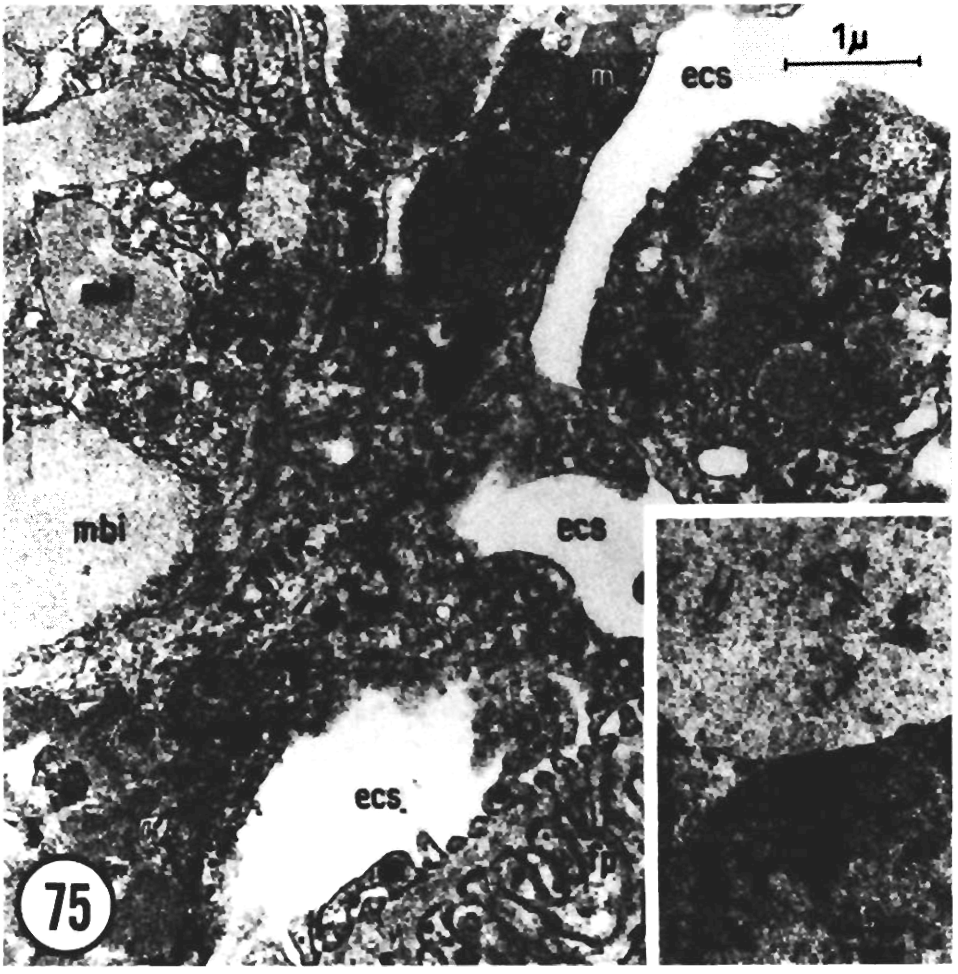

Fig. 75. Cytoplasmic inclusions of epithelial cell of maxillary gland end sac of *Artemia*. (From Tyson, 1968.) **A:** Area of cytoplasm containing several different inclusions. **B:** Part of two membrane-bound inclusions, each of which contains short, cylindrical elements (arrows). ecs, extracellular spaces; fp, foot processes, ger, granular endoplasmic reticulum; m, mitochondria; mbi, membrane-bound inclusions.

spaces that at times appear to be in direct contact with the end-sac lumen. The cell surface bordering these extracellular spaces often displays specializations such as shallow pits. Between adjacent foot processes are junctional specializations that Tyson (1968) felt were similar to the "filtration slit membranes" of the podocytes of vertebrate Bowman's capsules, strongly suggesting that primary urine formation is by ultrafiltration. There is typically one such junctional element visible between any two adjacent foot processes, although two, three, or more were reported by Tyson. The distance between the foot processes at the level of these junctional modifications is approximately 40 nm (Tyson, 1968). Nuclei of end-sac epithelial cells are irregular, often with deep clefts. Oval to rod-shaped mitochondria, often bearing one to a few electron-opaque granules, are present but not abundant. The most obvious inclusions in the end-sac epithelial cells are large, mem-

brane-bound bodies (Fig. 75A,B). These sometimes appear lobate, giving the impression that they formed by fusion of several smaller inclusions. Their origin and function are unknown. It is not clear whether they are all of one type vs. a variety of functionally unrelated membrane-bound bodies. Tyson (1968) noted that considerable variation exists in the size, shape, density, and texture of these inclusions, even within individual animals. Associated with these inclusions are Golgi elements, which may be found in the cytoplasm between the inclusions or (more often) in the cytoplasm surrounding a group of inclusions (Tyson, 1968). Granular endoplasmic reticulum (GER) is also found associated with these inclusions, and Tyson noted some similarities between the material in the lumen of the GER and that in the inclusions. Although that similarity might suggest that the inclusions contain some sort of secretory product, it is most likely that ultrafiltration is the method of urine formation. As is true of the decapod green gland, primary urine is a blood ultrafiltrate that is secondarily modified by reabsorption and perhaps secretion to form the definitive urine (Kirschner and Wagner, 1965; Tyson, 1968).

