermatozoa of the Nephropidae (*Nephrops*, *Homarus*) (see above) report or suggest that they are elongate and bear three rays at the end opposite the acrosome (Figs. 11e,f; 13e,f). There is no evidence that they are individually encapsulated as are those of the Astacoidea. The diameter of the three-rayed, cylindrical spermatozoa in my preparations of specimens of both genera is approximately 5 μm and the length, 16 μm . Excellent detailed descriptions of the spermatozoa are presented by Talbot and Chanmanon (1980a) and Jamieson (1991).

Among the best accounts of the male reproductive systems in other nephropoideans is that of Farmer (1974), who investigated reproduction in *Nephrops norvegicus*.

ASTACOIDEA

ASTACIDAE

Despite the numerous studies of spermatogenesis and spermatozoa in the Astacidae—those describing and/or

illustrating the gross morphology of the testis and those dealing with the structure of the sperm duct and spermatophore—the functional anatomy of the testis has been neglected. Among the earliest reports treating the male reproductive system were those of Willis (1672) and Porzio (1687) who, according to Brocchi (1875), noted the gonopores and described the testis of *Astacus fluviatilis* (Fabricius, 1775) (= *A. astacus* (Linnaeus, 1758)). Milne Edwards (1837a, b) also described and illustrated the testis and vas deferens but failed to recognize the acinar components. These units were apparently first observed by Lemoine (1868), who noted the rounded cul-de-sacs that were described as being composed of two types of cells. He found the number of rays of the spermatozoa highly variable, ranging from 2 to 13. Other early observations including those of Kölliker (1841) and Chantran (1872a, b)—the latter was said by Brocchi (1875) to have coined the term *spermatophore*—were primarily concerned with where fertilization takes place in the crayfish (probably *Astacus astacus* (Linnaeus, 1758)). Chantran (1872a, b) also described the spermatozoa, noting 5–7 rays. Brocchi himself presented an account of the testis and vas deferens, noting the superficial circular layer and deeper longitudinal fibers in the latter. Grobben (1878) included the testis of the crayfish among those species treated in his classical study of the male reproductive system in decapod crustaceans. Huxley (1880) briefly described and illustrated the testis, vasa deferentia, and spermatozoa of *Astacus astacus*; Nussbaum (1884) was concerned with the cytological aspects of spermatogenesis and spermatozoa. Carnoy (1885) investigated the multiplication of cells in the testis of this crayfish, elaborating on normal and direct cell division. Hermann (1883, 1884, 1890) presented detailed accounts of spermatogenesis and spermiogenesis. Sabatier (1885, 1893) also made observations on the sperm and spermatogenesis in this crayfish, noting spermatozoa with 8 rays. Gilson (1886) reported observations on the spermatogonia and spermiogenesis in *Astacus astacus*. St. George (1892) recorded the presence of developing ova in the testis of the same crayfish. In their discussions of reproduction in *Astacus*, Gerstaecker (1895) and Gerstaecker and Ortmann (1901) illustrated the male reproductive system and spermatozoa. Mrázek (1902), interested in cytological aspects of spermatogenesis, noted “abnormal” mitotic spindles in *Astacus*, describing and illustrating tripolar spindles with variations both on these and in the usual bipolar configurations. Others contributing to our knowledge of spermatogenesis and of spermatozoa of *A. astacus* and/or *A. leptodactylus* Eschscholtz, 1823, were Prowazek (1902a, b); Koltzoff (1906); Keppen

(1906); Retzius (1909), who illustrated 5–11 rays in the spermatozoa; Reinhard (1913, 1934), who reported 8–20 rays in the spermatozoa of *A. leptodactylus*; Grabowska (1927, 1929a, b), who illustrated as many as 15 rays on the sperm of *A. astacus*; and Tuzet and Bessière (1948), who also depicted sperm with 10–15 rays. Fasten (1914) reviewed the contributions made in many of the references cited above. Niiyama (1962) reported the large number of chromosomes ($N = 188$) he found in *Astacus trowbridgii* (Stimpson, 1857) (= *Pacifastacus leniusculus trowbridgii*). More recently the ultrastructural elements of the testis and spermatozoa attracted the attention of Meek and Moses (1961), Pochon-Masson (1965a, b), Eliakova and Gorichkina (1966), and Lopez-Camps et al. (1981). Studitskij and Eljakova (1972) were concerned with DNA in the sperm nucleus of *Astacus leptodactylus*. One of the most recent additions to our knowledge of the testis of astacids of which I am aware was that of Wielgus-Serafinska (1973), who compared the testis of *A. leptodactylus* with that of the cambarid *Orconectes (Faxonius) limosus* (Rafinesque, 1817). His descriptions of the gross features of the testis and observations on the sperm duct and histological features of the latter constitute important contributions. Unfortunately, however, Wielgus-Serafinska's partly erroneous interpretation of testicular structure, which was rectified in his (1976) detailed account of the male reproductive system of *O. limosus*, makes his description of the testis of *A. leptodactylus* difficult to interpret. The very brief account of the male reproductive system by Holdich and Reeve (1988) contains no new information. Obradovic (1989) reported seasonal differences in the testis of *A. astacus*, recognizing degeneration of acini following the maturation of the spermatozoa. As for the American astacids, Andrews' (1904a, b; 1931) careful observations on the sperm and spermatophores of *Pacifastacus leniusculus* are noteworthy. More recently, studies have been conducted on spermiogenesis (Dudenhäusen and Talbot, 1979) and on the structure (including ultrastructure) of the spermatozoa and spermatophore of the American astacid *Pacifastacus leniusculus* by Dudenhäusen and Talbot (1982, 1983). The following description of the spermatogenic cycle in this American astacid should provide a basis for comparing the structure and function of the various elements with those of members of the other astacidean families.

Pacifastacus leniusculus trowbridgii

The gross structure of the testis in this crayfish (Fig. 1e) resembles remarkably the tri-lobed organ of *Astacus asta-*

cus depicted by Huxley (1880), less like the photograph of the testis of *Astacus leptodactylus* presented by Wielgus-Serafinska (1973), and differs even more in mass and shape from the lobes of the testes of *A. astacus* illustrated by Milne Edwards (1837b) and Gerstaecker and Ortman (1901). The more robust anterior lobules are shorter than the long, comparatively slender posterior lobule, and as pointed out by Wielgus-Serafinska (1973:116), the three are "connected directly" rather than being joined by "long trunks." The absence of these trunks (= "stalks" of Word and Hobbs, 1958) reflect perhaps the major difference in the functional aspects of the testis in the astacid and cambarid (exclusive of the east Asian Cambaroidinae) testes (Fig. 3i).

The anterior lobules of the testis are directed anterodorsally, flanking the pyloric region of the stomach and abutting the anterodorsal part of the hepatopancreas. Beneath the pericardium the two lobules join the posterior lobe, and the latter extends posteriorly above the intestine into the first abdominal segment. Almost adjacent to the junction of the three lobules, the paired sperm ducts emerge ventrolaterally from the posterior lobule and turn posteriorly in massive coils that, lying on the posterior lobes of the hepatopancreas, reach the first abdominal segment before turning anteriorly and ventrolaterally to the phallic papilla on the ventromesial sides of the coxae of the fifth pereopods (Fig. 10e).

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. Compound multiple collecting tubules branching from the Y-shaped primary collecting duct have at their apices simple, sac-like acini that make up most of the mass of the three testicular lobules (Figs. 3h,i; 16a). The composition of the primary collecting duct and that of the collecting tubules (Fig. 17a) are basically identical: the acidophilic, glandular, epithelial lining consists of simple cuboidal to columnar cells with basally situated subspherical to oval nuclei ($12.4\text{--}17.7 \times 7.1\text{--}10.6 \mu\text{m}$) that are strongly basophilic and usually contain at least one basophilic element larger than the others. Lying basally and wedged between the epithelial cells is a scattering of cells with conspicuously larger, subspherical, more lightly staining nuclei ($15.9\text{--}17.7 \mu\text{m}$) lacking nucleolar-like bodies. Because of their resemblance to and the likelihood of their being antecedents of spermatogonia, they are identified herein as generative cells. The epithelial layer rests on a basement membrane intimately associated with a connective tissue layer with which are found longitudinally to obliquely oriented striated muscle fibers. The nuclei ($24.7\text{--}28.3 \times 1.8\text{--}5.3 \mu\text{m}$) associated with this compound

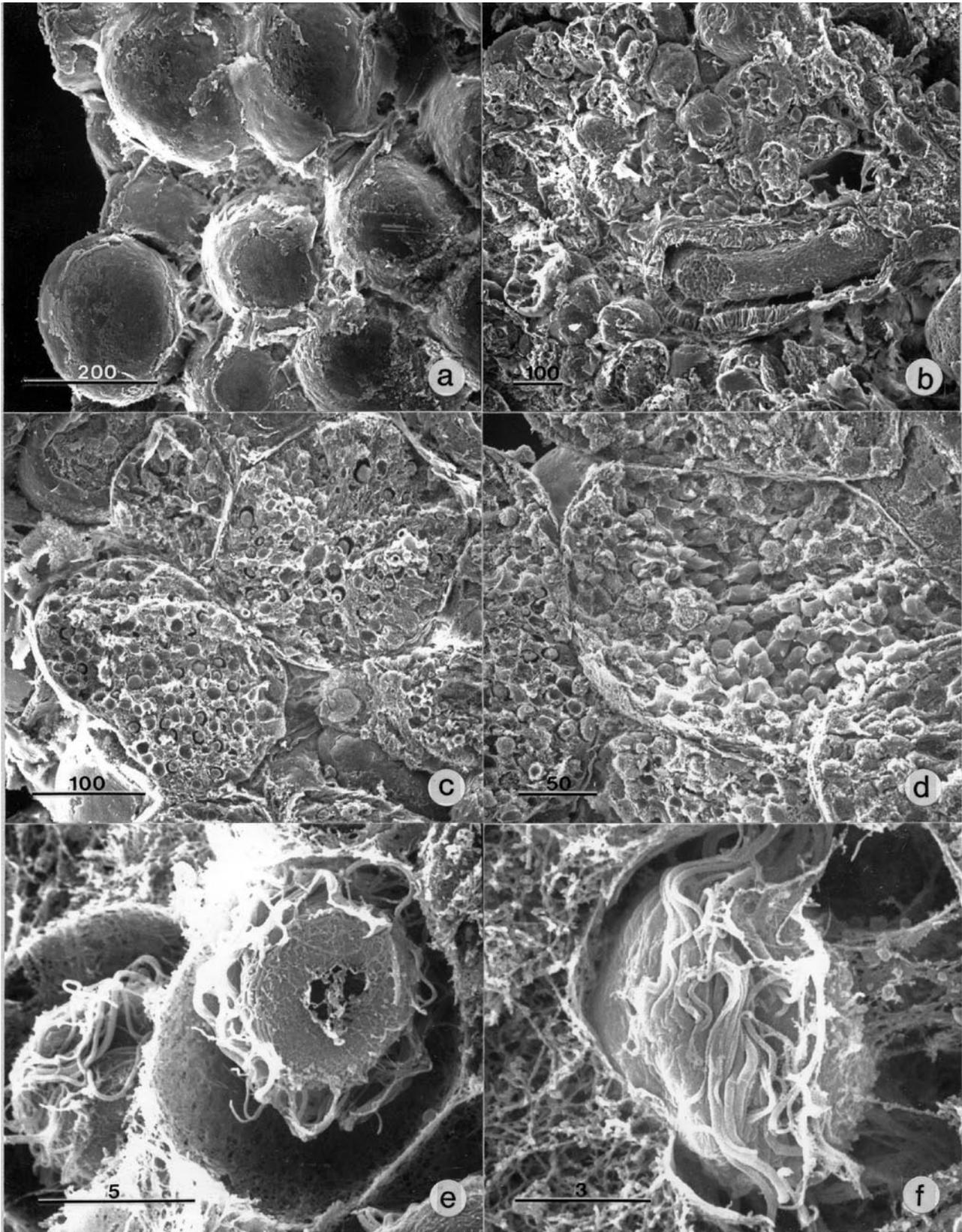


FIGURE 16. Scanning electron micrographs (μm) of testis of *Pacifastacus leniusculus trowbridgii*: a, acini ensheathed in connective tissue coat; b, acini surrounding primary collecting duct containing mass of spermatozoa; c, acini containing spermatocytes and spermatids; d, acini containing spermatids and ensheathed spermatozoa; e, f, ensheathed spermatozoa with encircling rays.

