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GONADOGENESIS IN THE SOLITARY ASCIDIAN CORELLA INFLATA, WITH A REVIEW OF THE LITERATURE ON ASCIDIAN GONADOGENESIS AND GONAD ORIGIN

by

KATHERINE PRESCOTT IRONS

A THESIS

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Supervisor

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Date June 30, 1986

Abstract

The process of gonadogenesis in the solitary phlebobranch ascidian, Corella inflata, was described using light and electron microscopy. Morphological criteria were used to identify seven stages of gonadogenesis in this species. Within three days after metamorphosis of the tadpole larva (at 10 to 12 degrees C), the initial stage of gonadogenesis consists of one or a pair of hemoblasts located in the gonad hemocoel in close proximity to the dorsal strand. These gonad-forming hemoblasts make contact with the dorsal strand during the next stage of gonadogenesis. After this association has been established, the ovotestis forms a solid, round cluster of gonial and somatic cells. The ovotestis subsequently cavitates to form a centrally placed lumen, and two types of somatic cells, designated Type I and Type II, are found. In the next stage, the germinal layer is localized in the peripheral portion of the ovotestis, and the remainder of the organ is composed of a squamous somatic region. The testicular rudiment subsequently differentiates from the anterior region of the ovotestis, and remains associated with the ovarian rudiment near its attachment with the dorsal strand. Finally, the testis and ovary form exit ducts, and the format of the adult reproductive system is essentially completed. This study has greatly enhanced our understanding of ascidian gonadogenesis, which had previously been based on light microscopic examination of living juveniles, and/or 5 to 10 μm paraffin sections.

The significance of these findings to other areas of ascidian reproductive biology are discussed. By observing individuals in earlier stages of gonadogenesis than had previously been examined, a type of circulating blood cell, the hemoblast, was implicated in the origin of the gonad and germ cells in Corella inflata. The dorsal strand, a posterior continuation of the neural gland in many ascidian species, was described; this structure was invariably found in close association with cells of the developing gonad, or with the oviduct in later stages. Without the association of the dorsal strand, the reproductive system does not develop, and it is

presumed that the dorsal strand plays a definite, albeit uncertain, role in gonadogenesis in Corella inflata. Germ cell-specific inclusions have been identified for the first time in gonial cells of the ovotestis, and the possible significance of these structures is discussed. The ultrastructural observations presented in this study suggest that cell divisions may occur cyclically in the developing reproductive system of this species. These observations also suggest that the follicle cells, which surround ascidian oocytes, may be derived from Type II somatic cells very early during gonadogenesis in Corella inflata. Finally, a synthesis of the literature on ascidian gonadogenesis, much of which is based on studies of the late nineteenth and early twentieth centuries, is provided. This synthesis documents our current knowledge of the subject of gonadogenesis, and focusses on questions that need to be addressed in future research.

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I. INTRODUCTION

Ascidians, or sea squirts, are sessile members of the subphylum Urochordata, and are conspicuous in the faunas of every marine geographic region (e.g. Herdman, 1882-1886; Arnback-Christie-Linde, 1922-1934; Hartmeyer, 1924; Huus, 1937; Van Name, 1945; Berrill, 1950; Millar, 1971; Plough, 1978). With few exceptions they are hermaphroditic (Berrill, 1975). Solitary forms, which reproduce sexually, and compound forms, which reproduce both sexually and asexually, are common. The classification is based largely on the structure of the branchial basket, or pharynx, and the location of the gonads within the body (Berrill, 1950; Monniot and Monniot, 1972; Plough, 1978).

The chordate affinities of the ascidians were first demonstrated by the Russian embryologist Kowalevsky (1866). This invertebrate group has subsequently received a tremendous amount of attention, especially in the areas of descriptive and experimental embryology. Numerous classical papers published in the late ninteeth and early twentieth centuries are notable, particularly those of Chabry (1887) on Ascidia scabra, Castle (1896) on Ciona intestinalis, Crampton (1899) on Molgula manhattensis, and Conklin (1905) on Styela partita. One direction which more recent studies have taken, building on Conklin's (1905) contribution, has been to identify the organ-forming regions of the egg and embryo (reviewed by Hirai, 1968; Berrill, 1950, 1975; Reverberi, 1971). It has been shown that these organ-forming regions are relevant only to the organization of the larva, rather than to the organization of juveniles and adults (e.g. Minganti, 1954; Ortolani, 1955; Cowden and Markert, 1961; Smith, 1967; Whittaker, 1973, 1979a, b; Whittaker et al., 1977).

A great deal of research has been done on the structure of the tadpole larvae of ascidians (Berrill, 1950; Millar, 1971; Cloney, 1978), and of the spectacular events of settlement and metamorphosis (Cloney, 1978). In ascidians, a sharp separation exists between the temporary structures of the larva and the rudiments of the permanent adult structures; the development of each proceeds independently until metamorphosis (Barrington, 1968).

Reorganization takes place within the individual following the initial stages of metamorphosis. The rudimentary adult organs reorient to a position 90 degrees from that at settlement, the cerebral vesicle retracts, and the visceral ganglion, sensory organs and axial complex of the larva are phagocytized. In addition, the rudiments of the adult organs are released from their arrested developmental state (Cloney, 1978).

Histogenesis of the adult viscera occurs rapidly, as a corollary to metamorphosis (Cloney, 1978). The digestive system is well enough developed for the juvenile to begin to feed within hours after metamorphosis in some compound forms like Distaplia occidentalis (Cloney, 1972), or days in solitary forms such as Corella willmeriana (Lambert, 1968). However, the differentiation of the adult organ systems, and particularly the reproductive system (one of the most conspicuous organs in the adult) have been largely ignored in the recent literature. Our understanding of ascidian reproductive biology is hampered by the relative lack of information on gonadogenesis. A study using modern microscopic techniques, to describe not only the morphological changes that take place during gonadogenesis, but also the development of the gonoducts, the origin of the gonad and germ cells, and the organization of the cells that form the developing gonad, is long overdue.

A. GONADOGENESIS

Most information on gonadogenesis in ascidians is found in monographs of the late nineteenth and early twentieth centuries. Most of these reports concern gonadogenesis in compound species, but there is a striking similarity in the sequence of development of the reproductive organs, regardless of taxon (Kowalevsky, 1874a, b; Van Beneden, 1881; Van Beneden and Julin, 1884, 1886; Hjort, 1896; Julin, 1893; Lefevre, 1897, 1898; Bancroft, 1899, De Selys Longchamps and Damas, 1900; Huus, 1924). The general pattern of gonadogenesis that emerges from this literature is that a solid clump of mesodermal cells, found in the "pre-gonadal region" of the body, cavitates to form a small lumen. This structure subsequently

thins on one side and elongates. The ovotestis now consists of a ventral region that is several cells thick, and a dorsal region composed of a squamous epithelium. In the next stage of gonadogenesis, the testicular rudiment differentiates from the ovotestis, as either a small bulge on the anterior portion of the ovotestis (Van Beneden and Julin, 1886; Lefevre, 1898; De Selys Longchamps and Damas, 1900; Aubert, 1954), or as a medial furrow which divides the ovotestis into two lobes (Van Beneden and Julin, 1886; Julin, 1893; Lefevre, 1897; Bancroft, 1899). The rudiments of the ovary and testis are initially connected by a small opening, but this subsequently closes as the gonoducts begin to form. These rudiments expand, and depending on the configuration of the adult reproductive system, may branch to form numerous lobes and lobules (Van Name, 1945; Berrill, 1950, 1975).

There are a few exceptions to this general pattern of gonadogenesis. The gonads of Distomus variolosus are of separate sex located in different regions of the body (Newberry, 1968). These organs develop from separate cell masses, each of which differentiates into an ovary or a testis. Initially, the sex of these individual masses cannot be determined, but the developing organs soon take on cellular characteristics that identify them by sex. Otherwise, the ovary and testis develop in a fashion similar to that described above. In botryllid ascidians, gonadogenesis is also somewhat different than that described above (Mukai and Watanabe, 1976; Sabbadin and Zaniolo, 1979). In these animals, after the single oocyte has attached in the gonadal space of the new blastozooid generation, some of the elements of the "loose cell mass", differentiate into the follicle stalk and an outer somatic cell layer surrounding the oocyte. Other elements of the "loose cell mass" differentiate into the testis, which develops in a fashion similar to Distomus variolosus.

These studies provide information on the sequence of events that occurs during gonadogenesis. They are, however, limited to gross morphological changes, and do not address the cellular organization of the developing gonad, or the structure of its cells.

B. GONODUCTS

Most reports that discuss gonadogenesis also discuss formation of the oviduct and sperm duct. In some species, a string of undifferentiated cells, the genital strand, is attached to the dorsal end of the ovotestis, and follows the intestine to the atrium. In a few of these species, the genital strand divides to form two ducts, each of which opens separately into the atrium (Kowalevsky, 1874a, b; Van Beneden and Julin, 1884, 1886; Lefevre, 1897). In other species, the genital strand remains attached to the dorsal end of the ovarian rudiment, and shortens and thickens as the ovarian rudiment elongates. Once the oviduct has reached the atrium, the genital strand is no longer visible (Van Beneden and Julin, 1886), and may therefore have a role in gonoduct formation. Bancroft (1899) observed that the genital strand is converted into the oviduct and sperm duct.

In one species, the posterior extension of the neural gland, the dorsal strand, has been implicated in gonoduct formation (Huus, 1924). Huus (1924) observed that the dorsal strand in Corella parallelogramma remains associated with the ovarian rudiment during gonadogenesis, and that as the ovarian rudiment elongates, it forms a tubular oviduct at its dorsal end. The testicular rudiment opens into the oviduct near the dorsal strand attachment, and the sperm duct is formed as the ovary elongates. Eventually, these two ducts open separately into the atrium. As the dorsal strand is present as only a vestige in the adult, Huus (1924) concluded that the gonoducts are formed from the dorsal strand. While other authors have disputed this observation (Brien, 1927; Aubert, 1954), it appears that the dorsal strand may play a role in gonadogenesis in some species of ascidians (Goodbody, 1974; Berrill, 1975).

In some other species, the gonoducts have been described as arising from the developing rudiments themselves (Van Beneden, 1881; Floderus, 1896; De Selys Longchamps and Damas, 1900; Aubert, 1954; Newberry, 1968). In still other species there is no oviduct, and mature oocytes appear to rupture the wall of the ovary into the atrium.

C. ORIGIN OF THE GONADS AND GERM CELLS

There is no evidence that the germ cells of ascidians belong to a cell lineage that can be traced back to the egg (Simkins, 1924; Nieuwkoop and Sutasurya, 1979; Berrill, 1975). Instead, the available evidence indicates that germ cells arise *in situ* from undifferentiated somatic cells such as those of blood or mesenchyme (Kowalevsky, 1866, 1874a, b; Van Beneden, 1881; Van Beneden and Julin, 1884, 1886; Lefevre, 1897, 1898; Newberry, 1968; Berrill, 1975; Sugimoto and Nakauchi, 1974). There is also some indication in a few species that the gonads and germ cells arise from regions of the dorsal strand (Brien, 1925, 1927), or the atrial epithelium (Berrill, 1941a, b; Sabbadin and Zaniolo, 1979). There are no reports, however, in which the origin of the germ cells has been experimentally examined.

Among compound ascidians, numerous authors have stated that the origin of the gonads and germ cells is undoubtedly mesodermal or mesoblastic; this is based on the structural similarity between the cells that form the developing gonad, and some of the circulating blood cells (eg. Kowalevsky, 1874a; Van Beneden and Julin, 1884, 1886; Lefevre, 1897, 1898; Newberry, 1968). Most of these studies have involved observations of animals in which the developing gonad was already present as a small clump of cells. The only authors that have examined sufficiently young specimens to observe formation of the gonad are Simkins (1924), Brien (1927), Newberry (1968), Mukai and Watanabe (1976) and Sabbadin and Zaniolo (1979). Newberry (1968) described small aggregations of lymphocytes (hemoblasts, according to the definition of Wright, 1981) in the pre-gonadal regions of the mantle sinus in Distomus variolosus. These aggregations become surrounded by a "stromal meshwork" of connective tissue, take on a fairly cohesive appearance, and divide mitotically, developing into the gonads. In the oozooid of Ecteinascidia turbinata, Simkins (1924) reported that the ovary and oocytes are derived from a mesodermal shelf that projects into the hemocoel. The testis of E. turbinata originates from a solid clump of cells near the ovarian rudiment. Brien (1927) reported that the gonad is derived from the dorsal strand in Aplidium zostericola. The dorsal strand divides in a

small localized region to form two tubes, the dorsal tube developing into the ovary, and the ventral tube into the testis. Mukai and Watanabe (1976) found that in Botryllus primigenus, the oocyte and its surrounding primary follicle cells are derived from a "loose cell mass", composed of hemoblasts. The oocytes are released into the circulatory system, migrate through the blood, and attach in the gonadal space of a subsequent generation. The testis is derived from elements of the "loose cell mass" of the new generation. Sabaddin and Zaniolo (1979) report a similar sequence of events, but considered that the "loose cell mass" consisted of cells already differentiated from hemoblasts.

All observations of gonad and germ cell origin in solitary ascidians are based on animals in which the developing gonad is already present in the pre-gonadal region (Kowalevsky, 1874b; Van Beneden and Julin, 1886; Julin, 1893; Floderus, 1896; De Selys Longchamps and Damas, 1900; Huus, 1924; Aubert, 1954). This information suggests that blood cells are involved in gonadogenesis, but it is of limited value in determining the origin of the gonad and germ cells in ascidians.

D. CHARACTERISTICS OF THE GONIAL CELLS AND SOMATIC CELLS

Several reports that describe the stages of gonadogenesis in ascidians also describe characteristics of the cells that form the developing gonad. However, these observations were all made on paraffin sections, and since gonadal cells are very small, little information other than cell shape and size exists which can be used to identify and compare these cells. While many reports on the characteristics of the mature gametes are available (see Kessel, 1983; Franzén, 1983), there have been no ultrastructural investigations on cells of the developing reproductive system during gonadogenesis.

Stage I oocytes (Cowden, 1961) are those that have differentiated from the germinal epithelium, and are beginning the initial phases of growth. The germinal vesicle in these cells is large, and contains an eccentric nucleolus (Reverberi, 1971; Mancuso, 1964; Kessel, 1983). The

cytoplasm is intensely basophilic (Cowden, 1961, 1962, 1967) due to the large number of ribosomes (Kessel, 1983). Kessel (1983) describes small dense aggregations, which probably represent ribosomes, and larger dense granular masses in regions of the perinuclear cytoplasm of these pre-vitellogenic oocytes. There is often a continuity of this material with the nucleoplasm through nuclear pores, and it appears that material is moved to the cytoplasm from the nucleus (Kessel, 1966a, 1983). The larger of these two inclusions has been variously named 'nuage', 'yolk nuclei', or 'vitelline bodies' (see Beams and Kesssel, 1974; Eddy, 1975; Kessel, 1983), and is a germ line specific inclusion. Nuage has been identified in a wide variety of animal germ cells (Beams and Kessel, 1974; Eddy, 1975; Kessel, 1983). Other organelles that are characteristic of Stage I oocytes are vesicles of RER, formed as blebs of the outer layer of the nuclear envelope, as well as lamellar RER (Kessell, 1983). There are relatively few mitochondria in these cells which are often associated with RER or lipid droplets, annulate lamellae, and a single small Golgi complex (Kessel, 1983).

At the end of Stage I of oogenesis, the oocytes become surrounded by a layer of primary follicle cells (e.g. Cowden 1961; Reverberi, 1971; Kessel, 1983), which contain both vesicular and lamellar RER, and a large Golgi complex. These primary follicle cells differentiate into the accessory cell layers which surround the mature oocyte. The origin, structure and function of these accessory cell layers have been the subject of intensive debate for nearly 100 years. They have been described to originate from the germinal epithelium (Pérès, 1954; De Vincentiis, 1962; Kalk, 1963a; Kessel, 1962, 1983), or from blood cells (Spek, 1927; Knaben, 1936; Mancuso, 1965). While there are numerous papers discussing the structure of the mature spermatozoa of ascidians (Franzén, 1983) there is almost no information on the structure of the developing germ and somatic cells of the testis. This has been briefly discussed by Georges(1969) and Tuzet et al. (1974).

Corella inflata is a beautiful, solitary phlebobranch ascidian found commonly on subtidal floats of the Pacific Northwest. Its distribution, abundance and general ecology have

been examined (Lambert, 1968; Lambert et al., 1981; Young, 1982), and its taxonomic status has recently been reviewed by Lambert et al. (1981). This species is readily available in the summer at snorkelling depths in the Friday Harbor vicinity, is easily maintained in the laboratory, and cultures of embryos and larvae are readily obtained. Corella individuals can grow to sexual maturity within less than three months (Lambert, 1968), and the gonads begin to differentiate within a few days of metamorphosis under laboratory conditions. The transparent tunic of Corella inflata allows direct observation of the various stages of gonadogenesis in intact, living animals.

II. MATERIALS AND METHODS

Adult and juvenile specimens of Corella inflata were collected intermittently from May to August, 1983 and 1984 by snorkelling and SCUBA from the undersurfaces of floats at Jensen's Marina, Friday Harbor Marina, and the breakwater at the Friday Harbor Laboratories in Friday Harbor, Washington. Intersiphon distance, and length from the posterior extremity (base) to between the siphons was measured with either Vernier calipers or an ocular micrometer in a Wild M-5 dissecting microscope. Specimens were subsequently set up in culture to obtain embryos, or fixed within three days of collection.

A. CULTURING

Spawning in Corella inflata occurs just after dawn, with first sperm and then eggs being released into the inflated atrium located uppermost on the animal under natural conditions. Self-fertilization appears to be common in this species, with fertilization taking place immediately after eggs are released into the atrium from the oviduct. The eggs are surrounded by follicle cells, and the inner layer of follicle cells contain ammonium ions (Lambert and Lambert, 1978). Consequently, the eggs float to the top of the atrium where they are retained until hatching. Embryos develop normally outside of the atrium, and it is therefore possible to obtain cultures of embryos by removing them from the atrium of the parent.

Some adult specimens were glued with Super Glue to Velcro strips, and placed, atrium uppermost, on floating strips of wood in a flow-through aquarium. These animals spawned daily for several weeks, and embryos were routinely collected by pipetting from the atrium of the parents. Other adult animals were placed individually or in pairs in 500 ml beakers of filtered seawater (FSW), which was changed twice daily. These adults released their embryos, which were pipetted directly from the water surface. Collected embryos were cultured in FSW until hatching. At that time, 200 to 300 tadpole larvae were collected with a fine bore breaking

pipette to avoid collection of the discarded follicle cells, and were placed in 5 cm diameter Falcon plastic petri dishes provided with either scored plastic or parafilm surfaces on which to crawl. Most tadpoles settled and metamorphosed after 6 to 12 hours. The petri dishes were then suspended from floating strips of wood in aquaria of FSW changed twice daily.

Juveniles, four days post-metamorphosis, were fed each day with cultures of the small single-celled flagellates, Dunaliella sp., Monochrysis sp., and Isochrysis sp., obtained from Carolina Biological Supply Co.

Adults, embryos and juveniles were maintained at ambient seawater temperatures which ranged from 10 to 16 degrees Centigrade during the course of the summer. Juveniles of the same post-settlement age from individual petri dishes were counted and measured with an ocular micrometer on either a Wild M-5 dissecting microscope, or a Wild M-20 compound microscope. Juveniles were easily removed from the scored plastic or parafilm surfaces for subsequent examination or fixation.

B. LIGHT MICROSCOPY

Positions and sizes of the developing organ systems of cultured juveniles relative to the developing reproductive system, were mapped with a camera lucida. Drawings of at least 4 live individuals of each stage of gonadogenesis were made with a Wild drawing tube fitted to a Wild M-20 compound microscope.

Live, unstained specimens were photographed using Nomarski differential interference contrast (DIC) techniques with a Nikon Optiphot microscope. Photographs were taken with Kodak Pan-X or Technical Pan 2415 film.

The reproductive systems of entire specimens were best visualized in whole mounts. These whole mounts were prepared according to one of two methods: a) small individuals (less

than 1.0 mm) were fixed in 0.5% osmium tetroxide in seawater, dehydrated through a graded series of ethanol, infiltrated and mounted on a microscope slide with an A:B ratio of 7:3 Epok 812 (E. Fullum & Co.), according to the technique of Cavey and Cloney (1973). b) Larger specimens (1.0 mm and larger) were fixed in Boum's fluid, washed in 70% ethanol, and stained in Grenacher's Alcoholic Borax Carmine for at least 5 hours. Specimens were dehydrated in a graded series of ethanol, infiltrated and mounted on a microscope slide in Permount (Gallagher and Kozloff, 1971).

Light micrographs of sectioned material were made from organisms fixed and embedded for transmission electron microscopy as described below. One micrometer sections were cut with glass or diamond knives on a Porter Blum MT-1 or MT-2 ultramicrotome and stained with methylene blue and Azure II (Richardson et al., 1960). Sections were photographed on a Zeiss Photomicroscope or a Nikon Optiphot using Kodak Technical Pan 2415 film at ASA 100.

C. TRANSMISSION ELECTRON MICROSCOPY (TEM)

Specimens larger than 2.0 mm were first removed from their tunics and placed in primary fixative; further dissection was done while the tissue was immersed in fixative. Animals smaller than 2.0 mm were not dissected prior to fixation, but were sliced open on the ventral surface within 15 minutes of immersion in fixative, to ensure adequate penetration.

Several fixation techniques were used in this study. The best results were obtained by initial fixation in a solution containing 2.5% glutaraldehyde, 0.2 M Millonig's phosphate buffer, and 0.14 M sodium chloride (Cloney and Florey, 1968) for 2 to 3 hours at room temperature. Specimens were then rinsed in a solution containing 0.2 M phosphate buffer and 0.34 M sodium chloride for 10 minutes, followed by post-fixation in 2% osmium tetroxide and 1.25% sodium bicarbonate (pH 7.2) for 1 to 2 hours at room temperature (Cloney and Florey, 1968).

A second fixation technique involved primary fixation in a solution containing 2% glutaraldehyde, 0.2 M sodium cacodylate, 0.275 M sucrose, and 1% tannic acid (Torrence and Cloney, 1981). Primary fixation was followed by a rinse of 0.27 M cacodylate and 0.37 M sucrose, and post-fixation in 2% osmium tetroxide buffered with 1.25% sodium bicarbonate. Best results for this fixation regime were obtained at 4 degrees Centigrade.

A third method was modified from Eisenmann and Alfert (1982), in which tissue was initially fixed for 1 hour over ice in a solution of 9 parts primary fixative (2% glutaraldehyde, 0.1 M sodium cacodylate, 0.05 M sodium chloride, and 0.175 M sucrose), and 1 part secondary fix (containing 1% osmium tetroxide, 0.3 M sodium chloride and 0.2 M cacodylate). This was followed by fixation in glutaraldehyde fixative without osmium for an additional hour, and osmication for 1 to 2 hours. The osmolality employed was approximately one half that recommended by Eisenmann and Alfert (1982).

After fixation, tissues were dehydrated through a graded series of acetone or ethanol, transferred through three changes of propylene oxide, infiltrated and embedded in flat planchettes in an A:B ratio of 6:4 Epok 812 (E. Fullam, & Co.), according to Luft (1961). Following polymerization, specimens were cut from the planchettes, mounted and oriented on an aluminum slug prior to sectioning.

Silver and silver-gold sections were cut on a Porter-Blum MT-2A ultramicrotome with a duPont diamond knife and mounted on uncoated 200 to 300 mesh copper grids. Sections were stained with 50% methanol saturated with uranyl acetate, washed in methanol, stained with lead hydroxide chelated with sodium citrate (Reynolds, 1963), and washed in 0.02 M sodium hydroxide. The sections were examined with a Phillip's EM 201 or EM 300 electron microscope.

D. SCANNING ELECTRON MICROSCOPY (SEM)

Gonads of several adult Corella inflata were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer and sodium chloride, and post-fixed in 2.0% osmium tetroxide and 1.25% sodium bicarbonate as described above for TEM. The tissues were dehydrated through a graded series of ethanol, tranferred to amyl acetate, and critical point dried. The gonads were then split open with a razor blade, coated with gold on a sputter coater, and viewed on a Cambridge Stereoscan-150 Scanning Electron Microscope.

III. RESULTS

A. GENERAL OBSERVATIONS

Tadpole larvae of Corella inflata generally swim for a period of 6 to 12 hours after they hatch and before they settle and metamorphose, although they are capable of postponing metamorphosis for several more hours. During the later stages of metamorphosis, the trunk and rudiments of the adult viscera rotate to a position 90 degrees from that at settlement, and phagocytosis of the larval nervous system and axial complex begins. Subsequent differentiation of the adult viscera is rapid, and within 3.5 to 4 days after metamorphosis (at 10 to 12 degrees C) the digestive system of the juvenile is functional and the organism is capable of feeding. Degenerating cells of the axial complex may be visible in the subendostylar sinus of the hemocoel for several days after the juvenile has begun to feed.

The orientation of the body of adult ascidians has been outlined by numerous authors; in this work, I refer to the description of Millar (1953) on Ciona. According to this plan, the branchial, or incurrent, siphon marks the anterior end; the basal attachment region, the posterior end. The ventral side is continuous with the branchial siphon and contains the endostyle. The side opposite to the ventral, in which the dorsal lamina is found, comprises the dorsal side. In addition, I occasionally refer to right and left sides of the body relative to this orientation (Figs. 1 & 2).

The gonad of adult Corella inflata is a compound, hermaphroditic organ located in the subendostylar blood sinus, or gonad hemocoel. The gonad hemocoel, like most of the blood channels in the ascidian circulatory system, has no endothelial lining. It is delimited posteriorly and laterally by the basal lamina of the epidermis, and anteriorly by the basal lamina of the atrial epithelium (Figs. 3 & 4), and therefore is part of the connective tissue compartment. It contains numerous circulating blood cells, and connective tissue fibers (Figs. 3 & 4).

The ovarian part of the gonad in adult Corella consists of numerous large lobes primarily concentrated in the loop of the intestine, but also ramifying over the lateral surfaces of the intestine and stomach (Fig. 5). Each lobe consists of a germinal layer containing oocytes in all stages of oogenesis. Each oocyte is surrounded by developing or fully formed layers of accessory cells, including follicle cells and test cells (Fig. 4). There is no distinct outer epithelial layer surrounding the lobes. Each ovarian lobe is continuous with the long oviduct, which is composed of a simple ciliated epithelium. The oviduct runs adjacent to the rectum, and opens into the atrium in close proximity to the anus.

The testicular part of the gonad consists of numerous smaller lobes or lobules. These lobules are positioned primarily on the left side of the animal, and external to the ovary (Figs. 3, 6 & 7). Each testicular lobule contains a germinal epithelium at its distal extremity (Fig. 7), and in mature animals, the lumen of the lobule is packed with spermatids and spermatozoa. Each lobule is continuous proximally with a short duct which opens into a single large, sparsely ciliated sperm duct composed of a simple epithelium. This duct in turn follows the rectum and opens into the atrium between the oviduct and anus.

The gonad of adult animals is found in the gonad hemocoel surrounded by the loop of the intestine. The position of the developing gonad relative to this loop serves as a convenient marker for both the location and the developmental stage of the reproductive system. In newly feeding animals, the intestine makes a short lateral loop to the right of and at the level of the stomach. In the earliest stages of gonadogenesis, the developing gonad is ventral and posterior to this loop of the gut (Illustration 1). In later stages of gonadogenesis, and in adults, the intestine forms a loop which curves around posterior to the stomach. In these stages, the gonad is surrounded by the gut loop and completes its development in this position (Illustration 2).

The extent of gonad development in juveniles is dependent on the size of the animal rather than its post-settlement age. Animals in culture grow at asynchronous rates depending on availability of food, position at settlement, and extent of fouling. Size has been found to be

an accurate indicator of gonad stage and will be used in the subsequent description of the stages of gonadogenesis.

B. DORSAL STRAND

The dorsal strand is a structure that appears to play a role in the development of the gonad in Corella inflata. In juveniles, 5 to 6 days post-metamorphosis, this thin strand of cells extends from the neural gland (Figs. 8 & 9) to the gonad hemocoel, running between the two atrial siphons along the epidermis of the dorsal side of the body (Fig. 9). It enters the gonad hemocoel and ends in contact with the developing gonad. After the fusion of the atrial siphons in older juveniles, the dorsal strand is found in the dorsal fold, the tissue that separates the branchial basket from the atrium and that forms the roof of the branchial basket between the neural complex and anus. The connection between the dorsal strand and the developing gonad is maintained during the further development of the reproductive system, but after the gonad is fully formed and the gonoducts are established, this association is lost. The dorsal strand is only vestigial in mature animals, ending adjacent to the opening of the sperm duct.

The dorsal strand can easily be seen and followed from the neural gland to the developing gonad in living animals using DIC optics. In whole mounts and in one-micrometer sections, however, it is very difficult to demonstrate due to both its thinness and its complex geometry. In longitudinal sections of the juvenile the dorsal strand is shown in grazing section only, making it difficult to demonstrate the relationship of the dorsal strand to other cells and organs of the body.

The dorsal strand is a cord of single cells, 2 μm in diameter, along its entire length during gonadogenesis (Fig. 10), except in the region where it contacts the developing gonad (Fig. 15). Occasionally, adjacent cells have overlapping extensions along their joining edges (Figs. 11 & 14). A thin external lamina surrounds the entire structure (Figs. 10 & 11), indicating it is epithelial in origin. In the gonad hemocoel, the dorsal strand may be separated

from the epidermis by as little as 150 nm (Fig. 12). Abundant connective tissue fibers are found in the region between the dorsal strand and the epidermis (Fig. 12).

The ultrastructure of the dorsal strand cells is comparable in all stages of gonadogenesis. Each cell has a large elliptical nucleus with dense peripheral heterochromatin (Figs. 10 & 11). The cytoplasm contains abundant free ribosomes and glycogen (Figs. 10, 11 & 16). There are scattered mitochondria, a large proximally positioned Golgi complex, occasional secondary lysosomes, and lamellar rough endoplasmic reticulum in the cytoplasm (Figs. 10, 11 & 16). Some localized regions of the cytoplasm, subjacent to the plasmalemma, contain bundles of microfilaments (Fig. 13). The diameter of these filaments is 4 to 7 nm, and is consistent with the interpretation that they are actin filaments. Some membrane-bounded vesicles, 120 to 200 nm in diameter and containing a core of moderately electron dense material, are present in the cytoplasm of some dorsal strand cells (Fig. 11). These vesicles are most abundant in the cells of the dorsal strand near the contact with the ovotestis. The membranes between adjacent cells of the dorsal strand are joined by desmosomes (Fig. 14).

The cells of the dorsal strand are virtually indistinguishable from the somatic cells of the developing gonad in the region where they make contact (Fig. 15). In animals 1.0 mm or smaller, the dorsal strand at the point of contact with the ovotestis is a single cell in diameter. In larger animals the end of the dorsal strand forms a blunt enlargement, 2 to 3 cells in diameter, at this point of contact (Fig. 15). The nuclei of the dorsal strand cells in this location are larger and more irregular in shape (Fig. 15) than those of either the distal dorsal strand or the developing gonad; otherwise the cells appear to be identical in structure.

In animals that are 0.6 mm in diameter and larger, another strand, or cord, of cells accompanies the dorsal strand in the vicinity of its contact with the developing gonad. In some areas, this cord is composed of multiple overlapping processes that are usually no more than 0.3 μm in diameter (Fig. 17). In other regions the cord is composed of a single process that varies from 0.3 μm (Fig. 12) to 2.5 μm in diameter (Figs. 15 & 16). The cord is separate from

the dorsal strand, although in some locations its plasmalemma directly contacts the plasmalemma of the dorsal strand cells (Fig. 16). In regions of close apposition, the external lamina surrounds both the dorsal strand and the cord (Fig. 16).