The efferent duct is divided into two morphologically and functionally distinct regions: a proximal efferent tubule, which connects to the end sac, and a distal terminal duct that leads from the efferent tubule to its opening on the maxilla (Tyson, 1969a). In *Artemia*, the efferent tubule departs from the ventral margin of the end sac. At the point where the efferent duct connects to the end sac, there are bundles of filaments that Tyson (1969a) interpreted as contractile elements. These are very likely what Cannon and Manton (1927) observed in *Chirocephalus* and referred to as a "very well defined muscular sphincter" valve of the end sac. These filaments are parallel to one another within a group, but no cross banding is evident. Tyson (1969a) suggested that these filaments act in some way like a sphincter muscle, similar to the valvelike apparatus that is seen in the segmental excretory glands of higher crustaceans (Goodrich, 1945).

The walls of the efferent tubule are lined with large, flattened epithelial cells (Fig. 80B,C). The tubule wall thickness (cell height) varies from about 5–25 μm. The most obvious features of these epithelial cells are their apical microvillous border (Fig. 77A) and, more basally, membranous folds and extensions (Figs. 76, 77B, 78, 80B,C). These basal processes may be of two types. The first type is large, deep infoldings of the basal plasma membrane within a single cell. The second type is interdigitations with these same processes from adjacent cells, and also with similar plasma membrane processes from lateral cell surfaces (Tyson, 1969a). All three of these features—microvillous border, basal infoldings, and basal and lateral interdigitations with adjoining cells—serve to markedly increase surface area of these cells. Cytoplasm near the basal infoldings and lateral processes contains large numbers of mitochondria. Elements of granular endoplasmic reticulum are found, usually in the form of vesicles scattered throughout the epithelial cells, although Tyson (1969a) cautioned that some of these vesicles could be artifacts of poor fixation. Golgi complexes are most often found in the apical part of the cells, and granular endoplasmic reticulum elements are often abundant in the nearby cytoplasm. Between these epithelial cells of the tubules are found two types of specialized zones of cell contact in the apical region. Zonulae adherentes of approximately 0.15–0.65 μm length occur most distally, and may form a continuous sheath around the perimeter of the apical part of the cell. Basal to these are found much longer (and possibly continuous) septate desmosomes (Fig. 78C) that may even extend completely around each cell (Tyson, 1969a).

After its final (third in *Artemia*) loop around the end sac, the efferent tubule passes through a plate or ring of connective tissue (Warren, 1938) before passing medially and