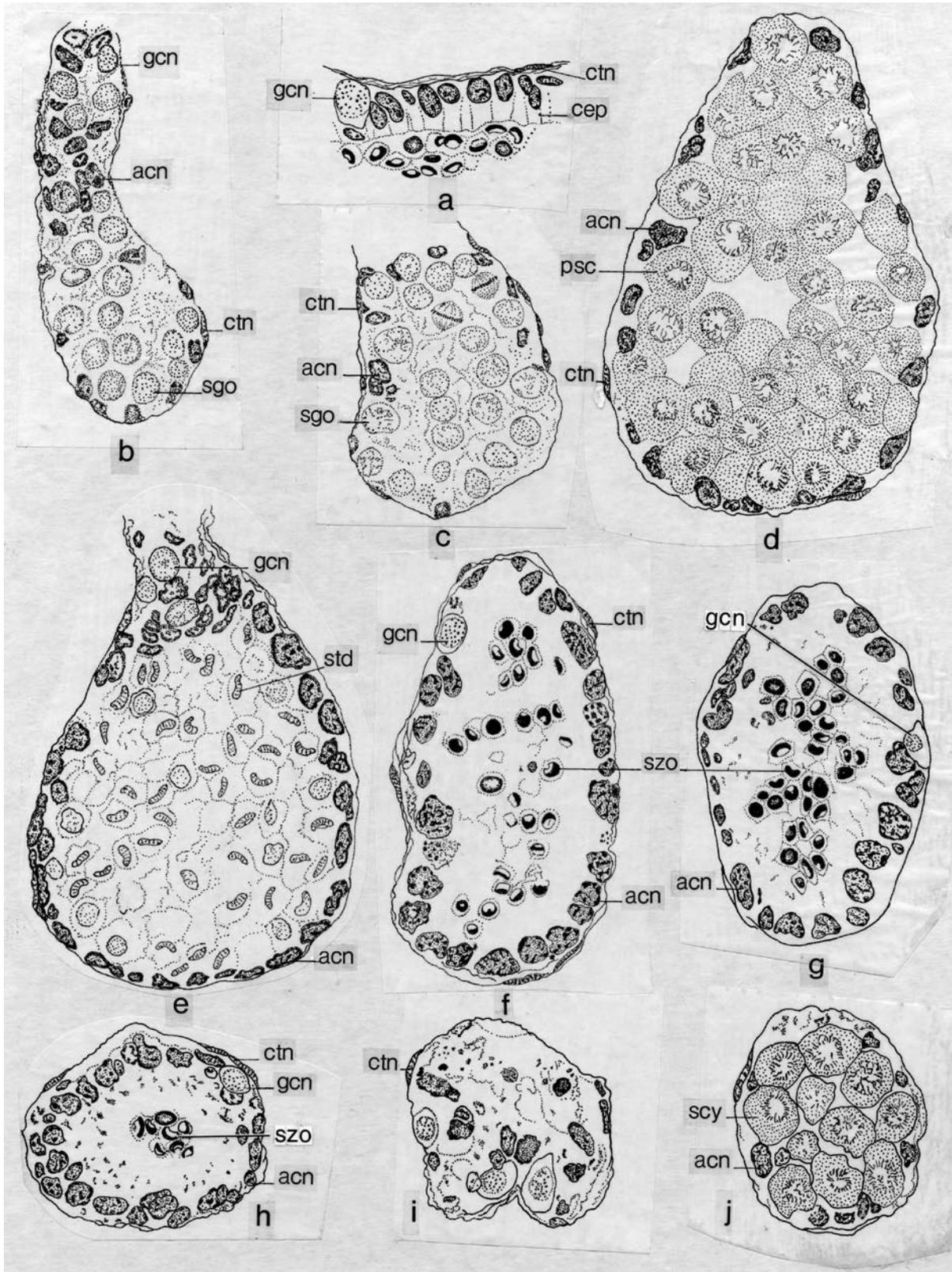


FIGURE 17. Elements of testis of *Pacifastacus leniusculus trowbridgii*: a, wall of collecting tubules with spermatozoa in lumen; b, young acinus with spermatogonia; c, acinus with multiplying spermatogonia; d, acinus with primary spermatocytes; e, acinus with spermatids; f, acinus with recently transformed spermatozoa; g, acinus with spermatozoa being moved centrally; h, acinus with spermatozoa crowded at center; i, degenerating acinus with few generative cells; j, regenerating acinus with primary spermatocytes.

layer are flattened and have been interpreted as being components of the connective tissue, for similar nuclei occur elsewhere in the testis in areas where muscular elements appear to be lacking. In tubules in which no spermatozoa are being transported, the lumen virtually disappears so that in section the core consists of a two-layered mass of columnar to cuboidal epithelial cells.

ACINI. Most of the variations existing in the acini are associated with the stage in the development of these spermatozoa-producing units (Fig. 16b–d), and to at least some extent, early in the transformation of spermatids to spermatozoa, those in comparable stages occur in corresponding regions of each of the lobules: the spermatozoa-filled units lie adjacent to the exiting vasa deferentia, next to and partly surrounding them are acini containing spermatids; more entally in each lobe are acini containing secondary spermatocytes, followed by those containing primary spermatocytes, and at the blind end of the lobe are the smallest acini, those containing spermatogonia. Not only are the latter acini smaller, but within them the subspherical nuclei of the latter are 15.9–19.5 μm in diameter and those of the accessory cells (the cell membranes of which cannot be determined in the material studied) are comparatively small (10.6–14.1 \times 7.1–8.9 μm) and few in number (Fig. 17b,c). With the approaching first meiotic division the acini become larger in diameter, and the nuclei of the accessory cells are more darkly stained, and more numerous (Fig. 17d), often two or three of them contiguous; by the second meiotic division the accessory nuclei are often quite irregular in shape and even more conspicuous (17.7–30.1 \times 7.1–17.7 μm); this irregularity is more apparent than real for it is the clumping that is responsible for most of the roughness. In the conversion of the secondary spermatocytes to spermatids (Fig. 17e), the heretofore cellular components of the acinus begin to disintegrate (Fig. 17i), and with the transformation of the spermatids to spermatozoa, each occupies a small vacuole in the resulting syncytial mass, where they accumulate (Fig. 17f–h) in the central core before being extruded to the collecting tubule. Accompanying the conversion of the spermatids to spermatozoa, the diameter of the acinus decreases and the nuclei of the former accessory cells (syncytia?) are brought closer together, often overlapping and forming a near-continuous layer under the superficial connective tissue coat of the acinus. Whereas the generative cells (forerunners of the spermatogonia, and perhaps the accessory cells) are not evident in the acini containing spermatocytes, a few have been seen in the superficial part of acini containing spermatids, and they have been observed in so many of those acini containing spermatozoa that it is

believed that they are present in virtually all of them (Fig. 17f–h). Accompanying the expulsion of the sperm from the acinus, except for the generative cells, there is a general deterioration and disappearance of the entire cellular content, including most, if not all, of the accessory cell nuclei (Fig. 17i). The generative cells that remain give rise to the new generation of spermatogonia (Fig. 17j), beginning a new spermatogenic cycle within the “shell” of the original acinus, in this respect differing conspicuously from the atrophy of acini having undergone a spermatogenic cycle in the Cambarinae and Cambarellinae. While there seems to be no direct evidence as to the source of the generative cells that appear in the acini containing spermatids or spermatozoa, their apparent absence in acini containing spermatocytes suggests that they might well have migrated from the “stock” housed within the walls of the collecting tubules (Fig. 18a). I am as mystified as were most of my predecessors as to the origin and multiplication of the accessory cells, particularly in view of their early appearance in regenerating acini (Fig. 17j). I have observed no mitotic figures that could be unquestionably associated with these nuclei; neither have I observed anything that might be construed as “direct division” reported by several of the earlier investigators.

MICROANATOMY OF THE SPERM DUCT

As in the Parastacidae, the entalmost testicular segment of the vas deferens is embedded in the posterior lobe of the testis and does not differ structurally from the primary collecting duct (Figs. 7a, 8b). As pointed out in the “Introduction,” the apparent differences in the dimensions of the glandular epithelial layer along the length of the vas deferens are apparently due to two factors. (1) The mass of the content: when the mass is comparatively small, it results in closely packed, tall, columnar cells, their bases so compacted that the nuclei in sectioned material render the appearance of a pseudostratified layer; when the mass is greater, the cells are less compacted and their nuclei are aligned at their bases. (2) In some areas that do not appear to be producing an abundance of secretion, the free borders of the cells are entire, but in areas where the secretion is abundant these borders are not discernible, the cytoplasm being continuous with, and its limits virtually indistinguishable from, the exudate within the lumen. Toward the ental end of the vas deferens the muscular layers are very thin, scarcely discernible in the connective tissue coat. Typical of all of the crayfishes, the muscular coat of the ejaculatory duct is conspicuously thickened (Fig. 9c), whereas the epithelial lining of a duct in active secretion

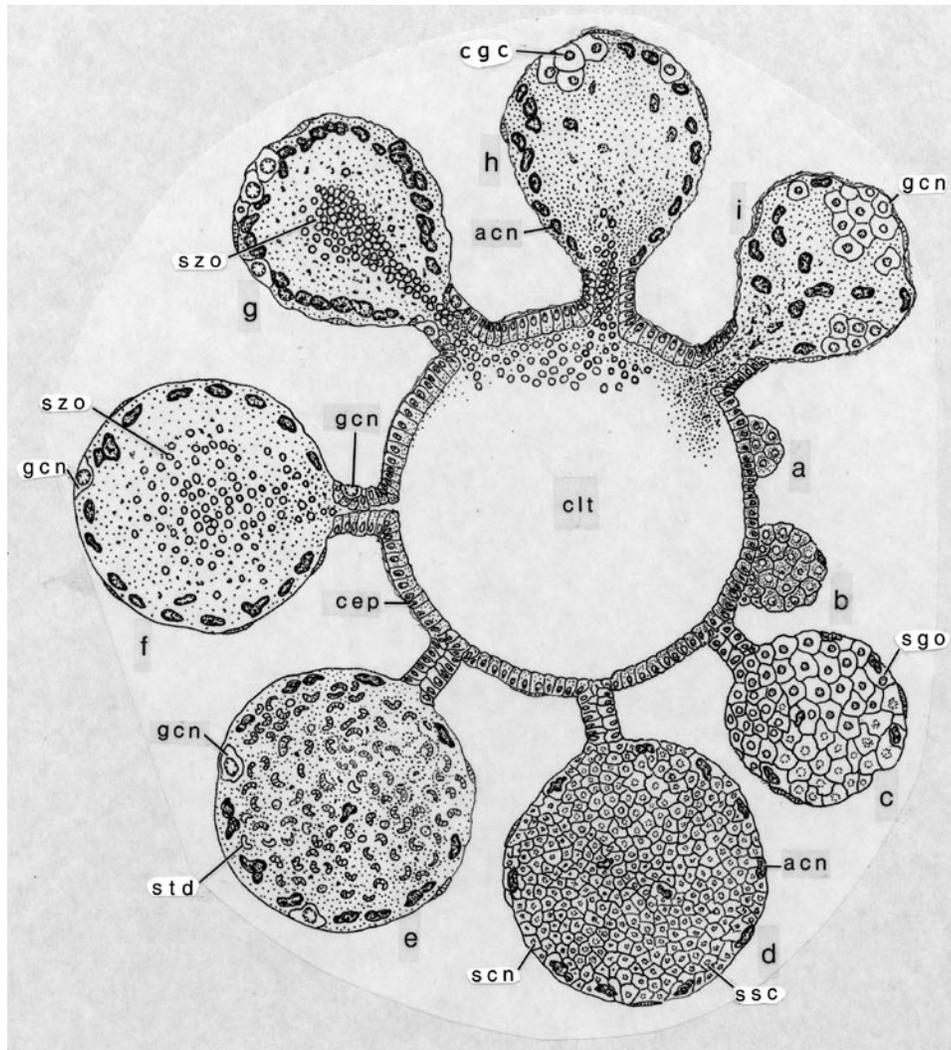


FIGURE 18. Schematic diagram of life cycle of acinus in *Pacifastacus leniusculus trowbridgii*: a, rudimentary acinus on wall of collecting tubule; b, young acinus; c, with spermatogonia; d, with secondary spermatocytes; e, with spermatids; f, acinus with spermatozoa; g, with spermatozoa concentrated in central area of acinus and being expelled into collecting tubule; h, degenerating acinus with cluster of generative cells; i, degeneration continuing but with regeneration progressing.

may be much reduced or virtually absent in some areas. Because of the oblique section of the ejaculatory duct in Figure 9c, the arrangement of the muscular layers appears to be reversed, circularly oriented fibers situated deep to the more superficial longitudinally dispersed ones. Many of the muscular bundles in the outer layer are actually obliquely oriented. As the ejaculatory duct approaches its junction with the cuticular-lined terminus it moves toward and comes to lie in contact with the wall of the coxa, connective tissue fibers apparently affixing it in place. On the opposite side of the coxa (Fig. 19b), a strong "retractor" muscle extends from the coxal wall to the distalmost

part of the duct where fibers join the intrinsic muscular mass and/or are inserted in the connective tissue layer just superficial to the epithelial lining for more than a third of the circumference of the duct. At this level the ejaculatory duct joins the terminus.

TERMINUS. The proximalmost part of the terminus is depicted in Figure 19b in this section appearing in paired diverticula from one side of the ejaculatory duct. Distally, as the diverticula lengthen (Fig. 19c), they form an arc adjacent to the lumen of the ejaculatory duct.