The cells of this cord have many features characteristic of axonal processes of nerve cells. Serial one-micrometer sections of whole animals have failed to reveal the perikarya of these cells, which are presumably located in the neural ganglion. The cytoplasm contains a few mitochondria, secondary lysosomes and lamellar rough endoplasmic reticulum (Figs. 11, 16 & 19). Numerous moderately dense, membrane-bounded vesicles, 100 to 150 nm in diameter, are conspicuous in the cytoplasm (Figs. 12, 15 through 19). Some of these vesicles are ovoid, with a long axis up to 250 nm in diameter (Fig. 18). There are also less numerous electron opaque vesicles 60 to 80 nm in diameter (Fig. 18). In addition, these cells contain occasional filamentous structures, 25 to 30 nm in diameter which are probably microtubules (Fig. 18). This morphological evidence suggests that this cord of cells is nervous tissue, and it is therefore named a nerve cord.

The nerve cord contacts the developing gonad close to its junction with the dorsal strand (Fig. 19). Some of the somatic cells of the developing gonad at this point of contact contain vesicles similar to those found in the nerve cord (Fig. 19). In addition, in certain areas of the developing gonad, cytoplasmic processes of the nerve cord contact and penetrate between cells of the developing gonad (Fig. 20). The contents of these short processes are similar to those of the nerve cord itself (Fig. 20). Junctions between membranes of the nerve cord and the developing gonad have not been identified.

C. STAGES OF GONADOGENESIS

There is a tremendous variety of terms in the literature used to describe the stages of gonadogenesis in ascidians, and much of it is confusing and misleading. I have chosen to use terms that are as accurate and clear as possible, to describe the morphological changes that take

place during gonadogenesis in Corella inflata. The term **ovotestis** is applied to the developing gonad before there is any indication of a separation of the organ into ovarian and testicular portions. Once the ovotestis has separated into two distinct regions of separate sex, the ovarian portion is called the **ovarian rudiment**, and the testicular portion is named the **testicular rudiment**. After these rudimentary organs have developed separate and distinct ducts, they are called the **ovary** and **testis** respectively. I have also used the term **germinal layer** to denote the region of the gonad in which gonial cells are found.

In Corella inflata, seven stages of gonadogenesis have been identified in juveniles during the establishment of the reproductive system: Stage I, in which the developing gonad consists of one or a pair of cells in close proximity to the dorsal strand; Stage II, where the pair of cells and the dorsal strand have made contact; Stage III, where the ovotestis consists of a small clump of cells; Stage IV, during which the clump of cells cavitates to form a lumen; Stage V, where the germinal layer of the ovotestis becomes localized in the ventral region of the organ; Stage VI where the testicular rudiment differentiates from the ovotestis ; and Stage VII, in which the ovary and testis have developing ducts, and take on the structural characteristics of the mature gonad. These seven stages will be described in the following sections using light and electron microscopic observations. A summary of the morphological changes that take place during gonadogenesis is available in Illustration 3, and in Table 1.

STAGE I

Examination with DIC optics shows that there are several types of circulating blood cells in the gonad hemocoel of animals that are less than 0.3 mm in diameter. A large cell, or pair of cells, morphologically identical both to circulating hemoblasts, and to the cells of the next stage of gonadogenesis, are found subjacent to the epidermis and posterior to the loop of the gut (Figs. 21 & 22). These hemoblasts are found in close proximity to the terminal end of the dorsal strand (Fig. 21), and my morphological observations indicate that they are not

circulating cells, but rather are anchored to the epidermis by short cytoplasmic extensions. No mitotic figures have been observed in either the hemoblasts, or the terminal cells of the dorsal strand.

During this stage of gonadogenesis, the dorsal strand grows posteriorly from the neural gland, along the epidermis between the atrial siphons to the gonad hemocoel. It ends posterior to the intestine, in the vicinity of the axial complex cells undergoing phagocytosis (Fig. 22), and near the pair of hemoblasts (Fig. 21). The dorsal strand is approximately 2 μm in diameter, and its terminal end is tapered (Fig. 21). In favorable optical sections, the tapered end of the dorsal strand is routinely associated with concentric rings of connective tissue fibers, approximately 8 to 10 μm in diameter (Fig. 21). There is every indication that the terminal end of the dorsal strand makes contact with the hemoblasts in the gonad hemocoel.

STAGE II

The Stage II ovotestis is first visible attached to the dorsal strand in the gonad hemocoel of animals only slightly larger than 0.3 mm (Figs. 23 & 24). The location of the ovotestis within the gonad hemocoel is somewhat variable, but it is always found close to the posterior extremity of the animal, well below the level of the intestine and on the right side of the body (Fig. 23). At this stage the ovotestis is 7 to 10 μm in diameter, and consists of two large cells which form a teardrop shape (Figs. 24 & 25).

The Stage II ovotestis is closely associated with the epidermis, but they do not make direct contact. There are abundant connective tissue fibers around the ovotestis (Figs. 23 & 25); the fibers are about 35 nm in diameter with a banding pattern similar to that of collagen (Fig. 26). The fibers may serve to anchor the ovotestis to the epidermis, or to prevent contact between the ovotestis and the numerous blood cells and cells of the degenerating axial complex that are present in the gonad hemocoel (Figs. 22 & 23).

The two cells of the Stage II ovotestis are morphologically identical. They generally form a regular, smoothly contoured outline (Fig. 25), although they may have short, blunt extensions. Individual cells are approximately 6 μm in their longest dimension. The nucleus contains a conspicuous nucleolus; the nucleoplasm is otherwise relatively diffuse, although there are localized regions of peripheral heterochromatin (Fig. 27). The cytoplasm contains few mitochondria, a Golgi complex, an occasional secondary lysosome, scattered profiles of lamellar rough endoplasmic reticulum (Fig. 27), and free ribosomes and glycogen rosettes. There are no specialized junctions between the cells of the ovotestis at this stage, nor is there any indication of a basal lamina around the cells (Fig. 27).

The cells of the ovotestis, in this stage of gonadogenesis, are virtually indistinguishable from hemoblasts of the circulating blood. The hemoblasts contain nuclear and cytoplasmic elements that are morphologically identical to those of the ovotestis (Figs. 27 & 28). The only way the ovotestis itself can be postively identified is by its location, and its attachment to the dorsal strand.

STAGE III

In animals which measure from 0.4 to 1.0 mm, the ovotestis is always found in the gonad hemocoel posterior to the intestine and ventral to the stomach. It consists of a clump of 15 to 20 cells, and measures 20 to 25 μm in length and 10 to 15 μm in width. The clump of cells is generally elongate in the smallest individuals of this stage (Fig. 29), although it is occasionally triangular. In larger animals of this stage, the ovotestis becomes a solid spherical clump of cells.

Two types of cells are easily distinguishable in the ovotestis of this stage. There are 2 to 3 large cells approximately 6.5 μm in diameter with a conspicuous nucleus 4.5 μm in diameter. These are the gonial cells. The remainder of the cells of the ovotestis are somatic cells; they are relatively squamous, and while variable in size, may be as much as 7 μm long by 1.5 μm wide.

The somatic cells surround the gonial cells.

Gonial Cells

The gonial cells are approximately spherical (Fig. 30), with short processes extending from several regions of the cell (Figs. 31 & 34). They contain a large nucleus with diffuse chromatin, little peripheral heterochromatin, and a prominent nucleolus (Fig. 30). Beaded granular clusters are occasionally visible in the nucleoplasm between the nucleolus and the nuclear envelope (Fig. 30). The cytoplasm of the gonial cells contains numerous free ribosomes and glycogen rosettes (Figs. 30 & 32). Small aggregations of granular material, which do not have a distinct structure, are found in localized regions of the perinuclear cytoplasm and associated with nuclear pores (Figs. 30 & 32). There are numerous mitochondria, most with a single matrix granule visible in section, a few cisternae of rough endoplasmic reticulum (Fig. 30), occasional secondary lysosomes up to 1 μm in diameter, and a small Golgi complex (Fig. 31) in these cells. Myeloid figures (Fig. 32), and spherical finely-grained inclusions which are 0.3 μm in diameter and not limited by membranes, are also found in the cytoplasm (Fig. 33). The organelles of each gonial cell are relatively more abundant in the region opposite to the point of contact with its neighboring gonial cell (Fig. 30). No specialized junctions were found between gonial cells at this stage, although there are infrequent membrane densities between the gonial cells and the somatic cells which surround them (Fig. 30).

Somatic Cells

The somatic cells of the Stage III gonad are variable in size and shape; some are elongate, and up to 7 μm in length with an elliptical nucleus (Figs. 30, 31 & 34). Others, particularly those at the extremities of the ovotestis, are cuboidal

with oval nuclei (Fig. 35). The somatic cells have long processes that completely surround the gonial cells (Fig. 31), separating them completely from the environment of the gonad hemocoel. A thin basal lamina, external to the somatic cells, is first visible in this stage (Figs. 34 & 35).

The nuclei of the somatic cells contain an eccentrically placed nucleolus and some peripheral heterochromatin (Figs. 34 & 35). The cytoplasm of a few of the somatic cells has vesicles of rough endoplasmic reticulum containing a diffuse matrix (Fig. 36), in addition to the flat cisternal form. A single cilium is found on the surface of other somatic cells; each projects into a small pocket between cell processes of both the gonial and the somatic cells (Figs. 30 & 36). Clusters of small clear vesicles are generally found subjacent to the plasmalemma near these intercellular pockets (Fig. 37). A coiling of the plasmalemma is also infrequently encountered along the borders between adjacent somatic cells (Fig. 38). This coiling may also be associated with clusters of vesicles. The basal portions of the somatic cells are joined by desmosomes (Fig. 39).

At the dorsal most aspect of the ovotestis, the somatic cells are continuous with the dorsal strand (Fig. 29); the dorsal strand at this point of contact remains a single cell in diameter, and is never expanded into a bulbous enlargement. The basal lamina of the somatic cells is continuous with the external lamina of the dorsal strand.

STAGE IV

Animals in this stage of gonadogenesis measure from 1.0 to 1.6 mm. The ovotestis is located in the same position as in Stage III, and it is now a hollow mass of cells, 25 to 30 μm in diameter, and consisting of 30 to 50 cells. The ovotestis is spherical to ellipsoidal in shape with a small irregular lumen in the smaller individuals, and with an extensive lumen in the larger

animals (Figs. 40 & 41). Localized regions of thinning are common in the ovotestis of the larger animals, and these are most extensive adjacent to the epidermis and in the region of attachment to the dorsal strand (Figs. 40 & 41). Stubby protuberances from some of the somatic cells extend towards the epidermis (Fig. 42), although none of these appear to make physical contact with it. Small intercellular pockets on the abluminal surface of the ovotestis are usually visible in live material with DIC microscopy. These are formed by processes of adjacent somatic cells, and they contain a diffuse matrix. In the dorsal most region of the ovotestis, the somatic cells are continuous with the dorsal strand (Fig. 15). The dorsal strand forms a bulbous enlargement that is two to three cells thick in this region (Fig. 15).

Gonial Cells

The gonial cells are spherical in shape, approximately 7.0 μm in diameter, and are only slightly larger in diameter than the gonial cells of Stage III. Judging from serial one micrometer sections, 10 to 12 of these cells are present, and they are surrounded luminally and abluminally by somatic cells (Figs. 42 & 43).

The nuclei of the gonial cells measure 4.5 μm in diameter. There are small regions of peripheral heterochromatin in localized regions of the nuclear envelope (Figs. 42, 43 & 45). There are also granular clusters in the peripheral nucleoplasm which are associated with nuclear pores (Figs. 43, 44 & 45). In certain favorable sections it appears that material is passing from the nucleus to the cytoplasm through the pores (Figs. 44 & 45); this is more frequently encountered than in the previous stage of gonadogenesis.

The difference in density between the gonial cells and somatic cells is less pronounced than in the Stage III gonad. The cytoplasm of the gonial cells contains ribosomes and glycogen rosettes (Figs. 43, 44 & 45). Mitochondria are more abundant in the gonial cells of this stage than in the Stage III gonad, particularly

peripheral to the nucleus. The gonial cells contain a few lamellae of rough endoplasmic reticulum which appears to encircle the cell (Figs. 43 & 45). Small vesicles containing flocculent material are associated with the luminal side of the plasmalemma (Fig. 45). Secondary lysosomes are conspicuous organelles in the cytoplasm (Fig. 43), as are myeloid figures (Figs. 43 & 46), and occasional small lipid droplets. In addition, the spherical fine-grained inclusions first seen in the gonial cells of the Stage III ovotestis (Fig. 33), are more abundant here, particularly in the perinuclear cytoplasm. There is one other cytoplasmic element in the gonial cells that is first observed in this stage of gonadogenesis and that is exclusive to the gonial cells. It is an inclusion, 0.3 to 0.4 μm in diameter, that lacks a membrane. It consists of dense, irregularly shaped clumps of electron-dense granular material (Figs. 43, 44 & 46). There are two or more such aggregations in each gonial cell. This inclusion, hereafter referred to as 'nuage', is often associated with nuclear pores (Fig. 44). Nuage is generally fairly diffuse near the nucleus (Fig. 45), and more compact further from the nucleus (Fig. 46), and it appears that nuage is transported from the nucleus to the cytoplasm through the nuclear pores. There are no specialized junctions joining the membranes of adjacent gonial cells to each other, but there are occasional membrane densities between the gonial cells and adjacent somatic cells (Figs. 42, 43 & 45).

Somatic Cells

There are two types of somatic cells in the Stage IV ovotestis, based on cell shape and characteristics of the nucleus. The Type I somatic cells are located both on the abluminal side of the gonial cells, and also in the thinner regions of the ovotestis, where gonial cells are absent (Fig. 47). These are squamous cells with elongate cytoplasmic processes which may overlap at their margins (Fig. 48). They

often extend for several micrometers in regions devoid of gonial cells (Fig. 47). These cells contain an elongate nucleus with fairly dense chromatin relative to the gonial cells (Fig. 47). The cytoplasm of the Type I somatic cells contains lamellar RER, and an infrequent vesicle of RER, a small Golgi complex and few mitochondria. Some of these cells bear a single cilium which usually projects into the lumen of the ovotestis, but occasionally into a pocket between processes of adjacent somatic cells. Zonulae adhaerentes bind adjacent Type I somatic cells (Fig. 48).

The remainder of the somatic cells of this stage are the Type II somatic cells. These are irregularly shaped low cuboidal cells, variable in size (up to 6 μm in length), with extensive cytoplasmic processes which surround adjacent cells (Fig. 42). The Type II cells are found primarily in the thicker regions of the ovotestis where they abut gonial cells. The cytoplasmic processes of the Type II cells surround the gonial cells , and the cell may span the entire distance from the basal lamina to the lumen (Fig. 42). The Type II somatic cells have basally to centrally placed nuclei, and the nucleoplasm is more dense than in the Type I somatic cells. The cytoplasm contains abundant vesicular RER with a diffuse intracisternal matrix (Figs. 42 & 50), and an extensive Golgi complex (Fig. 49). Large secondary lysosomes are also abundant in these cells, and mitochondria are more numerous than in the Type I somatic cells. These cells may also contain large vacuoles (Fig. 50), lipid droplets (Figs. 50), and small, spherical fine-grained inclusions similar to those found in the gonial cells (Fig. 33). The Type II somatic cells are bound to one another by desmosomes.

STAGE V

Animals in this stage measure from 1.8 to 2.4 mm. The intestine has descended to a more posterior position than in the previous stage, and it forms a distinct loop which nearly surrounds the ovotestis. The ovotestis is an ellipsoidal to fusiform structure constricted near the site of dorsal strand attachment (Fig. 51). It is approximately 35 μm in length in smaller animals of this stage, but grows into a crescent shape, approximately 75 μm in length by 45 μm in larger specimens, immediately prior to differentiation of the testicular rudiment in Stage VI. The lumen of the ovotestis is extensive (Figs. 51 & 52), and cilia project into it it from somatic cells. Pits can still be seen on the abluminal surface of the ovotestis, although the outer surface is more regular in appearance than in Stage IV. At its dorsal-most aspect, the ovotestis is attached to a blunt expansion of the dorsal strand (Fig. 51).

The primary difference between the Stage IV and Stage V ovotestis is that in this stage the germinal layer is confined to the ventral third of the organ (Figs. 51 & 52), a configuration that will persist during subsequent stages of gonadogenesis. In addition, the germinal layer becomes substantially thicker, with one or more gonial cells forming the wall of the germinal layer (Fig. 52). The remainder of the ovotestis consists primarily of Type I somatic cells, with long processes in the regions devoid of gonial cells (Figs. 51 & 52).

Gonial Cells

Coinciding with the thickening of the germinal layer during this stage, the gonial cells are more numerous (Fig. 53). Individual gonial cells may have short, blunt cytoplasmic extensions that project toward somatic cells on the abluminal surface of the ovotestis. No junctions between adjacent gonial cells have been observed, although there are membrane densities between gonial cells and the somatic cells that surround them. The gonial cells are comparable in size to those of the Stage IV ovotestis, as is the fine structure of the nucleus. In the cytoplasm, the

Golgi complex is prominent, probably indicating increased synthetic activity. Nuage is found dispersed in the perinuclear cytoplasm, and there is a noticeable increase in the abundance of this inclusion in the gonial cells of the Stage V ovotestis. It should be noted that the gonial cells in the ovotestis of each individual possess the same relative amounts of nuage, which may correspond to a cyclic production of this germ line specific inclusion. Vesicular RER has not been observed in any of these cells.

Some smaller (5 to 6 μm) gonial cells are found in the germinal layer of this stage (Fig. 53), although this is the only stage in which they have been observed. Nuage serves to distinguish the small gonial cells from the Type II somatic cells; it is otherwise difficult to distinguish them.

Somatic Cells

Both Type I and Type II somatic cells are more numerous in this stage than in the Stage IV ovotestis. There is an increase in the number of cilia projecting from the Type I cells into the lumen of the ovotestis. The Type I somatic cells are continuous with the dorsal strand at the dorsal end of the ovotestis. The Type II somatic cells are found almost exclusively in the germinal layer, associated with gonial cells. These somatic cells have conspicuously denser nucleoplasm than either the Type I somatic cells or the gonial cells (Fig. 54). Short processes extend from the Type II cells into the area between adjacent gonial cells and there may be several such processes in each cell (Fig. 54). The cytoplasm of the Type II somatic cells also contains occasional clusters of granular material which resemble aggregations of ribosomes (Fig. 55), as well as spherical fine- grained inclusions found in Type II somatic cells in the Stage IV ovotestis (Fig. 55).

STAGE VI

In this stage, animals measure from 2.6 to 2.7 mm. The ovotestis is located in the same position as in Stage V, although the gut loop has expanded as the intestine drops to a more posterior position. A slight bulge develops near the midline in the anterior region of the ovotestis early in Stage VI, and this is followed by a separation of the organ into two distinct portions. This process appears to be very rapid, because I have observed very few animals in which the bulge was in the process of forming.

The two portions of the developing gonad that result from this separation are the ovarian and testicular rudiments. The ovarian rudiment is similar to the ovotestis of Stage V. It is elongate, ellipsoidal or crescentic in shape, and measures approximately 80 μm by 40 μm (Figs. 56 & 57). The lumen is extensive. Gonial cells are concentrated in the ventral half of the organ, grading into a sparsely ciliated, dorsal somatic region. The dorsal strand attaches to the somatic cells of the ovarian rudiment at its dorsal most aspect. The testicular rudiment is oval in shape and approximately 20 μm in diameter. Gonial cells are relatively few in this organ, and they are evenly distributed. The testis is attached to the anterior portion of the ovary (Figs. 56 & 57).

1. *OVARIAN RUDIMENT*

The germinal layer of the ovarian rudiment, other than in the region of contact between the ovarian and testicular rudiments of the developing gonad, is 3 to 4 cells thick in the ventral most region, and includes 1 to 2 gonial cells and somatic cells in section (Fig. 58). This germinal layer is continuous with a thinner region containing a single gonial cell in section (Fig. 59), and comprising the remaining ventral half of the ovarian rudiment. This thinner region of the germinal layer is continuous with the squamous somatic cells that constitute the dorsal part of the organ (Fig. 60). The germinal layer in this stage is thicker than that of the Stage V gonad.

Gonial Cells; Oogonia

The gonial cells are more numerous in this stage of gonadogenesis, but they are similar in size to those of the Stage V gonad. Some gonial cells, however, have an enlarged, rounded nucleus with condensed chromatin localized in distinct clumps (Fig. 58); this does not appear to be a fixation artifact, and these cells may be entering into division stages. The cytoplasm of the gonial cells is similar to that of Stage V gonial cells, but there is yet more dispersed nuage, in addition to some perinuclear clusters of nuage. These cells also contain annulate lamellae, composed of 5 to 8 closely packed cisternae (Fig. 61). The gonial cells in the thickest region of the ovary have short cytoplasmic processes which extend between adjacent cells to contact the Type II somatic cells (Fig. 58). The gonial cells in the thinner portion of the germinal layer do not have processes at their points of contact with the somatic cells (Fig. 59).

Although there are considerably more gonial cells in this stage of gonadogenesis, no mitotic figures have ever been observed in these cells, or those of any of the preceding stages. As at least ten individuals of each stage have been sectioned and examined, I conclude that mitotic proliferation is a rapidly occurring, relatively synchronous event. In certain sections of this stage of gonadogenesis, a structure is present connecting adjacent gonial cells, close to the abluminal surface of the organ (Fig. 62). I interpret this to be a midbody. These cells, therefore, may be recently divided daughter cells.

Somatic Cells

The Type I somatic cells continue dorsally from the germinal layer to the attachment of the dorsal strand. These cells are identical in appearance to those of the previous stages. They form a single cell layer, but they overlap adjacent cells at

both ends (Fig. 63). Junctions between adjacent Type I cells are zonulae adhaerentes, found on both the luminal and abluminal surfaces of the cells. Several of these cells have cilia, which extend into the lumen of the ovarian rudiment (Fig. 63). These cells do not contain the vesicular form of rough endoplasmic reticulum.

Somatic cells still surround the gonial cells in the germinal layer of the ovarian rudiment, and nuclei of somatic cells are found near both the luminal and abluminal surfaces of the organ (Fig. 58). On the basis of shape, nuclear characteristics, and the presence of vesicular RER, it is apparent that most of the somatic cells in the germinal layer are the Type II somatic cell. In the thinner region of the germinal layer, which contains a single gonial cell in section, these cells span the entire distance between the luminal and abluminal surfaces; their processes surround the gonial cells on all sides (Fig. 59). In the thicker portion of the germinal layer it is more difficult to follow the processes of the somatic cells, but each gonial cell contacts a Type II somatic cell or cells in several places (Fig. 58). It appears that throughout this portion of the germinal layer, there is a somatic cell process extending between adjacent gonial cells, although somatic cells may not form a continuous layer around each gonial cell (Figs 58 & 59). Each gonial cell also contacts other gonial cells in one or more regions of its surface (Figs. 58 & 59). Type I somatic cells, with more diffuse chromatin, and cilia, are occasionally found on the luminal surface of the ovarian rudiment (Fig. 59).

2. *CONTACT BETWEEN OVARIAN AND TESTICULAR RUDIMENTS*

The testicular rudiment arises from a small region, approximately 20 μm in length, of the thin germinal layer in the anterior portion of the developing gonad (Figs. 64 & 65). It appears that the testicular rudiment forms as an evagination of the ovotestis, which initially communicates with the ovarian rudiment at a large opening. Somewhat later, this opening

decreases in diameter, so that the communication between the lumina of the rudiments is only approximately 5 μm in diameter. The basal laminae of the two rudimentary organs are continuous (Fig. 65). The cells in the region where the ovarian and testicular rudiments make contact form a multi-layered epithelium (Fig. 65). There are always 1 to 2 gonial cells in this region, which are recognizable by their nuage and the density of their cytoplasm (Figs. 65 & 66). In addition to the organelles typical of gonial cells, these cells also contain several small vesicles with a finely grained electron dense material (Fig. 67). These structures are typically found close to the abluminal plasmalemma.

Somatic cells or their processes surround the gonial cells in the region of contact between the ovarian and testicular rudiments. The somatic cells form a layer 2 to 3 cells thick in this region (Fig. 65). They are all similar, and appear to be Type II, based on the presence of vesicular endoplasmic reticulum. However, the nuclei of these cells contain relatively diffuse nucleoplasm, and one or two nucleoli (Figs. 65 & 66).

3. *TESTICULAR RUDIMENT*

The testicular rudiment is similar in structure to the ovarian rudiment. The gonial cells are present throughout the organ (Fig. 64), however, rather than being localized into a separate germinal region, as in the ovary.

Gonial Cells; Spermatogonia

There are relatively few gonial cells in the testicular rudiment of this stage. They are similar in size to the gonial cells of the ovarian rudiment, and contain identical organelles, including nuage, and in similar proportions (Fig. 68). The testicular gonial cells are also surrounded by somatic cells or their processes (Fig. 68), as is true in the ovarian rudiment.

Somatic Cells

The somatic cells of the testicular rudiment are are not separable into two distinct types as is the case in the ovarian rudiment. Some of the somatic cells are more squamous, with long cytoplasmic processes, while others are more cuboidal, particularly those somatic cells adjacent to the gonial cells. Each somatic cell extends from the luminal to the abluminal surface of the testicular rudiment (Figs. 65 & 68). Some of the somatic cells bear cilia which project into the lumen or into small intercellular pockets (Fig. 68), reminiscent of the Type I somatic cells of the ovarian rudiment.

STAGE VII

Animals in this stage measure from 2.8 to 4.0 mm. The intestine has descended further in a posterior direction and the gonad is now completely surrounded by the loop of the gut, and is situated between the stomach and intestine. This is the position in which the gonad is found in adults. The ovarian and testicular portions of the developing gonad continue to lengthen and increase in width after their separation in Stage VI. Stage VII marks the completion of the early stages of gonadogenesis, resulting in the establishment of the basic structure of the adult gonad. It includes what is assumed to be the initial stage of folliculogenesis and the establishment of the oviduct and sperm duct.

The ovary of this stage, as in Stages V and VI, is oval in shape with an extensive lumen. It is approximately 150 μm in length with the gonial cells localized in the ventral most region of the organ (Figs. 70 & 71). There is a thinner region, particularly on the lateral surfaces, which also contains a single layer of gonial cells. The remainder of the ovary consists of squamous cells, found adjacent to the testis (Figs. 70 & 71), and continuing into an elongate, sparsely ciliated tubular extension, the oviduct (Fig. 70), that follows along the intestine. This extension continues to grow towards the atrium. The oviduct remains continuous

at its tapered dorsal end with the dorsal strand.

The testis is initially located along the anterior region of the ovary, and it may either continue its development in this position (Fig. 70), or it may drop to a more lateral position on the right side of the ovary (Fig. 69). It is oval in shape, sparsely ciliated, and is approximately 60 μm in length (Fig. 70). The gonial cells are concentrated in the ventral part of the testis, and this germinal region is continuous with a squamous epithelium that forms the developing sperm duct (Figs. 69 & 70). The sperm duct opens into the oviduct close to the attachment of the dorsal strand (Fig. 70).

I. *OVARY*

The germinal layer in the ventral most portion of the ovary is 2 to 3 gonial cells thick (Fig. 71), and is continuous with a thinner region, containing a single cell in section. There are somatic cell nuclei along both the luminal and abluminal surfaces of the ovary, although they are more numerous on the luminal surface (Fig. 72). In addition, somatic cells are found penetrating between adjacent gonial cells (Fig. 71). The germinal layer is still continuous with the squamous somatic cells that form the oviduct (Fig. 70).

Gonial Cells

The gonial cells of this stage are considerably more numerous than in the Stage VI gonad (Fig. 71). They are approximately 7.0 μmn diameter, not noticeably larger than in previous stages of gonadogenesis. The cytoplasm of the gonial cells is very dense, containing large concentrations of glycogen and ribosomes (Figs. 72 & 73). There is also an increase in the number of granular clusters of nuage in the perinuclear cytoplasm (Fig. 72) in addition to the extensive amount of dispersed nuage. The mitochondria are still more abundant immediately surrounding the nucleus (Fig. 72), and the RER, Golgi complexes and occasional

vacuoles containing small vesicles are similar in distribution to the gonial cells of the Stage VI ovarian rudiment. Short cytoplasmic processes extend from gonial cells, to contact somatic cells. In addition, several of the gonial cells have the short connections between adjacent gonial cells, which I consider to be midbodies, as first seen in the Stage VI gonad. These short connections have densely staining membranes which extend approximately 0.3 μm from the base of the gonial cell. There are infrequently observed zonulae adhaerentes binding gonial cells to somatic cell processes. These junctions are probably quite localized and are seldom encountered in sections.

Somatic Cells

Type I somatic cells are still found primarily in the region of the ovary that is devoid of gonial cells, and they extend dorsally from the germinal layer to form the tubular developing oviduct (Figs. 70 & 71). They are also found on the luminal surface of the ovary. The nuclear and cytoplasmic characteristics of these cells are identical to those of the Stage VI gonad. The Type II somatic cells are numerous in the germinal layer of this stage, and can be identified in 1 μm sections by their shape and the dense staining of the nucleus (Fig. 71). In this stage, as in Stage VI, the nuclei of these cells are found primarily on the luminal surface of the ovary. These somatic cells have long cytoplasmic processes which extend between adjacent gonial cells, and which isolate the gonial cells from both the gonad hemocoel and the lumen of the ovary. The Type II somatic cells are also found between gonial cells in the thick portion of the germinal layer (Figs. 71, 73 & 74), where they conform to the contours of adjacent cells. Type II cells are also found on the dorsal edge of the germinal layer (Fig. 75), abutting the region of the ovary dominated by Type I cells. The cytoplasm of the Type II somatic cells is similar to

that described in previous stages. Vesicular RER is however, less abundant in the cells of this stage.

Oviduct

The developing oviduct is a wide (approximately 25 μm) tubular continuation of the squamous somatic region of the ovary that is devoid of gonial cells (Figs. 69, 70 & 71). There is no distinct separation of the oviduct from the ovary (Figs. 70 & 71). The dorsal strand attaches to the dorsal most aspect of the oviduct in a small conical projection that is 2 to 3 cells in diameter, not different from previous stages. The sperm duct opens into the oviduct near this site. The cells of the oviduct form a simple squamous epithelium, with overlapping processes that are bound together by zonulae adherentes. The nuclear and cytoplasmic characteristics of the oviduct cells are identical to those of the Type I somatic cells of the ovary. In areas where the oviduct runs adjacent to the testis or the sperm duct, a distinct basal lamina surrounds each of the two structures (Fig. 76).

2. *TESTIS*

The germinal layer of the Stage VII testis is localized in the ventral half of the organ, and is continuous with a simple squamous epithelium devoid of gonial cells which forms the remainder of the organ (Figs. 69 & 70). The germinal layer consists of a stratified epithelium comprising a luminal germinal layer, and a subjacent squamous somatic layer. At its dorsal most aspect the testis is constricted in diameter, and is continuous with a long narrow sperm duct (Fig. 70) which has no lumen at this stage.