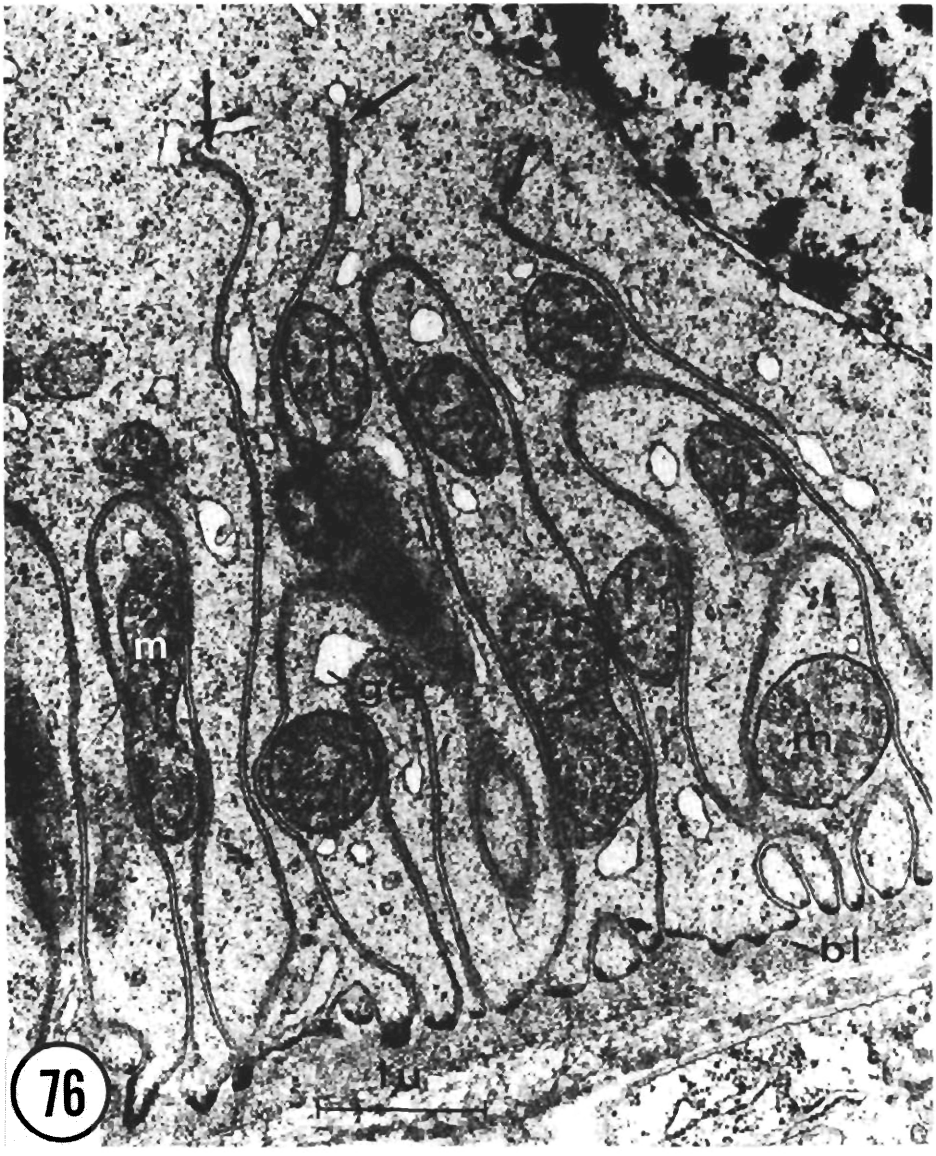

Fig. 76. Basal region of epithelial cell in proximal region (first coil) of efferent tubule, maxillary gland of *Artemia*. (From Tyson, 1969a.) Note extensive infoldings of basal plasma membrane associated with numerous mitochondria. bl, basal lamina; ger, granular endoplasmic reticulum; m, mitochondria; n, nucleus; arrows, blind endings of three infoldings of plasma membrane.

connecting with the terminal duct. The junction between the efferent tubule and the terminal duct is marked by a decrease in tubule diameter and a marked change in ultrastructure of the epithelium. The cells of the terminal duct have none of the surface-increasing specializations of the efferent tubule epithelium and lie beneath a thin (usually less than 0.5 μm), ectodermally derived cuticle that is similar in ultrastructure to the animal's exter-

Fig. 77. Apical region of cell of efferent tubule, maxillary gland of *Artemia*. (From Tyson, 1969a.) **A:** Microvillous border from distal region (third coil) of tubule. Note branched microvilli. **B:** Basal region of epithelial cell of third coil. Note electron density of cytoplasm of cell of third coil (cy^3) as compared to that of cell of first coil (cy^1). bl, basal lamina; m, mitochondria; mg, mitochondrial granules. Arrows denote course of convoluted basal cell membranes.

nal cuticle (Fig. 80D). Lateral cell margins are relatively simple, without infoldings or interdigitations with adjacent cell membranes. Mitochondria of these cells are smaller and less numerous than those of efferent tubule cells and are not found in close association with the plasma membrane. Golgi elements are present, but not near the apical border. Elements of granular and agranular endoplasmic reticula are found, with the former more abundant. Terminal duct cells also possess loose particles, which may be ribosomes or primary glycogen particles, and membrane-bound bodies, which may be oval,