Slightly more distally, the cuticular clusor completely surrounds the now arched lumen of the terminus (Figs. 10e,

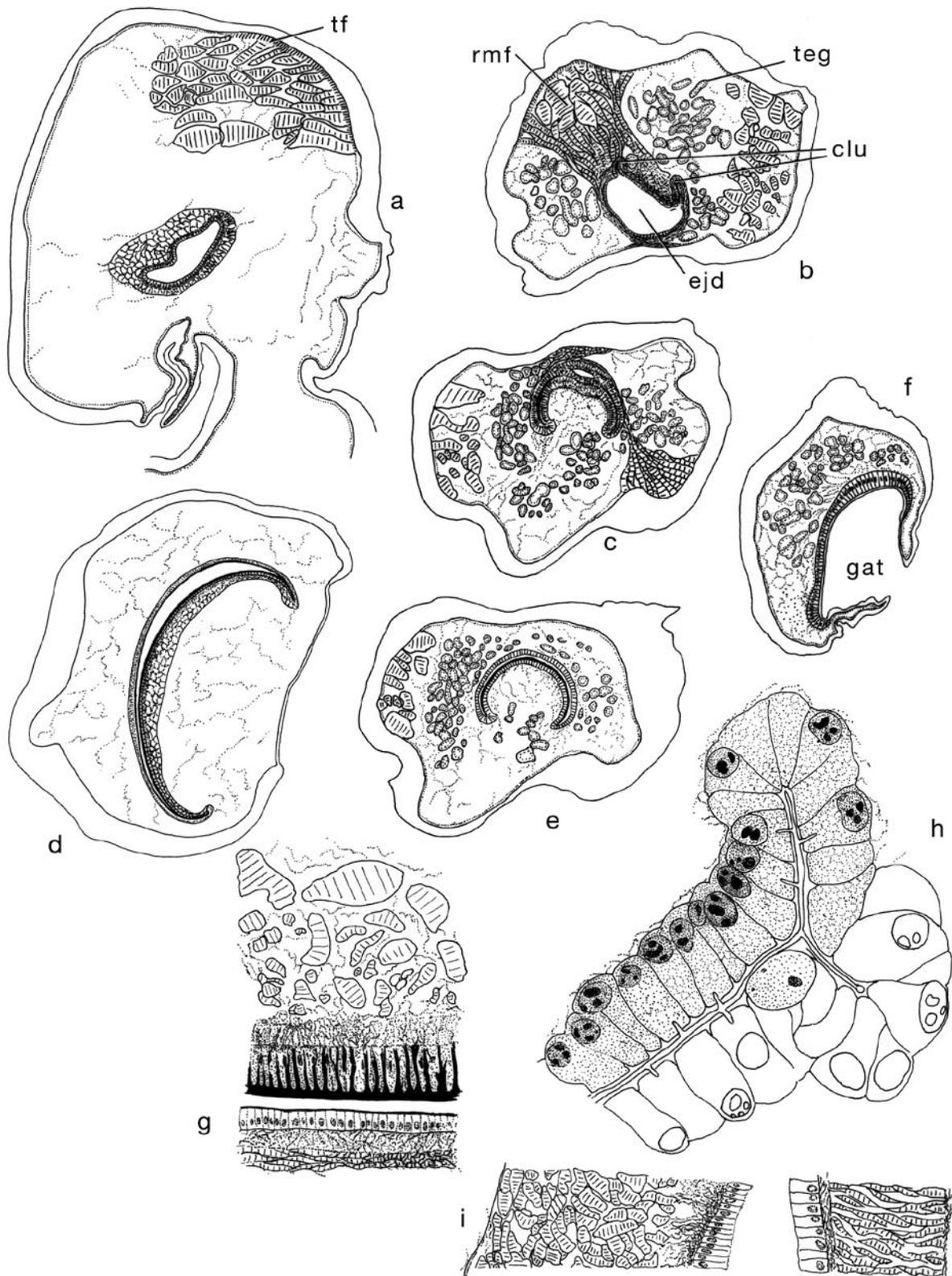


FIGURE 19. Ejaculatory duct and terminus of *Enoplometopus occidentalis* (a, d, g, i) and *Pacifastacus leniusculus trowbridgii* (b, c, e, f, h): a, proximalmost portion of the terminus; b, ejaculatory duct with paired diverticula; c, arc formed by lengthening diverticula; d, e, distalmost portion of the terminus; f, genital atrium; g, higher magnification of terminus in d; h, terminus glands (figure unfinished; HHHJr was working on at time of his death); i, higher magnification of ejaculatory duct in a.

19f). Typically the cuticle on the concave side of the arch is much thicker than that on the convex side. Underlying the cuticular lining is a tall columnar epithelium flanked by a basement membrane and connective tissue that is continuous with fibers that extend throughout the interior of the coxa, ones that are associated with haemocoelic spaces and skeletal muscles and others intimately encompassing elements of the terminus glands. In *P. l. trowbridgii*, except for a few fibers of the retractor muscle that are affixed to the bases of the proximalmost part of the diverticula, there are no muscle fibers associated with the terminus. The terminus glands, more abundant in *P. l. trowbridgii* than in any of the decapods examined in this study, extend from slightly proximal to the junction of the ejaculatory duct and the clusor for more than 300 μm beyond the junction of the latter with the genital atrium. They are larger and more massive than their counterparts in other species examined, but they differ little in basic structure from the “compound tubular glands” described by Johnson (1960:231–232, figs. 3, 4). In *P. l. trowbridgii*, the glands are indeed compound tubular ones but occur also in compound rosettes, each of which consists of an elongate branching core cell surrounded by clusters of peripheral cells each of which abuts the core cell. The nucleus of the core cell is conspicuously larger than those of the peripheral cells and has a single large chromatic body larger than the few smaller ones accompanying it. Within and extending the length of the core cell is a “cuticular duct,” which, like the duct in *Cambarus longulus* described by Johnson (1960), is joined by smaller radial “cuticular ductules” that extend into the basal part of the adjacent peripheral cells, the nuclei of which are smaller and usually contain two or three darkly staining masses. Ducts emerging from the rosettes join or extend separately between the epithelial cells abutting the cuticular lining of the clusor and proximalmost part of the genital atrium. Whereas only few openings have been found through the cuticle, enough have been identified to be reasonably certain that all of the ducts discharge into the lumen. The staining properties of the cytoplasm vary with the state of the development of the glands, ranging from basophilic early in their development to acidophilic later. There is evidence that these terminus glands, like those of the Cambaridae, having once completed a cycle of secretion, disintegrate, and in areas where their breakdown is occurring, granulocytes and other blood cells seem to congregate. Unfortunately, data are lacking that might correlate their development and disintegration with sperm production or the production of spermatophores.

SPERMATOPHORE AND SPERMATOZOA. In their excellent account of the spermatophore of *Pacifastacus*

leniusculus (probably the nominate subspecies), Dudenhausen and Talbot (1983) presented a brief account and “schematic diagram” of gross features of the male reproductive system. Their description of the “extruded” and “unextruded” spermatophore, utilizing the electron microscope, provides considerable information on the nature of the spermatophore wall and spermatozoa. My much more restricted observations, using only the light microscope, on the spermatophore and spermatozoa of *P. l. trowbridgii* are in agreement with their observations (Fig. 9b,c). The terminology used by them in describing the spermatophore has been adopted herein.

The spermatozoa-packed spermatophore receives its primary coat in the ental part of the coiled segment of the vas deferens and passes ectally as a simple, not convoluted, tubule, receiving the secondary granular coat more distally in the same segment. The outer globular layer is added farther along the vas deferens, and apparently secretions of the lining of the ejaculatory duct form the most superficial part of the coat.

Andrews (1904a:462) noted that the spermatozoa of *Pacifastacus leniusculus* (subsp.?) are twice the size of those of *Cambarus affinis* (Say, 1817) (= *Orconectes limosus*) and possess more than 20 rays. During this study, in the preparations of *P. leniusculus trowbridgii*, the spermatozoa were observed to possess diameters of 9.9–10.6 μm , and while I have been unable to determine the number of rays, in view of Figure 16e,f, Andrews’ estimate of more than 20 seems likely. This large number is quite different from reports on the numbers of rays recorded for the spermatozoa of some European members of the family: 2–15 (see above).

CAMBARIDAE

Previous Studies on the Cambarid Testis

The earliest account of the testis and vasa deferentia of members of the Cambaridae of which I am aware is that of Hagen (1870:22). In it he described in some detail or illustrated the external appearance of the testes of *Procambarus (Ortmannicus) acutus* (Girard, 1852), *P. (Scapulicambarus) clarkii* (Girard, 1852), *Cambarus (Cambarus) bartonii* (Fabricius, 1798), and *Orconectes (Gremicambarus) virilis* (Hagen, 1870), comparing them with that of *Astacus fluviatilis*. Hagen (1870) considered what is designated herein as the “tri-lobed testis” to consist of three testicles, the anterior pair joined “in a membranous hole a little shorter than the testicles, and connected with the third inferior lobe” (p. 22). While Hagen was not aware of the functional differences implied, the following observations proved to be most pertinent to this study: “The

three lobes of the testicles . . . are larger, rounded, and closely approximated in *Astacus*, the vasa deferentia longer than the body. In *Cambarus* the three lobes are small, elongated, and separated; the vasa deferentia shorter than the body" (p. 29).

Dimorphism in adult male cambarids was apparently first observed by Louis Agassiz (and H. J. Clark, according to Faxon, 1884:42), who recounted his observations to Hagen (Hagen, 1870:22), but that the two forms of the male represented alternating stages in the life cycle of a single individual was not realized until Faxon reported this discovery in 1884.

Harris (1901) endeavored to correlate testicular activity with the form of the male in *Orconectes (Gremicambarus) immunis* (Hagen, 1870) and *O. virilis* but found no differences between "testes of first and second form males taken at the same time of year, either as regards gross anatomy or microscopic structure" (p. 59). His samples were obtained in August, September, March, April, and May. Perhaps his major contributions were that (1) the alternation of the two forms of the male may be interrupted when, at least occasionally, a second form male molts to second, rather than to first form; and (2) the suggestion that the parallelism in spring and fall molts in the cambarids and astacids is perhaps not insignificant.

Andrews (1904a, b) presented a detailed description of the sperm of *Cambarus affinis*, noting the capsular mucous- or jelly-like coat "enveloping the bowl and the coiled-up arms" (1904b:461). He found that the number of rays in the sperm of this species to range from five to seven and those of *Cambarus bartonii*, from six to eight. The rays in *O. limosus* may be coiled to the right or left.

Fasten's (1914) study of the testes of *O. virilis* and *O. immunis* marked the first detailed histological and cytological study of cambarid testes and provides an excellent review of much of the literature treating the testes of astacids. Testicular activity throughout the year is summarized and correlated with periods of copulation. Emphasis is placed upon cytological aspects of spermatogenesis, and he reported the diploid number of chromosomes to be 200 for *O. virilis* and 208 for *O. immunis*. That his interpretation of the life cycle of the acini differs from that recorded by Word and Hobbs (1958) and most subsequent students is made clear in the statement that "The germinal epithelium can best be studied in tubules which are filled with ripe spermatozoa. In these it is found lining the walls of the cavity (Fig. 78)" (Fasten, 1914:605). His figure 78 is a photograph of a section through an acinus in which the spermatozoa are being concentrated centripetally. He did not realize that after the sperm have been moved to the

core of the acinus and expelled into the collecting tubule, the "cells" (designated herein as "secondary syncytia"), along with their large complex nuclei, disintegrate and the remains are evacuated to the collecting tubule, thereby contributing to the medium transporting the spermatozoa and leaving behind only the shrunken mass of connective tissue that had ensheathed the acinus. This study was followed by a cytological investigation of spermatogenesis in *Cambaroides japonicus* by Niiyama (1934) who reported the diploid chromosome number in that crayfish to be 196. Penn (1943) noted that the spermatozoa of *Procambarus clarkii* possessed four or five radiating arms.

McCroan (1940) reexamined spermatogenesis and spermioteleosis in *Cambarus virilis*, paying particular attention to Golgi material and mitochondria. Baron (1951) undertook a study of the cyclic behavior of the testis of *Cambarus longulus*, correlating activity of the testis with the seasons. She recognized two cycles of sperm production (May to September and October to May), with the greater amount of sperm produced by second form males in the fall. She found the diploid number of chromosomes in this crayfish to be 196. The same chromosome number was reported for *Cambarus (Puncticambarus) acuminatus* by Word, 1953.

Apparently unaware of the work of McCroan and Baron, Suko (1955) studied the testis of *Procambarus clarkii*, examining specimens collected from late July to early August, and from late March to early April. He described the testis of a "hatched larva with 4.5 mm body length," and those of specimens with body lengths of 20 and 25 mm as well as those of adults, comparing development with that of the ovary, pointing out that whereas the spermatogonia are usually located in the "medullary portion" of the gonad, the oogonia are found in the "cortical portion." Unfortunately, he reversed the orientation of the testicular lobes 180 degrees, but he made the important observation that "the fore-lobe [actually the posterior] is larger in size than the post-lobe. . . . Therefore, it seems to be formed by the fusion of two lobes in the embryonal stage as in the case of the ovary" (p. 40). Whether or not he was aware that the left vas deferens does not function in the transportation of sperm in this crayfish is not evident, but he remarked that "The right seminal canal is far longer, about twice the length of the left one" (Suko, 1955:40). His figures 8 and 11 and observations in this study do not seem to support his belief that "there occur the spermatogonia, each of which is surrounded by the intermediate cells among the old vacant cysts" (p. 43). Moreover, in describing the spermatozoa as being "of a hexahedrons form with four processes on each angle . . . but not an asteroid with

several long project[ion]s” (p. 43), he apparently was unaware that in his sectioned preparations the “projections” were fixed in the early stages of becoming unwound. Suko also stated that “at copulation the spermatozoa flow out by the destruction of the cyst at one part and by the contraction of the surrounding follicle layer” (p. 43). It seems unlikely to me that the exodus of spermatozoa from disintegrating acini is initiated by copulation.

The first account of testicular structure and activity based on sectioned material collected throughout the year was that of Word and Hobbs (1958) on *Cambarus acuminatus*. The emphasis in their study was in correlating changes in testicular anatomy with sperm production and, secondarily, in associating this activity with the form of the male. As Baron (1951) found in *C. longulus*, they noted a major annual cycle of sperm production beginning in May with maximum sperm-filled acini in the fall, accompanying the molt from second to first form males; also, they recognized a comparatively insignificant “winter cycle,” which appears to contribute little sperm. Anatomical features and changes in the spermatid cycle reported by them are reviewed below.

In Johnson’s (1960) investigation of the histology of the sperm duct of *Cambarus longulus*, he recognized three segments; the ectalmost, which he termed the “shaper,” lies within the phallic papilla; the adjacent segment, made most obvious by its heavy muscular coat, was designated the “ejaculatory duct,” and the entalmost segment, joining the testis, the “vas deferens.” He also described a three-layered spermatophore.

Moses (1961a) described and illustrated, by means of a micrograph, a phase micrograph, and electron micrographs, the structure of the mature spermatozoa of *P. clarkii* and, in a second article (1961b), presented a detailed account of spermiogenesis in this crayfish based on conventional light and electron microscopic studies. While there is no reason to question the validity of Moses’ description of what is taking place in his figures 3 and 4 (1961a:233), I do have reservations that the “discarded cytoplasm” of most of the spermatids is “engulfed” directly by the sustentacular cells (= accessory herein), for only those on the periphery of the core of spermatids are intact; the latter, accompanying spermiogenesis, amalgamate to form the primary syncytium supporting the encapsulated spermatozoa. For the same reason, the “finely granular material” forming what is termed in this study the “capsule” that envelops each spermatozoan, cannot be presumed to have been “laid down [directly] by the sustentacular cells,” rather, if not by the spermatids themselves, perhaps by the primary syncytium. There is every reason to believe that Moses was correct in

stating that “It is probably this shell that ruptures or is dissolved in water, releasing the arms” (op. cit.).

