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The Androgenic Glands of the Pacific Crayfish, *Pacifastacus leniusculus trowbridgii* Stimpson, 1859

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AN ABSTRACT OF THE THESIS OF Larry L. Sanders for the Master of
Science in Biology presented January 26, 1976.

Title: The Androgenic Glands of the Pacific Crayfish,
Pacifastacus leniusculus trowbridgii Stimpson, 1859.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Leonard Simpson, Chairman

Richard B. Forbes

Trygve P. Steen

The androgenic glands of the Pacific crayfish, Pacifastacus leniusculus trowbridgii Stimpson, 1859, were studied experimentally and by light and electron microscopy. The androgenic glands proliferate in the spring of the year concurrently with an increase in mitotic activity in the testes. Degeneration of a major portion of the glands occurs in the fall, when spermatogenic activity in the testes is near completion. Cellular development appears to progress from small undifferentiated cells through stages of vacuolization and hypertrophy and terminates with degeneration or lysis. The fine structure of the

vacuolated gland cells is indicative of increased metabolic activity and probable hormone secretion. However, experimental manipulation of the glands of P. leniusculus gave no evidence of their presumed endocrine function. A discussion of the similarities of the androgenic glands with other known arthropod endocrine glands is also included.

THE ANDROGENIC GLANDS OF THE PACIFIC CRAYFISH,

PACIFASTACUS LENIUSCULUS TROWBRIDGII

STIMPSON, 1859

by

LARRY L. SANDERS

A thesis submitted in partial fulfillment of the
requirements for the degree of

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TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
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INTRODUCTION

The androgenic glands of malacostracan crustaceans are epithelial endocrine glands which are the source of a hormone that is involved in the differentiation and maintenance of the primary and secondary sex characteristics of male animals (Charniaux-Cotton, 1960 and 1962; and Charniaux-Cotton and Kleinholz, 1964). First discovered in the amphipod, Orchestia gammarella (Charniaux-Cotton, 1954), the androgenic glands have since been identified in all major groups of malacostracan crustaceans (Charniaux-Cotton et al., 1966; and Zerbib, 1967). The location, morphology, and histology of the androgenic glands in various malacostracan groups has been reviewed by Charniaux-Cotton et al., 1966, and a number of ultrastructural studies of the glands have been reported (King, 1964a and 1964b; Meusy, 1965; Malo, 1970; Malo and Juchault, 1970; Payen et al., 1971; and Payen, 1970 and 1972).

The purpose of this study was to describe the morphology, histology, and fine structure of the androgenic glands of the Pacific crayfish, Pacifastacus leniusculus trowbridgii Stimpson, 1859, and to undertake a series of extirpation-implantation experiments using androgenic gland tissue, in order to determine the function of these glands. There is substantial evidence of the endocrine function of the androgenic glands in amphipods and isopods (Charniaux-Cotton, 1954, 1960, and 1965; Katakura, 1967; and others), but evidence of gland function in the decapods is not as well documented. Studies of decapod androgenic gland function, using extirpation-implantation techniques, have

been reported for both hermaphroditic (Charniaux-Cotton, 1959) and gonochoristic species (Puckett, 1964; Payen, 1968 and 1969; and others). Previous studies of the androgenic glands of various crayfish species have dealt with; gland vascularization (Graf, 1962), gland function (Puckett, 1964), and gland morphology and histology (Carpenter and deRoos, 1970). King, 1964b, has described the fine structure of degenerating cells in the glands of Pacifastacus.

REPRODUCTIVE CYCLE AND SEXUAL DIMORPHISM IN PACIFASTACUS

Mason, 1963, has reported the life history of Pacifastacus leniusculus. Important reproductive events are as follows. Females become sexually mature in the third or fourth year after hatching. Maturity is indicated by an increase in abdominal width following the summer moult. Male crayfish become sexually mature during the second or third year after hatching. Copulation takes place between September and late October, and is followed by spawning in October and early November. The eggs remain attached to the pleopods of the female during the winter, and hatching occurs in April and May.

Crayfish of the genus Pacifastacus exhibit external sexual dimorphism soon after hatching. Males differ from females in that the males possess five pairs of abdominal appendages, the pleopods, while females lack the first pair of pleopods. The 1st pleopods of males, (1^o gonopods), which eventually become modified for the transfer of sperm, are not present at hatching but appear when juvenile crayfish reach 14 mm to 16 mm total length. The 2nd pleopods, (2^o gonopods), are present at hatching and gradually differentiate to the adult form (Mason, 1963). The occurrence of a cyclic dimorphism of the gonopods, as observed in North American crayfish of the Family, Cambaridae, does not exist in Pacifastacus or other members of the Astacidae (Hobbs, 1974).

MATERIALS AND METHODS

Animal Collection and Maintenance

Crayfish were collected from small streams in Washington and Multnomah Counties in Northwestern Oregon. Animals were taken during the period from March 1973 to November 1974. Winter collections were small due to the disappearance of animals from the stream beds during the colder months of the year. Animals were maintained in the laboratory in a variety of temperature regulated containers. Oyster shell was used as a substrate, and larger rocks were added to provide cover for the animals. Tanks were aerated continuously and maintained at a variety of water temperatures, 8° to 20° centigrade. All animals were fed beef liver or frozen fish every three days. Food remaining in the tanks was removed the morning following the feeding period.