Gonial Cells

The gonial cells of the testis are smoothly contoured, and approximately 7.0 μm in diameter. They are similar in size and structure to the gonial cells of the

ovary (Figs. 77 & 78). The nucleus contains localized regions of condensed peripheral chromatin and small clumps of granular material near the nuclear pores (Figs. 78 & 79). The cytoplasm contains glycogen rosettes and numerous ribosomes (Fig. 79). There are a few clusters of nuage in the perinuclear cytoplasm (Fig. 79), identical to that of the gonial cells of the ovary, although not as numerous. In certain regions of the nuclear envelope, small granular clusters are found in the cytoplasm which appear to have passed through the nuclear pores (Fig. 79). A few mitochondria, short profiles of RER, infrequent myeloid figures, and a few vesicles containing flocculent material are also found (Figs. 78 & 79). The gonial cells of the testis rest on a single layer of somatic cells (Fig. 80) and border on and project into the lumen of the organ. They are not surrounded by somatic cells or their processes (Figs. 77 & 78). No junctions are found either between adjacent gonial cells or between gonial cells and the somatic cells on which they rest (Fig. 78). Some of the gonial cells are completely separated from the underlying somatic cells, and are free in the lumen of the testis (Fig. 77). These may be primary spermatocytes.

Somatic Cells

The somatic cells of the testis underlie the gonial cells in the germinal layer, and form the remainder of the organ. They form a simple squamous layer with overlapping processes, but without processes extending between adjacent gonial cells (Fig. 78). They are similar in structure to the Type I somatic cells of the ovary (Figs. 78 & 80). The cytoplasm is unremarkable, containing a large Golgi complex, few mitochondria and an occasional secondary lysosome (Fig. 80). Cilia project from most of the somatic cells into the lumen of the testis (Fig. 80). There appears to be only a single cilium per cell, and they are found in both the germinal

and somatic regions of the testis (Figs. 71 & 80). Zonulae adhaerentes bind adjacent somatic cells at their apical ends (Fig. 80), but they are not joined basally.

Sperm Duct

The lumen of the testis narrows markedly at its dorsal end to form a solid region several somatic cells in diameter (Figs. 70 & 76). This marks the beginning of the sperm duct, which has no lumen at this stage. The cells of this region are bound together with zonulae adhaerentes (Figs. 76 & 81). They may form an irregular contoured outline on their basal edges so that the basal lamina projects into spaces between adjacent cells (Fig. 82). Further from the testis, the sperm duct is only two cells thick, running adjacent to the oviduct, and without a lumen (Fig. 83). Near the attachment of the dorsal strand to the oviduct the sperm duct fuses with the anterior aspect of the oviduct. No special elaborations are visible in these cells at this point of contact. As the oviduct continues to grow towards the atrium, the sperm duct grows as well, maintaining its connection to the oviduct in relatively the same configuration.

IV. DISCUSSION

A. GONADOGENESIS

The development of the ascidian reproductive system from a small mass of mesodermally derived cells has received considerable attention, particularly in the older literature. Relatively little work has been done on gonadogenesis, however, in recent years, and most of the research on this subject has been based on the blastozooids of compound species (Kowalevsky, 1874a, b; Van Beneden, 1881; Seeliger, 1893-1906; Van Beneden and Julin, 1884, 1886; Maurice, 1888; Lefevre, 1897, 1898; Bancroft, 1899; Simkins, 1924; Brien, 1925, 1927, 1939, 1948; Berrill, 1941a, b; Aubert, 1954; Newberry, 1968; Izzard, 1968; Mukai and Watanabe, 1976; Sabbadin and Zaniolo, 1979). Only a few studies are available on gonadogenesis in a solitary species (Kowalevsky, 1866, 1871; Van Beneden and Julin, 1884, 1886; Julin, 1893; De Selys Longchamps and Damas, 1900; Huus, 1924; Aubert, 1954). These studies reveal that the stages of gonadogenesis among those ascidians which possess compound gonads are strikingly similar regardless of their taxonomic position. There is a great range of detail presented in these works, both on the stages of gonadogenesis and on the organization of the cells that form the developing gonad. My work on Corella inflata is the most thorough documentation of the morphological changes that take place during gonadogenesis, and is the only study that examines the organization and structure of the cells that constitute the developing gonad. These morphological changes are summarized in Illustration 3 and in Table 1.

I have observed the gonad in Corella inflata at an earlier stage of development than has been reported in any previous study of a solitary ascidian. In this animal, the first indication of the developing gonad is one or a pair of hemoblasts located subjacent to the epidermis in the gonad hemocoel, in close proximity to the dorsal strand. My observations suggest that these hemoblasts subsequently make contact with the terminal, tapered end of the dorsal strand.

because in the next stage, a pair of hemoblast-like cells is always found in contact with the dorsal strand. I have never observed animals in later stages of gonadogenesis without this association. The contact between the pre-gonadal hemoblasts and the dorsal strand appears to be critical to the initiation and the continuation of gonadogenesis in Corella inflata.

My observations indicate that the dorsal strand does not itself produce the first cells of the ovotestis, but rather that the dorsal strand makes contact with cells that are already present in the gonad hemocoel. The dorsal strand never bears a bulbous or swollen region at its tip before it makes contact with the hemoblasts, and mitotic figures have never been seen in these cells. It is reasonable to assume then, that in Corella inflata the first stage of gonadogenesis consists of an initial accumulation of a pair of hemoblasts, which subsequently becomes associated with the dorsal strand.

In Corella inflata, once this accumulation of hemoblasts is established, it constitutes a unique population of cells. The hemoblasts that form the initial accumulation of pre-gonadal cells are not augmented by an additional contribution of hemoblasts from the blood. This has also been observed in Distomus variolosus (Newberry, 1968), although the number of hemoblasts that forms the initial pre-gonadal accumulation is substantially greater than in Corella.

Most investigators describe the initial stage of gonadogenesis as a small cluster of mesodermally derived cells located in the gonad hemocoel of phlebobranchs and aplousobranchs (Berrill, 1950; Monniot and Monniot, 1972), and in the mantle or mantle sinus of stolidobranchs (Berrill, 1950; Monniot and Monniot, 1972; Newberry, 1968). In Corella inflata (a phlebobranch), however, the ovotestis at this point consists of two distinct cell types which are clearly discernible in TEMs; the gonial cells and the somatic cells. To a large extent, the gonial cells retain their hemoblastic morphology, although they are completely surrounded by somatic cells which isolate them from the gonad hemocoel. The ovotestis is attached to the dorsal strand at the dorsal most aspect, an orientation that is maintained during the entire

process of gonadogenesis. There is always a surrounding layer of connective tissue fibers which probably serves to anchor the ovotestis to the epidermis. I have frequently observed blood cells in the gonad hemocoel around the ovotestis, which probably have physiological functions here (Wright, 1981).

The small cluster of cells subsequently cavitates to form a spherical mass with an extensive central lumen in Corella inflata. The germinal layer of the ovotestis is a a single gonial cell in thickness during this stage, and there may be localized regions of thinning in small areas facing the epidermis which do not contain gonial cells. Nuage is first seen in the gonial cells of the cavitated ovotestis, and it is associated with material that appears to be moving from the nucleus to the cytoplasm through the nuclear pores. Nuage identifies the gonial cells specifically as part of the germ cell line (Beams and Kessel, 1974; Eddy, 1975; Kessel, 1983). These gonial cells are now completely isolated from both the internal and external environments of the ovotestis by somatic cell processes, which may be essential to the production of nuage. There are two different types of somatic cells identifiable in the cavitated ovotestis. The Type I somatic cells are more squamous, and their nuclei contain relatively diffuse chromatin. Some of these cells bear cilia which project into the lumen of the ovotestis, or into intercellular pockets. The Type II somatic cells are found consistently in association with gonial cells; they are more cuboidal, and possess nuclei with denser nucleoplasm. These characteristics of the gonial cells and somatic cells are found in all subsequent stages of gonadogenesis.

In Corella inflata the ovotestis is divisible into a peripheral or ventral germinal layer that is several cells thick, and a more elongate, dorsally placed somatic region during the next stage of gonadogenesis. While there are no substantial differences in the cells that form the ovotestis at this stage, the morphological organization that is established here persists throughout later development. In addition, the Type I somatic cells in the dorsal region extend further dorsally to form a tubular expansion that will ultimately become the oviduct. The dorsal strand maintains its attachment to the dorsal most aspect of this developing duct.

The ovotestis divides into rudiments of separate sex in the next stage of gonadogenesis. In Corella inflata, the testicular rudiment appears to form as a bulging, or evagination from a small region of the anterior part of the ovotestis that is a single gonial cell thick. The fine structure of the cells at the site of testicular rudiment formation is unremarkable although there is a considerable number of small vesicles whose contents are unknown, and which do not appear elsewhere in these cells. There are no other special cellular features, including contractile elements that have been observed at the ultrastructural level. An examination of the factors that control the process of testicular rudiment formation was beyond the scope of this research, but this is of considerable interest in understanding the nature of the hermaphroditic gonad. It should be noted that the differentiation of the testicular rudiment from the ovotestis is the stage of gonadogenesis that is the most variable according to accounts in the literature. In some species testicular rudiment formation is by budding of the ovotestis (Van Beneden and Julin, 1886; Lefevre, 1898; De Selys Longchamps and Damas, 1900; Aubert, 1954), while in others a furrow forms which divides the ovotestis into two separate rudiments (Van Beneden and Julin, 1886; Julin, 1893; Lefevre, 1897; Bancroft, 1899). It is possible, however, that these apparent differences result from different levels of investigation, and the mechanism of testicular rudiment formation requires a careful reevaluation in several species.

The organization of the ovarian rudiment of this stage is essentially identical to the ovotestis of the previous stage, although the germinal layer contains considerably more gonial cells. In addition, the oviduct continues to develop as an extension of the ovarian rudiment during this stage, growing in a dorsal direction toward the atrium.

The testicular rudiment has fewer gonial cells than the ovarian rudiment, and these are not localized into a distinct germinal layer. These gonial cells are indistinguishable from the gonial cells of the ovarian rudiment; they contain nuage, and are surrounded by somatic cell processes which isolate them from the external and internal environments of the organ. There is no distinction between somatic cells in the testicular rudiment; all somatic cells are similar to

the Type I somatic cells of the ovarian rudiment. The testicular rudiment opens at a small neck into the duct of the ovarian rudiment, close to the point of contact between the oviduct and the dorsal strand.

The establishment of the basic structure of the adult reproductive system is essentially completed during the next stage of gonadogenesis in Corella inflata. The ovary and testis are separate and distinct organs, and each has an exit duct which extends towards the atrium. The number of gonial cells, or oogonia, in the germinal layer has increased, and there are somatic cells in the germinal layer that probably represent developing follicle cells. These cells are similar to the Type II somatic cells, and it is likely that they differentiate from that cell type. The germinal layer of the ovary extends into the somatic region of the developing oviduct. The oviduct has lengthened, and it remains attached to the dorsal strand at the dorsal-most aspect of the ovary. There is never any evidence of either cell divisions or a bifurcation of the dorsal strand, that might suggest that the dorsal strand gives rise to the oviduct, as suggested by Huus (1924) in Corella parallelogramma. The periphery of the germinal layer or the margins of the duct itself probably proliferate to form the oviduct.

The testis also enlarges during this stage, and the germinal layer becomes localized in the ventral region of this organ. The gonial cells, or spermatogonia, of the testis are not noticabely different from the oogonia of the ovary. These cells rest on a somatic epithelium, and they are not surrounded by somatic cell processes on their luminal surface, but rather project into the lumen. The sperm duct develops from the wall of the testis, and opens into the lumen of the oviduct close to its attachment with the dorsal strtand. As the oviduct extends toward the atrium, the sperm duct also extends so that its position relative to the oviduct is maintained. The sperm duct is always found in close proximity to the oviduct, and it is always surrounded by a distinct basal lamina.

There are many questions concerning the process of gonadogenesis in ascidians that have been raised during this study of Corella inflata. These primarily concern the cellular

mechanisms that are involved in the morphological changes that take place during gonadogenesis. Of particular interest are the processes of cavitation, germinal layer localization, the differentiation of the testicular rudiment from the ovotestis, and formation of the gonoducts. Because the site of formation of the testicular rudiment appears to be constant in a given species, are there characteristics of the cells that participate in testicular rudiment formation that are different from other cells of the ovotestis? Finally, are the gonial cells and somatic cells, which are first distinguished as separate cell types in the ovotestis prior to cavitation, derived from the same cells? These questions have not been answered by histological or ultrastructural examination, and require an experimental approach to address them more fully.

B. SIGNIFICANCE TO OTHER AREAS OF ASCIDIAN REPRODUCTIVE BIOLOGY

This study of Corella inflata has provided new detailed information on the process of gonodogenesis in ascidians. In addition to addressing gonadogenesis specifically, several interesting questions concerning the development of the reproductive system have been formulated during this research. These include: 1) what is the origin of the gonad and germ cells?, 2) what is the role of the dorsal strand during gonadogenesis?, 3) when are germ cell line specific inclusions formed, how are they distributed, and what is their significance?, 4) do mitotic divisions occur cyclically in the cells of the developing reproductive system?, and 5) what is the origin of the follicle cells? In this section, I discuss these questions separately, referring specifically to Corella inflata.

ORIGIN OF THE GONAD AND GERM CELLS

My description of gonadogenesis in Corella inflata has included the establishment of the ovotestis, and has provided information on an earlier stage of development than has been reported in any previous study of a solitary ascidian. My observations demonstrate the striking

morphological similarity of circulating hemoblasts to the cell or pair of cells that form the first stage of gonadogenesis, prior to their attachment to the dorsal strand. The morphological similarity between hemoblasts and the gonad-forming cells remains visible in the next stage of gonadogenesis, after the dorsal strand attachment has occurred. It is clear from these observations that a pair of hemoblasts becomes associated with the dorsal strand once it has reached the gonad hemocoel, and these ultimately give rise to the reproductive system.

Several reports implicate hemoblasts in the origin of the gonad and germ cells in most ascidian species, regardless of the taxon (Newberry, 1968; Ermak, 1975, 1976, 1977; Sugimoto and Nakauchi, 1974; Mukai and Watanabe, 1976; Wright, 1981). The evidence for the hemoblastic origin of the gonad is, however, only suggestive rather than explicit. It is based on 1) the morphological similarity between the hemoblasts and the cells of the earliest stages of gonadogenesis, 2) the location of the developing gonad in regions of the body that are in contact with circulating blood cells, 3) the aggregating behavior of blood cells, and 4) the pleuripotent nature of hemoblasts.

Hemoblasts are large spherical cells approximately 5 to 6 μm in diameter, with a large nucleus and one or more nucleoli. They have a small amount of basophilic cytoplasm which contains polyribosomes, cisternal RER, several mitochondria, and a small Golgi complex (Ermak, 1977, 1982; Milanesi and Burighel, 1978; Wright, 1981; Rowley, 1982). The lymphocytes can be distinguished from hemoblasts by their smaller size (3 to 5 μm), the absence of discrete nucleoli, and the localization of peripheral heterochromatin (Pérès, 1943; Millar, 1953; Sabbadin, 1955; Goodbody, 1974; Ermak, 1976; Wright, 1981), yet numerous authors have apparently confused these two cell types (George, 1939; Andrew, 1961; Overton, 1966; Newberry, 1968; Smith, 1970; Freeman, 1964, 1969, 1971).

Newberry (1968) documented that the cells that form the gonad in Distomus variolosus are hemoblasts (*vide* lymphocytes). Similarly, in Symplegma reptans (Sugimoto and Nakauchi, 1974) and in Botryllus primigenus (Mukai and Watanabe, 1976), the size and morphology of

the cells that form the gonad appear to be identical to hemoblasts. Other observations, particularly those reported in the earlier literature, are less conclusive (Kowalevsky, 1871, 1874a, b; Van Beneden, 1881; Seeliger, 1893-1906; Van Beneden and Julin, 1886; Maurice, 1888; Lefevre, 1898; Bancroft, 1899), although they indicate that the cells that form the gonad are morphologically similar to amoeboid cells of the blood.

In all ascidians that have been studied to date, the gonads develop in regions of the body that are in contact with blood cells. In the phlebobranchs and aplousobranchs this region is the gonad hemocoel, and it contains numerous blood cells of all types, including hemoblasts (Millar, 1953; Wright, 1981). In the stolidobranchs the gonads develop either in the mantle (Berrill, 1975), which contains cells that have migrated into the connective tissue from the blood, or the mantle sinus (Newberry, 1968) which contains circulating blood cells. If hemoblasts are the cells that form the gonad and germ cells, they have access to the parts of the body in which the gonads develop. In Corella inflata, this site is located posterior to the loop of the gut, and is associated with both the epidermis and the dorsal strand. In Distomus variolosus the gonads develop in regions of the mantle sinus close to the peribranchial epithelium in which there is quieter blood flow (Newberry, 1968). In Symplegma reptans, the hemoblasts aggregate in the "genital tracts" (Sugimoto and Nakauchi, 1974). However, hemoblasts are not confined to these regions; they are found throughout the circulatory system of the ascidians. Because the gonads develop in specific sites of the body, there must be some mechanism responsible for promoting the accumulation of hemoblasts in the pre-gonadal region. Newberry (1968) suggested that the mantle specifically may promote the accumulation of pre-gonadal blood cells in that region. In Corella inflata there is no ultrastructural support for this hypothesis, although direct experiment is necessary to address this hypothesis explicitly.

All blood cell types of ascidians are capable of amoeboid movement and of forming aggregations (Hecht, 1918; Andrew, 1961; Wright, 1981). I have frequently observed this behavior by hemoblasts in many of the hemocoelic sinuses in Corella inflata. Most of these

aggregations are temporary in Corella, but there is a unique and persistent aggregation of only two hemoblasts that differentiates into the ovotestis; this occurs only in the presence of the dorsal strand. Newberry (1968) also noted frequent aggregations of hemoblasts in the mantle sinus of Distomus, consisting of a few to dozens of cells. Some of these aggregations along the "presumptive ovarian line and in the testicular region" (Newberry, 1968) subsequently become more encased in a connective tissue matrix and more compressed, then differentiate into the gonads. It is probable that the pluripotent hemoblasts of the first stage of gonadogenesis give rise to all of the cells of the ovotestis, because there is no evidence to suggest a further addition of hemoblasts to the developing gonad after its establishment. It appears then, that an aggregation of two to several hemoblasts is a prerequisite for gonad development in many ascidians. In species such as Corella inflata, this aggregation must be associated with the dorsal strand.

Hemoblasts are probably the source of most of the tissues of the asexual bud in members of those families (Styelidae, Perophoridae, and Clavelinidae) that multiply by vascular budding (Brien, 1939; Sabbadin, 1955; Oka and Watanabe, 1957, 1959; Milkman and Byrne, 1961; Freeman, 1964). Freeman (1964) found that hemoblasts (*vide* lymphocytes) are essential for budding in Perophora viridis and hypothesized that they supply an essential stem cell factor to the bud, without which other tissues cannot develop. There is evidence, then, that hemoblasts are a pluripotent cell type, and it is reasonable to assume that they are capable of differentiating into the gonad (Newberry, 1968; Ermak, 1975, 1976, 1977; Mukai and Watanabe, 1976; Wright, 1981).

There are three reports in the literature that do not support my hypothesis that the gonads of ascidians arise from hemoblasts. These reports are particularly difficult to interpret and assess, and it should be noted that these are based on studies of compound ascidians. First, in the Ecteinascidia turbinata oozooid, the ovary appears to be derived from mesodermal cells of the mantle by proliferation into the hemocoel (Simkins, 1924). Secondly, in Botryllus,

Berrill's (1941a, b) investigations led him to conclude that the germ cells are derived from the atrial epithelium. This conclusion is partially supported by Sabbadin and Zaniolo (1979), but disputed by Mukai and Watanabe (1976). Thirdly, in Aplidium zostericola, the dorsal strand was reported to give rise to the gonad (Brien, 1927). Brien (1948), however, does not discuss the issue of dorsal strand involvement in gonad origin in his later monograph on ascidian biology. It is clear that a reexamination of these species is essential to a thorough understanding of gonad origin in the ascidians.

These few microscopical studies provide morphological information on the similarities between hemoblasts and the cells of the first stages of gonadogenesis, and they implicate hemoblasts in the origin of the gonad and germ cells. Coupled with my observations on Corella inflata, this information indicates that hemoblasts probably give rise to the gonad in most species. However, there remain several important questions about the origin of the germ cells that a morphological study cannot address. First, are there characteristics of the gonad-forming hemoblasts that are unique to these cells, making them a distinct cell line? This has been suggested by Newberry (1968) and Sugimoto and Nakauchi (1974), but has not been addressed experimentally. Secondly, are the hemoblasts that form the gonad also found in the tadpole larva, or do they differentiate from hematopoietic tissues in the juvenile? After metamorphosis, degenerating cells of the axial complex are found in the gonad hemocoel of solitary ascidians and the oozooid of compound ascidians. This material may obscure the initial movement of hemoblasts to the site of gonad formation. Thirdly, are there characteristics of the mantle, or epidermis, that cause the hemoblasts to aggregate in the pre-gonadal regions of the body, and form the gonad?

It is quite obvious that the question of germ cell origin in this group of chordates has advanced very little in recent years, and remains a significant gap in our understanding of the reproductive biology of the ascidians. It should be noted that because there is considerable variation among species in the location of the gonads and in the presence and form of such

structures as the dorsal strand, there may be similar variation in the mechanism of gonad origin among different taxa of ascidians. This will only be elucidated by further investigation.

DORSAL STRAND

The dorsal strand is an enigmatic structure that has been reported in adults and juveniles of many ascidian species (Kowalevsky, 1874a; Metcalf, 1900; Huus, 1924; Brien, 1927, 1948; Millar, 1953; Bullock and Horridge, 1965; Goodbody, 1974). It develops soon after metamorphosis from a portion of the larval neural tube that also gives rise to the neural gland (Elwyn, 1937). In all animals that possess a dorsal strand, this structure is present as a posterior continuation of the neural gland (Metcalf, 1900; Bullock and Horridge, 1965; Goodbody, 1974). In Corella inflata, the dorsal strand is first visible growing towards the gonad hemocoel within 3 days after metamorphosis. It is always associated with the establishment of the gonad, and with the entire process of gonadogenesis. In many other species, the dorsal strand is also associated with the developing reproductive system (Kowalevsky, 1874a; Van Beneden and Julin, 1886; Huus, 1924; Brien, 1925, 1927, 1939; Aubert, 1954; Markman, 1958), although its role in gonadogenesis is entirely unknown.

My observations on Corella inflata are the first that describe the ultrastructure of the dorsal strand in juvenile ascidians. The dorsal strand is composed of a string of elongate cells attached at their ends by overlapping processes, joined by well-developed junctions, and surrounded by an external lamina. These cells contain microfilaments, probably actin, which may be important during the growth of the structure. Prior to the cavitation of the ovotestis, there are no special features of the cells at the point of attachment between the dorsal strand and the developing gonad.

After cavitation of the ovotestis has occurred, there is a blunt enlargement of the dorsal strand at its attachment to the developing gonad; these cells, and some of the adjacent somatic cells of the gonad contain moderately electron dense vesicles which are similar in size to

neurosecretory vesicles that have been described in other organisms (Baskin, 1976). This is the first observation that suggests a possible neurosecretory function of the dorsal strand and somatic cells of the gonad in any ascidian. The dorsal strand of Corella inflata remains a string of single cells during gonadogenesis, other than at its point of contact with the developing gonad. Later, after the developing gonoducts have reached the atrium, the dorsal strand loses its association with the reproductive system, becomes several cells in diameter and forms a lumen. This is the adult condition of most species (Metcalf, 1900; Huus, 1924; Mackie et al., 1974), although Millar (1953) reported that the dorsal strand remains associated with the ovary in adult Ciona.

During the initial stages of gonadogenesis in Corella inflata, there is no nervous tissue associated with either the ovotestis or the dorsal strand. Just prior to cavitation of the ovotestis, and during subsequent development, an irregular layer of cell processes, which I interpret to be axons, is found in close association with the dorsal strand in the region of the ovotestis. These axons contain moderately electron-dense vesicles which, on the basis of their size and distribution, are suggestive of a neurosecretory function (Baskin, 1976). The vesicles are also found in short extensions of these processes which extend into spaces between cells of the ovotestis. Although potential neurosecretory granules have been reported in some cells of the neural ganglion and the major nerve trunks leaving the ganglion (Dawson and Hisaw, 1964; Thiebold and Illoul, 1966; Chambost, 1966; Bouchard-Madrelle, 1967a, b; Aros and Konok, 1969; Lender and Bouchard-Madrelle, 1964; Georges, 1977; Sugimoto and Watanabe, 1980), this is the first observation of vesicles that may have a neurosecretory function, in processes of the peripheral nervous system of ascidians.

I interpret these potential neurosecretory cell processess to be elements of the dorsal fold plexus that has been described in the adults of various species (Huus, 1937; Fedele, 1938; Millar, 1953; Aubert, 1954; Mackie, et al., 1974), and in the juveniles of Ciona (Aubert, 1954; Markman, 1958) and Corella parallelogramma (Huus, 1924). The presence of nervous tissue

surrounding the dorsal strand led to the contention in the early literature that the dorsal strand was an element of the peripheral nervous system (Kowalevsky, 1871a; Maurice, 1886, 1888; Julin, 1881; Metcalf, 1900; Lorleberg, 1907). More recent work, however, indicates that the dorsal stand itself is entirely non-nervous and that the visceral nerve is a separate structure (Huus, 1924; Fedele, 1938; Brien, 1948; Millar, 1953; Markman, 1958; Mackie et al., 1974; Goodbody, 1974).

The association of the dorsal strand and the neurosecretory cells with the ovotestis during gonadogenesis leads to the question of the potential functions of these structures in the juvenile. I hypothesize that the dorsal strand may be a conduit for trophic factors, necessary for the initiation and/or continuation of gonadogenesis, to move from the neural gland to the developing gonad. In this way, the neural gland may actually control the establishment of gonadogenesis, and/or its continuation. This would explain why the gonad never develops without the dorsal strand attachment, and it may explain the presence of what appear to be neurosecretory vesicles in the cells of the dorsal strand. Goodbody (1974) reinterpreted the results of Carlisle (1951) and Bouchard-Madrelle (1967a, b) as indicating that the dorsal strand may serve as a pathway between the neural complex and gonads for the movement of endocrine products over a long-term reproductive cycle. However, because the dorsal strand does not connect the neural gland with the gonad in adults of most species, including Corella inflata, it is more likely that if the dorsal strand functions in endocrine control, it does so primarily during gonadogenesis. Such an explanation accounts for the continuation of the reproductive cycle in adult Chelyosoma productum even after neural complex removal (Hisaw et. al., 1966).

One major problem with this hypothesis is that the function of the neural gland is still unresolved. While some experimental results hint that the neural gland may function in some aspect of reproduction (Hogg, 1937; Carlisle, 1950, 1951, 1954; Sengal and Kieny, 1962, 1963a, b; Sengal and Georges, 1966; Bouchard-Madrelle, 1967a, b), there are no gonadotropic hormones known to be produced in this organ (Goodbody, 1974). In addition, the neural gland

is positioned directly subjacent to a substantial hemocoelic space, and such an endocrine pathway may therefore be superfluous. Ablation experiments, and the severing of the dorsal strand, would provide some information relevant to this hypothesis.

Alternatively, the dorsal strand may serve as a pathway directing growth of neurosecretory cells from the ganglion to the developing gonad. I suggest that, because the vesicles I have observed in the axons around the dorsal strand have never been observed in adults, they may be restricted to juveniles. Based on the presence of these vesicles both in the axons and in the processes that extend into intercellular pockets of the developing gonad, the vesicles may function in some aspect of the continuation of gonadogenesis in developing animals. However, because this nervous tissue is not present in the gonad hemocoel at the time gonadogenesis begins, some other mechanism must be involved in the initiation of gonadogenesis. At this time, there is no experimental evidence available to support this hypothesis, and there is insufficient ultrastructural information on the condition of the dorsal strand plexus in adults.

An alternative hypothesis for the function of the dorsal strand in juvenile ascidians is that it provides a template, or pathway, for the directional development of the oviduct and associated sperm duct, and gives way to the oviduct as it develops. This hypothesis is supported by the observation in Corella inflata that the dorsal strand is associated with the dorsal most aspect of the developing oviduct at all times during gonadogenesis, and that this association is lost by the time the oviduct reaches the atrium. Huus (1924) considered that the dorsal strand actually gives rise to the gonoducts, based in part on the loss of the association between the dorsal strand and the oviduct in adult Corella parallelogramma. Aubert (1954), however, states that in Ciona, the dorsal strand continues posterior to the ovotestis. In Corella inflata, I have never observed the dorsal strand in a postion similar to that described by Aubert (1954), but neither have I observed any indication that the dorsal strand gives rise to the gonoducts (Huus, 1924).

Finally, in those animals which possess a dorsal strand, the gonads may develop in a location that is determined by the dorsal strand. There may be features of the dorsal strand which promote the accumulation of hemoblasts at a particular site of the epidermis or mantle, which have not been revealed by my TEM observations in Corella inflata. The central issue of this hypothesis is the mechanism by which the dorsal strand grows from the neural gland to the pregonadal region of the animal, and the mechanism by which the dorsal strand and hemoblasts recognize each other, make contact, and initiate the establishment of the gonad. Two mechanisms by which the dorsal strand could be directed to its final destination are chemotaxis and contact guidance (Alberts et al., 1983). Neither the hemoblasts that form the the Stage I gonad nor the epidermal cells of the pre- gonadal region, have morphological evidence of the synthesis or secretion of chemotactic substances, although these substances would not necessarily be observed with TEM. It may be that molecules such as fibronectin or glycosaminoglycans, or even the orientation of the collagen fibers in the extracellular matrix may guide the dorsal strand to the pre-gonadal region in a fashion similar to the migration of neural crest cells (Alberts, et al., 1983). Membrane adhesiveness in the end cells of the dorsal strand might promote the accumulation of cells at this point, but further investigations, for example by selective staining for specific carbohydrates such as lectins on the cell surface (Alberts et al., 1983) are essential to demonstrate the mechanisms involved in this process.

Regardless of its role in Corella inflata, many ascidian species, including the majority of stolidobranchs, appear to lack a dorsal strand in the juvenile as well as the adult (Metcalf, 1900; Newberry, 1968; Goodbody, 1974). If this is indeed the case, then an entirely different mechanism of gonad formation must be proposed for those animals. I suggest that before a detailed hypothesis concerning gonad formation among members of the class can be proposed, a careful reexamination of those animals that reportedly do not possess a dorsal strand in the juvenile must be performed. It may turn out that the dorsal strand is a more universal structure in the ascidians than has been previously supposed. In any case, the above hypotheses apply

only to those animals in which a dorsal strand is present in the juvenile.

NUAGE

Many animal species possess certain cells, that on the basis of their cytoplasmic elements can be identified as constituting a germ cell line (Beams and Kessel, 1974; Eddy, 1975; Nieuwkoop and Sutasurya, 1979; Kessel, 1983). These cytoplasmic elements are sequestered during embryogenesis into those cells that will later differentiate into the germ cells (Eddy, 1975; Nieuwkoop and Sutasurya, 1979), and the destruction or removal of these cells will result in a sterile adult. In other species, the germ cells themselves contain a characteristic, densely-staining material in their cytoplasm, which I refer to as nuage after Eddy (1975). This nuage is found in young oocytes, and sometimes oogonia or nurse cells, and also occasionally in spermatogonia (Eddy, 1975). Although its specific role in determining germ cell formation is not known, nuage is a germ cell specific inclusion (Eddy, 1975; Nieuwkoop and Sutasurya, 1979).