Yasuzumi et al. (1961) conducted an electron microscopic investigation of nuclear and cytoplasmic differentiation in the development of sperm in *Cambaroides japonicus*. Kaye et al. (1961) were concerned with the endoplasmic reticulum during spermatogenesis in the same crayfish. Both of these studies are beyond the scope of this investigation. In his study of the life history of *Cambarus longulus*, Smart (1962) included observations on gross testicular development and found it to follow that reported by Baron (1951) and Word and Hobbs (1958). In Black’s (1966) study of the cyclic male reproductive activity in *Cambarellus (Pandicambarus) puer* Hobbs, 1945 and *Cambarellus (Dirigicambarus) shufeldtii* Faxon, 1884, he found the basic structure of the testis and sperm production to be virtually identical to that noted for the cambarids previously studied; his illustrations indicate that both vasa deferentia transport sperm. Anderson and Ellis (1967) were concerned with mitochondria and microtubules in the developing sperm of an unidentified member of the genus *Cambarus*.

Payne (1968), in investigating the life history of *Procambarus (Ortmannicus) hayi* (Faxon, 1884), depicted and briefly described gross morphological changes in the testis of this crayfish in a series of specimens having carapace lengths ranging from 23 to 42 mm. He noted that with the addition of new acini, the paired anterior lobes of the testis often became bifurcate, resulting in a testis with four or five lobes. He also pointed out that the right vas deferens was “larger, more highly convoluted, and generally of a white opacity not noted in the left” (p. 48).

Albaugh (1973) compared the life histories of *Procambarus acutus* and *P. (Capillicambarus) hinei* (Ortmann, 1905), and showed that the testes of these two crayfishes are similar in all respects to those of cambarids previously investigated; he also noted that the left vas deferens of the former species is not functional as a sperm conduit, but he noted sperm in the ental part of both vasa deferentia of *P. hinei*.

Some of the more recent studies of the testis of cambarids are those of Wielgus-Serafinska (1973, 1976), who was concerned with morphological and histological changes in that of *Orconectes limosus*. In preparing his detailed account (1976), he was apparently unaware of the similar previous study of Word and Hobbs (1958) on *Cambarus acuminatus*, but his presentation makes the two quite comparable. While the interpretations of structures and events in the two studies are strikingly similar in most respects, a few differences in terminology and detail exist, as apparently do some

in his 1973 and 1976 accounts. Of particular importance to the study reported here was his statement (1976:100), which is, in part, puzzling: "According to other investigators (Fasten 1914), this phenomenon [the lengthening of the testicular stalks with repeated seasons of sperm production] may be diagnostic for the subfamily Cambarinae." This is puzzling for I have been unable to locate any reference that proposes this apparently almost (the Cambarellinae must also be included) valid generalization. It could not have been Fasten, for the subfamily was not proposed until 1942! Smart (1962) is the only one among the references cited by Wielgus-Serafinska in which the testicular stalks are mentioned, and it should be pointed out that Smart's study was based on *Cambarus longulus*, not *Orconectes virilis* as noted by Wielgus-Serafinska.

Very brief accounts, containing no new information, of the male reproductive systems of the Astacidae and Cambaridae were presented by Huner and Barr (1981), Holdich and Reeve (1988), and Nishikawa et al. (1990).

In the most recent report on aspects of the male reproductive system in crayfishes (principally based on a study of the testis of *Procambarus clarkii*, Krol et al. (1992) reported interpretations that are at variance with those of Word and Hobbs (1958) and Wielgus-Serafinska (1976) on *Cambarus acuminatus* and *Orconectes limosus*, respectively, and with my observations on the testis of *Procambarus fallax*. They do not mention the trifoliate aspect of the testicular lobes, perhaps leading one to presume that the testes of astacids and cambarids do not differ from the H-shaped testis/es of most decapods. While they refer to paired "genital ducts" consisting of vasa deferentia and ejaculatory ducts, they indicate that it is the former that opens on the coxae of the fifth pereopods. Instead of recognizing the testis as a compound acinar gland, the sectioned acini are interpreted as seminiferous tubules, which clouds the meaning of the statement that "As testicular maturation progresses, a germinal layer of spermatogonia develops at one side of the periphery of the seminiferous tubule periphery. . ." (Krol et al. 1992:315). The explanations of the excellent micrographs (p. 322) do not agree entirely with my interpretations. In figure 42, the acinus labeled "CD" (aluminol cords) appears to me to contain spermatids and exhibits the nuclei of four accessory cells (their "nurse cells"). The acinus labeled "ST" (spermatids) according to my interpretation is one in which the spermatozoa have been concentrated in the central areas of the acinus, ready to be moved to a collecting tubule. Those acini immediately below the latter seem to be ones in a slightly earlier stage in the life cycle of the acinus, one in which the spermatozoa have not yet been concentrated in

the central area, as are those overlapped by the numbers "42" and "43." The triangular mass in figure 43 appears to consist of degenerating acini, their state following the expulsion of spermatozoa to the collecting tubules. Their figure 55 is magnificent but most puzzling to me. The cells that are identified as primary spermatocytes resemble spermatogonia, but the unidentified large peripheral nuclei are unlike those of accessory cells which, moreover, have never been observed by me to be so prominent in acini containing spermatogonia. The smaller nuclei on the periphery of the acinus resemble ones that I have associated with connective tissue. The cell underlying the "NC" is indeed a "nurse cell" (= accessory cell in this study) as is that immediately to the left, but notice the irregular, compound nature of the nucleus, which is strikingly unlike the large peripheral nuclei in the acinus below.

Although the cambarid testis has been described in some detail by Suko (1955), Word and Hobbs (1958), and Wielgus-Serafinska (1976), for comparative purposes it seems appropriate to review at least some of their observations here. Because a rather fundamental difference in testicular activity appears to exist among the subfamilies of the Cambaridae, a description of the testes of *Cambarus acuminatus* is presented to provide a comprehension of this organ in the Cambarinae and Cambarellinae. Observations on the testis and sperm duct in the Cambaroidinae were recounted above.

Unlike the testis of members of the family Astacidae and that of the cambarid subfamily Cambaroidinae, that of the other Cambaridae, although also tri-lobed, changes both seasonally and, even more spectacularly, at each breeding season. Word and Hobbs (1958, figs. 17–26) illustrated the gross changes that take place in the testis during the life cycle of *Cambarus acuminatus*. Prior to the first breeding season the testis is a rather compact tri-lobed organ that becomes altered in preparation for and during the first breeding season. While retaining the three lobules, they become displaced apically, joined by a short Y-shaped stalk and, a year later, with the approaching second breeding season the stalks begin to lengthen further. By the close of the third (and last in species investigated) breeding season, the length of each of the stalks, particularly the posterior one, much exceeds the length of the corresponding testicular lobule. As pointed out by Wielgus-Serafinska (1976) stalks do not develop in members of the genus *Astacus*. They have not been observed in *Pacifastacus l. trowbridgii* and probably do not occur in any members of the family Astacidae or in those of the Cambaroidinae. Assuredly they are not present in members of the Parastacidae, Nephropoidea, Enoplometopoidea, and Scyllaridea.

As for gross features of the sperm ducts, considerable variation occurs among the Cambaridae, ranging from the much looped, long, left duct in *Procambarus (Ortmanicus) zonangulus* (Fig. 1g)—the right is nonfunctional in conducting spermatozoa, shorter, and translucent—to those of the single-looped, very short ducts in *Orconectes rusticus* (Fig. 1h). The ducts observed in other members of the Cambarinae are somewhat intermediate in the degree of coiling and total length.

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. The arrangement and histological features of the primary collecting ducts

and the collecting tubules differ little from those of the Astacidae until the onset of the first breeding season. One of the more obvious differences is the lack of recognizable generative cells except in the enlarging blind ends of protubules, the first indication of a developing acinus. In preparation for the breeding season, the spermatozoa are transferred from the acini through the collecting tubules and, like the accompanying acini, the tubules degenerate (Fig. 20). This leaves an enlarged primary collecting duct embedded in the mass of atrophied acini and collecting ducts, which make up the major part of the stalk. In the meantime, the proximal part of the primary collecting duct and its adjoining tubules have “budded” a new generation of acini. In preparation for the next and subsequent

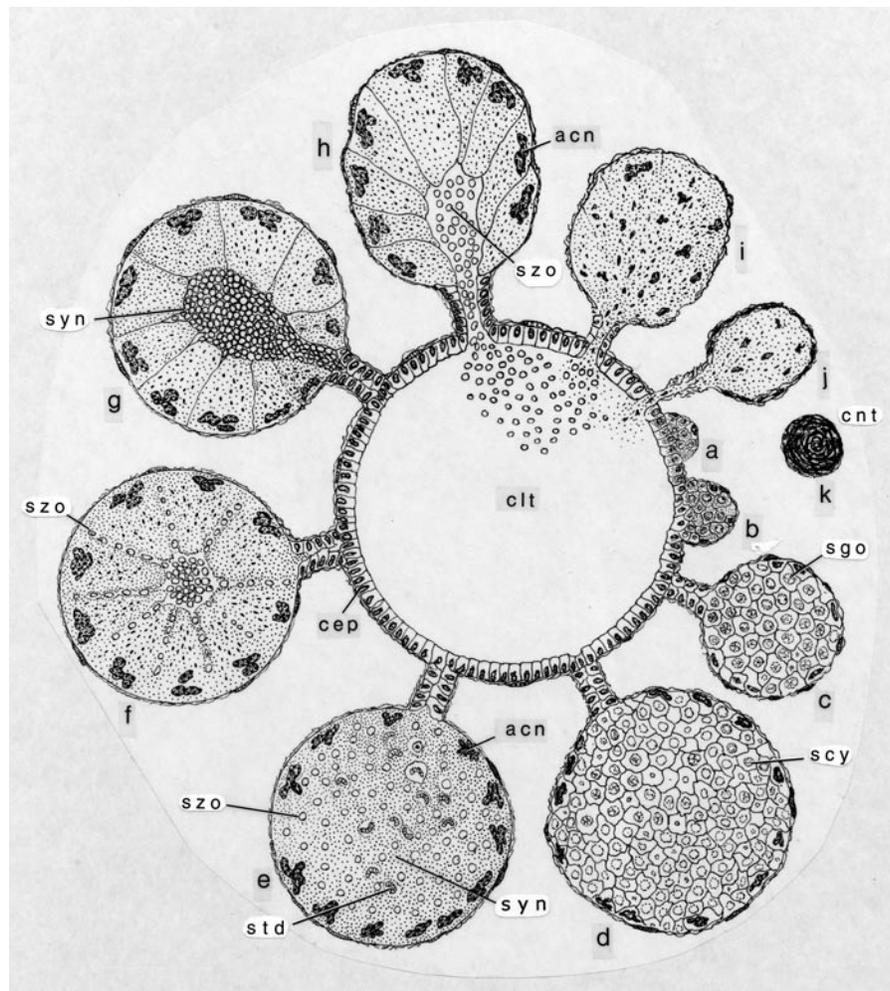


FIGURE 20. Schematic diagram of life cycle of acinus in *Cambarus acuminatus*: a, rudimentary acinus on wall of collecting tubule; b, young acinus; c, with spermatogonia; d, with spermatocytes; e, with spermatids and spermatozoa in massive syncytium; f, with spermatozoa being concentrated centrally; g, with secondary syncytia enveloping spermatozoa; h, with spermatozoa being expelled; i, j, degeneration of acinus in progress; k, completely degenerated with connective tissue remaining as remnant.

breeding seasons the same events are repeated, with a lengthening of the primary collecting duct (and stalk) and the addition toward the ental ends of the testis of a new generation of collecting tubules and acini. Because of the origin of acini from and close association with collecting tubules the early stages in the development of both are discussed together.

ACINI. The primordium of an acinus appears as a bud from the trunk of a collecting tubule, never from the wall of a preexisting acinus, near the periphery and toward the blind end of one of the three testicular lobules. Elongation with an apical increase in volume and number of cells results in a bulbiform mass of cells joined to the collecting tubule by a slender pedicel, protubule, in which no lumen is evident. Soon three types of cells are discernible in the bulbous area: spermatogonia and accessory cells surrounded by a thin connective tissue coat (Figs. 21a,b; 22a). Within the acinus, the spermatogonia, which occur in greatest numbers, have spherical or subspherical nuclei that are clearly larger (diameter 12.3–14 μm) than those of the accessory cells in which the finely divided chromatic elements are scattered. The nuclei of the accessory cells are elliptical in section (diameters 5.3–7.0 and 8.8–10 μm), and contain one or two prominent chromatic bodies. The nuclei of the connective tissue cells do not differ conspicuously from those of the accessory cells except generally they are more flattened, often distinctly less than 4.0 μm in thickness, but rarely more than 10 μm in length. Within the acinus mitotic figures are rather commonplace but seldom appear simultaneously in more than two or three cells in these “growing” acini. Until the spermatogonia have transformed into primary spermatocytes (Fig. 22b), initiating the first meiotic division, their cytoplasm has a greater affinity for basophilic stains and appears more dense than does that of the spermatocytes. Whatever triggers the onset of meiosis in the spermatocytes must stimulate most of them within an acinus (often within several adjacent and nearby acini as well) into action simultaneously, for there appears to be a high degree of synchronicity in the meiotic processes (Fig. 21c–d). Within the acini, those containing secondary spermatocytes can be distinguished rather easily from those with primary spermatocytes by the smaller size and larger number of generative products. And there is an added feature; an increase in the size and in the aggregation of the subperipheral accessory nuclei accompanies the approaching second meiotic division. Following the latter, the acinus becomes packed with spermatids that are recognizable by their peculiarly “banded” concavo-convex shape (Fig. 22c). With their transformation in spermiogenesis, the spermatozoa now situated in individual vacu-

olar spaces (Fig. 22d) are dispersed throughout the interior of the acinus, leaving a primary syncytial mass supporting them and clumps of accessory nuclei, the latter lying, for the most part, near the basement membrane adjacent to the connective tissue layer. In some unexplained manner, some of the sperm cells are brought together in a cluster near the center of the acinus, sharing a common cavity within the syncytium (Fig. 22e). A partitioning of the syncytium, which appears to progress centrifugally from the central cavity, into smaller pyramidal secondary syncytial units ensues (Fig. 22f), and the spermatozoa that were situated more superficially in the mass become shunted to lie along the developing partitions, afterward passing centripetally to the centrally located lumen (Fig. 21e, f).