Histological Methods

Paraffin sections of the testes, sperm ducts, androgenic glands, and ovaries of normal, experimental, and control animals were prepared. Ovaries were fixed in Bouin's fluid, dehydrated in ethanol, sectioned at 10 microns, and stained with alum hematoxylin and eosin. Testes were fixed in neutral formalin or Bouin's fluid, dehydrated in ethanol, and sections were cut at 7 and 10 microns. Sections of the testes were stained with alum hematoxylin and eosin or iron hematoxylin and orange G. The sperm ducts and androgenic glands were fixed in neutral formalin, Zenker's solution, or Helly's solution, and dehydrated in

ethanol. Sections were cut at varying thicknesses, 4 to 10 microns, and stained with alum hematoxylin and eosin, iron hematoxylin and orange G, or Mallory's triple stain.

The testes, sperm ducts, and androgenic glands were also embedded in Epon. Tissue removed from anesthetized or recently killed animals was placed directly in 2.5% glutaraldehyde buffered with phosphate at a pH of 7.4. After rinsing in buffer, the tissue was postfixed in 1% OsO_4 in an ice bath. Tissues were dehydrated in an ethanol series and embedded in Epon (Luft, 1961). Sections were cut on a Porter-Blum Ultramicrotome using glass knives. One micron sections were stained with Richardson's stain (Richardson et al., 1960) and with 1% basic fuchsin. Thin sections were picked up on uncoated 300 mesh copper grids, stained with uranyl acetate and lead citrate, and viewed with a Zeiss EM 9S-2 electron microscope.

Experimental Methods

Removal of the androgenic glands was accomplished as follows: Animals were anesthetized with CO_2 and secured to cork pinning boards ventral side up. The coxapodites of the fourth walking legs were then disarticulated from their connections with the ventral and branchial regions of the thorax and pulled away from the animal. This procedure exposed the sperm ducts which were cut with iridectomy scissors. The coxapodites were placed in crayfish saline (van Harreveld, 1936), and the terminal region of the sperm duct and its attached androgenic gland were then removed from the coxapodite. The wounds produced by the removal of the coxapodites were cauterized and covered with warm paraffin. The operated animals were allowed to recover from the operative pro-

cedure in individual recovery tanks, and were returned to the appropriate experimental tank after a sufficient recovery period. Control animals were subject to the same operative procedures as the experimental animals except that the coxapodites of the third walking legs were removed.

Androgenic gland tissue was implanted in females that varied in size from 15 to 30 mm carapace length. The gland tissue was implanted in the ventral abdominal sinuses through small incisions in the ventral exoskeleton. The incisions were sealed with warm paraffin. Females were not anesthetized, but were allowed a period of post-operative recovery in individual recovery tanks before being returned to experimental tanks. Female controls were given implants of muscle tissue from the third walking legs of male crayfish.

Androgenic glands were also implanted into the thoracic cavity of some females using the following method: Androgenic gland tissue recently removed from the coxapodites of male crayfish was drawn into 1/2 cc glass syringes along with 0.2 ml of crayfish saline. An 18 gauge hypodermic needle was attached to the syringe and the contents of the syringe were injected into the dorsal thoracic cavity, through the connective tissue which separates the thorax from the abdomen. This method minimized the loss of hemolymph and appeared to cause less shock to the animal than the previous method. Injected animals were then allowed a period of recovery, and were returned to experimental tanks.

Operated animals were observed regularly and at various intervals were sacrificed and examined histologically.

RESULTS

The morphology and histology of the reproductive systems of both male and female P. leniusculus vary according to the size and state of maturity of the individual animal. Seasonal differences in the form and structure of the reproductive organs correspond with the production of gametes and to a lesser extent with the moult cycle stage of the animal.

I. MALE REPRODUCTIVE SYSTEM

Light Microscopy

Testes. The testes of P. leniusculus are compound acinar organs which are located in the dorsal thoracic cavity below the pericardial cavity and dorsal to the posterior lobes of the digestive glands. The testes are trilobed structures with two anterior lobes extending to the region of the cardiac stomach and one posterior lobe which extends into the first or second abdominal segment (Fig. 1). Individual testicular acini, which are surrounded by a thin connective tissue sheath, consist of a variable number of germ cells and a peripheral layer of irregularly shaped accessory cells. Groups of acini are connected by ducts or collecting tubules which are composed of a cellular lining surrounded by connective tissue. Larger collecting tubules, which join to form the sperm ducts, are also surrounded by layers of circular muscle tissue.