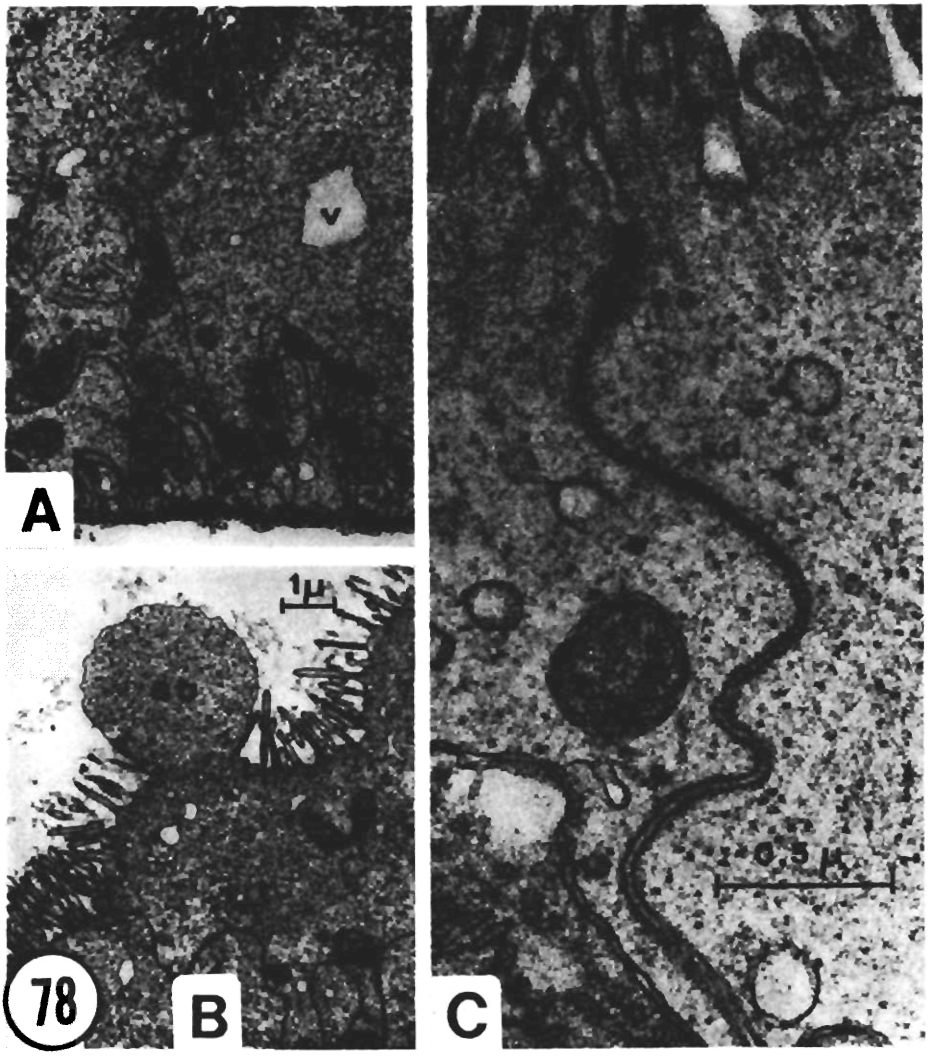

Fig. 78. Epithelial cells of efferent tubule, apical region, and cell junctions. (From Tyson, 1969a.) **A:** Lateral region of contact between two cells, one of which contains a vacuole (v). **B:** Apical bleb (ab) extruding into lumen of tubule. **C:** Septate desmosome (sd) and zonula adherens (za) between two cells of the first coil.

round, or irregular, containing a "flocculent material of low electron-density" (Tyson, 1969b: 159). Based on the nature of the epithelial cells of these two regions, and because ultrafiltration appears to take place in the end-sac cells of this gland, Tyson (1968, 1969a,b) postulated that selective reabsorption occurs in the efferent tubule, with the terminal duct acting more or less as a passive conduit for the final urine after modification by the efferent tubule. However, she noted also that the terminal duct may play some role in the synthesis of organic components of the overlying cuticle, based on the presence of well-developed RER in conjunction with conspicuous Golgi elements.