By this time, a lumen has appeared in the developing collecting tubule, which has also acquired a superficial thin layer of striated muscle fibers intimately associated with the connective tissue coat. With the passage of the spermatozoa into the collecting tubule, and often joining them, the entire body of the acinus within the connective tissue coat degenerates and accompanies the exodus of the spermatozoa (Fig. 22g,h). As the volume of the acinus begins to decrease, the connective tissue coat collapses and in so doing, becomes progressively more obvious. Most of the collecting tubules likewise degenerate, and with the acini, no longer recognizable as such, become a part of the spongy network, largely connective tissue, which surrounds the primary collecting ducts of the elongating trifurcate stalk joining the testicular lobes.

MICROANATOMY OF THE SPERM DUCT

VAS DEFERENS. Reflecting the degeneration of those acini that have produced a generation of spermatozoa and become atrophied, little is left behind in the stalk other than an enlarged primary collecting duct surrounded by masses of connective tissue. There is no testicular segment of the vas deferens. Rather, in the area surrounding the base of the vas deferens, the stretched primary collecting duct is surrounded by such a thin layer of connective tissue that there is no room within the stalk to accommodate the base of the vas deferens. The latter, extending posterolaterally from the wall of the testicular stalk, consists of an acidophilic, highly vacuolate, tall, columnar epithelium (35–90 μm in height), which in sperm-packed ducts is conspicuously thicker than the more superficial basement membrane and connective tissue-muscle fiber layer (nowhere more than 8 μm in thickness). In spermophore-bearing segments of the duct, generally the thickness of this connective tissue-muscle layer is only slightly greater

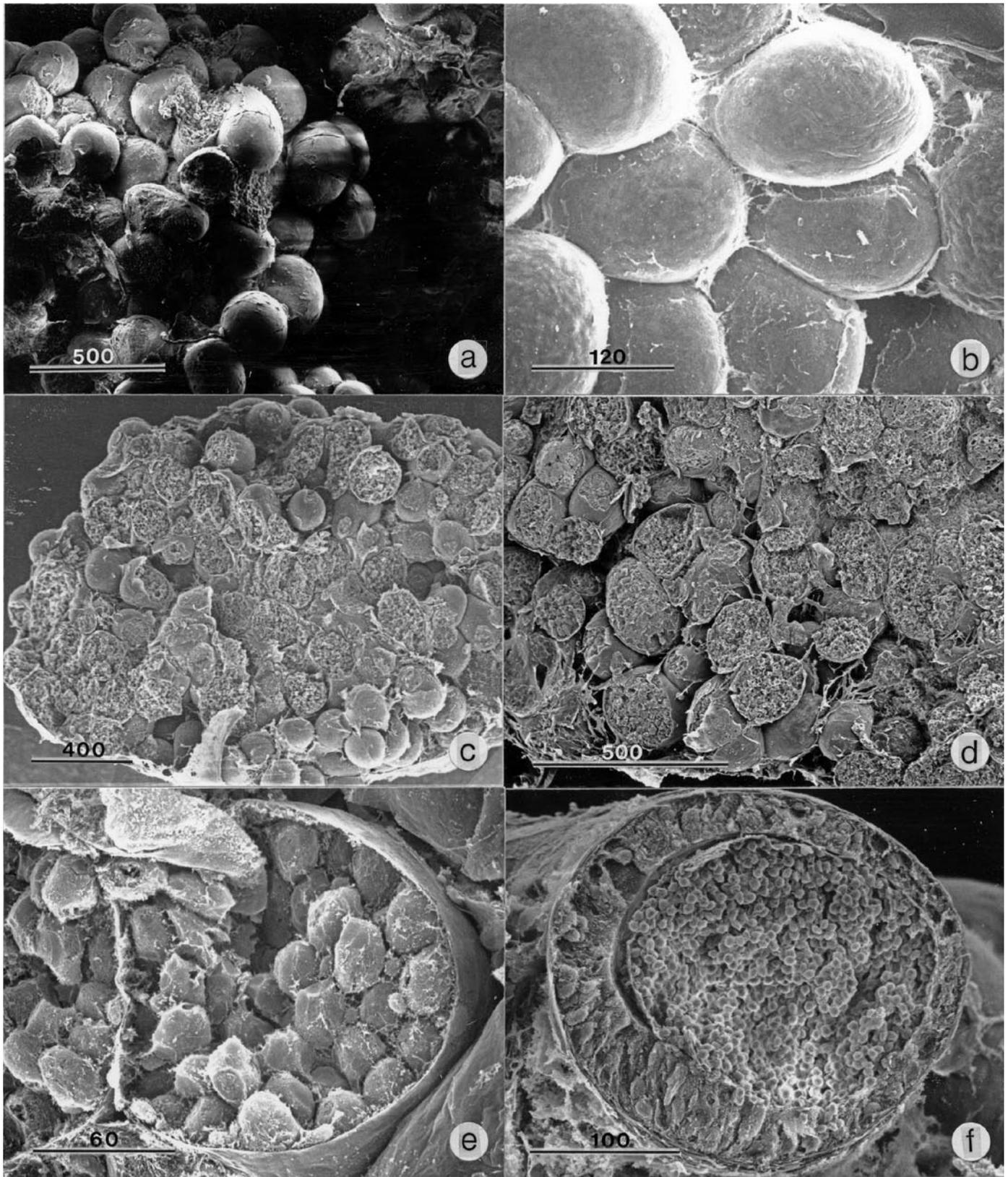


FIGURE 21. Scanning electron micrographs (μm) of testis of *Cambarus acuminatus*: a, b, simple acini ensheathed in connective tissue-epithelial coat; c, d, acini in different stages of spermatogenesis; e, acinus with ensheathed spermatozoa (?); f, proximal end of vas deferens at junction with testis (note lumen packed with ensheathed spermatozoa).

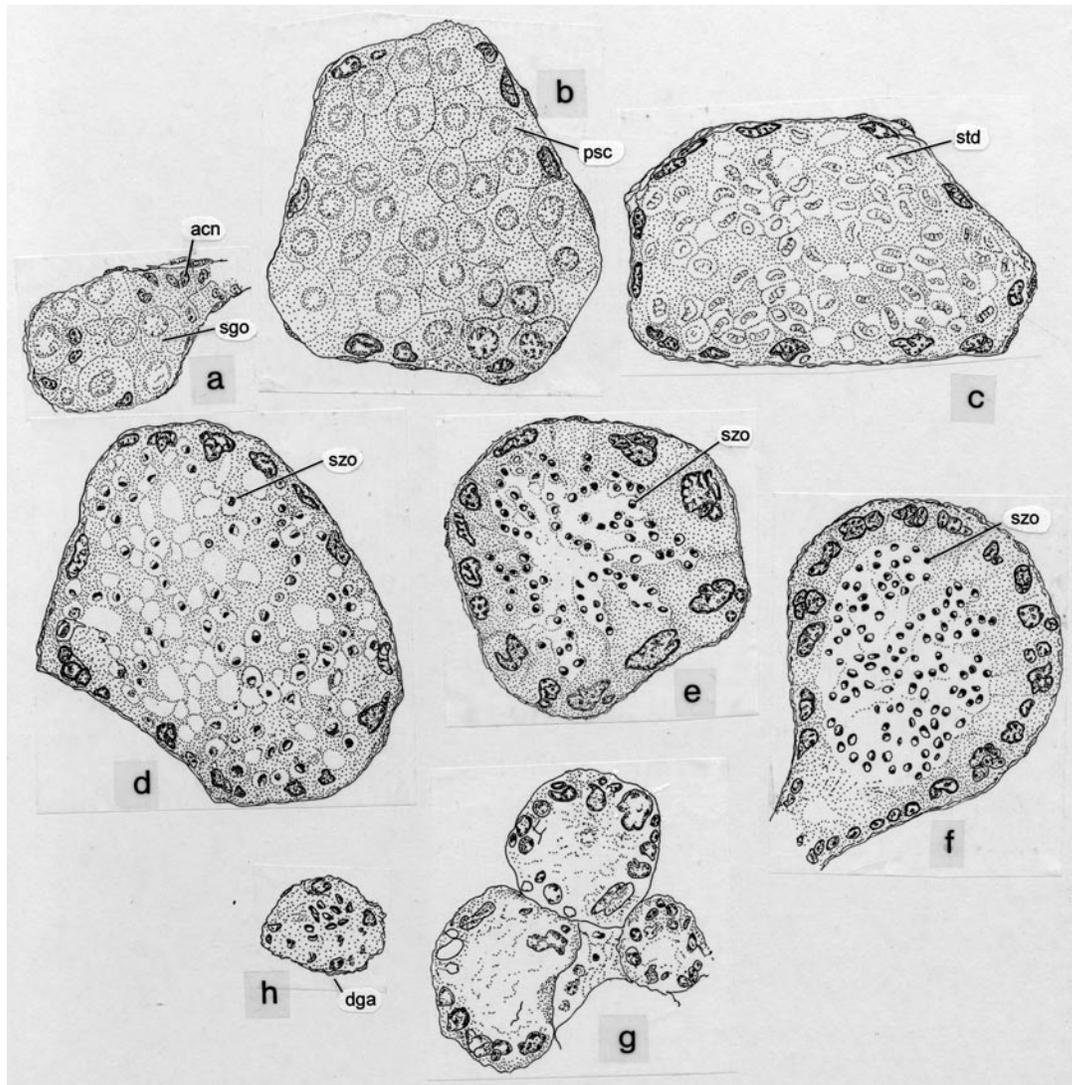


FIGURE 22. Elements of testis of *Orconectes rusticus*: a, young acinus with multiplying spermatogonia and nuclei of accessory cells (cell membranes not recognizable); b, acinus with primary spermatocytes; c, acinus with spermatids; d, acinus with spermatozoa in vacuolar spaces within continuous syncytial mass; e, acinus with spermatozoa being moved to central area as secondary syncytial elements develop; f, acinus with spermatozoa concentrated in central area surrounded by secondary syncytia; g, degenerating acini from which spermatozoa have been moved to collecting tubules; h, degenerate acinus with few remaining spermatozoa and broken-down nuclei of syncytia.

than the diameter of the muscle fibers within it, and thus the expected layering of deep longitudinal and more superficial circular fibers is not evident.

Along its length, there are no obvious changes in the anatomy of the vas deferens until the muscular wall begins to thicken, marking the transition to the ejaculatory duct, as the latter progresses toward the coxa of the fifth walking leg. In some areas, the epithelial lining may completely disappear, having disintegrated in the process of liberating its secretion/s.

EJACULATORY DUCT. Insofar as I am able to discern, the ejaculatory duct differs anatomically from the vas deferens only in possessing a much heavier muscular wall in which there is clearly an internal layer of fibers primarily directed in the longitudinal axis of the duct and a more superficial one in which the primary disposition is circumferential. The glandular epithelial lining is about the same height as that of the vas deferens, but largely basophilic and, especially in first form males, may be broken down in secretion that in Mallory's stain appears as blue

strands and small red globules. These products, which are obvious in the lumen prior to the arrival of a spermatophore from the vas deferens, appear not to be incorporated into the formation of the spermatophore wall, though they may well play a role in modifying the wall's consistency or facilitating the passage of the spermatophore through the duct and the adjoining cuticular terminus. In a preparation of the more distal part of the sperm duct of *Cambarus longulus*, for approximately 500 μm proximal to the junction of the ejaculatory duct and terminus, a strongly developed bundle of oblique and longitudinal muscle fibers along the side of the duct closest to the coxal wall anchors it to the latter; with little doubt, these extrinsic fibers serve in the withdrawal of that segment and the more distal part of the sperm duct. At about the distal end of this muscular bundle, terminus glands (see below) appear on the side of the duct where, slightly more distally, the concave side of the adjoining clusor occurs.

TERMINUS. The cuticular-lined terminal part of the sperm duct is quite variable in length, the longest observed was in *Procambarus zonangulus* (Fig. 10f). It is much shorter in the members of the genera *Cambarus* (Fig. 10h) and *Orconectes* that I have examined. Described here is that in *Orconectes rusticus* (Fig. 23).

The genital pore, which leads to the genital atrium, is located on the side, and some distance (as much as 400 or 500 μm) proximal to the tip of the phallic papilla (Figs. 10g, 23). The cuticular lining of the atrium differs in no conspicuous way, other than being slightly thinner, from the exterior cuticle of the papilla. Joining the genital atrium, the clusor (U-shaped concavo-convex cuticular lining of the pre-apical part of the sperm duct and associated tissues) extends proximally (Fig. 23) for about 450 μm , most of it situated within the phallic papilla. In the longer proximal segment, the clusor cuticle on the concave side is distinctly much thicker (averaging 8.9 μm) than that on the convex side (2.7 μm). It is laminated, for the most part acidophilic, but in the proximal part of its length the innermost lamina abutting the lumen is basophilic at least sometimes. Adjacent to the cuticle is a cuboidal to columnar epithelial layer intimately associated with a loose acidophilic connective tissue, strands of the latter extending to the wall of the phallic papilla. Around the more distal part of the clusor (Fig. 23a,b) the connective tissue divides the haemocoelic space into compartments that, with little doubt, retard the flow of the blood and, with an increase in pressure, brings about an extension of the phallic papilla and the eversion of at least a part of the wall of the genital atrium.