The annual production of spermatozoa can be separated into four

Figure 1. The male reproductive system of *P. leniusculus*. Diagram indicates the location of the testes (t), sperm ducts (s), and the androgenic glands (ag).

reasonably distinct stages which correspond roughly to the seasons of the year. In the late fall and winter months, following copulation, the testes contain a minimum number of germ cells and in most animals appear to be in a disorganized, nonfunctional state. Spermatozoa, and the mucous-like secretion of the epithelial lining of the collecting tubules, are occasionally found in the testes of mature crayfish captured after the copulatory period. However, copulation or the discharge of spermatozoa was not observed in any of the natural stream populations in the months following the normal copulatory period. During the later phases of the post-copulatory period, mitotically dividing germ cells are observed with increasing frequency, and a gradual reorganization of the testes tissue occurs. In the spring, March to May, testicular activity increases. Numerous mitotically dividing germ

cells are observed, and the size of the testes increases measurably in most animals (Fig. 2). Meiotic cell division is first observed in the

Figure 2. The testes of P. leniusculus captured in the spring. Mitotically dividing germ cells are numerous at this time of the year. Spermatogonia (s), dividing cells (d), accessory cells (a). 260 X.

early summer months, during the premoult phase of the yearly moult cycle of mature males. Spermatogenesis proceeds rapidly following ecdysis (Fig. 3). Spermiogenesis occurs in the acini and collecting tubules during late summer. Spermatozoa are present in the collecting tubules and sperm ducts in early fall, and at this time the testes and sperm ducts are the most obvious structures in the dorsal thoracic cavity.

Sperm Ducts. The sperm ducts arise near the junction of the

Figure 3. The testes of *P. leniusculus* captured in early summer. Spermatogenesis proceeds rapidly in the testes at this time of the year. Spermatogonia (sg), spermatocytes (sc), accessory cells (a). 400 X.

posterior and anterior lobes of the testes (Fig. 1). Histologically, two regions of the sperm ducts can be identified. In the dorsal thoracic cavity, the sperm ducts are highly coiled structures situated dorsal to the lobes of the testes. In this region, the ducts consist of an epithelial lining of densely staining secretory cells surrounded by layers of circular and longitudinal muscle fibers. The pseudostratified epithelial cells have large oblong nuclei with granular or ribbon like chromatin and diffusely staining cytoplasm. In the posterior region of the thoracic cavity, the sperm ducts become uncoiled and extend ventrolaterally into the coxapodites of the 4th walking legs. Upon entering the coxapodite, the sperm duct bends medially and opens toward

the ventral midline of the animal. The terminal region of the sperm duct is similar to the more proximal regions except that the muscular sheath is greatly enlarged in the terminal region. The enlarged muscular sheath of the terminal regions of the duct consists of circular and longitudinal muscle layers (Fig. 4).

Figure 4. Terminal region of the sperm duct of *P. leniusculus* with an associated strand of androgenic gland tissue. Lumen of the duct (l), secretory epithelial cell layer (e), circular muscle (c), longitudinal muscle (m), microvillar surface of epithelial cells (mv), androgenic gland tissue (ag). 160 X.

Maximum presecretory development of the epithelial cells appears to occur at the time of the yearly moult. At this time, nuclei stain intensely with hematoxylin and contain large spherical masses of chromatin. Microvilli stain with relatively strong intensity in iron hematoxylin and extend a maximum distance into the empty lumen of the duct. Cell division is observed infrequently in the epithelial cells, except in animals captured during the late spring and summer. In these ani-

mals, dividing cells are distinguishable in both regions of the duct. The epithelial cell lining of the entire duct is at the height of secretory activity in late summer and fall. In late fall, at the time of copulation, spermatozoa and the mucous-like secretion of the epithelial cells fill the lumen of the duct.

Androgenic Glands. The androgenic glands of P. leniusculus are located in the loose connective tissue which surrounds the terminal muscular regions of the sperm ducts. The morphology and histology of the androgenic glands vary according to the time of the year in which the animal is captured and to the size of the animal. The glands of mature crayfish undergo a yearly cycle of development which includes phases of proliferation and degeneration (Table I).

Androgenic gland tissue is difficult to locate in animals captured during the winter months. In these animals, the glands appear as small strands of tissue situated at the periphery of the sperm duct musculature (Fig. 4). Gland cells are small, 4 to 7 microns, with irregularly shaped nuclei. Small dense masses of chromatin are located throughout the nuclei of these cells. Mitotic figures are not observed, and cell boundaries are indistinct at this time of year.

Beginning in early spring, the androgenic glands are found in a majority of captured animals. Proliferation of the glands occurs at this time, and gland tissue is often observed as parallel and anastomosing strands of cells in the loose connective tissue of the coxopodites. Strands of gland tissue in sheet-like masses are also found near the muscular sheath of the sperm ducts. Individual strands of cells are surrounded by a thin layer of connective tissue, which is

also interspersed among the gland tissues in layer-like arrangements. The diameter of gland cells from animals captured in the spring is generally 2 to 5 microns larger than that of cells from winter animals, and the increase in gland size during this period is probably due to cell growth (Fig. 5). As spring progresses, dividing cells are observed in the larger strands of gland tissue. Cell division often occurs simultaneously in all the cells of a single strand of tissue, but mitosis is occasionally observed in single gland cells located in the connective tissue of the gland mass.