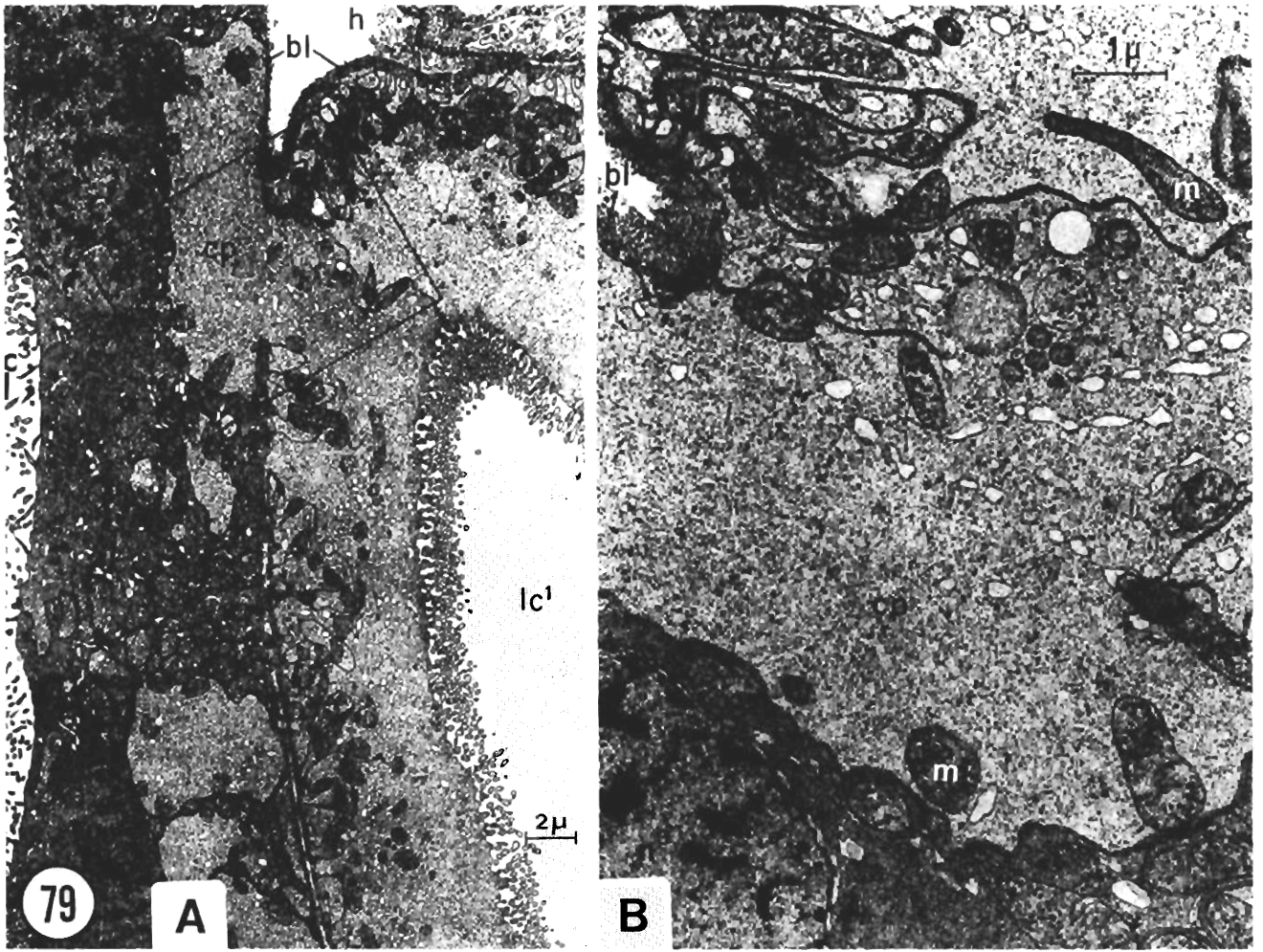

Fig. 79. Intercoil connections between epithelial cells in first and third coils of the efferent tubule, maxillary gland of *Artemia*. (From Tyson, 1969b.) **A:** Low-magnification montage of region of connection. Note difference in electron density of cytoplasm of cells of the two coils, and "islands" of lighter cytoplasm among the more dense cytoplasm of third coil cell. **B:** Higher magnification of area similar to that in rectangle in A. bl, basal lamina; cp, connecting process; h, hemocoel; lc^1, lumen of first coil; lc^3, lumen of third coil; m, mitochondrion; n^3, nucleus of cell in coil 3.

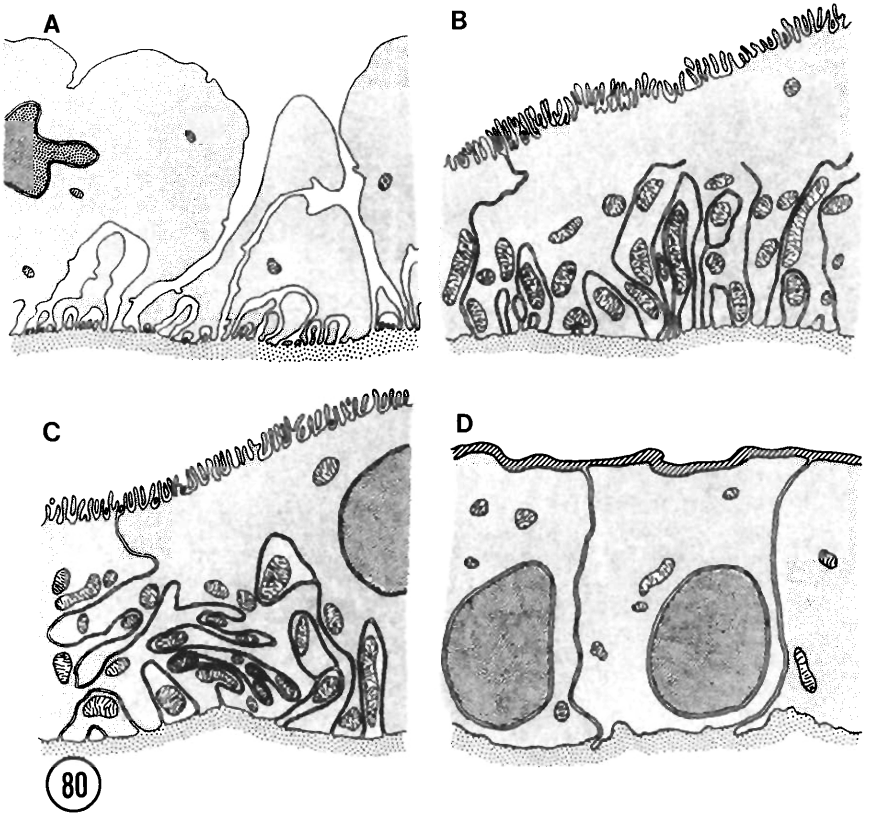

Fig. 80. Diagram of epithelial cells from various regions of the maxillary gland. (From Tyson, 1969b.) **A:** End sac. **B:** Efferent tubule, proximal region. **C:** Efferent tubule, distal region. **D:** Terminal duct (lined with cuticle). Apical end of cell is at top in each figure.