Nuage has been reported in young previtellogenic oocytes in several species of ascidian, both with the light microscope (Crampton, 1899; Conklin, 1905; Hirschler, 1917; Harvey, 1927; Jägersten, 1935), and with the TEM (Hsu and Cloney, 1958; Mancuso, 1964; Kessel, 1983). Because of its germ line specificity, I searched for nuage in the hemoblasts, and all stages of gonadogenesis in Corella inflata, to try to determine if nuage is found in any cells prior to the establishment of the gonad. The presence of nuage in circulating hemoblasts might indicate that all hemoblasts are capable of forming the gonad. The presence of nuage in only gonad-forming hemoblasts would provide evidence both that the germ line is determined fairly early in development, and that there are only a few cells capable of forming the gonad. In Corella inflata, nuage is first observed in gonial cells of the cavitated gonad (Stage IV), after the gonial cells have been completely isolated from the environments of both the lumen of the ovotestis and the gonad hemocoel by somatic cell processes. It is never found in any gonial cells of earlier

stages of gonadogenesis, or in any hemoblasts. This indicates that the hemoblasts that form the gonad are not unique by this morphological criterion, and that the gonial cells themselves begin the synthesis of nuage after cavitation of the ovotestis occurs. This is the first report on the presence of these germ line inclusions in the developing gonad of any ascidian.

Sections through the developing gonad of Corella inflata, demonstrate small granular aggregations in localized regions of the inner and outer membranes of the nuclear envelope. In favorable sections, it is possible to see this granular material extending through nuclear pores. There is therefore every indication that nuage is synthesized in the nucleus and is moved to the cytoplasm. This view is shared by Cowden (1967), Kessel (1966a, 1983) and Mancuso (1963, 1964, 1972). The material that passes through the pores consists of RNA and protein (Cowden, 1961, 1962, 1967; Cowden and Markert, 1961; Davenport and Davenport, 1965; Kessel, 1966a, 1983; Eddy, 1975). It appears that the frequency of nuage production decreases during growth of the oocyte, so that it is virtually absent in vitellogenic oocytes (Mancuso, 1964, Kessel, 1966a, 1983). Similarly, the granular aggregations that constitute the nuage appear to fragment as the oocyte grows, so that they are more numerous but smaller in larger oocytes (Mancuso, 1964; Kessel, 1966a, 1983). Kessel (1983) suggests that these aggregations may be masses of ribosomes which subsequently become dispersed in the cytoplasm and transformed into particulate ribosomes, accounting for the intense basophilia of previtellogenic oocytes.

It should be noted that my observations suggest that nuage may be produced cyclically during gonadogenesis in Corella inflata. The amount of nuage in the gonial cells of a given individual is relatively constant, as is the apparent movement of material from nucleus to cytoplasm. It is possible that nuage production corresponds to cell divisions taking place in the germinal layer, and that there is no nuage production during bouts of mitosis. As yet there is no experimental evidence to support this hypothesis, and carefully controlled experimental conditions, and TEM fixation of animals at all hours of the night, are required to address the issue more fully.

During gonadogenesis in Corella inflata, nuage is first visible in the ovotestis just after cavitation has occurred. This indicates that nuage is neither responsible for organogenesis, nor a determinant for the ovarian line. After the differentiation of the testicular rudiment from the ovotestis, nuage is still found in the male gonial cells, and ultrastructural evidence supports the conclusion that nuage is being produced in the testis, although it is not as abundant as in the ovarian portion of the gonad. Its structure is, however, identical in gonial cells of both the testis and the ovary. Developing male germ cells of other animals have been reported to contain nuage (Eddy, 1975; Kessel, 1983), but it has never been reported in the developing male germ cells of ascidians. This may partially result from a lack of research on the subject of spermatogenesis in ascidians (Georges, 1969; Tuzet et al., 1974).

CELL DIVISIONS

One problem that I have encountered in the study of gonadogenesis in Corella inflata is that cell division has never been observed in the gonadal tissues. This has made it difficult to determine the dynamics of gonial cell origin and abundance within the epithelium of the gonad. In the course of this study, sections of over 200 animals, fixed at various times of the day from early morning to late afternoon, showed no animals in which cells of the reproductive tissues were dividing. Because of the absence of mitotic divisions during these periods of the day, I suggest that cell division occurs rapidly and synchronously during gonadogenesis in ascidians.

Other events of the reproductive cycle, such as the release of gametes and tadpoles, are regulated by the perception of photoperiod in many species including Corella (Huus, 1937; Lambert and Brandt, 1967; Whittingham, 1967; Reese, 1967; Georges, 1968; Watanabe and Lambert, 1973; Woollacott, 1974, 1979; West and Lambert, 1976; Mukai and Watanabe, 1977; Woollacott and Porter, 1977). It is possible that photoperiod control of the cell cycle is exerted on the gonial cells so that, for instance, mitoses occur only very late at night. Other authors have also reported that mitotic figures are absent in the gonads of ascidians (Van Beneden and

Julin, 1886; Bancroft, 1899; Aubert, 1954; Hsu and Cloney, 1958). Tucker (1942) noted that in Styela clava, all observations concerning mitosis in the gonad were based on sections from a single animal. Tuzet et al. (1974) reported that while they did not observe mitoses, there were cytoplasmic bridges connecting adjacent spermatogonia in the testis. These bridges correspond to the midbodies that I have reported in the developing gonad of Corella inflata. The question of mitotic regulation during gonadogenesis is particularly intriguing, and it deserves further investigation.

ORIGIN OF THE FOLLICLE CELLS

One of the areas of ascidian reproductive biology that has historically received considerable attention is the question of follicle cell origin. While I have not addressed this question specifically during the course of my study on gonadogenesis in Corella inflata, I have made observations that provide some relevant information to this issue.

Somatic cells are first visible surrounding the gonial cells of the ovotestis in the clump stage (Stage III) of gonadogenesis in Corella inflata. After cavitation of the ovotestis has occurred, two types of somatic cells are distinguishable on the basis of their shape, size, and nuclear characteristics. These two types of somatic cells are present in the ovarian portion of the gonad throughout gonadogenesis.

In the ovotestis of Stages IV and V of Corella inflata, the Type II somatic cells are irregularly shaped low cuboidal cells, variable in size, and found primarily adjacent to the gonial cells. The nuclei contain dense nucleoplasm, and the cytoplasm contains conspicuous large vesicles of RER with a diffuse intracisternal matrix, and large Golgi complexes. These somatic cells are associated with gonial cells in the ovarian portion of the gonad after the ovarian and testicular rudiments have separated, but they are never found in the testicular portion of the developing gonad. In the Stage VII gonad, after the ovary and testis have completely separated and are forming their exit ducts, some of the Type II somatic cells appear

to establish a primary follicle cell layer around the gonial cells. I suggest that on the basis of these morphological observations, the Type II somatic cells are indeed the progenators of the follicle cells.

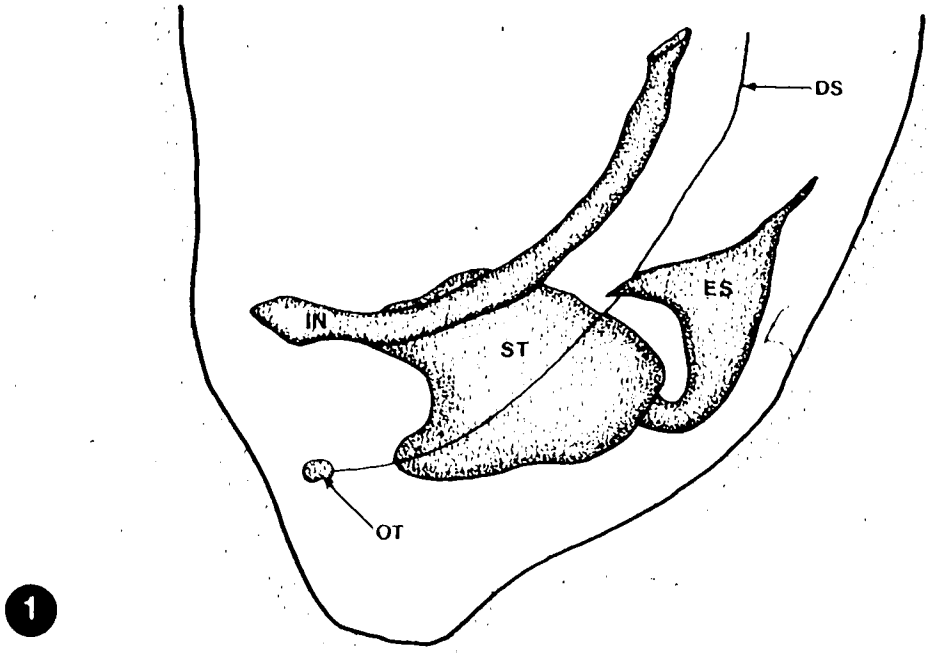
During the early stages of oogenesis, the oocyte of ascidians is surrounded completely by a layer of primary follicle cells (Cowden, 1961, 1962; Reverberi, 1971; Kessel, 1983). The follicle cells later divide to form first the test cells, and then both the inner and outer follicular layers (Huus, 1937; Tucker, 1942; Cowden, 1961, 1962; Kessel and Kemp, 1962; Kessel, 1962, 1983; Berrill, 1975). Work by Knaben (1936), Spek, (1927) and Mancuso (1965) points to a blood cell origin of the accessory cell layers around the oocyte. Pérès (1954), DeVincentiis (1962) and Kalk (1963a) believed that the follicle cells are germinal epithelium derivatives, and maintained that the test cells are derived from amoeboid cells of the blood. In Corella inflata, it appears that the follicle cell line is established very early during gonadogenesis, and that at least the primary follicle cells are derived from the Type II somatic cells. Because there is no evidence to suggest that blood cells contribute to the the gonad after it is established, these follicle cells are probably derived ultimately from the initial, unique accumulatian of hemoblasts of the first stage of gonadogenesis. These observations demonstrate the need for further specific investigation into the origin of the follicle cells in ascidians.

Table 1. The seven stages of gonadogenesis in Corella inflata, documenting the size of the juvenile and number of days after metamorphosis for each stage, as well as the shape of the developing gonad, the approximate number and size of the cells that form the gonad, and the features of the gonial and somatic cells of the gonad. The figures given for days after metamorphosis are the lowest observed for each stage. There is considerable variation in growth rate among individuals in each culture; but once a particular size is attained, the juvenile possesses the given stage of gonadogenesis. ann.lam = annulate lamellae, d.s. = dorsal strand, f.g.inclusion = fine-grained inclusion, loc.g.l. = localized germinal layer, peri.hetero. = peripheral heterochromatin.

STAGE	SIZE (mm)	AGE (days)	SHAPE REPRO SYST	SIZE REPRO SYST (μm)	APPROX # CELLS	FEATURES GONIAL CELLS	SOMATC CELLS TYPE I	SOMATC CELLS TYPE II
I: Ovotestis	<.3		2 cells, no d.s.	7-10	2	similar to hemoblast	none	
II: Ovotestis	0.3	7	2 cells, d.s.	7-10	2	similar to hemoblast	none	
III: Ovotestis	0.4-1.0	14	ellipsoidal cell cluster	20-25	15-20	f.g.inclusion	no difference	
IV: Ovotestis	1.0-1.6	25	spherical, w/- lumen	25-35	30-50	f.g.inclusion, nuage	peri.hetero. cilia	dense nuc.,VER, f.g.inclusion
V: Ovotestis	1.8-2.4	30	ellipsoidal w/ lumen, loc.g.l.	35-75	~100	f.g.inclusion, nuage	peri.hetero., cilia	dense nuc.,VER, f.g.inclusion
VI: Ov.Rudiment	2.6-2.8	75	ellipsoidal, w/ lumen, loc. g.l.	80-100	~200	f.g.inclusion, nuage, ann.lam.	peri.hetero., cilia	dense nuc.,VER, f.g.inclusion
Test.Rudiment			spherical w/ lumen	~20	20-25	nuage	no difference	
VII: Ovary	2.8-4.0	80	elongate, w/ oviduct,loc.g.l.	~150	~500	f.g.inclusion nuage, ann.lam.	peri.hetero., cilia	dense nuc.,VER, f.g.inclusion
Testis			elongate, w/ spermduct	~100	200	nuage	no difference	

Illustration 1. Drawing of the posterior region of a juvenile in Stage III of gonadogenesis. This shows the ovotestis attached to the dorsal strand, and the position of the ovotestis relative to the digestive system. DS= dorsal strand, ES= esophagus, IN= intestine, OT= ovotestis, ST= stomach.

Illustration 2. Drawing of the posterior region of a juvenile in Stage VII of gonadogenesis. This shows the oviduct attached to the dorsal strand, and the sperm duct opening into the dorsal region of the oviduct. The configuration of the digestive system is the same as that of the adult. DS= dorsal strand, ES= esophagus, IN= intestine, O= ovary, ST= stomach, T= testis.



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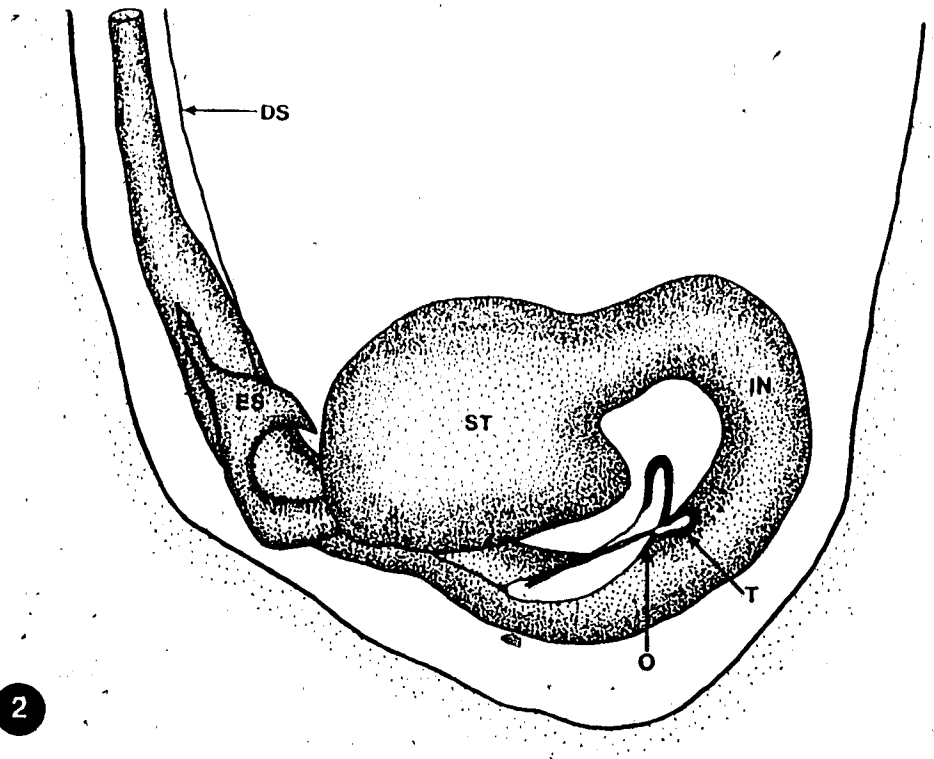
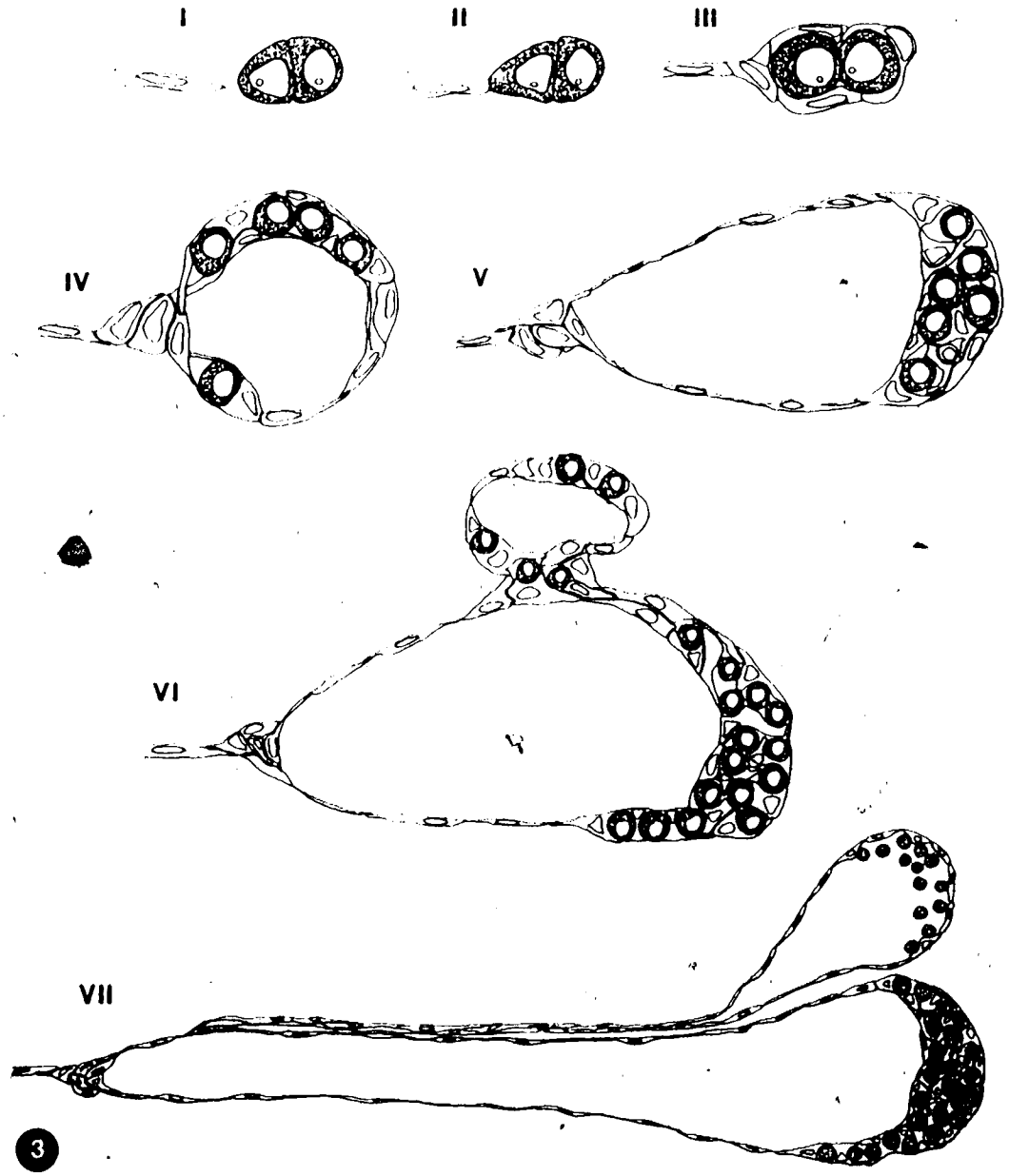


2

Illustration 3. Composite drawing of the seven stages of gonadogenesis in Corella inflata. This summarizes the morphological changes that take place during gonadogenesis, and includes changes in the relative numbers of cells that make up the developing gonad. The stippled cells represent the gonial cells.



3

Figure 1. Light micrograph (LM) of a young juvenile shortly after metamorphosis showing the right side of the body. A = anterior, AS = atrial siphon, BAR = basal attachment region, BS = branchial siphon, CA = caecum of the stomach, D = dorsal, EN = endostyle, EP = epidermis, ES = esophagus, IN = intestine, P = posterior, SI = stigmata, ST = stomach, TU = tunic, V ventral. 350X.

Figure 2. LM of a young juvenile showing the left side of the body. Labels are the same as above. A portion of the dorsal strand, attached to the ovotestis, can be seen in the posterior region of the animal (arrows). 350X.

Figure 3. Scanning electron micrograph (SEM) of a mature gonad. AE = atrial epithelium, BC = blood cell, OO= oocyte in the ovary, T = lobule of the testis. Scale bar = 40.0 μm, 430 X.

Figure 4. SEM of a portion of a mature ovary. AE = atrial epithelium, BC = blood cells, CT = connective tissue, FC = indentations of follicle cells around the oocytes, OO= oocyte. Scale bar = 20.0 μm, 530 X.

Figure 5. LM of a whole mount showing a developing ovarian lobe on the surface of the intestine. I = intestine, OO= oocyte. Scale bar = 50.0 μm, 260 X.

Figure 6. SEM of a portion of a lobule in the mature testis. BC = blood cell, CT = connective tissue. Scale bar = 110.0 μm. 110 X.

Figure 7. LM of a whole mount showing several lobules of the testis. GE = germinal epithelium, SD = sperm duct. Scale bar = 50.0 μm. 220 X.

Figure 8. DIC micrograph of the neural complex of a juvenile soon after metamorphosis, showing the location of the dorsal strand relative to the neural complex. CF = ciliated funnel, DS = dorsal strand, NG = neural ganglion, NL = neural gland, NV = nerve. Scale bar = 10.0 μm. 1,120 X.

Figure 9. DIC micrograph of the right side of a juvenile. The neural complex is positioned dorsally and anteriorly, and is subjacent to a hemocoelic space. BS = branchial siphon, DB = degenerating larval brain, DS = dorsal strand, EN = endostyle, EP = epidermis, HC = hemocoelic space with blood cells, NC = neural complex. Scale bar = 20.0 μm. 570 X.

Figure 10. Transmission electron micrograph (TEM) of the dorsal strand in the gonad hemocoel. EL = external lamina, ER = rough endoplasmic reticulum, M = mitochondrion, N = nucleus, SL = secondary lysosome. Scale bar = 2.0 μm. 8,500 X.

AF
M

Figure 11. TEM of the dorsal strand. EL = external lamina, N = nucleus, P = process, R = ribosomes, V = moderately electron dense vesicles. Scale bar = 0.25 μm. 31,400 X.

Figure 12. TEM of the dorsal strand along the epidermis in the gonad hemocoel. CT = connective tissue fibers, DS = dorsal strand, EP = epidermis, N = nucleus, NV = associated nerve axon. Scale bar = 0.5 μm. 22,300 X.

Figure 13. TEM of microfilaments along the edge of the plasmalemma in a dorsal strand cell. MF = microfilaments, N = nucleus. Scale bar = 0.25 μm. 37,700 X.

Figure 14. TEM of desmosomes joining overlapping processes of cells of the dorsal strand. D = desmosome. Scale bar = 0.25 μm. 46,200 X.

Figure 15. TEM of the region of contact between the dorsal strand and the developing gonad during Stage V. DS = dorsal strand, GS = somatic cells of the ovotestis, N = nucleus, NV = nerve, SL = secondary lysosome. Scale bar = 2.0 μm. 5,300 X.

Figure 16. TEM of the dorsal strand and associated nerve near their contact with the ovotestis. DS = dorsal strand, EL = external lamina which is continuous around the dorsal strand and nerve, ER = endoplasmic reticulum, M = mitochondrion, NV = nerve, V = moderately electron dense vesicles. Scale bar = 1.0 μm. 12,800 X.

Figure 17. TEM of the nerve in the gonad hemocoel, composed of multiple overlapping processes. CT = connective tissue, EP = epidermis, NV = nerve, V = moderately electron dense vesicles. Scale bar = 0.5 μm. 28,600 X.

DS
EL
ER
15

Figure 18. TEM of a cross section through the nerve near the ovotestis. MT = microtubules, V = moderately electron dense vesicles. Note the opaque vesicles in the nerve as well. Scale bar = 0.1 μm. 124,300 X.

Figure 19. TEM of the nerve near the contact between the dorsal strand and the ovotestis. DS = dorsal strand, GS = somatic cells of the ovotestis, SL = secondary lysosomes, V = moderately electron dense vesicles. Scale bar = 1.0 μm. 14,400 X.

Figure 20. TEM of a nerve process extending into the somatic region of the ovotestis. GS = somatic cells of the ovotestis, MT = microtubules, V = moderately electron dense vesicles. Scale bar = 0.25 μm. 47,300 X.

Figure 21. DIC micrograph of the dorsal strand close to a pair of hemoblasts in the gonad hemocoel during the first stage of gonadogenesis. BC = blood cells, CT = connective tissue, DB = degenerating larval brain, DS = dorsal strand, which has a tapered end, ES = esophagus, HB = hemoblasts. Scale bar = 10.0 μm. 1,340 X.

Figure 22. DIC micrograph of the dorsal strand in the gonad hemocoel during Stage I of gonadogenesis. AX = resorbed axial complex cells, DS = dorsal strand, EP = epidermis. Scale bar = 10.0 μm. 1,100 X.

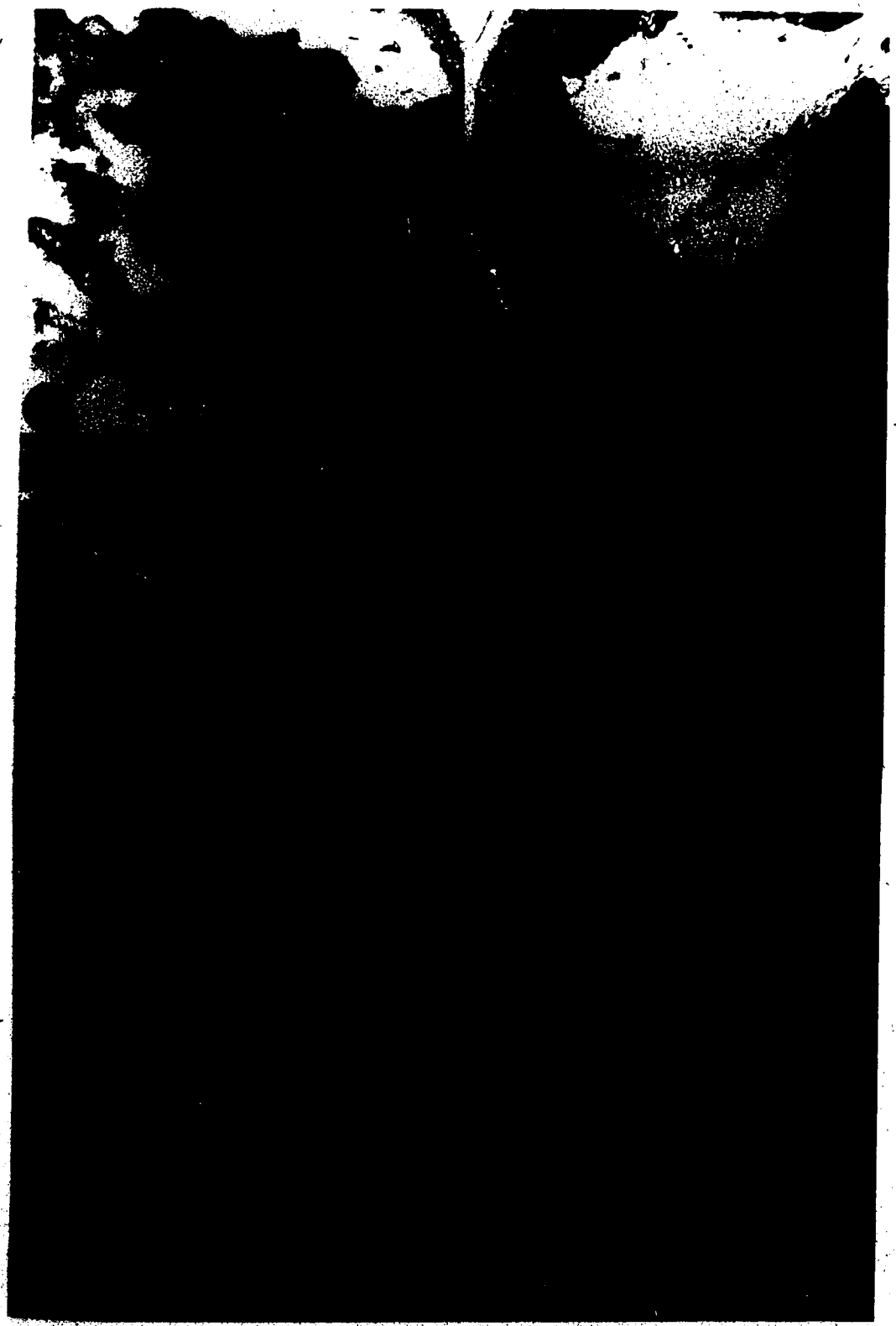


Figure 23. DIC micrograph of the Stage II ovotestis attached to the dorsal strand on the right side of the body. BC = blood cells, CT = connective tissue, DS = dorsal strand, EP = epidermis, OT = ovotestis. Scale bar = 20.0 μm. 890 X.

Figure 24. DIC micrograph of the Stage II ovotestis attached to the dorsal strand. CT = connective tissue, DB = degenerating larval brain, DS = dorsal strand, OT = ovotestis. Scale bar = 20.0 μm. 890 X.

Figure 25. LM of the Stage II ovotestis in the gonad hemocoel. AM = ampulla, CT = connective tissue, EP = epidermis, GH = gonad hemocoel, OT = ovotestis, PG = pyloric gland. Scale bar = 10.0 μm. 1,620 X.

Figure 26. TEM of collagen fibers around the ovotestis. Note the banding pattern of the individual fibers. Scale bar = 0.1 μm. 98,700 X.

Figure 27. TEM of the Stage II ovotestis. ER = endoplasmic reticulum, M = mitochondrion, N = nucleus, NU = nucleolus. Scale bar = 1.0 μm. 11,200 X.

Figure 28. TEM of circulating hemoblasts. Note the similarities between these cells, and the cells of the Stage II ovotestis. ER = endoplasmic reticulum, M = mitochondrion, N = nucleus, NU = nucleolus. Scale bar = 2.0 μm. 6,100 X. Inset, TEM of a midbody between two hemoblasts (arrows). 15,400 X.

21

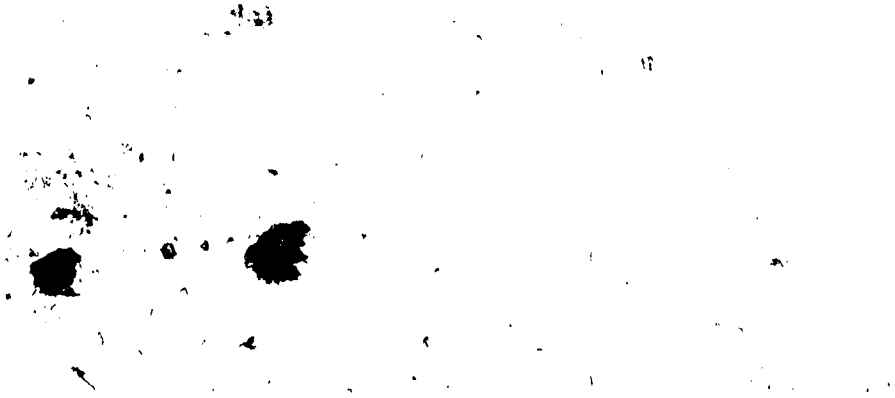


Figure 29. LM of the Stage III ovotestis attached to the dorsal strand. CT = connective tissue, DS = dorsal strand, EP = epidermis, GC = gonial cell, PG = pyloric gland, SC = somatic cell, TU = tunic. Scale bar = 10.0 μm. 1,690 X.

Figure 30. TEM of a gonial cell and somatic cells of the Stage III ovotestis. BL = basal lamina, C = cilium, ER = endoplasmic reticulum, GA = granular aggregations in the perinuclear cytoplasm, IP = intercellular pocket, M = mitochondrion with matrix granule, MD = membrane density, N = nucleus, NU = nucleolus, R = ribosomes, SC = somatic cell, SL = secondary lysosome. Scale bar = 1.0 μm. 18,500 X.

GC
GC

Figure 31. TEM of gonial cells surrounded by somatic cells in the Stage III ovotestis. ER = endoplasmic reticulum, EP = epidermis, G = Golgi complex, GC = gonial cell, GH = gonad hemocoel, SC = somatic cell. Scale bar = 2.0 μm, 8,500 X.