Figure 5. Strands of androgenic gland tissue from *P. leniusculus* captured during the spring. The increase in gland size at this time is due mainly to cell growth. Androgenic gland tissue (ag), connective tissue (c), granular hemolymph cells (g), hypodermis of coxapodite (h). 400 X.

In late spring, at the time of the yearly moult of mature males,

cellular diversity is at a maximum within the proliferating gland tissue. In addition to strands containing the developing cells previously described, three other cell types are commonly observed in this proliferating tissue. Larger cells, 12 to 16 microns in diameter, with highly vacuolated cytoplasm, are found in strands of 60 to 80 microns in diameter (Fig. 6). Cells of this type are characterized by irregularly shaped nuclei with peripheral chromatin. Vacuolization of the cytoplasm is extensive, and strands of cells are encountered in which the central region of the strand is filled with large vacuoles. The cell boundaries of this cell type are indistinct in paraffin and Epon

Figure 6. Androgenic gland tissue from P. leniusculus captured at the time of the annual moult in late spring. Cells in various stages of development are present in this proliferating tissue. Dividing cells (d), vacuolated cells (v), degenerating cells (p). 400 X.

sections. The fourth recognizable cell type is found in strands of maximum diameter, 70 to 90 microns. Cells in this stage of development are of maximum size, 14 to 18 microns in diameter, and have spherical nuclei. Cell boundaries are usually distinct in paraffin and Epon sections, and granules and inclusions are observed in the cytoplasm. The extensive cytoplasmic vacuolization described in the previous developmental stage does not occur in this cell type. Instead, cellular hypertrophy appears to be occurring. The fifth cell type, observed during the late spring and encountered with increasing frequency throughout the following months, is the terminal stage of cellular development. Cells of this type have intensely staining pycnotic nuclei, and highly disrupted vesicular cytoplasm (Fig. 6). Inclusions and granules are often found in the cytoplasm, and the connective tissue sheath of strands of these cells is fragmented and discontinuous.

In the summer months the androgenic glands reach their maximum size and volume. For the most part, the glands consist of strands of hypertrophied and degenerating cells. Following ecdysis, strands of cells in earlier stages of development are observed infrequently, if at all. In the fall, gland size diminishes rapidly. Nuclear pycnosis is commonly observed in the gland mass of mature males, and in most animals the glands appear to be degenerate and nonfunctional.

Electron Microscopy

Androgenic Glands. As with paraffin sections, observation of androgenic gland fine structure reveals a yearly cycle of cellular proliferation and degeneration. Cellular development, however, is not synchronous throughout the entire gland mass. During most of the

TABLE I

CORRELATION OF ANDROGENIC GLAND MORPHOLOGY AND HISTOLOGY
WITH REPRODUCTIVE MORPHOLOGY AND ACTIVITY
IN ADULT MALE P. LENIUSCULUS

	Spring	Summer	Fall	Winter
...	Premoult - Ecdysis	Postmoult	Intermoult	Copulation ...
<u>Testes</u>	Increase in size from winter. Mitotic activity increases.	Spermatogenesis proceeds rapidly. Sperm in acini.	Cessation of meiotic activity. Sperm in acini and tubules.	Reorganization of tissues. Gradual increase in germ cell numbers.
<u>Sperm Ducts</u>	Usually empty of sperm. Small number of dividing cells in epithelial lining.	Increase in number of dividing cells. Secretion of mucous-like material from epithelial lining.	Sperm and epithelial cell secretion in duct lumen. Mitosis not observed in cellular lining.	Usually contain some sperm. Epithelial cell division not observed.
<u>Androgenic Glands:</u>	 Proliferation Degeneration			
<u>Morphology</u>	Parallel and anastomosing strands of cells. Larger diam. strands usually some distance from ducts.	Similar to spring, also in sheet-like masses. Maximum gland size.	Decreasing number of large diam. strands. Strand degeneration prevalent.	Small number of cellular strands in muscle and connective tissue of sperm ducts.
<u>Histology</u>	Structurally diverse cellular strands. Cell division occurring. Vacuolated and degenerating cells present.	Vacuolated and hypertrophied cells predominant. Degenerating cells with pycnotic nuclei.	Strands consist of hypertrophied and degenerating cells.	Undifferentiated cells in small diameter strands.

year, strands of cells differing structurally are observed in the glands. Structural variation in neighboring strands of cells is most often observed in the glands of late premoult animals.

The cellular developmental cycle, in the glands of P. leniusculus, appears to be initiated in early spring (Table II). The majority of cells, at this time of year, have irregularly shaped nuclei and uniformly dense cytoplasm. Cytoplasmic organelles, such as mitochondria and Golgi bodies, are few in number and scattered throughout the cytoplasm. A relatively small amount of endoplasmic reticulum, both granular and agranular, is also observed in these cells.

As cellular development proceeds, individual cells increase in size. Cell growth is accompanied by an increase in the number and complexity of cytoplasmic organelles. Large numbers of tubular or spherical mitochondria with transverse cristae are found in these cells, and a large amount of sac-like or vesicular agranular endoplasmic reticulum is frequently observed. Cell division is observed most often in cells of this type. A common feature of developing gland cells is an extensive infolding of the cell membrane, especially in cells at the periphery of the strands of gland tissue. These so called "interdigitating membranes" are a feature of all cellular stages except the terminal degenerative stage. In some cells, the infolding membranes separate the peripheral cytoplasm from the more central regions of the cell.