Holliday et al. (1990) found high Na^+/K^+–ATPase enzyme-specific activity, indicating that the maxillary glands in *Artemia* function also to some extent in osmoregulation. This finding lends support to Potts and Barry's (1964) statement, based on a study by Medwedwa (1927), that *Artemia* may produce a hyperosmotic urine. However, Conte and coworkers (see Conte, 1984) have found that the urine is always isosmotic, and believe the maxillary glands incapable of producing hyperosmotic urine (Conte, 1984). If true, then their role in salt regulation must be aided by other organs (thoracic epipods and larval salt glands) (Conte, 1984). *Daphnia* and other freshwater taxa take in much water by oral and anal drinking, and probably by osmosis, so it is difficult to believe that the large amount of urine that must be produced is always isosmotic.

Phagocytic Storage Cells

There is also a possibility that the large phagocytic storage cells circulating in the open hemocoel aid in the excretory process. Lochhead (1950) credited these cells with "uptake of pigment granules and other foreign particles." Lochhead and Lochhead (1941) credited these cells with phagocytosis, and Criel (1991a) considered them the functional equivalent of the combined nephrocytes and fat bodies of insects (see section on connective tissue and musculature).

REPRODUCTIVE COMPONENTS

Branchiopod reproduction is an extremely complex subject and has been the subject of numerous studies. The following is a very brief review.

Most populations of anostracans reproduce sexually, although parthenogenesis and her-

maphrodism are known in some species of *Artemia* (Wolfe, 1971; Fryer, 1987c; Belk, 1991; Criel, 1991a). Wolfe's (1971) statements concerning parthenogenesis and hermaphrodism in genera other than *Artemia* apparently are incorrect (D. Belk, personal communication). Polyploidy is fairly common. Sexual populations of *Artemia* have a chromosome number of 42 (except for one diploid species), whereas parthenogenetic populations have diploid, triploid, tetraploid, and pentaploid forms, with several ploidy numbers and even aneuploidy known from a single population (Abreu-Grobrois, 1987; Browne and Bowen, 1991). Notostracans are either sexual (habitually so in some species) or hermaphroditic (Fig. 88), and in some of the hermaphroditic species the reproductive mode can vary as a function of geography (Longhurst, 1954, 1955; Fryer, 1987c, 1988). Although most previous accounts of parthenogenesis in the Notostraca are probably incorrect (Fryer, 1987c, 1988), Zaffagnini and Trentini (1980) provide evidence for automictic parthenogenesis in European populations of *Triops cancriformis* that are classified by them as "rudimentary hermaphrodites." In part, their reasoning was based on previous evidence for automictic parthenogenesis in another branchiopod, the spinicaudatan *Limnadia lenticularis*, a species that, like notostracans, has testis lobes present in the female reproductive system (Zaffagnini, 1969). Most spinicaudatan populations reproduce sexually, although it is not uncommon to find highly skewed numbers of males and females (usually with many more females), and both selfing hermaphroditism and sexual outcrossing are known in the Limnadiidae (Sassaman, 1989). In some taxa (e.g., *Limnadia, Cyclestheria*) males are either extremely rare or unknown. For example, there are no known males in any populations of *Cyclestheria* in Australia (Timms, 1986) or India (e.g., Nair, 1968), although males are known from North America (Sissom, 1980). *Cyclestheria* is known to exhibit sexual reproduction (resulting in the production of resting eggs) or parthenogenesis (resulting in eggs that develop in a dorsal brood pouch), depending on the population and possibly on the time of year, and the same may be true for populations of *Limnadia lenticularis* (but with resting eggs always the result). Laevicaudatan populations always have both sexes, as far as is known, and reproduction is assumed to be sexual. In the Ctenopoda, diploid parthenogenesis is universal. In anomopods, onychopods, and haplopods, diploid parthenogenesis resulting in brooded eggs is nearly universal (Fryer, 1987c). Some Caspian Sea populations of cercopagid onychopods lack males and sexually reproducing females, and many cladocerans (especially among the daphniids) are obligate parthenogens (Hebert, 1987; Zaffagnini, 1987), but sexual reproduction resulting in "resting" eggs often occurs, and many populations are known to alternate between parthenogenetic and sexual reproduction within a single annual cycle (e.g., *Leptodora* and many anomopods, e.g., see Hebert, 1987, reproduce parthenogenetically during the summer but sexually, producing resting eggs, in the fall).