Proximally, layers of striated muscle fibers embedded in fibrous connective tissue are added (Fig. 23c): an inner

one of largely obliquely longitudinally disposed ones and a more superficial, primarily encircling layer. The longitudinal layer is not of uniform thickness, tending to be concentrated in bands, the heaviest one lying within the concavity of the clusor. In contrast, the band of circular fibers, also intimately associated with connective tissue, is more uniform in thickness, although the layer is interrupted at intervals by short stretches of connective tissue, and along some of the length of the clusor thicker on the concave side. Many, if not most, of the longitudinal fibers are affixed to the cuticle by tonofibrils that extend from the muscle fibers between the epithelial cells. These are evident along the proximal two-thirds to three-fourths length of the clusor but are sparse or even absent along the distalmost part. This description of the epithelium and tonofibrils needs further elaboration for, particularly along the proximal third of the length of the clusor, not even a trace of the epithelium persists; only a dense mat of tonofibrils interspersed with granulocytes lies adjacent to the cuticle. More distally, nuclei of epithelial cells are discernible among the fibrils, and, as just stated, in the distalmost part tonofibrils are wanting, although the epithelial layer is traversed by more delicate connective tissue fibers. This variation along the length of the clusor provides a probable panorama of the sequence of events occurring in the formation of proximalmost part of the clusor. In the early stage of cuticular secretion the epithelium consists of tall columnar cells on the concave side and cuboidal ones on the convex side; delicate connective tissue fibers extend through the layer and are anchored in/on the cuticle. As the ultimate laminae are added, the epithelial cells become completely broken down in secretion (perhaps a crowding factor is involved) while the attendant connective tissue fibers are being modified to or replaced by the much more prominent tonofibrils. The presence of numbers of granulocytes among the tonofibrils seems to be indicative of the "cleaning" needed following the destruction of the epithelial elements. As the muscle layers diminish distally such crowding is diminished and the epithelial cells are interspersed with tonofibrils, replacing the latter along the distalmost part of the clusor.

Associated with the clusor is the presence in the adjacent haemocoelic space of a number of terminus glands, the ducts of which permeate the cuticle. At least structurally, these complex epithelial masses seem to be typical of "tegumental glands" (Fig. 23c-e) that have been reported to be associated with a wide variety of cuticular invaginations. Their presence in *Cambarus longulus* was described in detail and illustrated by Johnson (1960:231, figs. 1, 3, 4). He pointed out their similarity to those found in the

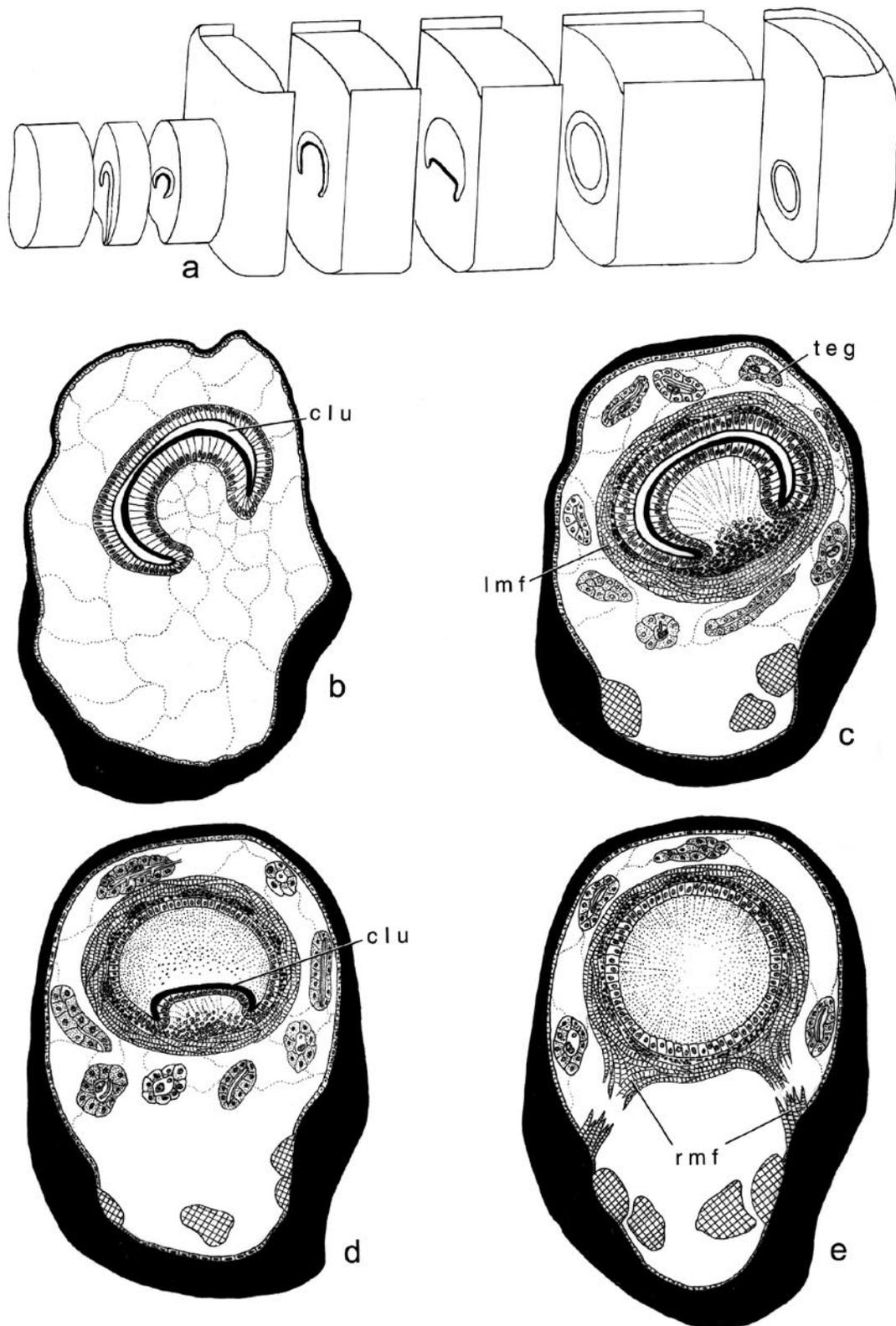


FIGURE 23. Diagrammatic representation of coxa and phallic papilla of a cambarid, based on *Orconectes rusticus*: a, caudoventral view of coxa and phallic papilla containing ectal part of ejaculatory duct and terminus (cluser and genital atrium); b, basal part of phallic papilla with cluser; c, basal part of cluser with strongly developed longitudinal muscle at base of concave side and flanked superficially by “tegumental glands”; d, entalmost part of cluser where cuticle is limited to thickened concave side; e, ectal part of ejaculatory duct; retractor muscles.

fore- and hindguts of the crayfish described by Richards (1951) but questioned the limited function noted by Richards (i.e., its role in forming the epicuticle). Johnson found no glands in the second form male of *Cambarus longulus*, and I have noted in *Orconectes rusticus* a marked reduction in the number and sizes of these glands in a second form male. Inasmuch as spermatophores are not known to be shed by second form males, spermatozoa apparently not reaching the ejaculatory duct until just before the crayfish molts to first form, I join Johnson in suggesting that these tegumental-like glands are more likely to function in some capacity associated with the production or expulsion of spermatophores. Is it possible that their function is primarily concerned with lubrication or in hardening the outer secondary spermatophore layer rather than, or perhaps in addition to, contributing to the epicuticle? I have noted no structural difference in the cuticular lining of the terminal part of the sperm duct in first and second form males. There is good reason to believe that in *O. rusticus* these glands are at least in part ephemeral, shedding their secretion and then becoming atrophied. The nuclei of the secretory "peripheral cells," after the latter have become packed with acidophilic granules, become irregular and appear shriveled, and these and the "central cells" seem soon thereafter to disintegrate. In the second form male examined by me, collected a month or so before the anticipated molt to first form in the fall, the comparatively very small secretory cells of these glands lack the granular elements that are characteristic of the larger secretory cells present in first form males collected in November. Moreover, there is no evidence of degradation among them.

The presence or absence of a genital atrium and, if present, its length are dependent upon the extent to which the terminal part of the phallic papilla is inverted. If the clusor joins the genital aperture directly, the genital atrium, as such, does not exist; if, however, the clusor is retracted, then the length of the atrium depends on the length of the inverted segment. Like the integument elsewhere, when recognizable it consists of a thin cuticle underlain by a cuboidal to squamous epithelium overlying a loose connective tissue continuous with that permeating the haemocoelic space within the papilla.

Unfortunately, I have been unable to observe the action of the clusor in its postulated function as a substitute for a sphincter or during the passage through it by a spermatophore. I suspect that obstruction (reduction or obliteration of the lumen by the approximation of the thickened concave wall and the opposing thinner convex one) is brought about largely by its basic structure but made more secure by the contraction of the circular muscle layer surrounding

its proximal part and, more distally, by those stretched between and across the extremity of the arms of the U-shaped cuticle. Opening of the lumen almost certainly results from force supplied by peristaltic action of the muscular wall of the ejaculatory duct, assisted by contraction of the longitudinal musculature of the clusor, particularly the band on the concave side that serves to depress the thickened concave element. There is no such assistance, however, along the distal part of the clusor. Consequently, I suspect that the spermatophore would become stalled in its passage through the clusor without the pressure provided by the ejaculatory duct.

SPERMATOPHORE AND SPERMATOZOA. The first stage in the production of the primary wall of the spermatophore appears about 35 μm from the junction of the vas deferens and the wall of the testicular stalk. There, it consists of an amorphous, pale tan (in Mallory's Triple Stain) secretion that flows between the more superficial elements of the core of spermatozoa and envelops a part of the spermatophore mass. A short distance distally this secretion completely surrounds the core, thicker on one side than on the other. Embedded within the amorphous material is a dense layer of many rod-like bodies of varying lengths, closely packed in a layered fashion and oriented with their long axes in the longitudinal plane of the spermatophore. On the thicker side of the developing spermatophore the wall is some 53 μm : the inner amorphous layer, 21 μm in thickness, the middle rod-bearing layer, 19 μm , and the superficial amorphous layer, 13 μm . In Mallory's Triple Stain, the rods stain dark brown to bright red. In haematoxylin and eosin, the rod-like bodies are less conspicuous, the layer containing them appearing almost red and the other layers pale pink. In many areas the inner amorphous layer is not continuous, so that the overall impression is a dark, rod-bearing inner layer and an outer amorphous one. The ruptured encapsulated spermatozoa in which the rays are partly "unfurled" reveal rudiments of five or six rays.

Comments on Related Aspects

In describing amplexus in *Cambarus affinis* [= *Orconectes limosus*], Andrews (1904a) pointed out that one or the other of the fifth pereopods is brought to lie subperpendicular to the longitudinal plane of the body, appressed to its ventral surface, projecting from the opposite side, and supporting the partly extended, interlocked first and second pleopods. The function of supporting the pleopods during amplexus was emphasized by Andrews, but he may have overlooked an equally important function related to the extension of the phallic papilla. In the astacid and

cambarid crayfishes the ventral surface of the coxa of the fifth pereopod is membranous and, with total adduction of the appendage at the coxa-basis articulation, the basis is pressed against the ventral coxal membrane effecting, I suspect, a rise of the blood pressure within the podomere and a concomitant extension of the phallic papilla with an eversion of at least a part of the genital atrium. Retraction is brought about by two or three groups of muscle fibers (Figs. 19b, 23e) extending from the muscular coat of the ejaculatory duct to thickened areas on the cuticle of the coxal wall. In Figure 23b, a pair of these muscular bundles is labeled *retractor muscles*. Although in section the bundles appear to emerge from the circular layer, some of the bundles are enmeshed with the conspicuous longitudinal muscular band situated on the concave side of the clusor. Elements of the band are anchored along the superficial side of the thickened invaginated cuticular wall.

For some years I have been aware of the probable correlation of a single functional sperm duct and asymmetry in the first pleopods of the male (the base of the sinistral member situated markedly to slightly posterior to that of the dextral member of the pair). Payne (1968) and Albaugh (1973) reported the absence of spermatozoa in the sinistral sperm ducts of *Procambarus hayi* and *P. acutus*, respectively, both of which have asymmetrical first pleopods, and in all of the crayfishes with such pleopods that I have examined, the sinistral sperm duct has not been observed to carry spermatozoa. In all except one of those species with symmetrical pleopods in which the sperm ducts have been examined, both ducts were opaque, denoting the presence of spermatozoa. That exceptional species is *P. zonangulus*. This finding was not surprising because the latter species is definitely otherwise allied to species in which the pleopods are asymmetrical, a feature that in this crayfish is believed to represent a genetic reversal from the advanced asymmetrical arrangement back to the more primitive symmetrical one; the sperm ducts, however, have maintained the advanced condition: only the dextral member transports spermatozoa. As mentioned above, Andrews found that during amplexus in *Orconectes limosus*, either the right or the left fifth pereopod may be adducted to support the first pleopods, it seems likely that only the dextral member might be employed in successful sperm transfers to the female in those species with asymmetrical pleopods and in the closely related *P. zonangulus*.

CAMBAROIDINAE

The testes of specimens of *Cambaroides japonicus* (Fig. 1f) and *C. similis* (Fig. 1j) available to me are more

distinctly Y-shaped than are those of members of either the Cambarinae or Cambarellinae. Moreover, unlike other cambarids but like the astacid *Pacifastacus leniusculus trowbridgii*, they do not become stalked with the passage of their first breeding season, reflecting the absence of a massive permanent degeneration of the acini that participated in the production of spermatozoa in the breeding season just passed. Perhaps partly responsible for the slender appearance of the three tapering lobules is the stage of testicular activity at which the specimens were sacrificed, all either at the close of, or at an early stage in, the spermatogenic cycle. The sperm ducts, emerging from the testis very close to the junction of the three lobules, are comparatively looped loosely along the flanks of the posterior lobule, in this respect differing from the corresponding tightly coiled section of the sperm duct of the astacid *P. l. trowbridgii* and more like those of other Cambaridae (c.f. Figs. 1e,g,i). The gross appearance of the comparatively straight, more distal part of the sperm ducts is unremarkable. (Perhaps the differences noted may be related to the absence of form alternation [Kawai and Saito 1999, 2001 (Appendix 2)].)