Vacuolization of the cytoplasm of gland cells is a normal stage in the cellular developmental cycle. Vacuolated cells are observed regularly in the glands of crayfish in late premoult phases. Cells in this stage of development, contain numerous tubular and spherical mito-

chondria with transverse cristae (Fig. 7). In addition, extensive fields of agranular endoplasmic reticulum are observed, often surrounding vacuoles of various size. The nuclei of these cells are often pear-shaped with densely staining peripheral chromatin and a single, more centrally located nucleolus. In non-localized regions, the periphery of the nucleus is scalloped in appearance, with numerous closely spaced indentations. Cell membranes, with numerous infoldings, are usually distinct, and electron dense regions between neighboring membranes are commonly encountered. As mentioned above, vacuoles are often associated with fields of agranular endoplasmic reticulum. In some regions of these cells, however, vacuoles appear to be closely associated with swollen distorted mitochondria. The vacuoles are of varying size and shape in these cells. Smaller, spherical vacuoles are usually membrane bound and located in intracellular regions. Larger, irregularly shaped vacuoles lack membranes, and often extend into the cytoplasm of adjoining cells.

In cells undergoing extensive vacuolization, a number of unique structures are observed (Fig. 8). Larger vacuoles, up to 5 microns in diameter, are often surrounded by layer-like arrangements of electron dense, agranular membranes. The cytoplasm immediately surrounding these electron dense membranes is often filled with small tubular and spherical vesicles. Electron dense, membrane bound granules, characteristic of Golgi systems, are not encountered in these cells. Mitochondria are characteristically swollen and distorted, and in various phases of vacuolization. Spherical clusters of membranes, in concentric layer-like arrangements, are also found in these cells. These

multi-lamellar bodies are similar in size to the spherical mitochondria but lack an electron dense matrix.

Figure 7. Stage of cellular vacuolization in the androgenic glands of *P. leniusculus*. This stage of cellular development coincides with the annual moult of mature males. Mitochondria (m), endoplasmic reticulum (e), vacuoles (v), nucleus (n). 5000 X.

Figure 8. Vacuolated androgenic gland cell from *P. leniusculus* showing the structural relationship of the lamellar membrane systems (e), mitochondria (m), multi-lamellar bodies (mb), and vacuoles (v). 8500 X.

Following the stage of vacuolar activity, androgenic gland cells reach their maximum size. These cells, characteristically, have spherical nuclei and diffuse, vesicular cytoplasm. Mitochondria and endoplasmic reticulum are difficult to identify, although, in some cells,

agranular membrane systems, in lamellar arrangements, do exist. Golgi bodies and vesicles, though never abundant in androgenic gland cells, are observed most frequently in cells of this type. Membrane bound granules are also found in these cells, but never in great numbers.

Following the stage of maximum cell size or hypertrophy, the terminal degenerative stage of cellular development occurs. Cells in this stage of development have spherical, uniformly dense nuclei and numerous cytoplasmic vesicles and granules. The nuclear membranes of degenerating, pycnotic nuclei are often swollen and distorted, and in later phases of degeneration, fragmentation of the nuclei occurs. Mitochondria and fields of endoplasmic reticulum are rarely distinguishable, although Golgi systems are observed in some cells. The connective tissue sheath, surrounding strands of these cells, is often fragmented, and frequently, vesicles and granules are observed in regions peripheral to the strands.

TABLE II

THE SEQUENCE OF CELLULAR DEVELOPMENT IN
THE ANDROGENIC GLANDS OF ADULT MALE
P. LENIUSCULUS.

Stage	Cell Diameter	Cell Ultrastructure	Time of Year Observed	Remarks
I	4-7 μ	Uniformly dense cytoplasm, with few mitochondria and a small amount of endoplasmic reticulum. Nuclei are irregular in shape with densely staining masses of chromatin.	Winter and Spring	Undifferentiated cells.
II	8-12 μ	Increase in the number of cytoplasmic organelles. Golgi bodies and vesicles not frequently encountered. Nuclei are irregular in shape with a centrally located nucleolus. Cell division is common in cells of this size.	Early Spring to Early Summer	Stage of cell growth and division.
III	12-16 μ	Mitochondria and agranular endoplasmic reticulum associated with vacuoles, both membrane bound and lacking membranes. Nuclei with numerous indentations and peripheral masses of chromatin. Nucleoli regularly observed.	Spring and Summer	Stage of cytoplasmic vacuolization.
IV	14-18 μ	Mitochondria, when observed, are swollen or vacuolated. Golgi systems more frequent than in earlier stages. Nuclei are spherical with electron dense chromatin throughout. Cytoplasm is vesicular and contains some granules.	Spring to Late Summer	Continuation of vacuolar processes. Cellular hypertrophy.
V		Cytoplasm is vesicular, with granules commonly observed. Nuclei are uniformly dense and in some instances fragmented. Cell membranes are often discontinuous and cell contents are observed in surrounding regions.	Spring to Fall	Cell degeneration and lysis.