Female System

The ovaries are typically paired, tubular organs located on either side of the gut, often just dorsal or even slightly ventral to it, and usually originating anterior to the genital somite. In many taxa, they extend posteriorly from the genital somite into at least some of the "abdominal" somites (e.g., Fig. 81A,B). In notostracans, the ovaries (or "ovatestes" in some species or populations) extend along nearly the entire length of the body (Wingstrand, 1978; Tommasini et al., 1989). In ctenopods, the ovaries consist of a row of large cells; these cells originate anteriorly, and extend posteriorly as long, single-cell rows, with each cell being flanked by one nurse cell in front and two behind, or vice versa (Fryer, 1987a). In the anomopods, the ovarian cells originate posteriorly, are not in a single uniseriate row, and each cell is found in a cluster with three nurse cells (Rossi, 1980; Fryer, 1987c). In haplopods, the ovary, which is a single (unpaired) lobular organ, is confined to the postgenital somites (Wingstrand, 1978; Rossi, 1980).

Oviducts lead from the ovaries to openings at the base of thoracic limbs (Notostraca, Spinicaudata), to openings within a dorsal "brood pouch" formed by the (sometimes reduced) carapace (Anomopoda, Ctenopoda, Onychopoda, Haplopoda), to an ovisac (sometimes termed the "uterus") within a large ventral brood pouch (Anostraca, Fig. 81A,B), or to the base of flaplike lamellar extensions of the body wall (Laevicaudata). In anostracans, a unique ventral brood pouch is present for storage of shelled eggs. In other taxa, storage is accomplished via modified limbs (Notostraca and at least some Spinicaudata) or within the confines of the carapace chamber, which may be modified for this purpose.

Formation of the outer (tertiary) egg membrane occurs via a variety of mechanisms, including secretion by epithelial cells lining the ovaries or oviduct and from specialized shell glands adjacent to the ovisac (Anostraca only).

Oogenesis

A comparison of oogenesis in the major orders provides a striking example of branchiopod diversity and of how misleading generalizations on their anatomy can be. Although the resulting egg is often rather similar in the major groups, formation of the egg follows different pathways in different taxa. Generally the ovarian cells begin as a cluster of four follicular cells, three of which become "alimentary" or "nurse" cells, aiding in the development of the fourth cell, the true oocyte, but further details vary with the taxa.

Anostracans have been studied extensively with regard to oogenesis and other aspects of the female reproductive system but should not be considered representative for all branchiopods. Most information stems from studies on *Artemia, Chirocephalus, Eubranchipus, Streptocephalus,* and *Tanymastix* (e.g., Fautrez-Firlefyn, 1951; Linder, 1959; Anteunis et al., 1966; Lochhead and Lochhead, 1967; Garreau de Loubresse, 1974; Criel, 1980a,b, 1989, 1991a; Munuswamy and Subramoniam, 1985, and papers cited therein). In these taxa, the germinal zones are located in a ventral strip along the tubular gonad, i.e., they are not confined to discrete "follicular" areas.

In *Artemia,* and probably in most other anostracans, the cells of the ovaries show cyclical changes correlated with events of vitellogenesis (Criel, 1980a, 1989, 1991a). Early in vitellogenesis the ovaries appear translucent, becoming opaque and more granular as development proceeds. Two different cell types, the somatic cells (Lochhead and Lochhead, 1967; Criel, 1989, 1991a) and germ cells, are identifiable (Fig. 82). Germ cells include both oogonia and cells that later differentiate into oocytes and nurse cells (Criel, 1989, 1991a). The somatic cells, some of which contain irregular dense inclusions, form "an interrupted layer of clear cells facing the gut and separating the clusters of germ cells" (Criel, 1989: 100, fig. 1) and are found closer to the gut. Oogonia, forming islands between the vitellogenic oocytes, are found more lateral to the gut. Germ cells between the oogonia and somatic cells are more mature closer to the gut (Criel, 1989). Early previtellogenesis is marked by an increase in cell size and the transformation of the cluster of young oocytes into a long "ribbon" (Fig. 83) (Criel, 1989), each of which consists of a maximum of about 32 oocytes (Criel, 1989).

Differentiation between oocytes and nurse cells is obvious by the increase in cytoplasm of one oocyte of the ribbon toward the termination of previtellogenesis. The nucleus of that oocyte is transformed into a large eccentric "germinal vesicle," and a "yolk nucleus" appears in the cell's center (Fig. 84A) (Criel, 1989, 1991a). Smaller "accessory nuclei" can then be found spread over the surface of the oocyte (Criel, 1991a). Four yolk products have been reported (Fig. 84B): protein yolk platelets, lipid droplets, vesicular bodies, and small intracisternal granules (Criel, 1991a). A continuous layer of somatic cells underlies the basal lamina, and more or less surrounds the oocyte—nurse cell complexes. These cells actively phagocytose the degenerating nurse cells and probably play a role in