Figure 32. TEM of a myeloid figure in a gonial cell of the Stage III ovotestis. AG = granular aggregations that appear to be in transit to the cytoplasm through the nuclear pore, GR = glycogen rosettes, MY = myeloid figure, NE = nuclear envelope, R = ribosomes. Scale bar = 0.25 μm, 59,200 X.

Figure 33. TEM of fine-grained inclusions in a gonial cell of the Stage III ovotestis. FI = fine-grained inclusion, NE = nuclear envelope, NP = nuclear pore. Scale bar = 0.1 μm, 92,700 X.

Figure 34. TEM of a somatic cell of the Stage III ovotestis. BL = basal lamina, G = Golgi complex, GC = gonial cell, N = nucleus with peripheral heterochromatin, SC = somatic cell. Scale bar = 1.0 μm, 21,300 X.

GH
31
34

Figure 35. TEM of somatic cells of the Stage III ovotestis. Note that the somatic cells at the extremities of the organ are more cuboidal in shape. BL = basal lamina, GC = gonial cell, N = nucleus with peripheral heterochromatin. Scale bar = 1.0 μm. 15,400 X.

Figure 36. TEM of somatic cells of the Stage III ovotestis, showing a cilium projecting into an intercellular pocket. C = cilium, ER = endoplasmic reticulum, which has a vesicular form, IP = intercellular pocket, SC = somatic cell. Scale bar = 0.5 μm. 17,600 X.

Figure 37. TEM of vesicles associated with the plasmalemma along an intercellular pocket of a somatic cell. GC = gonial cell with irregular processes, IP = intercellular pocket, SC = somatic cell, V = vesicles. Scale bar = 0.5 μm. 30,900 X.

Figure 38. TEM of a somatic cell membrane specialization, which is coiled, and contains some vesicles similar to those in Figure 37. Scale bar = 0.1 μm. 79,700 X.

Figure 39. TEM of desmosomes joining adjacent somatic cells of the Stage III ovotestis. Scale bar = 0.25 μm. 39,100 X.

Figure 40. DIC micrograph of the Stage IV ovotestis, with localized regions of thinning, particularly in areas facing the epidermis. The dorsal strand attachment is out of focus in this micrograph. EP = epidermis, L = lumen of the ovotestis. Scale bar = 10.0 μm. 1,170 X.

Figure 41. LM of the Stage IV ovotestis, showing the large gonial cells and squamous somatic cells. AE = atrial epithelium, EP = epidermis, GC = gonial cell, L = lumen of the ovotestis, SC = somatic cell. Scale bar = 5.0 μm. 1,890 X.

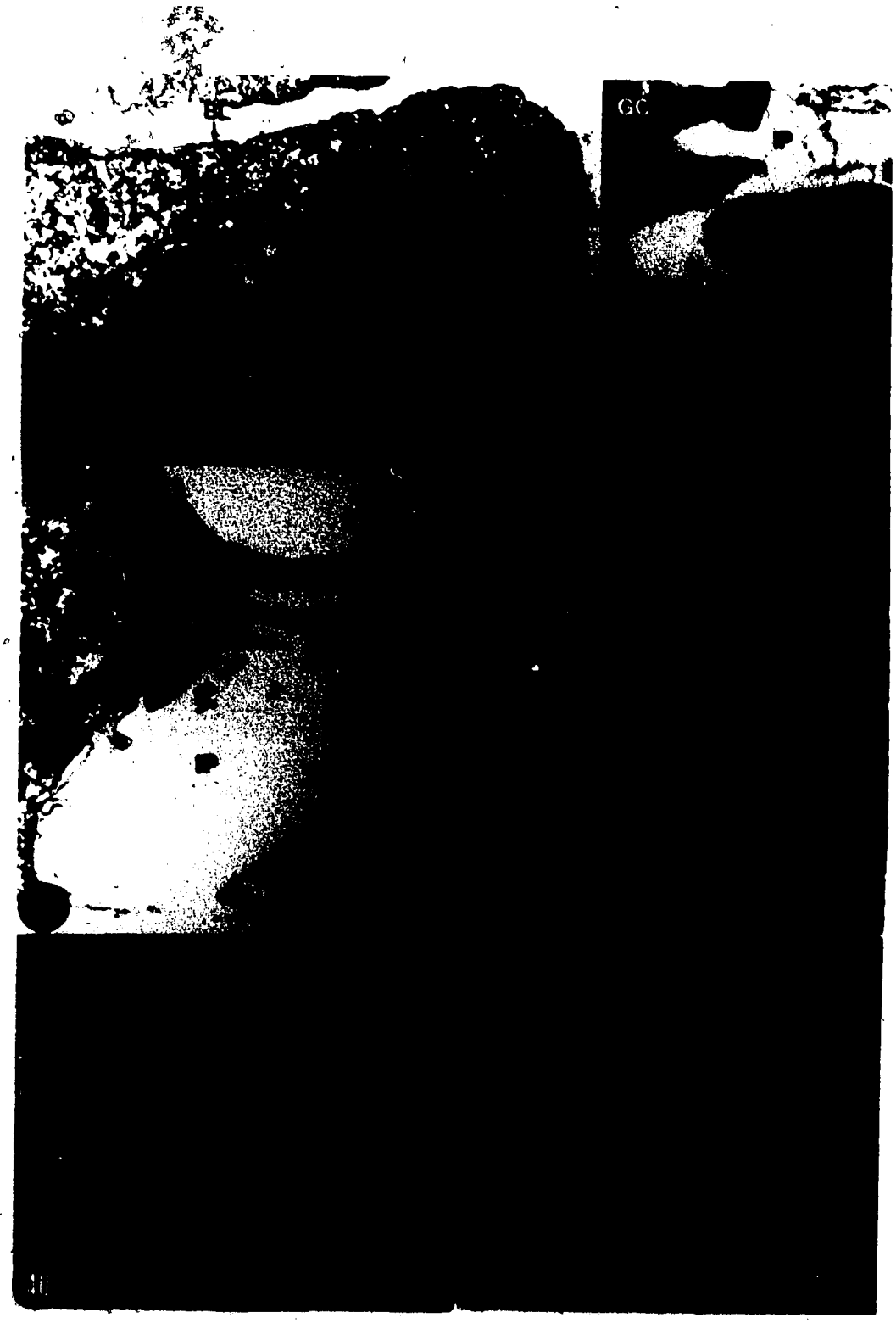

GC

Figure 42. TEM of a gonial cell surrounded by Type II somatic cells in the Stage IV ovotestis. The Type II somatic cells can be distinguished by their dense nucleoplasm, and the vesicular endoplasmic reticulum. EP = epidermis, ER = vesicular endoplasmic reticulum, GC = gonial cell, MD = membrane density, N = nucleus, P = process of somatic cell, SC2 = Type II somatic cell. Scale bar = 2.0 μm. 8,300 X.

Figure 43. TEM of a gonial cell of the Stage IV ovotestis. n. = nucleus with peripheral heterochromatin, AG = granular aggregations in the perinuclear cytoplasm, ER = endoplasmic reticulum, L = lumen of the ovotestis, M = mitochondrion, MD = membrane density, MY = myeloid figure, N = nucleus with peripheral heterochromatin, NA = nuage, NU = nucleolus, SC1 = Type I somatic cell, SL = secondary lysosome. Note the vesicular form of endoplasmic reticulum in the Type II somatic cell adjacent to the gonial cell. Scale bar = 0.5 μm. 18,400 X.

Figure 44. TEM of nuage in the cytoplasm of a gonial cell. This appears to be associated with nuclear pores. N = nucleus, NA = nuage, NP = nuclear pore, R = ribosomes. Scale bar = 0.25 μm. 66,700 X.

Figure 45. TEM of a gonial cell of the Stage IV ovotestis. AG = granular aggregations, ER = endoplasmic reticulum, MD = membrane density, N = nucleus, V = vesicles associated with the plasmalemma. Scale bar = 0.5 μm. 24,700 X.

Figure 46. TEM of myeloid figures and nuage in a gonial cell. MY = myeloid figure, NA = nuage. Scale bar = 0.5 μm. 23,000 X.

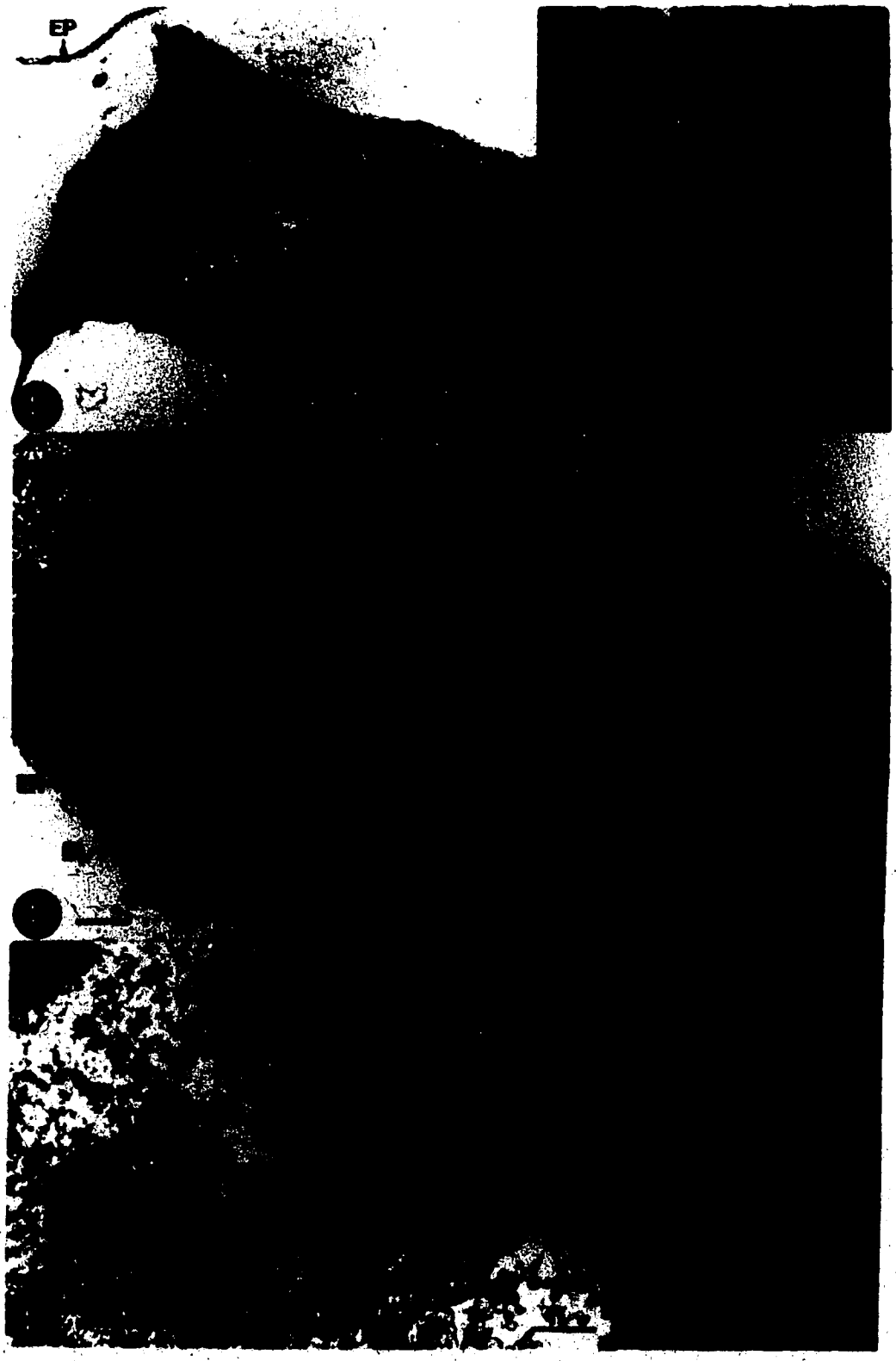

EP

Figure 47. TEM of the Stage IV ovotestis, showing Type I somatic cells with extensive processes in the region that is devoid of gonial cells. EP = epidermis, GH = gonad hemocoel, L = lumen of the ovotestis, P = somatic cell process, SC1 = Type I somatic cell. Scale bar = 5.0 μm. 1,950 X.

Figure 48. TEM of cytoplasmic processes of Type I somatic cells. P = somatic cell process, ZA = zonula adhaerens. Scale bar = 0.5 μm. 25,300 X.

Figure 49. TEM of a Golgi complex, with a large number of vesicles, in a Type II somatic cell. G = Golgi complex, V = vesicles. Scale bar = 0.5 μm. 31,000 X.

Figure 50. TEM of a Type II somatic cell. BL = basal lamina, ER = vesicular endoplasmic reticulum, LI = lipid droplet, VA = vacuole. Scale bar = 0.5 μm. 31,000 X.

Figure 51. DIC micrograph of the Stage V ovotestis, showing the attachment of the dorsal strand at a blunt expansion, and the localization of the germinal layer. DS = dorsal strand, GL = germinal layer, L = lumen of the ovotestis, SC = squamous somatic cells. Scale bar = 20.0 μm. 840 X.

Figure 52. LM of the Stage V ovotestis, showing the gonial cells in the germinal layer and the squamous somatic region that constitutes the remainder of the organ. GC = gonial cells, GL = germinal layer, L = lumen of the ovotestis, SC = somatic cells. Scale bar = 10.0 μm. 1,260 X.

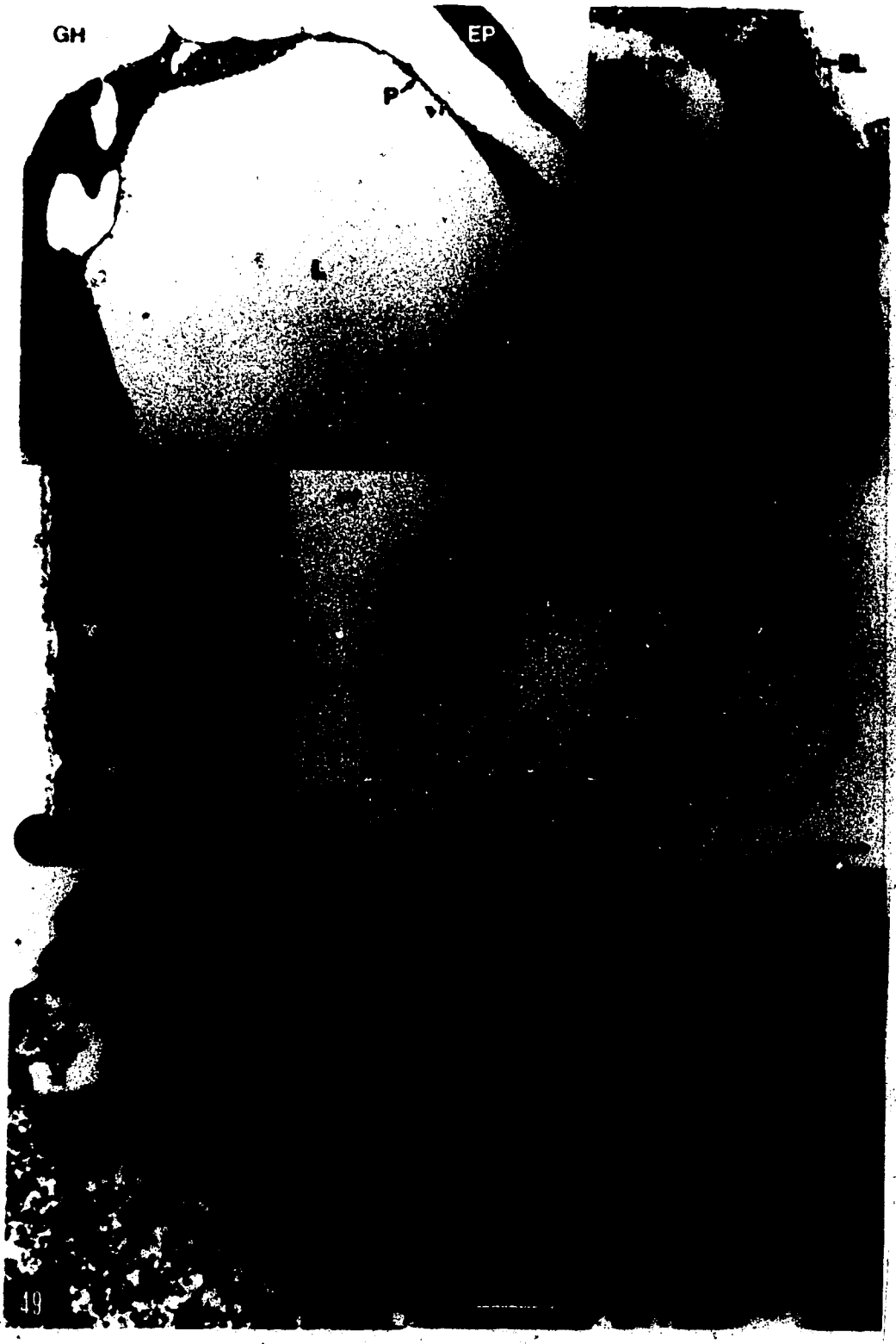

GH
EP
49

Figure 53. TEM of the germinal layer of the Stage V ovotestis, including gonial cells and somatic cells. GC = gonial cell, SC1 = Type I somatic cell, SC2 = Type II somatic cell, SGC = small gonial cell. Scale bar = 2.0 μm. 9,100 X.

Figure 54. TEM of a Type II somatic cell in the germinal layer of the ovotestis. P = process from the Type II somatic cell that extends around adjacent gonial cells. SC1 = Type I somatic cell. SC2 = Type II somatic cell. Scale bar = 1.0 μm. 11,800 X.

Figure 55. TEM of a Type II somatic cell. FI = fine-grained inclusions, GR = granular clusters that resemble ribosomes. Scale bar = 0.2 μm. 79,100 X.

Figure 56. LM of the Stage VI gonad, showing the ovarian rudiment with localized germinal layer, and the attached testicular rudiment. GL = germinal layer of the ovarian rudiment, OL = ovarian lumen, SC = somatic cells of the ovarian rudiment, TL = lumen of the testicular rudiment, TR = testicular rudiment. Scale bar = 10.0 μm. 950 X.

Figure 57. DIC micrograph of the Stage VI gonad showing the testicular rudiment as a bulge extending from the anterior portion of the ovarian rudiment. The testicular rudiment opens into the lumen of the ovarian rudiment. OR = ovarian rudiment, TR = testicular rudiment. Scale bar = 10.0 μm. 980 X.

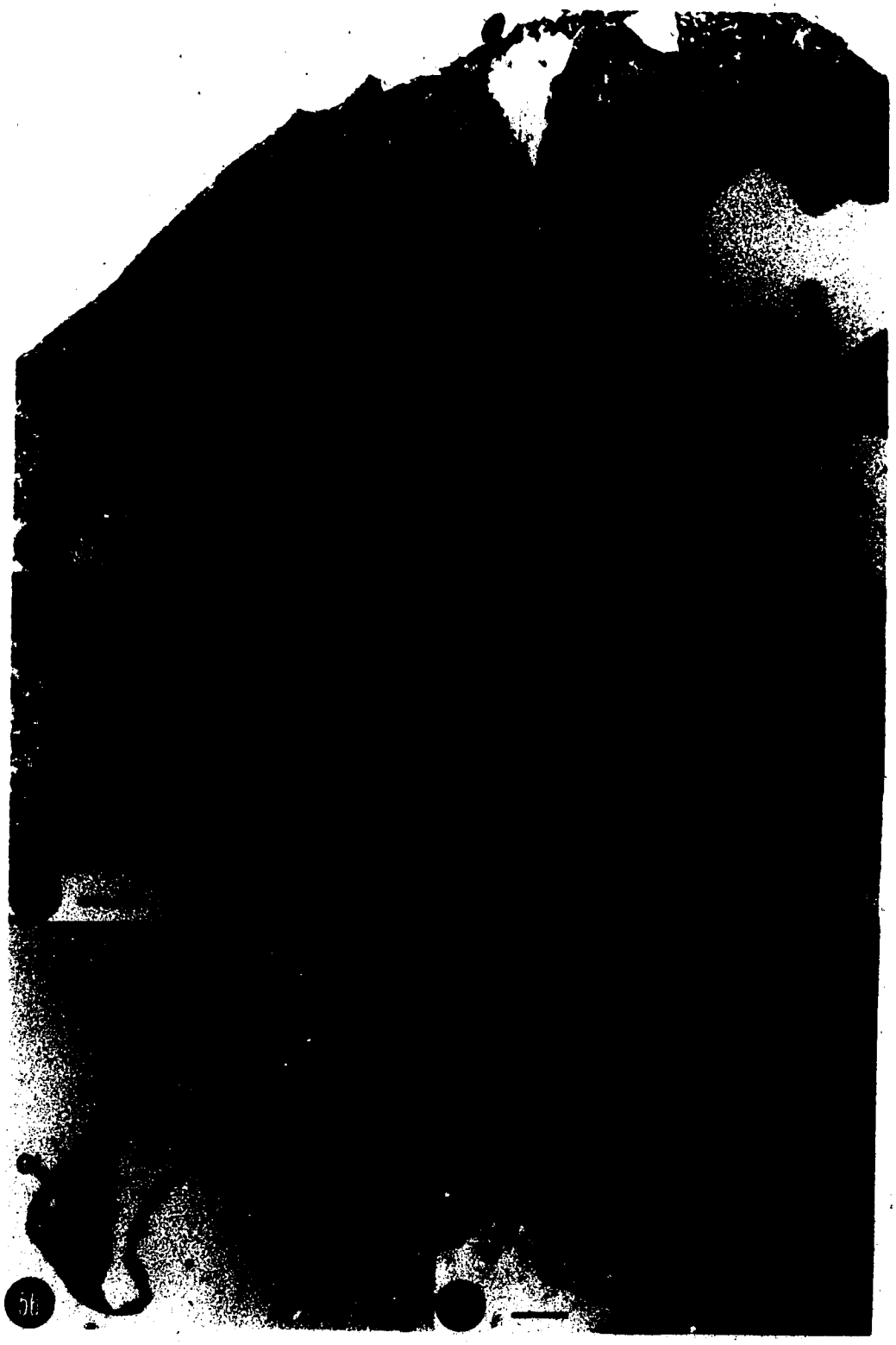


Figure 58. TEM of the germinal layer of the ovarian rudiment. ER = vesicular endoplasmic reticulum in the Type II cells. GC = gonial cell. GH = gonad hemocoel. OL = ovarian lumen. OO = primary oocyte. SC2 = Type II somatic cell. Scale bar = 2.0 μm. 5,600 X.

Figure 59. TEM of the thinner region of the germinal layer. GC = gonial cell. SC1 = Type I somatic cell. SC2 = Type II somatic cell. Scale bar = 2.0 μm. 8,750 X.

Figure 60. TEM of the region of the germinal layer that grades into the squamous somatic region in the ovarian rudiment. Note the large vesicles of endoplasmic reticulum in the Type II somatic cell. GC = gonial cell. SC2 = Type II somatic cell. Scale bar = 1.0 μm. 11,800 X.

Figure 61. TEM of annulate lamellae in a gonial cell of the ovarian rudiment. AL = annulate lamellae. FI = fine-grained inclusion. Scale bar = 0.5 μm. 31,600 X.

Figure 62. TEM of a midbody between adjacent gonial cells of the ovarian rudiment. GC = gonial cell. MB = midbody. Scale bar = 0.5 μm. 26,300 X.

Figure 63. TEM of a Type I somatic cell. Note the overlapping processes, and the cilium projecting into the lumen of the ovarian rudiment. GH = gonad hemocoel, OL = ovarian lumen, P = somatic cell process. Scale bar = 0.5 μm. 25,000 X.

Figure 64. TEM of the testicular rudiment of the Stage VI gonad. The germinal layer is not localized in this stage. GC = gonial cells of the testicular rudiment, OL = ovarian lumen, OR = ovarian rudiment, SC = somatic cells of the testicular rudiment, TL = testicular lumen, TR = testicular rudiment. Scale bar = 5.0 μm. 2,300 X.

Figure 65. TEM of the region of contact between the ovarian and testicular rudiments in Stage VI. BL = basal lamina which is continuous around the two organs, GC = gonial cell, GH = gonad hemocoel; OL = ovarian lumen, OR = ovarian rudiment, TR = testicular rudiment. Scale bar = 2.0 μm. 7,800 X.

Figure 66. TEM of a grazing section through a gonial cell in the region of contact between the ovarian and testicular rudiments. N = nucleus, NA = nuage, SC2 = Type II somatic cell. Scale bar = 1.0 μm. 11,400 X.

OL
CH
TR
SC
TL
OL
BL
CH
OL

Figure 67. TEM of a grazing section through a gonial cell in the region of contact. GC = gonial cell, V = vesicles containing electron dense material, VA = vacuole with small vesicles. Scale bar = 0.5 μm, 19,800 X.

Figure 68. TEM of a gonial cell in the testicular rudiment. BL = basal lamina, N = nucleus, NA = nuage, SC = somatic cell with a cilium projecting into an intercellular pocket. Scale bar = 1.0 μm, 10,700 X.

Figure 69. DIC micrograph of the Stage VII gonad. The testis is positioned laterally to the ovary. O = ovary, OD = oviduct, PG = pyloric gland, SD = sperm duct, T = testis. Scale bar = 50.0 μm, 260 X.

Figure 70. LM of the Stage VII gonad. The testis here is anterior to the ovary. AE = atrial epithelium, EP = epidermis, GH = gonad hemocoel, GL = germinal layer of the ovary, O = ovary, OD = oviduct, PG = pyloric gland, SD = sperm duct, T = testis. Scale bar = 50.0 μm, 260 X.


BL
T
SD
GL
O
OD
GH
EP

Figure 71. LM of the ovary of Stage VII. GC = gonial cells, GL = germinal layer, SC1 = Type I somatic cells, SC2 = Type II somatic cells, SD = sperm duct. Scale bar = 10.0 μm, 1,080 X.

Figure 72. TEM of the thinner region of the germinal layer of the ovary. GC = gonial cell, NA = nuage, OL = ovarian lumen, SC2 = Type II somatic cell. Scale bar = 1.0 μm, 11,200 X.

Figure 73. TEM of a region of the thick germinal layer showing a Type II somatic cell between adjacent gonial cells. GC = gonial cell, SC2 = Type II somatic cell. Scale bar = 1.0 μm, 11,200 X.

Figure 74. TEM of a Type II somatic cell in the germinal layer. GC = gonial cell, SC2 = Type II somatic cell. Scale bar = 0.5 μm, 32,100 X.

Figure 75. TEM of the germinal layer where it grades into the squamous somatic region that forms the oviduct. GC = gonial cell, OL = ovarian lumen, SC2 = Type II somatic cell. Scale bar = 2.0 μm, 7,800 X.

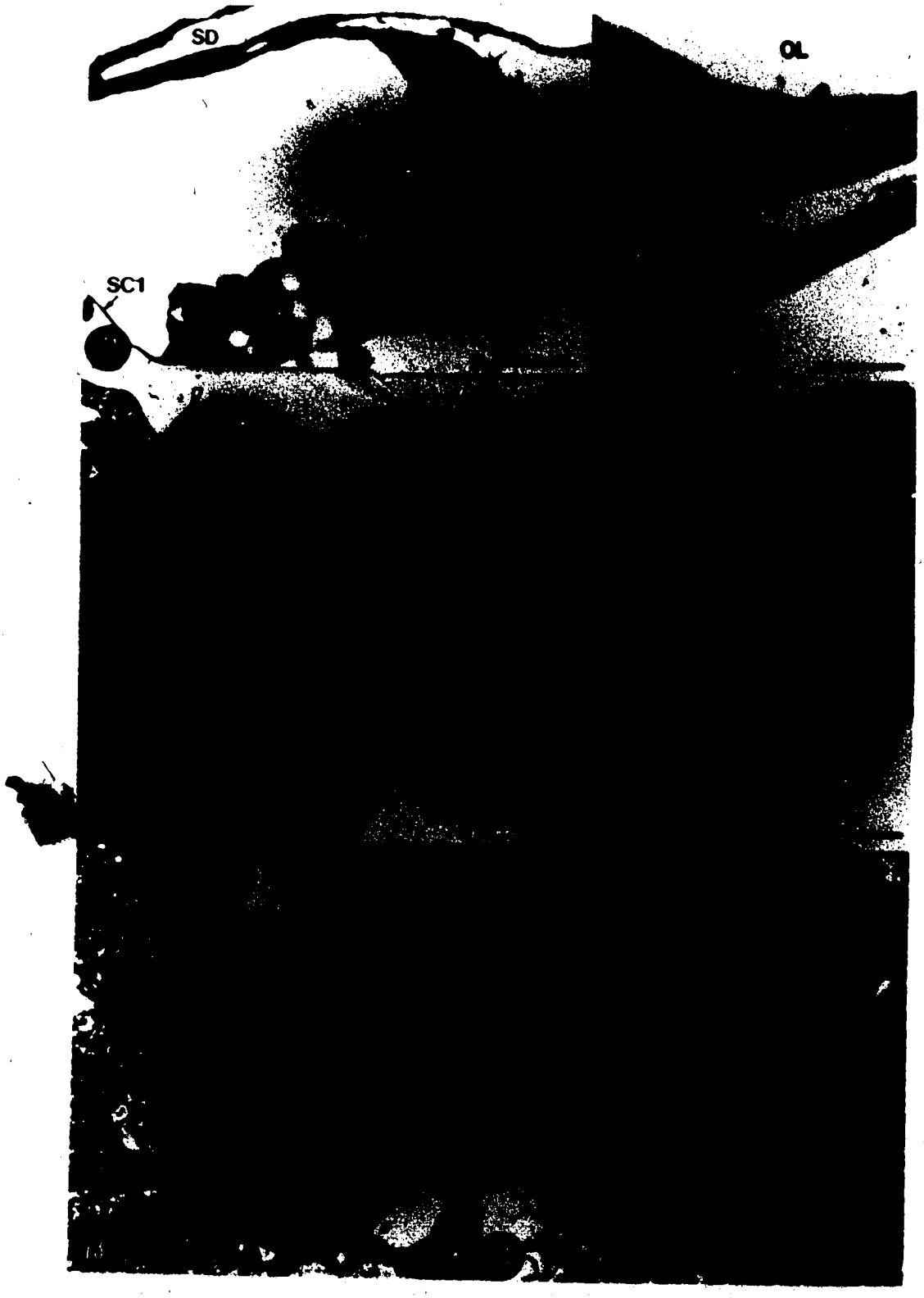

SD
OL
SC1

Figure 76. TEM of the oviduct adjacent to the developing sperm duct. BL = basal lamina between the sperm duct and oviduct, OD = squamous cells of the oviduct, OL = ovarian lumen, SD = sperm duct. Scale bar = 2.0 μm. 7,800 X.

Figure 77. LM of the testis of Stage VII. GC = gonial cells, GH = gonad hemocoel, SC = somatic cells, TL = testicular lumen. Scale bar = 10.0 μm. 1,330 X.

Figure 78. TEM of gonial cells of the testis. AG = granular aggregations, N = nucleus, SC = somatic cell. Scale bar = 2.0 μm. 7,700 X.