II. FEMALE REPRODUCTIVE SYSTEM

The ovaries of female P. leniusculus are similar to the testes in shape and location. Paired oviducts arise near the juncture of the two anterior lobes of the ovaries and extend ventrolaterally into the coxapodites of the 2nd walking legs. The oviducts open on the medial surface of the coxapodites, toward the ventral midline of the animal.

The ovaries of all animals examined contained ova in various stages of development and yolk storage. The ovaries of females nearing sexual maturity, 25 to 30 mm carapace length, contain numerous yolk filled ova, with a maximum diameter of 1 to 2 mm. Smaller females have correspondingly fewer ova of this type. Vitellogenic ova are surrounded by a single layer of follicle cells (Fig. 9). In cross section, individual follicular cells are oblong in shape and contain spherical, densely staining nuclei. The nuclei of vitellogenic ova stain with varying intensity and granular inclusions are often observed in the spherical nuclear mass. The yolk of these ova is often pigmented, and yolk near the periphery of the ova is globular in appearance.

Germ cell development, preceeding the stage of yolk storage, occurs in the central region of each of the ovarian lobes. As would be expected, the ovaries of smaller, immature crayfish contain greater numbers of meiotically dividing germ cells than do those of females nearing sexual maturity.

Figure 9. Vitellogenic and developing ova in the ovaries of P. leniusculus. Yolk filled ova are numerous in the ovaries of females nearing sexual maturity, while the earlier developmental stages of crayfish ova are more commonly encountered in smaller, immature animals. Vitellogenic ova (v), developing ova (d), follicular lining (f). 160 X.

III. EXPERIMENTAL RESULTS

Androgenic Gland Extirpation

Androgenic glands were removed from male crayfish ranging in size from 18 to 40 mm carapace length. Crayfish of this size range include those in their second through fourth years. Glands were removed from two groups of crayfish during the spring and late summer, and animals were maintained for periods varying in length from 4 to 60 days. Mortality rates for operated males were relatively high, 20%, with death usually occurring in the first post-operative day. Surviving animals recovered rapidly, and wound tissue was observed on the base of the excised coxapodites within 5 days. Moulting in operated and control animals occurred at approximately the same time. However, the moult cycle stage of controls from natural stream populations was found to vary from that of laboratory animals during both spring and late summer. In most cases, the laboratory animals were found to be in an advanced stage of the moult cycle.

As described previously, the testes of normal, spring animals contain numerous mitotically dividing germ cells, and by late spring meiotically dividing cells are observed. The sperm ducts of these animals have a well developed epithelial cell lining, and the androgenic glands are proliferating (Table III). The testes of experimental animals are similar to those of the controls. Germ cell activity is high, and meiosis is observed in animals sacrificed later in the spring. Sperm duct histology is much like that of the controls except that in

some operated animals the tissue adjacent to the surgical site undergo degeneration. Tissue in degenerating regions contains numerous granular hemolymph cells and layers of dense, brittle material. Degeneration of the ducts of experimental males is observed to proceed from the severed end of the duct toward the more proximal regions. Degeneration of sperm duct tissue occurred in only a small percentage of operated animals, and it probably was caused by infection or mechanical damage from the operative procedure. Differences in the behavior of operated and control animals were not observed.

The testes of animals captured in late summer contain germ cells in the later stages of spermatogenesis, and spermatozoa are found in the collecting tubules of most animals. The sperm ducts of most control animals have an actively secreting epithelial cell layer, and androgenic gland volume is at its maximum. Experimental animals exhibit similar histological features, with few exceptions. Spermatozoa are found in the collecting tubules of some animals, and the sperm ducts of these animals contain secretory material. The testes of some experimental animals contain germ cells in less advanced stages of spermatogenesis than those of the majority of controls. Qualitative differences in testes histology are difficult to assess, however, as spermatogenesis proceeds rapidly once primary spermatocytes are present in the testes. In paraffin sections, the sperm ducts of experimental and control animals appear similar and no differences in histology are observed. As in the spring, no behavioral differences are observed in control and experimental crayfish.

Androgenic Gland Implantation

Two groups of female crayfish, 17 to 30 mm carapace length, received androgenic gland implants during the spring and late summer. Each female was implanted with a pair of androgenic glands, the full gland complement of a normal male. Animals were maintained for periods of up to 60 days. Mortality rates for operated females were lower than for experimental males. Five percent of the operated females were lost due to the operative procedure or other causes during the course of the experimental period. As with the males, moulting in operated females was similar to that of controls.

The ovarian histology of both experimental and control females was similar. During both seasons, vitellogenic ova are present in the ovaries, and germ cells in earlier stages of development are also observed. Observations of ovarian tissue from numerous experimental females yielded no evidence of male germ cell development. Simple visual observation of paraffin sections revealed no major differences in the yolk storage of ova from experimental and control females of the same size. The oviducts of experimental and control females were similar in both seasonal groups.