MICROANATOMY OF THE TESTIS

DUCTS AND TUBULES. The disposition and cellular components of the compound collecting tubules and their relationship to the primary collecting duct (Fig. 24a) differ in no conspicuous aspects from those occurring in *Pacifastacus leniusculus trowbridgii* and diagrammed in Figure 18.

ACINI. The basic structure and the life cycle of the acinus of the two species of *Cambaroides* studied by me do not differ from those recorded herein for *P. l. trowbridgii*, and surprisingly markedly less like those of members of the other two subfamilies of the Cambaridae. Even the dimensions of the nuclei of the cellular components of the acini are virtually identical. Because descriptions of the acini examined in the testes of the two species of *Cambaroides* would be little more than a repetition of observations made on these structures in *P. l. trowbridgii*, the reader is referred to the discussion of the acini of the latter above.

MICROANATOMY OF THE SPERM DUCT

VAS DEFERENS. In the testicular segment of the vas deferens there is a rapid transition of the lining epithelial layer from a comparatively thin cuboidal-subcolumnar layer (approximately 35 μm in height with basally situated

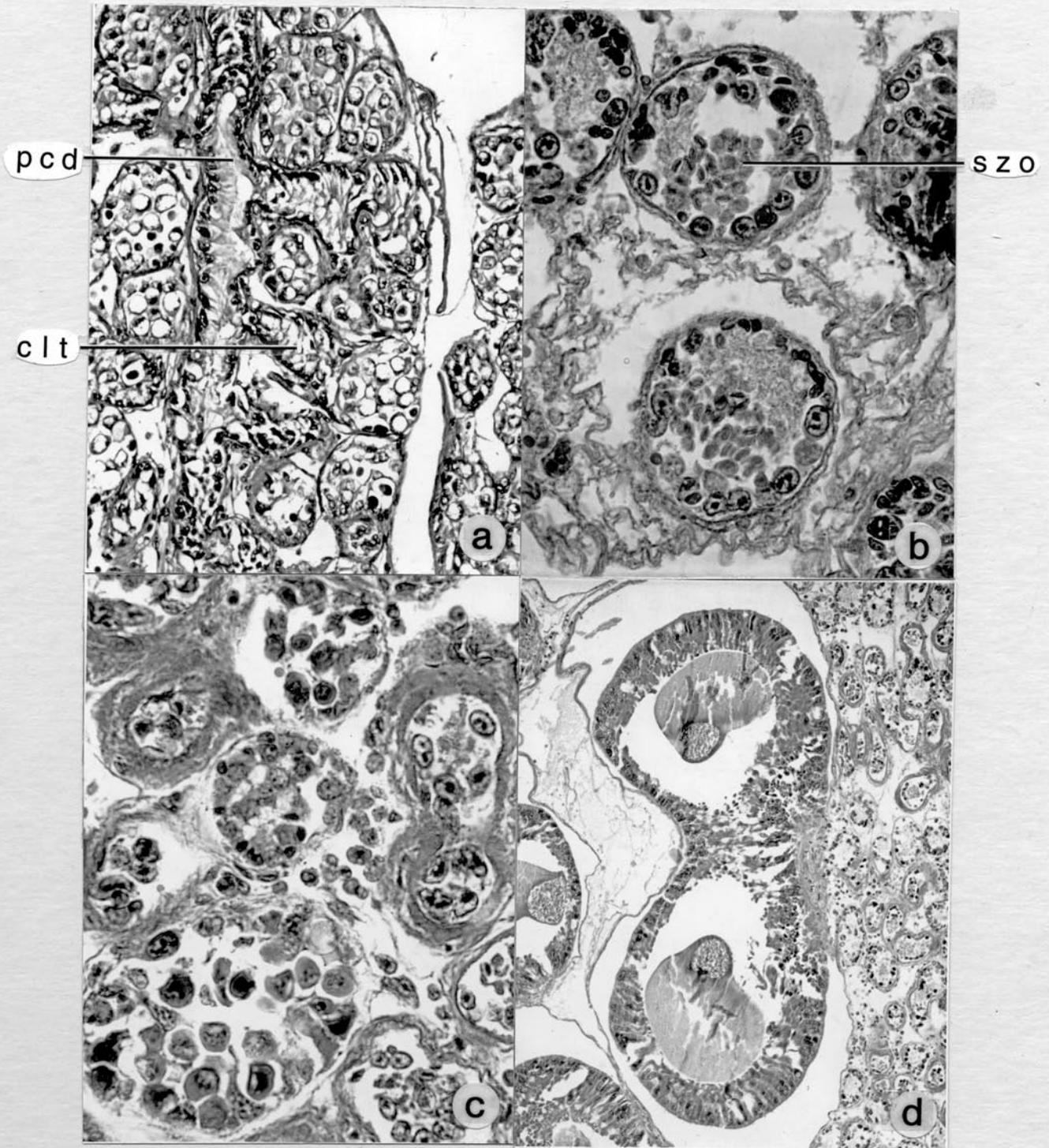


FIGURE 24. Testis and sperm ducts of *Cambaroides japonicus* (a) and *C. similis* (b–d): a, primary collecting duct, collecting tubules, and simple acini with spermatogonia; b, acini with spermatozoa; c, spent and regenerating acini; d, asymmetrical spermatophore in loop of vas deferens.

ovate- to subspindle-shaped nuclei ranging from 10 to 17 μm in length) to a tall columnar one (approximately 115 μm in height with nuclei attaining lengths of as much as 53 μm), typical of the comparatively empty looped segment. In actively secreting sections of the vas deferens the acidophilic cytoplasm may be reduced to such an extent that the cells become subcuboidal with heights of even less than 35 μm and the nuclei, correspondingly reduced in height, becoming subspherical. Despite the apparent functional differences in sectors of the vas deferens, histologically, except for alterations in the cytoplasmic mass, there appear to be no consistent noteworthy differences along its length.

EJACULATORY DUCT. Ectally, the vas deferens acquires a thicker muscular coat and is transformed imperceptibly into the ejaculatory duct. In the transitional zone the duct bears a distinct, evaginated (into the lumen), longitudinal fold that has been designated by some authors studying other decapods as the “typhlosole” (Fig. 25d,e). Although I have observed such folds in all of the *Cambaroides* materials sectioned during this study, at first I questioned whether or not this is a permanent feature of the duct. Possibly the fold is formed by a contraction of a small segment of the circular muscle fibers spanning the base of the invagination. Such would

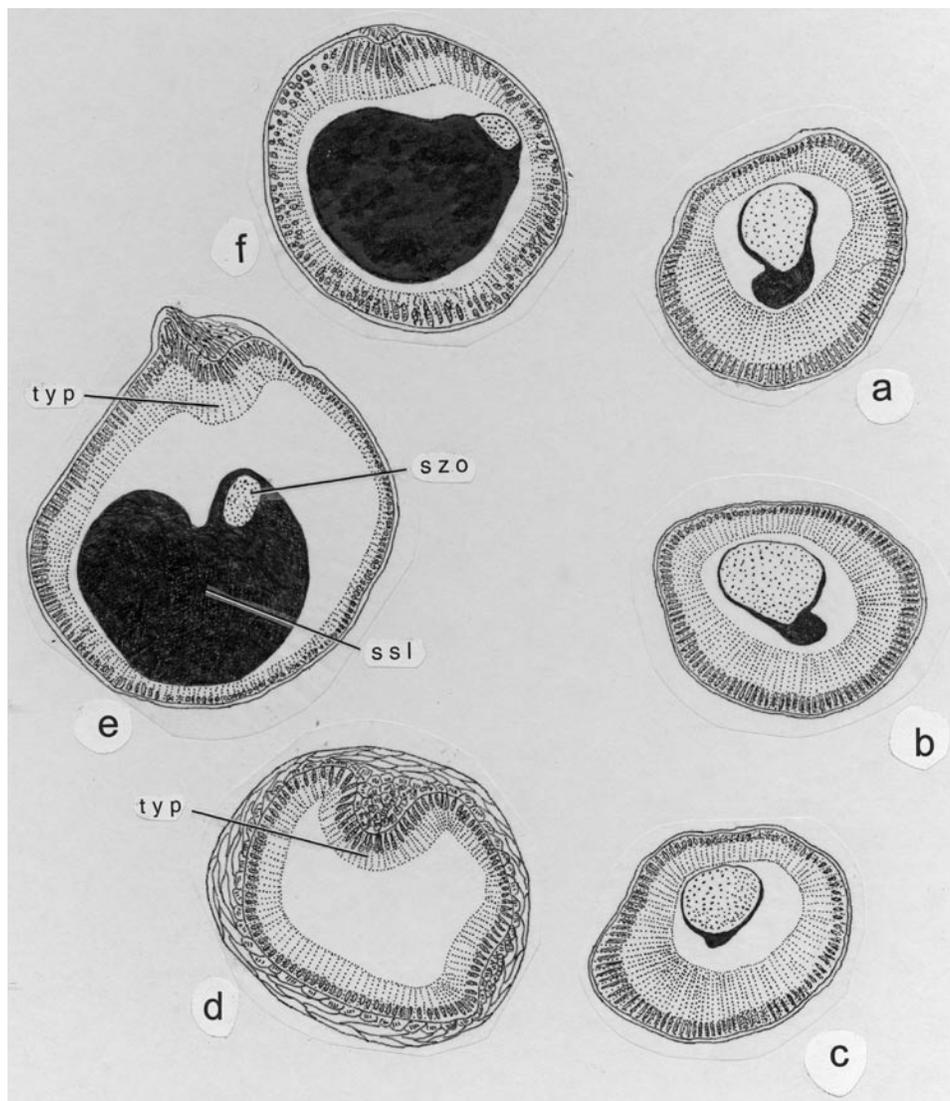


FIGURE 25. Sperm ducts of *Cambaroides similis* (a-c, e) and *C. japonicus* (d, f): a-c, series of sections from proximal to more distal portions of the sperm duct showing development of spermatophore; d, transitional zone with typhlosole; e, ejaculatory duct with typhlosole and raised area; f, section through distal portion of sperm duct.

bring about a bulge in the epithelial layer, compacting the underlying longitudinal muscle fibers and connective tissue. Suggesting the possibility of the ephemeral nature of the fold is the presence of other similar, although less prominent bulges, that have been noted in the same area of the duct. But after noting the orientation of the spermatophore in relation to the typhlosole, it is apparent that it is a characteristic feature that plays an important role in shaping the spermatophore.

Within the coxa, a short distance before the duct joins the terminus, it courses toward the coxal wall and becomes affixed to it (Fig. 26a,b), both through connective tissue fibers and a conspicuously thick band of muscle fibers radiating from that side of the duct that more distally joins the concave side of the clusor. I found no other muscle extending from another part of the coxal wall to the ejaculatory duct as is present in both *P. l. trowbridgii* and in members of the Cambaridae. (While this cluster of muscle fibers may serve as a “retractor muscle,” there is little reason to assume it to be a homologue of this muscle in *P. l. trowbridgii*, which is affixed to the opposite side of the coxa.) From its contact with the coxal wall, the more distal part of the ejaculatory duct veers away from the wall amidst a network of connective tissue fibers that partition much of the haemocoel into smaller sectors. The muscular coats, particularly the band just mentioned, continue toward the junction with the terminus.

TERMINUS. At the union of the ejaculatory duct and the cuticle-lined terminus (Fig. 26c), except for the muscle band on the side of the duct corresponding to the concave side of the clusor, the other muscular elements are virtually absent, and even the band has decreased in volume but, for a short distance, fibrillar elements connecting them to the thickened concave side and angles of the clusor are clearly defined. The U-shaped clusor differs in no conspicuous way from that in most of the decapods examined. Although in material observed no terminus glands could be found, evidence of their presence at seasons other than when the specimens were fixed was discovered in the remains of what appear to be ducts leading toward the clusor in the haemocoelic spaces. The mergence of the latter with the thin-walled genital atrium is unremarkable.

SPERMATOPHORE AND SPERMATOZOA. Almost immediately upon entering the looped segment of the vas deferens, rudiments of the primary spermatophore layer, appear along one side of the sperm mass, thus initiating the formation of the ultimate strongly asymmetrical spermatophore. As the sperm mass passes through the looped section of the vas deferens the primary layer is completed as elements of the granular layer are being added.

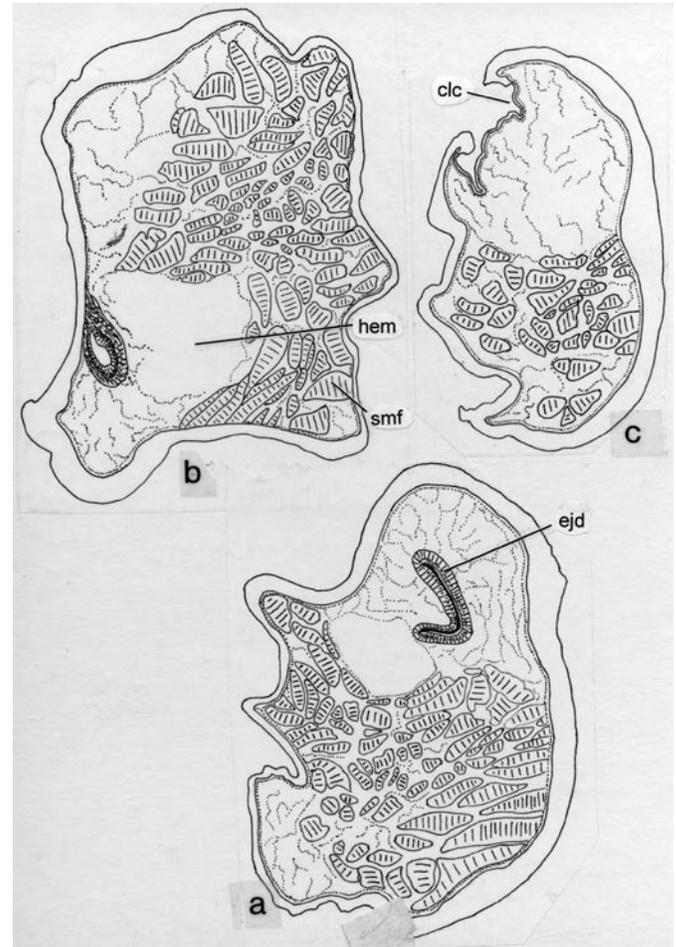


FIGURE 26. Ejaculatory duct and terminus of *Cambaroides*: a, b, distal portion of the ejaculatory duct; c, terminus and clusor.