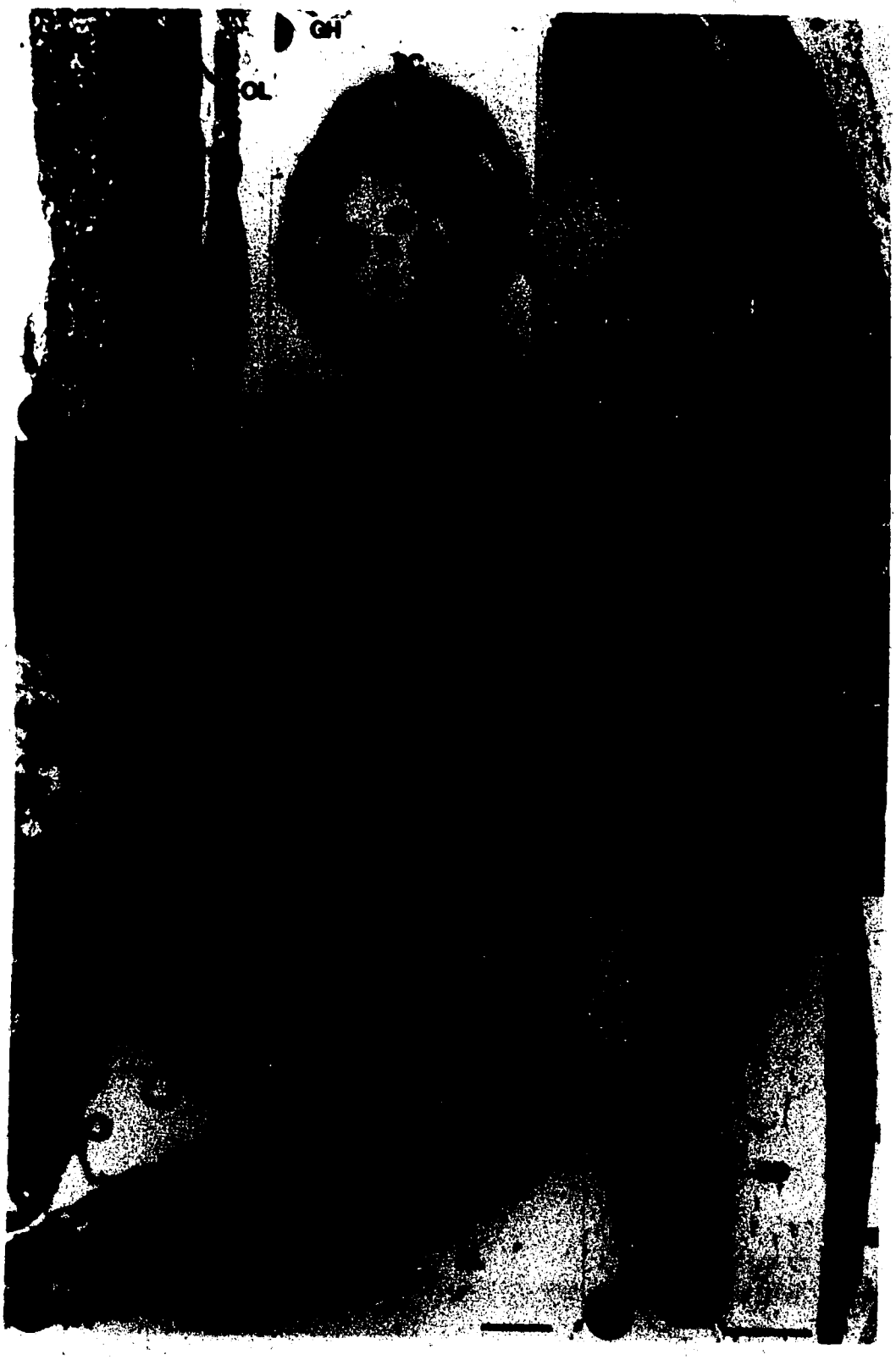
Figure 79. TEM of a gonial cell of the testis. AG = granular aggregations in the perinuclear cytoplasm, N = nucleus, NE = nuclear envelope. Scale bar = 0.5 μm. 35,100 X. Inset, nuage of a testicular gonial cell. 34,600 X.

Figure 80. TEM of a somatic cell of the testis. BL = basal lamina, C = cilium, G = Golgi complex, GC = gonial cell, N = nucleus. Scale bar = 0.5 μm. 24,500 X.

Figure 81. TEM of of cells of the developing sperm duct. ZA = zonula adhaerens. Scale bar = 0.5 μm. 31,600 X.

Figure 82. TEM of the developing sperm duct. The cells are irregular in shape and surrounded by a basal lamina. BL = basal lamina. Scale bar = 1.0 μm. 11,300 X.

Figure 83. TEM of a grazing section through the end of the developing sperm duct. OD = oviduct, SD = sperm duct. Scale bar = 2.0 μm. 7,900 X.


GH
OL

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VI. APPENDIX: A REVIEW OF THE LITERATURE ON ASCIDIAN GONADOGENESIS AND GONAD ORIGIN

The purpose of this appendix is to provide a synthesis of the literature that is available on ascidian gonadogenesis, and those related topics that I have addressed during the course of this research. Much of the information discussed in the literature is confusing and misleading, in part because the terms used have reflected an attempt to find homologies between structures of ascidians and those of other chordate groups. In addition, the microscopic techniques available to investigators in the late nineteenth and early twentieth centuries limited the completeness and clarity of their observations. Finally, I have found that many of the early observations have been misinterpreted in more recent discussions of these topics.

I begin this appendix with a summary of the anatomy of the adult reproductive system in members of both orders of ascidians. This provides a background for comparisons between the morphological changes that take place during gonadogenesis, and the organization of the mature gonad. Secondly, I discuss the available information and hypotheses that deal with the origin of the germ cells and gonads in ascidians, and compare this information with my observations on Corella inflata. Thirdly, I summarize published observations on the stages of gonadogenesis that have been identified in both solitary and compound species, concentrating on those forms in which the testis and ovary develop from a common organ rudiment, the ovotestis. Published results are compared with my observations on the stages of gonadogenesis in C. inflata. This is followed by a discussion of the characteristics of the germ and somatic cells of the gonad, with emphasis on the early developmental stages. Finally, I discuss the association of the neural complex with the reproductive system, and its implications in the development of the reproductive system.

A. REPRODUCTIVE SYSTEM: GENERAL CHARACTERISTICS

All ascidians, with the possible exception of three genera, are hermaphroditic (Berrill, 1975). In the Order Enterogona, the single compound gonad is positioned within the loop of the intestine and consists of numerous ovarian lobes surrounded on all sides, but particularly on the right lateral surface, by a large number of smaller testicular lobules. The gonad in some representatives of the Suborder Aplousobranchia, namely in the family Polyclinidae (Monniot and Monniot, 1972), is located in the postabdomen, below the digestive system. The members of the Order Pleurogona possess two or more gonads associated with the mantle on either side of the branchial basket. These gonads may be compound, or they may exist as distinct ovaries and testes located in different regions of the body (eg Newberry, 1968). An oviduct and sperm duct extend from the gonad along the intestine to open into the atrial cavity or atrium near the base of the atrial siphon in oviparous animals. Most compound species are ovoviviparous, and in species such as Distaplia (Berrill, 1948), parts of the oviduct may be modified into a brood pouch. An oviduct may be entirely absent in other species, such as the Didemnidae (Berrill, 1950). In many of the stolidobranchs there is a tendency for numerous small sperm ducts to open separately into the atrial cavity (Berrill, 1975).

There have been many studies on the gross morphology of the reproductive system in several species of ascidian, particularly with reference to gametogenic cycles, gamete maturation and spawning cycles (see Millar, 1971; Berrill, 1975 for review). These studies indicate that while the structure of the adult gonad is similar in most oviparous species, there is considerable variation in the seasonality of the gonads (e.g. Millar, 1952, 1954, 1958; Lützen, 1960; Wallace, 1961). In general, each lobe of the ovary contains a "germinal epithelium", from which oocytes and probably also the follicle cells differentiate. All stages of oogenesis and folliculogenesis are present in each lobe (Tucker, 1942; Pérès, 1954; Millar, 1953; Ermak, 1976). As it is difficult to ascertain the histological nature of this tissue, the "germinal epithelium" of the ovary is referred to in this work as the "germinal layer". The ciliated

epithelial lining of the oviduct is continuous with the germinal layer (Tucker, 1942; Millar, 1953). The structure of the ovary in ovoviviparous forms is often greatly modified due to the production of a small number of large eggs, which are usually brooded (Berrill, 1950, 1975). The testicular lobules are composed of a relatively squamous germinal epithelium which gives rise to spermatogonia and spermatocytes (Tucker, 1942; Tuzet et al. 1974; Millar, 1953). The structure of the adult gonad of Corella inflata is essentially the same as in other oviparous phlebobranch ascidians.

B. ORIGIN OF GONADS AND GERM CELLS

Most work on the origin of ascidian gonads is based on an examination of juveniles in which the developing gonad is already present. Most of this work focusses on the asexually budded blastozooids of compound species, although there are a few reports on gonad origin in solitary species. Little work concerning the origin of the germ cells or gonads has been done in recent years, and no experimental analysis of this subject is available. Finally, there have been no ultrastructural studies on the cells of the developing gonad during gonadogenesis.

Berrill (1975) states in his monograph on the reproductive biology of ascidians that "there is no germ line traceable back to the egg and the gonadal rudiment may derive from dorsal cord, atrial epithelium, mesenchyme, or lymphocytic tissues according to the nature of the reproductive process". Berrill (1975) cites few specific references in support of this ambiguous statement, and these he interprets incorrectly (Van Beneden and Julin, 1886; Simkins, 1924; Huus, 1937). Apparently he has not carefully examined those works that have provided a foundation for the study of gonad and germ cell origin in the ascidians. Here, I outline the patterns of origin that have been implicated in the literature to date, and summarize my observations on Corella inflata.

BLOOD CELLS

The ascidian circulatory system is essentially a hemocoel (Berrill, 1950; Millar, 1953; Burighel & Brunetti, 1979; Mukai et al., 1978). It contains several cell types referred to historically as blood cells, as well as plasma. The embryonic origin of the blood cells has been investigated only in Clavelina picta where a small mass of mesodermal cells, proliferated from the archenteron, develops into hematogenic tissue (Cowden, 1968). These early blood-forming cells of the embryo (hemocytoblasts) are undifferentiated, and are similar in appearance to the hemoblasts of the adult hematogenic tissue (Ermak, 1977). In tailbud stage embryos, some hemocytoblasts differentiate into adult blood cell types, while others retain their embryonic nature. After metamorphosis, some of the hemocytoblasts migrate to hematopoietic sites in various parts of the body. These include the pharyngeal wall around the gill slits, and the connective tissue and blood channels around the intestine (Kowalevsky, 1871; Millar, 1953; Ermak, 1977, 1982). Still other hemocytoblasts may become lodged in discrete nodules in certain areas of the body wall, and, in some stolidobranchs, near the gonads (Ermak, 1977; Wright, 1981).

Numerous investigators have reported on the morphology of the blood cells in adult ascidians using both light and electron microscopy (Hecht, 1918; George, 1939; Pérès, 1943; Millar, 1953; Endean, 1955, 1960; Andrew, 1961; Kalk, 1963a; Smith, 1970; Overton, 1966; Ermak, 1977; Milanesi and Burighel, 1978; Rowley, 1982). Six categories of blood cell have been identified: hemoblasts, lymphocytes, leukocytes, vacuolated cells, pigment cells, and nephrocytes (see Wright, 1981). Recent work indicates that all adult blood cells are derived from hemoblasts (Goodbody, 1974; Milanesi & Burighel, 1978; Wright, 1981). These stem cells are found primarily in the hematogenic tissue, but also in circulation in the blood (Ermak, 1977; Wright, 1981).

Hemoblasts are large spherical cells approximately 5 to 6μm in diameter, with a large nucleus and one or more nucleoli. They have a small amount of basophilic cytoplasm which

contains polyribosomes, cisternal RER, several mitochondria, and a small Golgi complex (Ermak, 1977, 1982; Milanesi & Burighel, 1978; Wright, 1981; Rowley, 1982). These cells are amoeboid, as are all blood cell types (Hecht, 1918; Andrew, 1961; Wright, 1981). The lymphocytes can be distinguished from hemoblasts by their smaller size (3 to 5 μm), the absence of discrete nucleoli, and the localization of peripheral heterochromatin (Pérès, 1943; Millar, 1953; Sabbadin, 1955; Goodbody, 1974; Ermak, 1976; Wright, 1981); yet numerous authors have apparently confused these two cell types (George, 1939; Andrew, 1961; Overton, 1966; Newberry, 1968; Smith, 1970; Freeman, 1964, 1969, 1971).

Hemoblasts are probably the source of most of the tissues of the asexual bud in members of those families (Styelidae, Perophoridae, and Clavelinidae) that multiply by vascular budding (Brien, 1930; Sabbadin, 1955; Oka and Watanabe, 1957, 1959; Milkman and Byrne, 1961; Freeman, 1964). Freeman (1964) found that hemoblasts (*vide* lymphocytes) are essential for budding in Perophora viridis and hypothesized that they supply an essential stem cell factor to the bud, without which other tissues cannot develop. It has been suggested that the hemoblast is also the multipotential cell type responsible for gonad formation (Newberry, 1968; Ermak, 1975, 1976; Mukai & Watanabe, 1976; Wright, 1981).

Compound Species

In an elegant study of gonad formation in Distomus variolosus (in the pleurogonid family Styelidae), Newberry (1968) showed that circulating "lymphocytes" form aggregations in various regions of the mantle sinus. These cells are amoeboid, contain a large nucleus and nucleolus, and probably correspond to the hemoblasts of Wright (1981). Some of the hemoblast aggregations, those in the presumptive ovarian and testicular regions, give rise to the gonads, which are of separate sex. In his examination of 5μm sections of the hemoblast aggregations, Newberry (1968) concluded that the cells of the pre-gonadal clumps in Distomus

are undifferentiated, and probably do not have cytological features that set them apart from other hemoblasts. Ultrastructural studies might demonstrate differences in the cytoplasmic architecture of the cells in these gonad-forming clumps.

In other compound ascidians, the evidence for a hemoblastic origin of the gonads is less conclusive. The observations of Lefevre (1897) on Ecteinascidia blastozooids, indicate that a clump of cells derived from a region of the inner vesicle of the bud becomes surrounded by blood cells and constitutes the gonad rudiment. In Perophora viridis (Lefevre, 1898), P. listeri (Kowalevsky, 1874b; Van Beneden, 1881; Van Beneden and Julin, 1886), Clavelina rissoana (Seeliger, 1893-1906; Van Beneden and Julin, 1886), Distaplia occidentalis (Bancroft, 1899), and Symplegma reptans (Sugimoto and Nakauchi, 1974) the gonad first appears as a small clump of cells. These cells are morphologically similar to amoeboid cells of the blood. Sugimoto and Nakauchi (1974) state that these cells in the compound stolidobranch Symplegma reptans are "lymphocytes", but they are probably hemoblasts by Wright's (1981) definition. They are located among other blood cells in the "genital tracts", or presumptive gonad region of the blastozooid, and the germ cells are derived from them.

The gonad probably also originates from blood cells in other compound ascidians. Mukai and Watanabe (1976) found that clusters of hemoblasts form a "loose cell mass" in the gonadal space of Botryllus schlosseri blastozooids. This "loose cell mass" gives rise to the oocytes and a surrounding primary follicle layer which are subsequently released into the blood. These cells then migrate via the blood to the gonadal space of a new blastozooid generation in the growing colony. Individual oocytes become associated with the "loose cell mass" of the new generation. The somatic cells of the ovary, and also the testis, subsequently differentiate from cells of the "loose cell mass". The research of Mukai and

Watanabe (1976) confirms the earlier report of the blood cell origin of the gonad and the migration of the oocytes in the blood in B. schlosseri (Izzard, 1968). Sabaddin and Zaniolo (1979), however, considered that the cells of the "loose cell mass" (or worse, "blastema") had already differentiated from the blood cell line, and were not, in fact, hemoblasts. They do not, however, rule out the possibility that the "loose cell mass" is ultimately derived from hemoblasts.

Solitary Ascidians

The single gonad in the phlebobranch and aplousobranch ascidians develops in the large subendostylar sinus of the hemocoel. Numerous blood cells are found in this area in the juvenile. Van Beneden and Julin (1886) working with Phallusia scabroides, and Kowalevsky (1866, 1871) with Ciona intestinalis noted that the first indication of the developing gonad is the presence of a small accumulation of several mesoblastic cells similar to those in the hemocoel which surrounds it. Other than in their superficial resemblance to blood cells, however, there is no evidence that clearly indicates the origin of the cells that form this small pre-gonadal clump. This lack of evidence is probably because no individuals were examined in which the clump was absent, and because the microscopic techniques used by the authors were limiting. It is likely that at the stage described by the authors, the developing gonad contains some cells that are distinct from the blood cells. This is true in Corella inflata, where the clump of cells that forms the Stage III gonad consists of two distinct types of cells, gonial and somatic cells, clearly distinguishable only with TEM.

I have observed the developing gonad in Corella inflata at an earlier stage of development than has been reported in any previous study of a solitary ascidian. My observations demonstrate the striking morphological similarity of the

circulating hemoblasts and the two cells that form Stages I and II of gonadogenesis, as revealed by the transmission electron microscope. The morphological similarity between the gonad- forming cells and hemoblasts continues to be apparent in the gonial cells of Stage III. In Stage IV the gonial cells contain nuage, an inclusion unique to this population of cells. In other respects, the gonial cells continue to resemble hemoblasts. The origin of the somatic cells of the gonad is not known. As there is no evidence that suggests a further addition of hemoblasts, or other blood cells, to the developing gonad after its establishment, it may be that the pluripotent hemoblasts of the first stage of gonadogenesis give rise to all of the cells, both gonial and somatic, of the Stage III gonad.

There is further evidence for the hemoblastic origin of the gonad, found in the first stage of gonadogenesis in Corella. Before the gonadal material is identified, by its location and its association with the dorsal strand, a cell or pair of cells that resemble hemoblasts is visible in the pre-gonadal region of the gonad hemocoel, subjacent to the epidermis and in close proximity to the dorsal strand. Although I have been unsuccessful at obtaining sections of this very transient association, it has been observed in at least 20 individuals using DIC microscopy.

My observations indicate that before gonadogenesis can begin in Corella, the pre-gonadal hemoblasts must be associated with the dorsal strand. No animals in Stage II or Stage III of gonadogenesis have ever been observed without this dorsal strand attachment. The dorsal strand never bears a bulbous or swollen region at its tip during the Stage I. This indicates that the dorsal strand does not itself produce the first cells of the ovotestis, but rather that the dorsal strand makes contact with cells that are already present in the gonad hemocoel.

MESENCHYME CELLS

The term "mesenchyme" has traditionally been rather a catch-all term applied to various wandering or fixed cellular elements of the body. Mesenchyme cells are mesodermally derived cells that are not joined to one another. In stolidobranch ascidians the gonads develop in some portion of the mantle, which consists of an outer epidermis, an inner atrial epithelium, with connective tissue, muscle, nerves, and blood spaces in between. In Molgula ampulloides (De Selys Longchamps and Damas, 1900) and Styelopsis grossularia (Julin, 1893) the gonads are first visible as a small clump of mesenchyme, (or mesoblastic cells), located in the mantle wall. As blood cells are capable of wandering from the hemocoel into the mantle tissue (Newberry, 1968; Wright, 1981), and as the cells of the clumps of gonadal tissue are similar in appearance to the amoeboid cells that surround them in the mantle, it is a reasonable assumption that the gonad in these species is also a blood cell derivative.

MESODERMAL PLATE

In most compound ascidians the oozooid has no gonads, and reproduces only asexually (Sugimoto & Nakauchi, 1974; Nakauchi, 1982). Gonads of both sexes develop, however, in the oozooid of Ecteinascidia turbinata. Simkins (1924) described the ovary of the oozooid as derived from a simple mesodermal shelf-like structure that projects from the left body wall into the "coelom" (actually the hemocoel) of the animal. According to Simkins (1924) the cells of this projection are derived from the "splanchnoderm" - an inappropriate term as the cavity is not a coelom. It is likely, however, that certain undifferentiated mesodermal or mesenchymal cells of the mantle proliferate into the hemocoel and form the ovarian rudiment. While the cells of the early ovarian rudiment stain similarly to somatic cells, they are identifiable due to their location and their larger, germinal vesicle-like nuclei. The multiple testes develop, probably at the same time as the ovarian rudiment, from small inconspicuous clusters of cells which are similar to the cells of the surrounding tissue (Simkins, 1924). Simkins (1924) provides no

information in his account on the presence of blood cells in the gonadal region, and it may be that the testis arises from blood cells. It is clear that the origin of the gonad in the oozooid of E. turbinata is different from the blastozooid, because in blastozooids the gonads develop in the hemocoel, presumably from blood cells, as reported by Lefevre (1898).

DORSAL STRAND

Morphological study implicates the dorsal strand as the direct source of the gonads in only one ascidian species (Brien, 1925, 1927). In individual blastozooids of Aplidium zostericola, the dorsal strand expands distally, and then separates in the region of the esophagus, to form two tubes which are histologically different. The ventral most of these two tubes maintains the same undifferentiated characteristics of the dorsal strand prior to its separation, and it develops into the testis. The dorsal most of the tubes becomes surrounded by larger cells, and develops into the ovary. Brien (1927) clearly states that the dorsal strand develops into the ovarian and testicular rudiments of the gonad, but his description does not make the origins of the germ cells clear. It is possible that blood cells aggregate around the dorsal and ventral components of the dorsal strand, and these blood cells may be the source of the germ cells, or at least contribute to the formation of the gonad. Brien (1948) does not discuss the issue of dorsal strand involvement in gonad origin in his later monograph on ascidian biology.

Aubert (1954) did not rule out the possibility that the gonad in newly metamorphosed Ciona is derived from the dorsal strand. His observations were hindered by the large accumulation of cells from the degenerating axial complex, which obstructed the earliest stages of gonadogenesis. Based on the undifferentiated nature of the dorsal strand cells in the bud of Diazona violacea, Aubert (1954) suggested that the gonads originate in this species as they do in Aplidium zostericola (Brien, 1927). The descriptions of dorsal strand involvement in gonad origin in these species are inconclusive at best, and this topic merits further attention and

reexamination.

Huus (1924, 1937) maintains that the dorsal strand gives rise to the gonoducts in Corella parallelogramma. However, his work has been misinterpreted by Aubert (1954) and Berrill (1975) who imply that his results show that the gonad itself is derived from the dorsal strand.

ATRIAL EPITHELIUM

Berrill (1941 a, b) observed that the germ cells of both the ovary and testis form very precociously during bud development in Botryllus schlosseri. He found that the germ cells are derived from the region of the atrial epithelium, in "sphere", that pinches off to form a new bud during blastogenesis. He indicates that the germ cells, including a few large oocytes and numerous smaller, presumptive testicular cells, arise by a "delamination or extrusion" from this region of the atrial epithelium. It is possible that the cells are in fact pinched off the atrial epithelium, and are released into the blood, to form the loose cell mass, as suggested by Sabaddin and Zaniolo (1979). If this were true, distinct histological differences between the hemoblasts and the germ cells should exist, reflecting their different origins. Mukai and Watanabe (1976), however, have shown that the germ cells are morphologically very similar to the hemoblasts.

C. STAGES OF GONADOGENESIS

The stages of gonadogenesis outlined in the literature appear to be strikingly similar among the ascidians which possess compound gonads, regardless of their taxonomic position. In this section I discuss the literature that is available on the structure of the developing gonad in ascidians, and relate it to the stages of gonadogenesis I have identified in Corella inflata.

STAGES I AND II

These stages have not been identified in any previous study of gonadogenesis in a solitary ascidian. In Corella inflata, Stages I and II have been observed only in very small animals raised from embryos through metamorphosis, and they are extremely difficult to find in sectioned material.

The first indication of the developing gonad in Corella inflata is one or a pair of cells located in the gonad hemocoel of very small animals, in close proximity to the termination of the dorsal strand. These cells are identical in their morphology to hemoblasts of the circulating blood, and there are no differences between the two cells. This stage is followed by Stage II, during which the pre-gonadal hemoblasts make contact with the end of the dorsal strand. I have never observed Stage II, or any other stage of gonadogenesis, without the contact with the dorsal strand. On this morphological evidence, I suggest that in Corella inflata, the association of the hemoblasts with the dorsal strand is essential to the initiation of gonadogenesis.

It is reasonable to assume that in most ascidian species the first stage of gonadogenesis consists of an initial accumulation of hemoblasts, that precedes the slightly differentiated cluster of cells in Stage II. In the compound stolidobranchs, the accumulation of hemoblasts in the mantle sinus has been clearly documented in Distomus variolosus (Newberry, 1968), and suggested in Symplegma reptans (Sugimoto and Nakauchi, 1974). In Distomus, those cells that form the initial accumulation are not augmented by an additional contribution of hemoblasts from the blood. This is also true in Corella inflata, although the number of cells that forms the Stage I gonad is substantially fewer than in Distomus. It appears that once the developing gonad is established, it constitutes a unique population of cells.

In those species which possess a dorsal strand or genital cord, these structures serve as convenient markers for locating this first stage of gonadogenesis. In those animals that do not have either a dorsal strand or a genital cord (see Metcalf, 1900), the cells that will form the developing gonad may passively aggregate in the "pre-gonadal region" of the body, such as the

mantle, as suggested by Newberry (1968).

STAGE III

This stage has been identified in most previous studies as the first stage of gonadogenesis. It consists of a small cluster of cells located in the gonad hemocoel in phlebobranchs and aplousobranchs, and in the mantle or mantle sinus in the stolidobranchs. In Corella inflata, the Stage II ovotestis consists of two distinct cell types which are clearly discernible in transmission electron micrographs. These cells are the gonial cells and somatic cells. To a large extent, the gonial cells retain their hemoblastic nature. They are completely surrounded by somatic cells, and are therefore isolated from the environment of the gonad hemocoel. The ovotestis is attached to the dorsal strand, and always appears to be surrounded by connective tissue fibers, which may anchor the ovotestis to the epidermis, preventing it from floating free in the blood. I have frequently observed blood cells in the gonad hemocoel around the ovotestis, which may be important in carrying out various physiological functions of the cells of the ovotestis (Wright, 1981).

In a comprehensive study of gonadogenesis in Corella parallelogramma, Huus (1924) was unable to find animals in which the ovotestis was not present. He described the first stage of gonadogenesis as consisting of a small compact mass of cells located between the stomach and intestine, and near the caecum of the stomach. This corresponds to Stage II in C. inflata. Some of the cells of the ovotestis in C. parallelogramma have large spherical nuclei and little cytoplasm, and there are smaller nuclei interspersed among the larger ones. The smaller nuclei do not, however, form a distinct outer layer. Huus (1924) states that although the larger nuclei of the ovotestis are noticeably different from the smaller nuclei, the difference in size is not great enough to suggest that there is more than one cell type at this stage. Cell membranes were apparently not discernible at the light microscope level, as Huus (1924) based his description on nuclei rather than on individual cells. The dorsal strand is always attached to the dorsal end of

the ovotestis. It is thicker near this attachment, and contains 4 to 5 rounded nuclei. This thickened region of the dorsal strand extends into a single-celled region, which contains spindle-shaped nuclei. No other free mesenchyme or blood cells are present in the immediate vicinity of the ovotestis.

The other comprehensive study of gonadogenesis of a compound gonad is that of Van Beneden and Julin (1886). In the aplousobranchs, Perophora listeri, Clavelina rissoana and Phallusia scabroides, they describe the first stage of gonadogenesis, corresponding to Stage II in Corella inflata, as being composed of a small cluster of undifferentiated mesoblastic cells identical to blood cells. The cell mass is loosely held together, and in P. listeri and C. rissoana it is spherical, while in P. scabroides it is triangular. Individual cells in P. scabroides have occasional projections that touch neighboring cells. The ovotestis of these animals is attached to a thin strand of cells, the genital cord. This cord is composed of single cells joined end to end. It is not continuous with the neural gland, and therefore is not a morphological equivalent of the dorsal strand. Lefevre (1898) confirmed Van Beneden and Julin's (1886) account of this stage of gonadogenesis in P. viridis, but observed that the genital cord arises *in situ* from free spindle-shaped cells around the ovotestis. These cells join end to end to unite with the ovotestis. Lefevre (1897) similarly confirmed Van Beneden and Julin's (1886) report on Clavelina rissoana. He describes the ovotestis of this stage as composed of an "irregular mass of cytoplasm containing a few scattered nuclei", and attached to the genital cord.

In very young specimens of the solitary phlebobranch, Ciona intestinalis, the ovotestis consists of a small mass of cells, found in the gonad hemocoel, near the degenerating cells of the axial complex (Aubert, 1954). The cells of this mass are indistinguishable from mesenchyme cells, corroborating Floderus (1896). No further details on the cells are available. Among the solitary stolidobranchs, De Selys Longchamps and Damas (1900) found that in Molgula ampulloides a small mass, approximately 10 μm in diameter, of rounded cells is found in the mantle wall on each side of the body. This probably corresponds to Stage II of Corella

inflata. There is neither dorsal strand nor genital cord in this species. In Dendrodoa grossularia, the Stage II gonad (the earliest stage observed in this animal) consists of a small irregularly-shaped mass of mesodermal cells, abutting the peribranchial epithelium (Julin, 1893). The ovotestis subsequently forms a peripheral flattened layer, and a syncytial inner layer with rounded nuclei. At this point, before cavitation of the ovotestis occurs, a genital cord develops from the ovotestis. It is likely that the syncytial mass in the ovotestis of D. grossularia is an artifact of fixation. In no other ascidian is there a description of a syncytial layer during any stage of gonadogenesis.

In the compound stolidobranch Distaplia occidentalis, Bancroft (1899) was unable to find a stage in which the ovotestis was not present. In the earliest stage he observed, corresponding to Stage III in Corella inflata, the ovotestis is situated on the flattened inner vesicle of the bud. The ovotestis consists of a compact mass of cells, with small "oogonia", or gonial cells, in the anterior region of the organ, intermingled with primordial follicle cells. The less differentiated, presumptive testis cells are more posterior relative to the "oogonia". The ovotestis fuses with the dorsal strand (dorsal tube) under most conditions. Bancroft (1899) believed that this union was not significant, as he occasionally found no sign of a dorsal strand. In the absence of a dorsal strand, the ovotestis produces a genital cord along the anterior end. This is a difficult observation to interpret, and it is likely that in some of his study animals, Bancroft (1899) missed the association between the dorsal strand and the ovotestis.

STAGE IV

The small cluster of cells that constitutes Stage III of gonadogenesis cavitates and forms a centrally placed lumen in Stage IV. This stage is probably universal among ascidians, although most workers do not distinguish between this stage and Stage V. In Corella inflata the ovotestis is a regular spherical shape with a fairly extensive lumen. There are regions of thinning, localized in small areas facing the epidermis; these thinner areas do not contain gonial

cells. The ovotestis consists of gonial and somatic cells, and the wall is never more than a single gonial cell thick. In this stage, the gonial cells have distinct germ cell characteristics, visible only with TEM, which include the first indication of nuage. The somatic cells have extensions that completely surround the gonial cells, isolating them from both external and internal environments. In the Stage IV ovotestis there are two types of somatic cells. Type I somatic cells are more squamous, and their nuclei have relatively diffuse chromatin. Some of these cells bear cilia which project into the lumen of the ovotestis, or into intercellular pockets. The Type II somatic cells are found consistantly in association with gonial cells; they are more cuboidal, and possess nuclei with denser nucleoplasm.

In Corella parallelogramma the ovotestis also cavitates, and forms a small eccentrically placed lumen (Huus, 1924). Huus (1924) does not, however, describe the Stage IV ovotestis as a separate stage of gonadogenesis. Similarly, Van Beneden and Julin (1886) do not describe this stage in Perophora listeri, Clavelina rissoana and Phallusia scabroides. They discuss the cavitation of the ovotestis as associated with the formation of a distinct germinal layer, several cells in diameter, on the ventral side of the organ. I consider this localization of the germinal layer to be separate stage, Stage V, of gonadogenesis. Lefevre (1898) describes the Stage IV gonad in Perophora listeri corresponding to my criteria, but does not discuss Stage V. In Distaplia occidentalis, lumen formation usually does not occur before the ovotestis differentiates into ovarian and testicular portions (Bancroft, 1899).