During the spring, implanted gland tissue removed from female recipients was observed to be similar to gland tissue from normal males. Masses of gland tissue consisting of cellular strands were recovered from a number of experimental females. The implanted muscle tissue from control females also appeared normal when compared to muscle tissue from unoperated controls. In some females, however, the implanted gland tissue was invaded with granular hemolymph cells and tissue

degeneration was apparent (Table III). Implanted gland tissue could rarely be identified in late summer experimental animals. When found, implanted tissue consisted of masses of cells with pycnotic nuclei, diffusely staining cytoplasm, and indistinct cell boundaries. This tissue was often surrounded by dense, brittle concretions, and contained numerous granular hemolymph cells.

TABLE III

A SUMMARY OF THE RESULTS OF ANDROGENIC GLAND
REMOVAL AND IMPLANTATION IN P. LENIUSCULUS.

ANDROGENIC GLAND REMOVAL					
	Time of Year	Gland Histology	Testes	Sperm Ducts	Remarks
Control Males 18-40 mm c.l.*	Spring	Proliferation. Dividing, vacuolated, and degenerating cells.	Increasing spermatogenic activity.	No secretory activity. Epithelial cell division.	
Experimental Males 18-40 mm c.l.	Spring	-----	Same as above.	Same as above.	
Control Males 18-40 mm c.l.	Late Summer	Maximum gland size. Hypertrophied and degenerating cells.	Spermatogenesis near completion.	Secretion of mucous-like material occurring.	
Experimental Males 18-40 mm c.l.	Late Summer	-----	Same as above.	Same as above.	
ANDROGENIC GLAND IMPLANTATION					
	Time of Year	Gland Histology	Ovarian Histology	Remarks	
Control Females 17-30 mm c.l.	Spring	-----	Vitellogenic and developing ova present.		
Experimental Females 17-30 mm c.l.	Spring	Recovered glands similar to those of control males.	Same as above.	No differences in pleopod morphology or behavior after moulting.	
Control Females 17-30 mm c.l.	Late Summer	-----	Vitellogenic and developing ova present.		
Experimental Females 17-30 mm c.l.	Late Summer	Recovered glands degenerated. Invaded with hemolymph cells.	Same as above.	Some evidence of an immune response to implanted tissue.	
* Carapace Length					

DISCUSSION

As stated in the introduction, the malacostracan androgenic glands are thought to be the source of a hormone that maintains the form and sexual capability of male animals. Specific knowledge of the endocrine function of these glands has come from studies involving the removal and implantation of gland tissue, and also from studies correlating gland structure with male reproductive activity (Adiyodi and Adiyodi, 1970).

Removal of the glands of sexually mature P. leniusculus, during both proliferative and degenerative phases of gland development, resulted with little, if any, information concerning their endocrine function (Table III). Since androgenic gland removal has been shown to cause a decrease in germ cell division and a cessation of spermatogenic activity in some amphipod and decapod species (Charniaux-Cotton, 1955; Puckett, 1964), the possibility exists that gland removal in P. leniusculus was incomplete or was carried out during post critical periods of the reproductive cycle.

Although a number of gland implants were maintained successfully in female recipients, no effects on ovarian histology or female secondary sex characters were noted (Table III). Previous studies of decapod glands, using implantation techniques, have shown that implanted glands are capable of inhibiting vitellogenesis, and also of having a masculinizing effect on female recipients (Payen, 1969; and others). Puckett, however, noted no changes in the ovaries or secondary sex

characters of female crayfish which received implants of both testes and androgenic glands (Puckett, 1964). Since female P. leniusculus were implanted with two glands, the full male complement, the lack of observable results could be due to an insufficient quantity of actively secreting gland tissue, or to implants of tissue in a nonsecretory phase. The experimental results are further complicated by the possibility of an immune response to the implanted gland tissue. Implanted gland tissue was often invaded with granular hemolymph cells and surrounded by layers of dense brittle material, especially in females nearing maturity. In addition, it is possible that tissue differentiation, in the experimental females, had proceeded beyond the stage of being capable to respond to the masculinizing, androgenic hormone.

Although experimental studies of the androgenic glands of P. leniusculus yielded little information concerning gland function, the tissue and cell structure of the glands is indicative of a yearly pattern of activity and secretion. A comparison of androgenic gland tissue and cell structure with reproductive morphology and activity gives the following information (Table I). Proliferation of androgenic gland tissue occurs during early premoult phases of the moult cycle. This increase in gland size, through mitosis and cell growth, coincides with an increase in mitotic activity in the testes. As proliferation continues, strands of cells of varying structure can be identified. These include; strands of dividing cells, vacuolated cells, and cells in various stages of degeneration. At the time of the yearly moult of mature animals, the androgenic glands contain numerous strands of cells with highly vacuolated cytoplasm, and spermatogenesis is occurring in the

testes. Following ecdysis, spermatogenesis proceeds rapidly, and spermatozoa are present in the sperm ducts within a short period of time. The glands are at their maximum volume at this time, and consist primarily of strands of hypertrophied and degenerating cells. Degeneration of a major portion of the glands occurs prior to copulation, in the fall of the year. In the post-reproductive period following copulation, the glands are composed of a small number of cellular strands located in the muscle and connective tissue of the sperm ducts. At this time the testes are depleted of germ cells and are in a disorganized, nonfunctional state.