The primary layer, which is only 1–3 μm in thickness, does not completely surround the mass before beginnings of the granular layer are added on the side where first the primary layer appeared. It is the granular layer that makes up the bulk of the spermatophore. In material examined, the outer globular layer is comparatively thin and not clearly set off from the granular layer. The average diameter of the subcircular to elliptical (in cross section) sperm mass is approximately 84 μm , and in spermatophores approaching the ejaculatory duct, the mass is strongly eccentrically situated (Fig. 24d), often only a few microns from one side and more than 315 μm from the opposite. The sperm mass is strikingly more eccentrically located and comparatively smaller in diameter than it is in the spermatophores of other members of the Cambaridae examined. The encapsulated spermatozoa are much like those of the other Cambaridae, having a diameter of approximately 7 μm and bearing four

to six rays. Information on the ultrastructure of the testis and features of the spermatozoa of *Cambaroides japonicus* are presented by Yasuzumi et al. (1961) and Yasuzumi and Lee (1966).

SUMMARY AND DISCUSSION

SECONDARY SEXUAL CHARACTERISTICS

The Parastacoidea differ from the Astacoidea in lacking pleopods on the first abdominal segment, and those of the second are not modified in the male for transferring spermatophores to the female. In the Astacoidea, the Nephropoidea, and the Enoplometopoidea the first pleopods of the male are present and modified for conducting spermatophores to the female. Among the Astacoidea, only in the Astacidae and Cambaridae are the second pleopods also involved in transferring the spermatophores. In the Nephropoidea and Enoplometopoidea the paired first pleopods function together in funneling the spermatophore to the female, whereas in the Astacidae and Cambaridae the first and second pleopods on one side function together, but independently of those on the other side, in this process.

At least two other secondary sexual features of the male set the family Cambaridae apart from all of the other Astacoidea. One is the presence of “copulatory hooks” on the ischia of one or more pairs of the second through fourth (erratically on the fifth) pereiopods. The other is a cyclic dimorphism (not demonstrated in the East Asian genus *Cambaroides*, Kawai and Saito, 2001 [Appendix 2] involving the secondary sexual features that, at the last juvenile molt prior to the first breeding season, brings about conspicuous changes in structure of the first pleopods (and, to some extent, texture of the latter) and copulatory hooks that mark the adult state (referred to as “Form I” or “first form” male). Following the breeding season, a molt results in a return of these elements to a quasi-juvenile condition, at which stage the animal is referred to as a “Form II” or “second form” male. With the approach of the next breeding season, a molt returns the crayfish to the adult, “first form” condition. This alternation of states, usually—but not invariably—separated by single molts, continues through the adult life of the crayfish. For reasons not understood, however, sometimes a molt of the quasi-juvenile does not result in a transformation of the animal to the adult, “first form” state. Because the molt to “first form” appears to be correlated with a mass production of spermatozoa and its expulsion from the testis into the vas deferens, one might

suspect that relative inactivity of the testis (whatever the factors involved) may well be responsible for the irregularity of the usual rhythm. To my knowledge, only one North American cambarid has been reported not to undergo such cyclic dimorphic changes: Taylor (1985) reported that in a population of *Procambarus (Pennides) spiculifer* (LeConte, 1856) investigated by him the adult males do not return to the quasi-juvenile state.

Patterns of the Astacidean Testis

For reasons pointed out in the section on “Terminology” above, the male gonad of the Astacoidea is considered by me to be a single organ and occurs in two basic forms referred to herein as “H-shaped” or “Y-shaped.” The former, which is considered by the few of those who have expressed an opinion to be the more primitive, is typical of the testes of the Parastacoidea, Nephropoidea, and Enoplometopoidea (Figs. 3d–g, 27). It consists of a pair of longitudinal lobes joined anterior to their midlength by a transverse bridge. The shorter segments anterior to the bridge are designated anterior lobules and the longer ones, posterior lobules. The derived “Y-shaped” pattern, that of the Astacoidea, was probably formed by a fusion of the paired posterior lobules into a single one.

Acini

The spermatozoa-producing units of the astacidean testis are the sac-like acini that develop primarily as buds from the stem-like primary collecting duct (and later from secondary collecting tubules), lying in the axes of the testicular lobules. Each of these increases in size as spermatogenesis proceeds, produces a generation of spermatozoa and, accompanying spermiogenesis and the expulsion of the spermatozoa into the collecting ducts, undergoes one or two of three fates: (1) regeneration of the acinus occurs and another cycle of sperm production ensues; (2) secondary acini develop in the wall of the existing acinus, converting the primary acinus into a passageway to the collecting tubules; or (3) the acinus degenerates and new acini arise from the more superficial collecting tubules situated toward the blind ends of the lobules. The first of the alternatives is employed exclusively by only the Astacidae and Cambaroidinae; the first and second fates have been adopted by the Parastacoidea, Nephropoidea, and Enoplometopoidea; and the third only by the Cambarinae and Cambarellinae. Since sperm production occurs in cycles, the volume of the testicular lobules is subject to rather striking change (e.g., ranging in the Parastacoidea from plump H-shaped

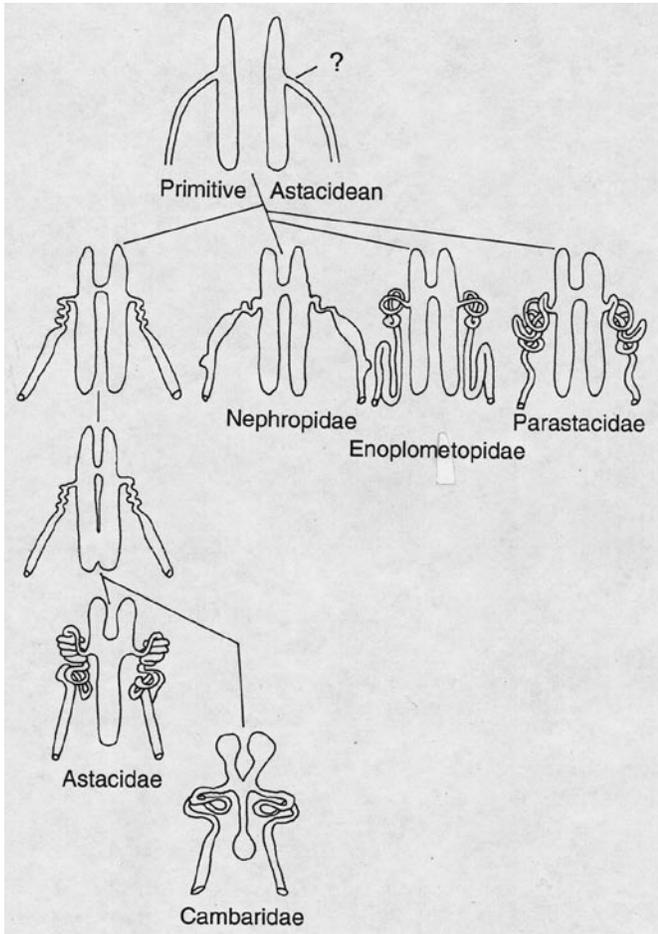


FIGURE 27. Diagrammatic representations of gross structure of Astacidean testis and sperm ducts in dorsal aspect, demonstrating proposed development of the male reproductive systems in the superfamilies Parastacoidea, Nephropoidea, Enoplometopoidea, and Astacoidea.

sausage-like bodies with the approach of the breeding season to H-shaped threads at the close). But with the degeneration of the acini in the testis of the Cambarinae and Cambarellinae the testis becomes stalked (three lobules joined by a slender Y-shaped stalk) at the end of the first breeding season, the stalk becoming longer toward the end of each succeeding period of sperm production.

Associated with the three alternative fates of the acini is the disposition of the germinal cells. In the Cambarinae and Cambarellinae—in which the acini degenerate following sperm production—they are recognizable only at two points: (1) in the acinar buds from the collecting tubules—in the smallest, probably youngest buds only one cell type is recognizable and these exhibit neither the characteristics

of generative cells nor accessory cells, rather resembling the epithelial cells of the collecting tubule—and (2) as they assume the role of spermatogonia—the nuclei of germinal cells and spermatogonia appear to me to be indistinguishable. They are not evident along the lengths of the tubules, nor are they present within an acinus after the onset of spermatogenesis. In sharp contrast, germinal cells seem to be always present in the collecting tubules of all of the other astacideans examined. Moreover they appear in the walls (i.e., between the superficial layer of accessory cells and the superficial connective tissue) of acini by the time the spermatogenic elements are being converted to spermatids, often having formed clusters, the primordia of secondary acini in the Parastacoidea, Nephropoidea, and at least sometimes in the Enoplometopoidea. Within the walls of an acinus they may be disposed in a partial layer or scattered, constituting the initial spermatogonia of a new cycle of sperm production. Such occurs in the acini of the Astacidae, Cambaroidinae, Parastacoidea, Nephropoidea, and Enoplometopoidea. I have been unable to discover the precursor of the generative cells in the testes of any of the Astacidea studied. For example, they suddenly appear in the wall of an acinus in which all cells of the interior are undergoing spermatogenesis or are accessory cells, so whether they have migrated there from elsewhere (the walls of the collecting tubule?) or have become differentiated in situ from epithelial cells or from accessory cells is not known. (The latter source seems most unlikely.) The fate of those generative cells that reach the interior of an acinus seems surely to be conversion to the role of spermatogonia and/or perhaps to becoming progenitors of the accessory cells.

Equally enigmatic are not only the immediate ancestry and multiplication of the accessory cells, but also the breadth of their functional roles. Soon after the primordium of an acinus becomes recognizable, two types of cells are recognizable within the connective tissue coat: germinal cells (spermatogonia) and accessory cells, both readily recognizable by their distinctive nuclei. Nuclei in various stages of the mitotic process have been often observed in the material examined, but there is every reason to believe that all of them are spermatogonia (germinal cells). Like all students concerned with histological and cytological events in the functioning of the astacidean testis of which I am aware, I have failed to find any evidence of a division of an accessory cell! Several of the earlier workers claim that divisions of these cells are accomplished through amitosis, but the current observations neither support nor refute such a conclusion. I simply have seen no evidence of these cells in division. That their numbers increase and that the number of their nuclei within a single cytoplasmic

mass characterically become compounded, the sources of the cells and of the mechanisms bringing about the clustering of the nuclei in multinucleate masses remain obscure. At one time I suspected that the latter might be the products of those "irregular" cell divisions involving tripolar spindles and other spindles sharing common centrioles but, for example, nuclear clusters and irregular nuclei are in the testis of *Pacifastacus*. After searching, no "irregular" spindles in any of the sectioned material at hand have been found. In contrast, "irregular" spindles are not uncommon in the testis of *Homarus*, yet the nuclei of the accessory cells in acini containing spermatids do not occur in clusters or appear abnormal.

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Horton H. Hobbs Jr.

Appendix 1: Abbreviations Used in Figures of Testis Study

acl	accessory lobule
acn	accessory cell nucleus
al	acidophilic ligament
anl	anterior lobule
asd	atrophied sperm duct
bep	cuboidal epithelium
cep	columnar epithelium
cgc	cluster of generative cells
clc	cuticular lining of clusor
clt	collecting tubule
clu	clusor
cmf	circular muscle fibers
cnt	connective tissue
cpa	compound acinus
ctn	connective tissue nucleus
dga	degenerating/degenerated acinus
ejd	ejaculatory duct
gat	genital atrium
gcn	generative cell nucleus
gls	glandular segment
hem	haemocoelic space
lmf	longitudinal muscle fibers
pcd	primary collecting duct
pol	posterior lobule
ppa	phallic papilla
prs	proximal segment
psc	primary spermatocyte
psl	primary spermatophore layer

rmf	retractor muscle fibers	stk	stalk
rsa	rudiment of secondary acinus	syn	syncytium
scn	spermatocyte nucleus	szo	spermatozoa
scy	spermatocyte	tco	transverse commissure
sgo	spermatogonia	teg	tegumental gland
smf	striated muscle fiber	ter	terminus
spd	sperm duct	tf	tonofibrils
spm	sphincter muscle	typ	typhlosole
ssc	secondary spermatocyte	vdf	vas deferens
ssl	secondary spermatophore layer	vel	velum
std	spermatid		

Appendix 2: Additional References

Only literature published prior to 1993 are included in the manuscript. Clearly, numerous publications appeared after 1993 that are relevant to the comparison of crayfish reproductive systems and a representative list is offered below.

The detailed comparative morphological analyses of testes, sperm ducts, and associated organs allows the use of these characters for phylogenetic and evolutionary deductions. In light of recent discoveries, it is unfortunate that the parastacoid condition is presented herein as the ancestral one in relation to that of the Nephropoidea, Enoplometopoidea, and Astacoidea. Present views of freshwater crayfish relationships, based on morphological and molecular data, suggest that they are monophyletic and that lobsters are plesiomorphic in many characters. However, data from this study are consistent with this interpretation.

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