STAGE V

During this stage in Corella inflata the ovotestis becomes structured into a peripheral or ventral germinal layer that is several gonial cells in diameter, and a more elongate, dorsally placed somatic region. In all cases, the ovotestis is larger in Stage V than in Stage IV, and has a more extensive lumen. Many authors have referred to this stage as the stage during which cavitation of the ovotestis occurs. I have found that in Corella inflata, it is a distinct stage, and

always follows Stage IV, in which the germinal layer is not localized. While there are no substantial differences in the cells that form the ovotestis at this stage, the morphological organization will persist throughout later development. In addition, the Type I somatic cells in the region devoid of gonial cells extend further to form the beginnings of the oviduct.

In Corella parallelogramma the ovotestis enlarges by forming a larger lumen (Huus, 1924). The lateral walls of the ovotestis are a single cell in diameter, and consist only of squamous cells in this region. The front, or ventral side, becomes several cells thick and contains spherical nuclei. Huus (1924) termed the thicker region of the ovotestis the undifferentiated germinal epithelium, which also contains flattened or squamous somatic cells similar to those in the region devoid of gonial cells. The connection between the ovotestis and the dorsal strand occurs on the dorsal side of the thin-walled portion of the organ, and there are several rounded nuclei in this region of connection.

In Perophora listeri (Van Beneden and Julin, 1886; Lefevre, 1897), Clavelina rissoana and Phallusia scabroides (Van Beneden and Julin, 1886), the ovotestis becomes ovoid by forming a small eccentrically placed lumen. The ovotestis is delimited on the left (dorsally) by a single cell layer, and on the right (ventrally) by several layers of cells. The tip of the ovotestis extends into the genital strand. In Ciona intestinalis, the ovotestis is also spherical during this stage, with an eccentrically placed lumen (Aubert, 1954).

STAGE VI

This is the stage of gonadogenesis that is the most morphologically variable according to accounts in the literature. During this stage the ovotestis divides to form rudiments of separate sexes. In some species testicular rudiment formation is by budding of the ovotestis, while in others the ovotestis forms a furrow, which divides the ovotestis into two separate organ rudiments.

In Corella inflata the testicular rudiment always forms as a bulging, or evagination of the anterior wall of the ovotestis. This occurs along a small region of the ovotestis that is a single gonial cells in section, rather than in the thicker germinal layer, or the squamous somatic region. The testicular rudiment may later drop to a more lateral position relative to the ovarian rudiment. The fine structure of the cells at the site of testicular rudiment formation is unremarkable, although there is a considerable number of small vesicles whose contents are unknown. No contractile elements have been observed in the cells at the periphery of the bulging. As there are no special cellular features, and as the location of the testicular rudiment formation is not variable, it is likely that the factors that control testicular rudiment formation may be elucidated only experimentally.

A distinct basal lamina is found around each of the rudiments, which is continuous until the two rudiments separate. An epithelium is never formed between the two rudiments at any stage in C. inflata. The ovarian and testicular rudiments maintain contact for some time during which the small testicular rudiment opens into the larger ovarian rudiment. This opening always occurs in the somatic region composed of squamous cells.

The ovarian rudiment in Corella inflata is essentially identical to the ovotestis of Stage V, although the germinal layer contains considerably more gonial cells. Type I and Type II somatic cells continue to be found in the same orientation, surrounding the gonial cells and isolating them from both the gonad hemocoel and the lumen of the organ. The testicular rudiment has fewer gonial cells, and these are not localized into a distinct germinal region in this stage. The gonial cells are indistinguishable from those of the ovarian rudiment. They are also surrounded by processes of somatic cells, and are therefore isolated from the external and internal environments of the testicular rudiment. There is no distinction between somatic cells in the testicular rudiment, and all somatic cells are similar to the Type I cells of the ovarian rudiment.

During Stage VI in Corella parallelogramma, Huus (1924) describes a distinct depression that forms on the ventral side of the ovotestis. This observation is different from C. inflata, both in the site of testicular rudiment formation, and in the manner by which the testicular rudiment develops. The ovotestis in C. parallelogramma subsequently divides into a larger lateral rudiment, and a smaller medial one closely applied to the ventral surface of the lateral rudiment. This larger is the ovarian rudiment, while the smaller is the testicular rudiment. The testicular rudiment is formed along the middle portion of the stratified germinal layer of the ovotestis. The ovarian rudiment at this site is reduced to a single cell layer. This observation in Corella parallelogramma is also different from that in C. inflata, where the testicular rudiment forms from a thin region of the germinal layer. The ovarian rudiment is crescent-shaped in C. parallelogramma, with a simple epithelium of low flat cells on the lateral surfaces, and a compound germinal layer on the medial surface, containing cells with large, spherical chromatin- rich nuclei. There are two distinct germinal layer zones, separated by the simple epithelium at the site where the testicular rudiment was formed. Huus (1924) describes a small structureless "membrane", which is probably the basal lamina, separating the ovarian rudiment from the testicular rudiment. The testicular rudiment is smaller than the ovarian rudiment, and is composed of a stratified epithelium of cells similar to those of the ovarian rudiment. A blindly ending sac, to which the dorsal strand attaches, forms a tubular continuation of the ovarian rudiment.

In Perophora listeri, the formation of the testicular rudiment occurs on the side of the ovotestis opposite to the genital cord (Van Beneden and Julin, 1886). In this location, a furrow divides the ovotestis into two incomplete lobes that communicate with each other. The walls of the ventral ends of these lobes are considerably thicker than at the tapered dorsal end where the wall is reduced to a simple flattened epithelial layer. The genital cord is attached to the ovotestis in this region. Soon after the furrow forms in Perophora, the two lobes become more distinctly separate (Van Beneden and Julin, 1886; Lefevre, 1898). The ovarian rudiment is the more

external, situated immediatly below the epidermis. It has a large ovoid lumen, and is continuous with the genital cord. The testicular rudiment is more deeply placed, subjacent to the ovarian rudiment. The lumen of the testicular rudiment is continuous with the ovarian lumen at a very large opening. Testicular rudiment differentiation in C. rissoana is similar to that described for P. listeri (Van Beneden and Julin, 1886; Lefevre, 1898). Kowalevsky (1874b), however, described the ovarian and testicular rudiments of P. listeri as arising from two separate clumps of cells, rather than from a common ovotestis. His work has been supersceded by that of Van Beneden and Julin (1886).

In Phallusia scabroides, the ovotestis divides into two unequal parts, by the formation of a small epithelial bulge on the wall of the ovotestis (Van Beneden and Julin, 1886). The testicular rudiment is perpendicular to the axis of the ovotestis, and opens at a large orifice into the lumen of the larger ovarian rudiment. The structure of the two rudiments is the same as that described for Perophora listeri (Van Beneden and Julin, 1886). These authors suggest that the dorsal strand is responsible for the formation of the large common duct, which is continuous with the ovarian rudiment.

The ovotestis separates to form two distinct cellular regions in Ciona intestinalis, which take on a Y-shaped configuration (Aubert, 1954). The two ventral regions, or bulges, develop into the ovarian rudiment, and consist of a germinal epithelium continuous with the thin epithelium of the developing oviduct. The single medial bulge develops into the testicular rudiment. This organ also contains a germinal epithelium that is continuous with the oviduct. Aubert (1954) remarked that the dorsal strand coexists with the beginnings of the gonoducts, and that the gonoducts therefore must form from the ovotestis rather than from the dorsal strand.

In Ecteinascidia turbinata blastozooids, Lefevre (1898) briefly mentions that the ovotestis divides into two lobes. At first these two lobes communicate, but they subsequently separate and give rise to the ovary and testis. The genital cord maintains its attachment to the

ovarian lobe, but it subsequently splits to form the oviduct and sperm duct. In Distaplia occidentalis, the differentiation of the testicular rudiment begins with the formation of a small notch on the ventral side of the ovotestis (Bancroft, 1899). The two rudiments separate in an anterior direction, accompanied by a small structureless "membrane" (a membrana propria) which forms between the two masses. The two rudiments subsequently become separate, and the genital cord continues to the atrial epithelium. The ovotestis in Molgula ampulloides also divides into two incompletely separated lobes, although the mechanism is not discussed (De Selys Longchamps and Damas, 1900). The small testicular rudiment is found in a more posterior position, and is covered by the larger ovarian rudiment. These rudiments are composed of layers similar to those described by Huus (1924). In Dendrodoa grossularia the ovotestis divides into ovarian and testicular rudiments by the formation of a very thin, transverse furrow. This begins in the middle of the ovotestis and progresses towards the two extremes. The central syncytium proliferates, and the group of spherical cells subjacent to the peribranchial epithelium develop into the ovarian rudiment. The more superficial cells develop into the testicular rudiment. The rudiments have the same structure, and are composed of peripheral flattened cells, and large gonial cells.

STAGE VII

The basic structure of the adult reproductive system is essentially completed during Stage VII. The ovary and testis become distinct separate organs, and each develops an exit duct. Further development involves the elaboration and enlargement of lobes in both the ovary and testis, and the differentiation of the mature gametes.

In Corella inflata, the ovary maintains the same morphology as the ovarian rudiment of Stage VI. The germinal layer continues to be thickest in the ventral-most region of the organ, and the thinner germinal layer continues into the somatic region which extends to form the developing oviduct. The number of gonial cells continues to increase during this stage of

gonadogenesis. I have observed no direct evidence of cell division in this or any other stage of gonadogenesis, but indirect evidence in the form of midbodies between gonial cells has been observed. This indicates that cell division is a synchronous and relatively rapid event in Corella inflata. The fine structure of the gonial cells is not noticeably different from those of Stage VI. They continue to be separated by somatic cell processes from both external and internal environments of the ovary. Vitellogenesis does not begin until a later time. Developing follicle cells are, however, apparent in the germinal layer of the ovary. Based on size and shape, and on nuclear and cytoplasmic characteristics, these appear to be similar to the Type II somatic cells. These follicle cells extend into regions of the germinal layer and probably are derived from those cells that give rise to the gonial cells. I have observed no evidence supporting the blood cell origin of follicle cells at any stage in C. inflata, as suggested by Spek (1927) and Knaben (1936).

The developing oviduct is a tubular extension of the ovary, and it remains attached to the dorsal strand at the dorsal-most aspect of the ovary. There is never any sign of a bifurcation of the dorsal strand, or of cell divisions at the end of the dorsal strand, that might indicate that the dorsal strand gives rise to the oviduct, as was suggested by Huus (1924). Although no cell divisions have been observed in the somatic epithelium of the ovary, it seems likely that the periphery of the germinal layer, or the margins of the duct itself, proliferate to form the oviduct.

The testis also enlarges during this stage, and the germinal layer becomes localized in the ventral region of the organ, continuous with a somatic region, as in the ovary. The germinal layer, however, is never as thick as that of the ovary. The gonial cells of the testis are not noticeably different from those of the ovary. They contain nuage and the appearance of nuclear-cytoplasmic exchange that is typical of the gonial cells in all stages beginning with Stage IV. The gonial cells of the testis rest on a somatic epithelium. They are not surrounded by somatic cell processes on their luminal surfaces, but rather project into the testicular lumen.

The sperm duct appears to develop from the wall of the testis. This continues to open into the lumen of the ovary close to the attachment with the dorsal strand. As the oviduct extends towards the atrium, the sperm duct also extends, so that its position relative to the oviduct is maintained. The sperm duct is always found in close proximity to the oviduct, and it is always surrounded by a distinct basal lamina.

The ovarian rudiment lengthens and widens in Stage VII of Corella parallelogramma (Huus, 1924). Two separate, symmetrical germinal zones are found on the lateral surfaces in the ventral portion of the organ. The nuclei of the cells of the germinal epithelium are spherical, and they all appear to be similar. Huus (1924) does not discuss somatic cells, in particular the follicle cells, although he mentions that individual oocytes begin to grow and have distinctly different nuclear characteristics from other cells of the germinal epithelium. The remainder of the ovary consists of a flattened simple epithelium, both in the region between the germinal zones, and in the area of the developing oviduct. The testis also elongates in Stage VII of C. parallelogramma, and forms two small lobes along the dorsal side of the ovary. Huus (1924) states that the dorsal growth of the testis probably involves a differentiation of cells from the medial wall of the ovary and oviduct, and the ventral growth is probably the result of an increase in the number of cells in the germinal epithelium. The ends of each testicular lobe contain a stratified germinal epithelium composed of cells that are all alike, and resemble those of the germinal layer of the ovary. The area between the ends of the testicular lobes is composed of a simple, flattened epithelium. At the dorsal end of the testis a narrow duct is found next to the ovary; this has its origin from the oviduct.

Huus (1924) describes the development of the gonoducts in Corella parallelogramma, which occurs during this stage. The oviduct, which he terms the "primary duct" (as he believes that both oviduct and sperm duct are derived from this structure), lengthens. A "tissue band" is found between the ovary and testis. This tissue band becomes less extensive in a dorsal position, and yet more dorsally, there is a small closed duct which appears in the wall of the

ovary. This corresponds to the communication between the ovarian and testicular rudiments found in Corella inflata, although in C. inflata, the two rudiments communicate via a lumen. A faint bulge, composed of cuboidal cells, subsequently appears on the medial side of the oviduct wall. A lip-shaped projection forms in the lumen of the oviduct, and a distinct tube subsequently forms as a direct continuation of the testis, and represents the developing sperm duct. The origin of the sperm duct is therefore partly from the testis itself, and partly from the oviduct. The sperm duct remains in intimate contact with the oviduct on the medial side. In C. parallelogramma, the dorsal strand is still continuous with the dorsal end of the oviduct. As the gonoducts develop, the dorsal strand progressively shortens, and when the ducts reach the atrial epithelium the dorsal strand is present only as a vestige. Huus (1924) concludes that the gonoducts are therefore formed from the dorsal strand. This issue is discussed further in a later section.

In Perophora listeri, the ovary of this stage has an extensive lumen, and the dorsal and lateral portions of the ovary are composed of a flat, thin epithelium (Van Beneden and Julin, 1886). The ventral end of the ovary is considerably thicker, and consists of large cells, with large, clear nuclei, formed from a germinal epithelium. These cells, and associated small nuclei that may represent the developing follicle cells, constitute the "primordial follicles" of the ovary. The ovary elongates during this stage to form a long cylindrical tube, the developing oviduct, that continues into the genital cord on the dorsal side. The genital cord thickens and shortens as the oviduct elongates. Late in this stage, the oviduct opens into the atrium, and the genital cord is no longer visible.

The ventral end of the testis in P. listeri expands to form two incompletely separated lobes, each with an external epithelium of flattened cells, and subjacent to this, a thicker spermatogenic layer. This layer contains spherical cells of different sizes, which have few points of contact between them. There are numerous free cells in the lumen. The developing sperm duct is found between the testicular lobes, and opens into the developing oviduct near the

connection with the genital cord. The wall of the sperm duct is composed of a cuboidal epithelium continuous with the epithelia of the testis, and the ovary. As the oviduct elongates, the sperm duct maintains its position. Ultimately, the sperm duct opens into the atrium. Further development of the ovary and testis involves the formation of lobes, but the basic histology of these organs does not change.

In both Clavelina rissoana and Phallusia scabroides, Stage VII is essentially identical to that described for P. listeri, except that the ovary in C. rissoana does not form lobes (Van Beneden and Julin, 1886). Lefevre (1898), working on P. viridis, confirmed the report of Van Beneden and Julin (1886). In Ecteinascidia turbinata blastozooids, the genital cord is transformed into the oviduct and sperm duct during this stage (Lefevre, 1897). In Distaplia occidentalis, the ovary cavitates during this stage (Bancroft, 1899). The gonial cells of the ovary are situated on the ventral side of the lumen. The testis also enlarges, becomes crescent-shaped, and covers the ovary on the ventral surface. Later the testis divides into several smaller lobes. The oviduct and sperm duct are differentiated from the dorsal strand. As the ducts reach the atrial epithelium the dorsal strand probably disappears, although its fate is not discussed (Bancroft, 1899).

Aubert (1954) did not continue his observations in Ciona beyond Stage VI, although he did observe that the gonoducts are formed by the ovarian and testicular rudiments themselves. In this stage of Molgula ampulloides, a superficial flat epithelium is visible around the ovarian rudiment (De Selys Longchamps and Damas, 1900). This epithelium takes on a bumpy appearance due to irregular enlargement of the germinal epithelium. The testis elongates and becomes bilobed. It does not open into the ovarian lumen, but rather forms small individual sperm ducts which open separately into the atrial cavity. In Dendrodoa grossularia, the germinal layer of the ovary is located ventrally and contains primary oocytes surrounded by follicle cells (Julin, 1893). The remainder of the ovary consists of a thin epithelium, which Julin (1893) terms a superficial limiting epithelium. The cells of this epithelium divide to form

the oviduct, which eventually communicates with the atrial cavity. The smaller testis is surrounded by a flat epithelium containing spermatogenic cells. The testis also consists of a superficial epithelium and a germinal layer. It divides to form lobes, each with its own duct continuous with a common sperm duct. The sperm duct consists of a thin epithelium and it also eventually opens to the atrial cavity.

OTHER PATTERNS OF GONADOGENESIS

There are a few descriptions in the literature of gonadogenesis in ascidians that do not develop gonads from an ovotestis. In these animals, the stages of gonadogenesis differ somewhat from that described above.

In the oozooid of Ecteinascidia turbinata, the ovary differentiates from the "mesodermal plate" which projects into the hemocoel (Simkins, 1924). The mesodermal cells are said to form the germinal epithelium. The ovary cavitates, and consists of a thick region containing large, oval nuclei, and a thin region composed of ellipsoid nuclei. Oogonia and follicle cells differentiate from the germinal epithelium. The multiple testes originate from a separate, solid mass of cells at about the same time as the ovary. The testes are never connected in any way to the ovary. Each testis consists of two layers of cells, an outer epithelium and an inner spermatogenic layer. The testes elongate and branch, and may completely surround the ovary.

In blastozooids of Aplidium zostericola, the distal extreme of the dorsal strand enlarges and then differentiates into a dorsal and a ventral tube, which are intimately joined (Brien, 1927). The dorsal most of these structures develops into the ovary which is composed of a very thin dorsal wall and a thicker ventral wall. The ventral wall becomes the germinal epithelium, from which both oocytes and follicle cells differentiate. The ventral most of the two structures expands laterally and differentiates into the testis, maintaining its connection to the ovary. The testis is rounder than the ovary, and is composed of numerous layers of undifferentiated cells

in the ventral portion of the organ. The testis maintains contact with the dorsal strand in this animal, a situation that is uncorroborated by investigations on other ascidians. There are gonoducts, which extend to the atrium, in association with a thin remnant of the dorsal strand, but Brien (1927) does not discuss the structure of these ducts.

In Distomus variolosus , the multiple ovaries and testes develop from separate clusters of cells (Newberry, 1968). In each ovarian rudiment, some of the peripheral lymphocytes form an epithelium which delimits the ovary. Certain cells become substantially larger, and some of these larger cells develop into oocytes. Others of the larger cells, "aborted oogonia", along with lymphocytes, later develop into the follicle cells. A cavity forms eccentrically in the ovary, with the germinal layer localized on the peribranchial surface. Oocytes are randomly distributed within this germinal layer, and these are isolated from the mantle sinus and the lumen of the ovary by developing follicle cells. The oocytes become aligned in a zig-zag fashion in maturing ovaries. An oviduct extends from each ovary to the atrial epithelium, but Newberry (1968) was not able to follow its development. Each testis cavitates by what appears to be autolysis of the cells in the central part of the organ. The cells of the testis are uniform in size, and these proliferate and differentiate into gametes. During cavitation, a solid column of cells extends from each testis to the atrium. The sperm duct is formed by the extension of the testicular lumen into the column of cells. It is composed of a simple ciliated epithelium, and remains closed at its distal tip until sexual maturity.

In Botryllus primigenus, the usually single oocyte becomes partly surrounded by some cells of the "compact cell mass" soon after its arrival in the gonadal space of the developing bud (Mukai and Watanabe, 1976). The cells immediately surrounding the oocyte differentiate into the accessory cell envelopes and the follicle stalk. The follicle stalk cavitates, and is analogous to the oviduct. The resultant "egg follicle" matures with further elaboration of the accessory cells, and the oogonium divides meiotically to form the oocyte. The testis is also derived from cells of the "compact cell mass". It has a peripheral epithelium, and cavitation of

the central cells forms a lumen. The peripheral cells presumably constitute a germinal epithelium. The organ opens to the atrium via a short sperm duct. In Botryllus schlosseri, Sabaddin and Zaniolo (1979) agree in most respects with the description of Mukai and Watanabe (1976). They emphasize, however, that the germinal epithelium is localized in the regions opposite the ducts in both the ovary and testis. In addition, The testis forms several lobes in B. schlosseri.

SUMMARY

Gonadogenesis in those ascidians that possess compound gonads appears to follow a similar pattern of morphological changes despite substantial differences in approach and extent of examination by investigators. There is an initial establishment of gonadal cells associated with a dorsal strand, genital cord, or some particular region of the mantle wall. Once this initial cluster of cells is established, the developing gonad constitutes a unique population of cells. The cluster of cells then differentiates into somatic cells and gonial cells. The organ subsequently cavitates, and the cells that form the ovotestis can be isolated into gonial cells and two types of somatic cells; the Type II cells probably representing the first stage of folliculogenesis. The following stage involves the localization of the germinal layer into the ventral most region of the ovotestis. The ovotestis divides into rudiments of separate sex during the next stage. The formation of the testicular rudiment is morphologically variable from accounts in the literature, but in general it appears to be formed either as a bulge on the anterior portion of the ovotestis, or as a medial division of the organ into right and left portions. The two resultant organ rudiments contain gonial cells that are essentially identical, and Type I somatic cells. Type II somatic cells are not found in the testicular rudiment. During the final stage of gonadogenesis, the ovary and testis complete the differentiation into the form found in the adult. The ovary consists of a substantial germinal layer composed of gonial cells isolated from both the external and internal environments of the organ. Type II somatic cells, or follicle cells are more

apparent, and the oviduct develops and expands, attached to the dorsal strand from the dorsal most region of the ovary. The testis is also organized into a ventral germinal layer. There are no follicle cells in the testis, and the gonial cells are not isolated by somatic cells from the luminal surface. The sperm duct opens directly into the oviduct.

The observations on the organization of the cells that compose the developing reproductive system are diverse. The cells of ascidians are small, and the relationships among them are not clearly visible with the light microscope. In addition, the cells and tissues of ascidians are difficult to fix, and it is likely that some of the observations made by previous authors are artifacts of fixation. An example is Julin's (1893) observation that in Dendrodoa grossularia there is a central syncytial mass in the ovotestis that develops into the germinal layer. I have found that TEM fixations and observations are essential to understanding the relationships between the cells in all stages of gonadogenesis. This study of Corella inflata is the first to use these techniques to describe the stages of gonadogenesis. Further comparisons of the process of gonadogenesis among the groups of ascidians must involve TEM.

D. CHARACTERISTICS OF GONIAL CELLS AND SOMATIC CELLS

There is an enormous literature describing the germ cells of ascidians. These studies have concentrated on the structure of the gametes in mature animals, and on gametogenesis, which generally continues throughout the life cycle after maturity is attained (Millar, 1971; Berrill, 1975; Kessel, 1983). Many of these studies have also concentrated on the origin and development of the accessory cell layers surrounding the oocyte (Reverberi, 1971; Kessel, 1983). While most of the early monographs on gonadogenesis have described the general sizes and shapes of the cells that form the developing gonad, these descriptions are of limited value regarding the nuclear and cytoplasmic characteristics of the cells during gonadogenesis. There are no ultrastructural studies available on the cells that form the developing gonad during gonadogenesis.

In this section, I review what is known of the ultrastructure of small, developing oocytes and their accessory cell layers, and of the cells of the testis, within the established organs. These published observations are compared with the characteristics of the gonial and somatic cells of the developing gonad in Corella inflata.

GONIAL CELLS

The gonial cells of Corella inflata do not undergo any substantial growth during the stages of gonadogenesis I have described in this study. There are, however, some changes that take place in the ultrastructure of these cells during gonadogenesis. In Stages I and II, the ovotestis consists of two cells which are morphologically identical to hemoblasts. These cells are spherical in shape, and each has a large nucleus with some peripheral heterochromatin. The cytoplasm is not unique. In Stage III, the gonial cells are separated from the environment of the gonad hemocoel by somatic cell processes. In this stage there is some indication that material is moving from the nucleus to the cytoplasm, through the nuclear pores. In Stage IV, the gonial cells of the ovotestis are separated from both the luminal and gonad hemocoelic environments by somatic cell processes. These cells have relatively more mitochondria and lamellar RER, and there are also small vesicles on the luminal surface of the plasmalemma, and myeloid figures. Nuage first appears in this stage, primarily in regions close to the nucleus, and associated with what may be material moving into the cytoplasm through nuclear pores. In Stage V gonial cells, the Golgi complex is more prominent than in previous stages, and there is also more nuage in the cytoplasm. The nuage is usually more dispersed, and located in regions further away from the nuclear envelope. After the differentiation of the testicular rudiment from the ovotestis in Stage VI, the gonial cells of the ovarian rudiment are similar to those of the Stage V ovotestis, but here there is yet more dispersed nuage, and there are annulate lamellae in the cytoplasm. Occasional large cells, which may represent primary oocytes are also seen. The gonial cells of the testicular rudiment are similar to those of the ovarian rudiment,

although they never have any annulate lamellae. During Stage VII, the cytoplasm of the ovarian gonial cells becomes more dense with a relative increase in the number of ribosomes and glycogen. No secondary lysosomes have been seen in these cells, and the nuage, mitochondria, and annulate lamellae are more abundant in this stage. The gonial cells of the testis in Stage VII are not separated from the luminal surface of the organ by somatic cell processes. These cells are similar to the ovarian gonial cells although the nuage is not as abundant, and there are no annulate lamellae.

Ovarian Portion of the Gonad

Cowden (1961) has divided oogenesis into three stages in Ascidia nigra. Stage I consists of small oocytes, approximately 50 μm in diameter, which have differentiated from the germinal epithelium and are beginning their initial growth phase. Although these Stage I oocytes are substantially larger than the gonial cells of Corella inflata during gonadogenesis, they are still the smallest that are available for ultrastructural comparisons. During this stage of oogenesis, the oocytes increase in size and become surrounded by a layer of primary follicle cells (Cowden, 1961). The nuclei of these oocytes have a large nucleolus, and some peripheral heterochromatin (Cowden, 1961). The cytoplasm is intensely basophilic, correlated with the large number of ribosomes in the cytoplasm (Cowden, 1961, 1967; Hsu, 1962; Kessell, 1966b, 1983). There is similarly a large number of ribosomes in the gonial cells in all stages of Corella inflata, with the largest relative numbers found in Stage VII. The number of ribosomes in these gonial cells compared with Stage I of oogenesis is unknown. Small amounts of cytoplasmic glycogen are found in the Stage I oocytes (Cowden, 1961), as well as in the gonial cells of Corella inflata.

Several investigators have reported on nuclear-cytoplasmic exchange as a characteristic feature of Stage I oocytes in ascidians (Kessel, 1966a, 1983; Mancuso

1963, 1964, 1972). This takes the form of small granular aggregations associated with nuclear pores in localized regions of the inner and outer membranes of the nuclear envelope. In favorable sections it is possible to see this granular material extending through the pores, and there is therefore reason to believe that this material originates in the nucleus and moves to the cytoplasm. The material that passes through the pores consists of RNA and protein (Cowden, 1961, 1962, 1967; Cowden and Markert, 1961; Davenport and Davenport, 1965; Kessel, 1966a, 1983; Eddy, 1975). It appears that the frequency of nuclear-cytoplasmic exchange decreases during growth of the oocyte, so that it is virtually absent in vitellogenic oocytes (Mancuso, 1964; Kessel, 1966a, 1983). Similarly, the granular aggregations associated with this nuclear-cytoplasmic exchange appear to fragment as the oocyte grows, so that they are more numerous but smaller in larger oocytes. The aggregations are also virtually absent in vitellogenic oocytes (Kessel, 1966a, 1983; Mancuso, 1964).

Kessel (1983) describes two types of aggregations, composed of material that originates in the nucleus and transported to the cytoplasm, although it is difficult to distinguish between them based on his descriptions and photographs. The first type of aggregation consists of small masses of densely packed granules associated with the perinuclear cytoplasm. Kessel (1983) suggests that these aggregations may be masses of ribosomes which subsequently become dispersed in the cytoplasm and transformed into particulate ribosomes, accounting for the intense basophilia of the Stage I oocytes. I have frequently encountered these small dense aggregations in localized regions of the perinuclear cytoplasm, beginning in Stage III and continuing through gonadogenesis in Corella inflata. They are not as large or as abundant in the small gonial cells as is indicated in the published descriptions of Stage I oocytes. The relative number of ribosomes in the gonial cells

during gonadogenesis is probably much smaller than in the Stage I oocytes, after significant growth has occurred.

The other type of aggregation that appears to be transported from the nucleus to the cytoplasm in Stage I oocytes is larger, variable in size and shape, and is distributed throughout the ooplasm. These aggregations correspond to nuage (Eddy, 1975; Kessel, 1983), and probably to the yolk nucleus (associated with mitochondria) that has been described by several authors with light microscopy (Crampton, 1899; Conklin, 1905; Hirschler, 1917; Harvey, 1927; Jägersten, 1935) and with the TEM (Hsu and Cloney, 1958; Mancuso, 1964). These inclusions have been identified in the germ cells of a variety of animals, and generally appear as discrete aggregations (Beams and Kessel, 1974; Eddy, 1975; Kessel, 1983). I have found nuage, corresponding to these dense granular aggregations, beginning at Stage IV of gonadogenesis in Corella inflata. These germ cell specific inclusions are probably synthesized in the nucleus of the gonial cells and are transported to the cytoplasm at an early stage, even before the establishment of the ovarian and testicular portions of the gonad. Nuage is not confined to the ovarian germ cells. It is also present in the gonial cells of the testis after the differentiation of the testicular rudiment from the ovotestis. My morphological observations indicate that nuage may be produced cyclically during gonadogenesis, and it is possible that this corresponds to cell divisions taking place in the germinal layer.

Some studies have described the endoplasmic reticulum in developing oocytes of ascidians (Kessel, 1965, 1966b, 1983). In Stage I, the RER is found in both vesicular and lamellar form (Kessel, 1983). These vesicles of RER arise as blebs of the outer layer of the nuclear envelope, and they are widely dispersed in the ooplasm, particularly in vitellogenic oocytes (Kessel, 1965, 1966b, 1983; Mancuso, 1963). The vesicles may fuse to form the lamellar form of RER (Kessel,

1983), which is less frequently encountered than the vesicular form in the Stage I oocytes. The lamellae have been observed in close association with mitochondria, although the significance of this association is not known (Kessel, 1966b, 1983). Both the vesicles and lamellae of RER contain a diffuse filamentous material. Reverberi (1971) states that RER is only found in oocytes that have begun oogenesis. I have, however, observed some RER in the gonial cells of Corella inflata during gonadogenesis, although these cells do not contain the vesicular form of this organelle. The vesicular RER appears to be associated exclusively with the Type II somatic cells during gonadogenesis in Corella inflata. There are a few profiles of lamellar RER around the circumference of each gonial cell, but these are not associated with mitochondria as reported by Kessel (1966b, 1983).