During the proliferative phase of gland development, in late pre-moult P. leniusculus, structural diversity among neighboring strands of cells is at a maximum. This condition, which indicates asynchronous cellular development, occurs in the glands of a number of decapod species (Charniaux-Cotton et al., 1966; Carpenter and deRoos, 1970; Payen, 1972; and others), and has been cited as evidence of the secretory nature of these glands (King, 1964a). In addition, the presence of degenerating cells in non-localized regions of supposedly functional glands has been considered by some to indicate a holocrine secretory process (Charniaux-Cotton, 1960; Tcholakian and Reichard, 1964; and others). Holocrine secretion, however, has not been confirmed by histochemical or ultrastructural studies (King, 1964a; Hoffman, 1969; and Payen, 1972).

Since gland proliferation in P. leniusculus coincides with the initiation of spermatogenesis in the testes, it seems likely that maximum secretory activity occurs at this time. The ultrastructure of a

majority of non-dividing cells, especially the vacuolated cells, would seem to substantiate this hypothesis. Cells undergoing vacuolization contain numerous mitochondria and a large amount of sac-like agranular endoplasmic reticulum. The nuclei of these cells are irregular in shape, highly indented at the periphery, and nucleoli are regularly observed. Vacuoles, both lacking membranes and membrane bound, are often closely associated with mitochondria, or are surrounded by endoplasmic reticulum. In some cells, lamellar arrangements of electron dense agranular membranes are in close proximity to large vacuoles, indicating a high amount of metabolic or synthetic activity. Vacuolated cells are most often encountered during gland proliferation, and are observed with much less frequency in other seasons of the year. Structurally similar cells have been described in the glands of a number of malacostracan species (Charniaux-Cotton et al., 1966; Hoffman, 1969; and Carpenter and deRoos, 1970), and have previously been observed in the glands of Pacifastacus (King, 1964b). Cells of this type are common in the glands of the crayfish, Orconectes nais, at the time of the moult from the sexually inactive form to the active sexual form (Carpenter and deRoos, 1970).

The cellular fine structure of various malacostracan glands has been reported to be similar to that of known protein synthesizing and secreting cells (King, 1964a; Meusy, 1965; and Malo and Juchault, 1970). However, certain features of protein secreting cells are lacking in the gland cells of P. leniusculus. Electron dense Golgi bodies and vesicles, and membrane bound protein or polypeptide granules are not present in the cytoplasm of gland cells during active phases of

development, both of which are characteristics of known protein secreting cells (King, 1964a). Furthermore, granular endoplasmic reticulum, which is a diagnostic feature of protein synthesizing cells, is never abundant in the gland cells of P. leniusculus.

Although androgenic gland cells do not resemble known vertebrate steroid secreting cells, certain features of the glands are similar to those of arthropod endocrine glands thought to be involved in the synthesis and release of lipid hormones. The insect prothoracic glands and the crustacean Y organs, which are thought to be the source of steroid moulting hormones, and the insect corpora allata, which are the source of the lipid Juvenile Hormone, have been observed to undergo cyclic changes in cellular structure that can be correlated with hormonal activity. Increased secretory activity in all of these glands corresponds with an increase in cytoplasmic vacuolization, nuclear irregularity, and an increase in the number of organelles such as mitochondria (Gabe, 1953; Waku and Gilbert, 1964; Scharrer, 1964; and Herman, 1967). Similar processes are observed in the glands of P. leniusculus, especially during the proliferative phase in late spring. This suggests not only a similarity of the secretory processes, but also that the androgenic gland hormone is lipid in nature.

Although the vacuolar contents of the gland cells of P. leniusculus were not identified, lipid vacuoles have been described in the hypertrophied glands of the crabs; Carcinus maenas, Callinectes sapidus, and Rithropanopeus harrisii (Meusy, 1965; Payen et al., 1971). Further evidence of the lipid nature of the androgenic gland hormone has recently been reported (Berreur-Bonnenfant et al., 1973). These

workers have extracted and partially purified a biologically active factor from the androgenic glands and hemolymph of the crab, Carcinus maenas. The active factor, which is in the lipid fraction of the extract, is capable of inhibiting vitellogenesis in the ovaries of Orchestia.

Thomsen and Thomsen, 1970, have suggested a working hypothesis for the synthesis and release of the lipid Juvenile Hormone, and it is possible that these processes occur in the same manner in the androgenic glands of P. leniusculus. These authors hypothesize that the Juvenile Hormone is synthesized in the agranular endoplasmic reticulum and collected in membrane bound vacuoles. They also suggest that dissolution of the vacuolar membranes will result in the formation of lipid droplets in the cytoplasm, which subsequently will be released to intercellular spaces and the hemolymph (Thomsen and Thomsen, 1970). The androgenic glands are similar, in that vacuolization occurs in cells with large amounts of agranular endoplasmic reticulum and numerous mitochondria. Furthermore, cellular vacuolization is at a maximum when spermatogenesis is occurring in the testes, a time at which the androgenic hormone titer would be expected to be high.

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