Cytoplasmic annulate lamellae have been observed in previtellogenic and vitellogenic oocytes of ascidians (Hsu, 1963; Mancuso, 1964; Kessel, 1964, 1965, 1966b, 1983). They consist of from 3 to 4 and up to 36 lamellae, arranged in stacked arrays and distributed in a more or less random fashion throughout the ooplasm (Hsu, 1963; Kessel, 1983). There do not appear to be more than 3 or 4 such organelles per cell (Kessel, 1965), and these are more abundant and contain the greatest number of lamellae in vitellogenic oocytes (Kessel, 1965, 1983). The membranes of the lamellae may have attached ribosomes, and ribosomes may also be present in the cytoplasm between the lamellae (Kessel, 1965). The ends of individual lamellae may be connected with lamellar RER (Kessel, 1965, 1983). It is thought that annulate lamellae form by a blebbing process of the nuclear envelope. These blebs subsequently detach from the nuclear envelope and then differentiate into lamellae by a specialized fusion process (Kessel, 1983). It should by noted that intranuclear forms of annulate lamellae have been reported in ascidian oocytes (Hsu, 1963; Kessel 1964, 1965, 1968; Evringham 1965). Annulate lamellae were not

seen in any of the earliest stages of gonadogenesis in Corella inflata. They were observed in the gonial cells of the Stage VI and Stage VII gonad, only in the cells of the ovarian portion of the gonad. There does not appear to be a characteristic location or orientation of these organelles in the gonial cells, and there does not appear to be more than one per cell. The function of the annulate lamellae is not known, although they may be responsible for the storage or activation of developmental information (Kessel, 1968, 1973; Franke, 1974).

Very small oocytes contain relatively few mitochondria, located in the perinuclear cytoplasm (Mancuso, 1964; Kessel, 1966b, 1983). During growth of the oocyte, the mitochondria increase in number, although this has not been quantified (Kessel, 1966b, 1983), and prior to vitellogenesis, the mitochondria become widely distributed in the ooplasm. There are usually two or more matrix granules, approximately 500 nm in diameter, which are characteristic of ascidian mitochondria (Kessel, 1966b, 1983; Mancuso, 1966; Beams and Kessel, 1976). These granules may represent the accumulation of divalent cations (Kessel, 1983; Bloom and Fawcett, 1982). Mitochondria may be surrounded by a lamella of RER, and there may be a close association between mitochondria and lipid droplets (Kessel, 1966b, 1983). In Corella inflata, the mitochondria are slightly concentrated in the perinuclear cytoplasm during all stages of gonadogenesis, although this has not been quantified. They appear to increase in number during gonadogenesis.

Mancuso (1964) reported that a Golgi complex is not found in Stage I oocytes. Kessel (1983), however, reported that in small oocytes a Golgi complex is usually located in the ooplasm near the nuclear envelope. There appears to be an increase in the number of Golgi complexes as the oocyte grows (Kessel, 1983). These organelles consist of a variable number of cisternae arranged in a stacked

parallel array (Kessel, 1983). I have not made a quantitative study of the presence and location of the Golgi in the gonial cells of the developing gonad in Corella inflata, but it appears that they increase in size during gonadogenesis. I have never seen more than one Golgi complex per cell, although it is possible that there are more. The Golgi complex appears to be important in yolk formation (Hsu, 1962; Mancuso, 1964; Kessel, 1966b, 1983).

In addition to the cytoplasmic organelles of the developing oocyte described above, there are also numerous secondary lysosomes which are characteristic of all cells in the ovotestis after metamorphosis. In gonial cells of the early gonad rudiment, these are large and among the most conspicuous components of the cells. Later in development they become less numerous and smaller.

Testicular Portion of the Gonad

There is substantially less information available on the ultrastructure of the gonial cells of the developing testis. Available information on the male reproductive system is based primarily on the structure of the male gametes (Franzén, 1983). A description of the ultrastructure of the spermatogonia of the testis is presented in only two reports (Georges, 1969; Tuzet et al., 1974). The spermatogonia are large cells, approximately 7 μm in diameter (Georges, 1969), with a large nucleolated nucleus. The nucleolus is often situated subjacent to the nuclear envelope (Georges, 1969). The nucleus contains diffuse granular chromatin. The cytoplasm of the spermatogonia contains a large Golgi complex associated with small osmiophilic vesicles (Tuzet et al., 1974). There are small mitochondria with few cristae (Georges, 1969; Tuzet et al., 1974), vacuoles, some sparse ribosomes, and little ER (Tuzet et al., 1974). Tuzet et al. (1974) also reported that while they did not observe mitoses, some adjacent cells had connecting cytoplasmic bridges.

The testicular gonial cells of the developing gonad in Corella inflata are very similar ultrastructurally to those of the ovary. The cells of the testis are more elliptical, and are not separated from the luminal environment of the organ by somatic cell processes. Some of these cells, in Stage VII, are separated from the underlying epithelium and extend into the lumen. There are relatively fewer ribosomes, on a qualitative basis, in the testicular gonial cells. In Stage VII, there is some nuclear-cytoplasmic exchange taking place, as seen in the small granular aggregations on both sides of the nuclear envelope. This is less abundant than in the ovarian gonial cells. Similarly, there is nuage in the testicular gonial cells, and the structure of this nuage is identical to that of the ovarian gonial cells. Developing male germ cells of other animals have been reported to contain nuage-like material (Eddy, 1975; Kessel, 1983), but this has never been reported in developing male germ cells of ascidians. There are fewer mitochondria and profiles of RER in the testicular gonial cells than in the ovarian gonial cells in Corella inflata, and these gonial cells do not contain annulate lamellae. No cilia are found in Stage VII gonial cells, as have been reported in secondary spermatocytes and spermatids (Georges, 1969; Tuzet et al., 1974). Prior to the onset of growth, there do not appear to be major differences that distinguish between the gonial cells of the male and female portions of the developing reproductive system.

SOMATIC CELLS

The somatic cells of the ovotestis are first visible surrounding the gonial cells in Stage III of gonadogenesis. In Stage IV of gonadogenesis there are 2 types of somatic cells in the ovotestis, which are distinguished on the basis of their shape and size, and on the characteristics of the nucleus. These 2 somatic cell types continue to be present in the ovarian portion of the gonad during gonadogenesis, but after the testicular rudiment has differentiated from the

ovotestis. Type II somatic cells are never found in the testicular portion of the gonad.

Ovarian Portion of the Gonad

The mature ascidian oocyte is surrounded by several layers of accessory cells prior to spawning. These include a layer of test cells which are found in indentations in the oocyte surface, a layer of inner follicle cells, and a layer of outer follicle cells. These follicular layers are separated from the test cells and oocyte by an acellular chorion (Reverberi, 1971; Berrill, 1975; Kessel, 1983). Much of the research on oogenesis in ascidians has been devoted to the origin, structure, and function of these accessory cell layers. There are no ultrastructural studies on the follicle cell layers during gonadogenesis.

The ovotestis of Stage IV in Corella inflata consists of gonial cells and two types of somatic cells. The Type II somatic cells are irregularly shaped low cuboidal cells, variable in size, and found primarily adjacent to the gonial cells. The nuclei contain dense nucleoplasm, and the cytoplasm contains conspicuous large vesicles of RER with a diffuse intracisternal matrix, and large Golgi complexes. These somatic cells continue to be found associated with gonial cells in subsequent stages of gonadogenesis. After the differentiation of the testicular rudiment from the ovotestis, the Type II somatic cells are found only in the ovarian portion of the gonad. In Stage VII, the Type II somatic cells appear to form the beginnings of a primary follicle cell layer. Later in development, during Stage I of oogenesis, the oocyte is completely surrounded by a layer of primary follicle cells (Cowden, 1961, 1962; Reverberi, 1971; Kessel, 1983). This primary follicle cell layer later divides to form the test cells, and then the inner and outer follicular layers (Huus, 1937; Tucker, 1942; Cowden, 1961, 1967; Kessel and Kemp, 1962; Kessell, 1962, 1983; Berrill, 1975).

Work by Knaben (1936), Spek (1927), and Mancuso (1965) points to a blood cell origin of the accessory cell layers. Pérès (1954), DeVincentiis (1962), and Kalk (1963a) believed that the follicle cells are germinal epithelium derivatives, while the test cells are derived from amoeboid cells of the blood. While this work on Corella inflata has not specifically addressed the question of accessory cell origin, it appears that the follicle cell line is established very early in gonadogenesis, and that the primary follicle cells at least are derived from the same cells that give rise to the gonial cells during gonadogenesis. Kessel (1967, 1983) and Beams and Kessel (1974) state that among the first organelles to become active in the follicle cells are the ER (found in both vesicular and lamellar form in these cells), and the Golgi complex. It is possible that even early in gonadogenesis, these organelles are large and well-developed in preparation for their secretory function in later stages of oogenesis.

Testicular Portion of the Gonad

The ultrastructure of the testicular somatic cells have been briefly described by Georges (1969). These cells are found on the peripheral margin of the testicular lobes. They are relatively elongate cells with an irregularly shaped nucleus, and heterogenous cytoplasm. Georges (1969) terms this the germinal epithelium. Occasional larger cells have been described among the squamous cells of the germinal epithelium (Georges, 1969). These cells are laden with heterogenous granules reminiscent of nuclear debris, and she suggests that they are phagocytic cells.

I have observed a similar external epithelium in the testis of Stage VII of gonadogenesis in Corella inflata, composed of a single type of squamous somatic cells surrounded by a basal lamina. These cells contain elongate nuclei with little

peripheral heterochromatin, and unremarkable cytoplasm. There are occasional cilia projecting into the testicular lumen from these somatic cells. These cells are continuous with the squamous epithelium of the sperm duct. I have not seen such phagocytic cells in the epithelium of the testis as reported by Georges (1969).

E. NEURAL COMPLEX AND DORSAL STRAND

Adult ascidians possess a neural complex consisting of the neural ganglion and closely apposed neural gland. The neural complex is embedded in the body wall midway between the siphons. It is surrounded by a connective tissue sheath, and it borders on a hemocoelic space (eg Millar, 1953; Bullock and Horridge, 1965; Goodbody, 1974). In Corella inflata, the association of extensions of the neural complex with the developing gonad is conspicuous soon after metamorphosis, and this association appears to be essential for the initiation of gonadogenesis. In this section, I therefore discuss the origin, anatomy, and potential functions of the various components of the neural complex, concentrating on the structure and potential functions of the dorsal strand.

ORIGIN OF THE NEURAL COMPLEX

Most of the existing work on the ganglion and gland have been directed toward an attempt to homologize the structure of the ascidian brain with that of the vertebrates, or to provide evidence against such an homology. Julin (1881) first suggested the homology of the ascidian neural gland with the vertebrate pituitary, based on his belief that the neural gland arises as an independent outgrowth from the stomodeum, or prebranchial portion of the pharynx. Subsequent works, however, by Salensky (1893), Willey (1893), Hjort (1896), Seeliger (1895-1906), Garstang and Garstang (1928) and Elwyn (1937) have shown that both the ganglion and gland are derivatives of the neural tube of the embryo.

The close physical association of the neural ganglion and neural gland is emphasized by their origin during embryogenesis. Elwyn (1937) summarized earlier work on the origin of the the neural complex in Ciona intestinalis, Clavelina lepadiformis, and Distaplia magnilarva. The anterior end of the neural tube divides longitudinally into two tubes, after closure of the neuropore. The right tube enlarges to form the cerebral vesicle of the larva, and the left forms the hypophyseal duct (neurohypophyseal duct of Willey, 1893). These two structures are continuous with the neural tube of the trunk and tail of the larva. Subsequently, the communication between the cerebral vesicle and the hypophyseal duct is lost; the former develops into the functional central nervous system of the larva, and the latter grows anteriorly and ventrally to establish communication with the prebranchial portion of the pharynx (stomodeum), anterior to the cerebral vesicle. Elwyn (1937) showed that the anterior opening of the hypophyseal duct is not a stomadeal invagination in Ecteinascidia turbinata, and an homology of the ascidian neural complex with the vertebrate brain is inappropriate.

The anterior opening of the hypophyseal duct into the pharynx persists, and develops into the ciliated funnel. The duct extends dorsally and posteriorly, where in the region of the cerebral vesicle, the dorsal wall proliferates a mass of cells that develop into the neural ganglion. Glandular folds form from the ventral wall of the duct in this region, and these develop into the neural gland. In most stolidobranchs, the ganglion develops from the ventral wall of the duct, and in some the ganglion develops laterally (Elwyn, 1937; Metcalf, 1900). The hypophyseal duct continues posteriorly from the neural complex, develops into a solid cellular strand variously named the "cordon ganglionnaire-visceral" (Van Beneden and Julin, 1884), the "dorsal cord" (Markman, 1958), the "ganglio-genital-strang" (Huus, 1924), or the "dorsal strand" (eg Millar, 1953; Goodbody, 1974). Therefore, the neural ganglion, neural gland proper, and the anterior and posterior continuations of the neural gland are all derived from the same portion of the embryonic neural tube.

NEURAL GANGLION AND NERVES

The neural ganglion in adult ascidians consists of an outer cortex containing unipolar neurons and what may be neurosecretory cells, and an inner fibrous medulla (Bullock and Horridge, 1965; Goodbody, 1974). In most species, five major nerves arise from the ganglion; two anterior nerves that innervate the branchial siphon and associated musculature, two posterior nerves that innervate the atrial siphon and associated musculature, and a visceral nerve that extends posteriorly to the visceral mass (eg Millar, 1953; Bullock and Horridge, 1965; Goodbody, 1974). In Corella, there are two visceral nerves that arise as numerous small branches of the posterior nerve roots. These nerves pass into the roof of the branchial basket, the dorsal fold, and continue to the viscera, with offshoots leading to the branchial basket (Huus, 1924; Mackie et al., 1974).

There are numerous unanswered questions about the nature of visceral innervation in ascidians (Goodbody, 1974). Regarding the reproductive system, several authors (eg Kowalevsky, 1874a; Millar, 1953; Goodbody, 1974) state that the visceral nerve extends to the region of the gonads, although there is vitually no information concerning gonadal innervation. Goodbody (1974) states that neurosecretory fibers may innervate the gonad, and the visceral nerve may innervate the sphincter muscle of the gonoducts. Bullock and Horridge (1965) report that the visceral nerve ramifies particularly over the oviduct, and less extensively over the rectum, intestine, stomach and esophagus. At least in the region of the dorsal fold there is a nucleated nerve plexus that is associated with the visceral nerve and dorsal strand (Mackie et al., 1974; Fedele, 1923a, b, 1927). In young Ciona, this plexus continues to the gonad (Markman, 1958), while in older Ciona, the plexus is associated with the gonoducts (Bone, 1959).

In ascidians, the nerves from the ganglion appear to be mixed, containing both sensory and motor elements, and associated with a "reticulum of supporting material" and connective tissue cells (Bullock and Horridge, 1965). Although some information exists on the structure of

peripheral nerves at the light microscope level (eg Hunter, 1898a, b; Hilton, 1913), there is little information concerning the fine structure of the peripheral nerves. Mackie et al. (1974) describe the ultrastructure of axons as containing numerous distinctive neurotubules and a cytoplasm that is "characteristically rather featureless". The perikarya of the visceral nerve axons are located in the neural ganglion (Markman, 1958; Mackie et al., 1974). Mackie et al. (1974) also point out the possibility of mistaking certain cells of the connective tissue and blood for nerves. Very little is known of the development and distribution of the nerves in newly metamorphosed animals.

Neurosecretory cells have been reported in the neural ganglion, although actual secretory products have not been identified. In a histochemical study, Dawson and Hisaw (1964) reported that there are cells containing granules scattered among other nerve cell bodies in the peripheral region of the neural ganglion in ten species of ascidians. In the stolidobranchs, they observed that the cell bodies contain "finely granulated, densely stained material, sometimes regionally displaced by clear, unstained vacuoles of varying size and number", while in the phlebobranchs the granular material was generally localized in a perinuclear position. The granules in these two ascidian groups stain differently, indicating that their secretory products are different (Dawson and Hisaw, 1964). In at least one species, Chelyosoma productum, the stained granules were best visualized in animals with mature gonads, and Goodbody (1974) suggests that they may be associated with reproduction. Aros and Konok (1969), in a light microscope histochemical study of the neural ganglion, found granules, which by their size (250 nm) and staining properties they suggest might be neurosecretory granules. They found these granules in both the perikarya of neurons in the ganglion, and in neurites in each of 12 species studied. Ultrastructural observations by Thiebold and Illoul (1966) and Chambost (1966) also demonstrate granules, which appear to be neurosecretory, in the perikarya of some cells in the cortical region of the ganglion. In three species, Lane (1972) found small membrane-bounded, dense-cored granules (10 nm in diameter) which appeared to be elaborated by the Golgi

complex. Similarly, Georges (1977) described perinuclear granules in the ganglion which were greatly reduced in number following the removal of the ovary. Bouchard-Madrelle (1967a, b) and Lender and Bouchard-Madrelle (1964) also observed a variation in neurosecretory granule abundance during gonad development. Sugimoto and Watanabe (1980) described the ultrastructure of two types of neurosecretory cells in the neural ganglion and in axons in the immediate vicinity of the ganglion. One of these cell types contained electron-dense granules 100 to 140 nm in diameter, and the other contained electron-opaque granules 240 to 300 nm in diameter. These granules were found to increase in number as the oocytes began vitellogenesis. It should be noted that the emphasis in the study of potential neurosecretory cells in ascidians has been confined exclusively to cells in the neural ganglion, and has not included the peripheral nervous system.

Several investigators have searched for neurotransmitters, or putative transmitter substances in extracts of ganglia. Acetylcholine (Florey, 1963), the amino acid precursors, GABA and taurine (Osborne, 1971; Osborne et al., 1979), glycine, glutamate, and aspartate (Osborne et al, 1979) have been reported. The catacholamines, dopamine and noradrenaline (Osborne et al., 1979) have also been reported in small quantities. These findings indicate that neurotransmitters are probably synthesized, although what they are, and their potential function, is still unknown.

NEURAL GLAND

The neural gland of adult ascidians consists of a highly folded glandular region, an anterior duct which opens into the branchial basket, and a posterior extension, the dorsal strand (Bullock and Horridge, 1965; Millar, 1953; Goodbody, 1974). The anterior duct is lined with a ciliated cuboidal epithelium, and opens into the branchial basket at the ciliated funnel. The cilia may beat inward from the branchial basket to the gland (Georges, 1970, 1971), although Millar (1953) believed that water circulates around the funnel and is not pulled into the region of the

duct.

Numerous glandular evaginations form the body of the neural gland. The morphology of the gland in most species varies, apparently with physiological function, ranging from a compact structure to a loose mass of vacuolated cells (Metcalf, 1900; Pérès, 1943; Brien, 1948; Georges, 1970, 1971; Lane, 1971; Goodbody, 1974; Godeaux and Beros-Debrous, 1979). Numerous functions have been ascribed to the neural gland since it was first described by Hancock (1868), with particular emphasis on possible sensory or secretory processes, and also to its connection with the dorsal strand. In over 100 years of examination, however, our understanding of its role is still far from complete.

One possible function of the neural gland that has been considered recently, is a role in digestion, due to the association of the neural gland with the food-collecting apparatus, and the variable condition of the neural gland cells (Lane, 1971; Goodbody, 1974). Another potential function is the secretion of hormones like oxytocin and vasopressin (Butcher, 1930; Bacq and Florkin, 1935, 1946). Pérès (1943, 1947a, b), Sawyer (1959) and Dodd and Dodd (1966) concluded that these pituitary hormones are most likely not secreted by the gland, although a smooth muscle contractant similar to oxytocin may be secreted in low concentrations by the gland and/or the tissues surrounding the neural complex (Dawson and Hisaw, 1964; Lane, 1968; Goodbody, 1974). If such a smooth muscle contractant is secreted by the gland, it might serve to maintain the contracted state of the sphincters of the gonoducts, thereby preventing the premature release of gametes (Goodbody, 1974). Pestarino (1984) found prolactin-like peptides in the neural gland of one species, and hypothesized an osmoregulatory role.

Most recent research efforts on neural gland function have concentrated on its possible role in gonadotropin secretion. Huus (1937) suggested that the gland may control reproduction by first collecting stimulatory substances, such as gametes from conspecifics, in the ciliated funnel. These substances might then stimulate the neural gland to secrete a substance into the blood stream that causes gamete release. In an effort to identify gonad stimulating activity in

the neural gland. Hogg (1937) and Carlisle (1950) injected neural gland extracts into mice, and found that their gonads increased in size and weight relative to controls. Similar experiments by Benazzi (1939) and Dodd (1955), however, did not support this conclusion. In a series of experiments designed to test for neural gland control of spawning, Carlisle (1951, 1954) concluded that gametes of conspecifics are actively taken into the gland, causing it to secrete a hormone that excites the ganglion. The release of gametes is then stimulated via the "gonadal nerve". In studies of neural complex removal, Bouchard-Madrelle (1967a, b) concluded that the number of young oocytes in the ovary is controlled by the neural gland, while the neural ganglion influences the number of mature follicles and oocytes in previtellogenesis. Experiments of Sengal and Georges (1966) indicate that spawning may be inhibited by the neural gland. In addition, Sengal and Kieny (1962, 1963a, b) found that oocytes in whole cultured gonads continued to mature only when in the presence of explanted neural complex. They conclude that some component of the neural complex is essential to gonad maturation. Contradictory results were obtained by Hisaw et al. (1966), who found that removal of the neural complex in Chelyosoma productum has no effect on gametogenesis, even after one year.

DORSAL STRAND

The dorsal strand has been described in a large number of ascidian species since Kowalevsky's (1874a) work on Didemnum styeliferum, Phallusia mamillata and Ascidia (Metcalf, 1900; Huus, 1937; Brien, 1947; Goodbody, 1974 for summary). It is a fine strand, or cord, of cells that continues dorsally from the neural gland, and runs along the dorsal wall of the branchial basket close above the dorsal lamina and dorsal blood vessel. In Corella inflata, which has an expanded atrium, the dorsal strand runs in the dorsal fold between the anterior dorsal edge of the branchial basket and the atrium (Mackie et al., 1974).

The length of the dorsal strand is extremely variable in adults. It may extend only a short distance from the neural gland, it may end near the esophogeal opening (Metcalf, 1900),

or near the caecum of the stomach (Van Beneden and Julin, 1884). In some species, there is no dorsal strand at all in the adult (Metcalf, 1900). Millar (1953) reported that the dorsal strand in Ciona intestinalis extends to the gonad where it terminates abruptly as a slight swelling in the wall of the ovary. Metcalf (1900) and Aubert (1954), however, did not find this structure in the gonad region of adult Ciona. In Corella parallelogramma (Huus, 1924), and C. inflata, the dorsal strand is rudimentary in the adult, and is not associated with the gonad.

Most reports on the histology of the dorsal strand have been limited to adults. In Ciona the dorsal strand is a hollow cylinder 10 to 15 μm in diameter, and composed of relatively elongate, spindle-shaped cells (Millar, 1953). Brien (1925, 1927, 1939) observed that in Aplidium zostericola, Clavelina and Distaplia magnilarva blastozooids, the dorsal strand is irregular in section, and composed of undifferentiated cells similar to mesenchyme cells. Huus (1924) reported that in adults of Corella parallelogramma, the dorsal strand is composed of rounded cells with irregularly scattered nuclei arranged around a lumen, and with 6 cells in diameter. Van Beneden and Julin (1884, 1886) described the cells of the dorsal strand as being similar to those of the neural ganglion, around a small central lumen in Molgula ampulloides. Mackie et al. (1974) depict the dorsal strand in adults of Corella willmeriana as approximately 15 μm in diameter, and composed of several cells surrounding a small lumen. Some species, such as Ascidia atra, have a dorsal strand without a lumen (Metcalf, 1900).

Relatively little work has been done on the length and structure of the dorsal strand in juvenile ascidians. In Corella inflata, the dorsal strand extends to the gonad hemocoel, where it makes contact with the pre-gonadal hemoblasts. The association between the dorsal strand and the developing gonad is maintained during gonadogenesis, but after the gonoducts reach the atrium, the dorsal strand loses its association with the gonad, and is present as only a vestige in the adult. Huus (1924) described a similar association between the dorsal strand and the developing gonad in C. parallelogramma, which is maintained during gonadogenesis, and subsequently lost in the adult. The association has also been described in Didemnum styliferum,

Phallusia mammillata, Ciona intestinalis (Kowalevsky, 1874a), Aplidium zostericola, Clavelina, Distaplia magnilarva (Brien, 1925, 1927, 1939) and Ciona intestinalis (Markman, 1958). Aubert (1954), however, believed that the dorsal strand continues past the ovotestis in Ciona intestinalis juveniles, and does not make contact with gonadal material. His description of the dorsal strand in this location indicates he may, in fact, be describing the visceral nerve. Still other authors were unable to find the termination of the dorsal strand in the juvenile (eg Van Beneden and Julin, 1886; Maurice, 1886, 1888; Julin, 1892, Damas, 1902).

In juveniles, the dorsal strand consists of a cord of single cells attached end to end between the neural gland and its point of termination (Huus, 1924; Van Beneden and Julin, 1884, 1886; Aubert, 1954). In many cases the cells of the dorsal strand have been described as relatively undifferentiated, resembling blood cells (Van Beneden and Julin, 1886; Brien, 1927). Maurice (1886) describes it as composed of nerve fibers, Van Beneden and Julin (1886) as identical to the cells of the neural gland, and Fedele (1938) describes the cells as similar to neuroblasts, giving rise to the nerve cells that are found near the dorsal strand.

My observations on Corella inflata are the first that describe the ultrastructure of the dorsal strand in juvenile ascidians. The dorsal strand is composed of a string of elongate cells attached at their ends by overlapping processes, joined by well-developed junctions, and surrounded by an external lamina, giving it an epithelioid configuration. The cells contain microfilaments, probably actin, which may be important during the growth of the structure. At the point of attachment between the dorsal strand and the developing gonad, there are no special features of the cells. In Stage IV of gonadogenesis, and during subsequent development, there is a blunt enlargement at this point of attachment, but here too, the cells of the dorsal strand are virtually identical to the somatic cells of the developing gonad where the attachment occurs. The dorsal strand remains a string of single cells during gonadogenesis, other than at this point of contact, and it is likely that the dorsal strand becomes several cells thick and forms a lumen only after it is no longer associated with the gonad. I have not followed this later in

development, however, in Corella inflata. Kowalevsky (1874a) first described the dorsal strand as an extension of the neural ganglion, ending in a visceral ganglion in the region of the stomach. Many of the earlier descriptions of the dorsal strand similarly conclude that the dorsal strand is an element of the peripheral nervous system (Maurice, 1886, 1888; Julin, 1881; Lorleberg, 1907). Metcalf (1900) described varying degrees of association between the dorsal strand (rapheal duct) and the visceral nerve (rapheal nerve). He could not distinguish the visceral nerve from the dorsal strand in many species, and concluded that at least in their posterior extremities, they were parts of the same structure. Other more recent works, however, conclusively demonstrate that the dorsal strand itself is entirely non-nervous, and that the visceral nerve is a separate structure (Huus, 1924; Brien, 1925, 1927, 1948; Fedele, 1938; Millar, 1953; Aubert, 1954; Markman, 1958; Mackie et al., 1974; Goodbody, 1974).

The dorsal strand is certainly associated with nervous elements for most of its length, in a structure variously called the "dorsal cord plexus" (Markman, 1958), "dorsal cord sheath" (Millar, 1953), or "dorsal fold plexus" (Mackie et al., 1974). This consists of an irregular network of bipolar and multipolar neurons surrounding the dorsal strand and visceral nerves. Processes of these neurons form a sheath-like plexus around the dorsal strand (Fedele, 1938; Millar, 1953; Aubert, 1954; Markman, 1958; Mackie et al., 1974). Some of these processes may extend to the branchial basket, portions of the intestine, endostyle, and pericardium (Fedele, 1938; Markman, 1958; Mackie et al., 1974). The dorsal fold plexus has been reported in young juveniles of Ciona (Markman, 1958; Aubert, 1954) and Corella parallelogramma (Huus, 1924).

Immediately after the dorsal strand has made contact with the developing gonad in Stage II, there are no nerve cells or processes associated with either structure in Corella inflata. However, in Stage III, and during subsequent development, an irregular layer of cell processes is closely associated with the dorsal strand in the region of the ovotestis. I interpret these to be axons, and elements of the dorsal fold plexus. These axons contain moderately electron dense vesicles which, on the basis of their size and distribution, are suggestive of a neurosecretory

function. The vesicles are also found in short extensions of these processes which extend into spaces between cells of the ovotestis. In addition, vesicles are occasionally seen in the cytoplasm of somatic cells of the ovotestis, and in cells of the dorsal strand near the attachment between the ovotestis and the dorsal strand. There have been no previous reports of these vesicles in the cells of the developing gonad, the dorsal strand, or the nerve plexus. I suggest that the vesicles in the nerve cell processes may be confined to juveniles, and that they may function in the differentiation of the reproductive system in developing animals. There is no experimental evidence to support this claim, and a great deal more work is needed to address this question thoroughly.

Potential functions of the dorsal strand have been proposed, but it remains an enigma. The studies of Fedele (1938) and Mackie et al. (1974) suggest that the dorsal strand has a functional relationship with the visceral nerve, and that the associated plexus may be important in branchial innervation. In this context, the dorsal strand itself may supply nutrients to the nerve cells in the manner of glial cells (Goodbody, 1974). Brien (1927) found that the dorsal strand gives rise to new neural tissue and the gonads during blastogenesis in Aplidium zostericola. Huus (1924) concluded that the dorsal strand probably gives rise to the gonoducts, based in part on the absence of the dorsal strand in adult Corella parallelogramma. Aubert (1954), however, asserts that the dorsal strand continues past the gonad rudiment in young Ciona, and could therefore not be involved in gonoduct formation. In Corella inflata, I have never observed the dorsal strand in a position similar to that described by Aubert (1954) in Ciona, but neither have I observed any indication that the dorsal strand tissue gives rise to the gonoducts. The dorsal strand does, however, remain associated with the developing oviduct during gonadogenesis. I consider it more likely that the dorsal strand provides a template for the directional development of the oviduct, and gives way to the oviduct as it develops.

The association between the neural gland and the developing gonad during development implies that the dorsal strand may be a conduit for trophic factors necessary for gonadogenesis

to move from the neural gland to the gonad. In this way, the neural gland may actually control the establishment of gonadogenesis, and/or its continuation. This would explain why the gonad never develops without the dorsal strand attachment. Goodbody (1974) reinterpreted the results of Carlisle (1951) and Bouchard Madrelle (1967a, b) as indicating that the dorsal strand may serve as a pathway between the neural complex and gonads for endocrine products over a long term reproductive cycle. Because the association of the neural gland with the gonads in adults is questionable, it is more likely that if the dorsal strand functions in endocrine control, it does so primarily during gonadogenesis. Such an explanation accounts for the continuation of the reproductive cycle in adult Chelyosoma productum even after neural complex removal (Hisaw et al, 1966). However, because the neural complex is directly subjacent to a substantial hemocoelic space, such an endocrine pathway seems superfluous. It may also be true that the dorsal strand serves as a pathway directing the growth of neurosecretory cells from the ganglion to the developing gonad, and that these substances are important in the continuation of gonadogenesis. These hypotheses apply only to those animals in which a dorsal strand is found early in gonadogenesis. The dorsal strand in many species, particularly stolidobranchs, is not associated with the developing gonad, and a different mechanism of gonad initiation and development must be proposed for these animals.