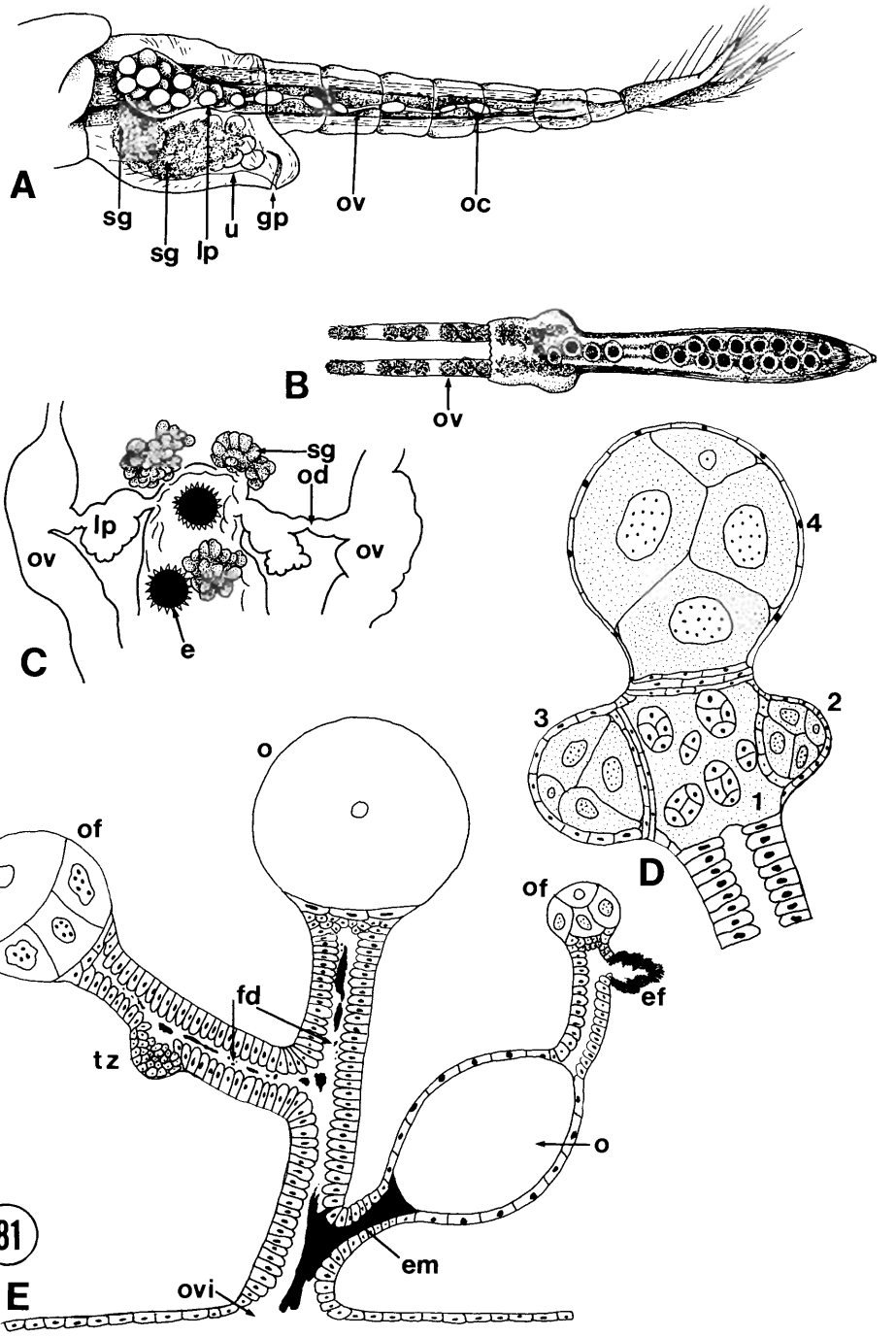

Fig. 81. Aspects of the female reproductive system in Anostraca (**A–C**) and Notostraca (**D,E**). **A:** Lateral view of female *Eubranchipus bundyi*. Note "uterus" (ovisac) within median ventral brood pouch. (After Linder, 1959.) **B:** Ventral view of ovaries and long ovisac within brood pouch of *Branchinecta paludosa*. (After Sars, 1896.) **C:** Relationships of ovaries, oviducts, lateral pouches, shell glands, and median ovisac (here containing shelled eggs) in *Chirocephalus nankinensis*. (After Hsü, 1933.) **D:** *Triops cancriformis* "germarium" showing stages in nurse cell development. Note gradual increase in size of three nurse cells as compared to relatively smaller oocyte from stage 1 to 4. (After Trentini and Sabelli Scanabissi, 1978.) **E:** Diagram of *Triops cancriformis* gonad with three follicle ducts (fd). (After Trentini and Sabelli Scanabissi, 1982.) e, egg; ef, empty follicle; em, eggshell material; gp, genital pore; lp, lateral pouch of oviduct; o, oocyte; oc, od, ovi, oviduct; of, oocyte follicle; ov, ovary; sg, shell glands; tz, testicular zone of notostracan follicular duct; u, "uterus" (ovisac).

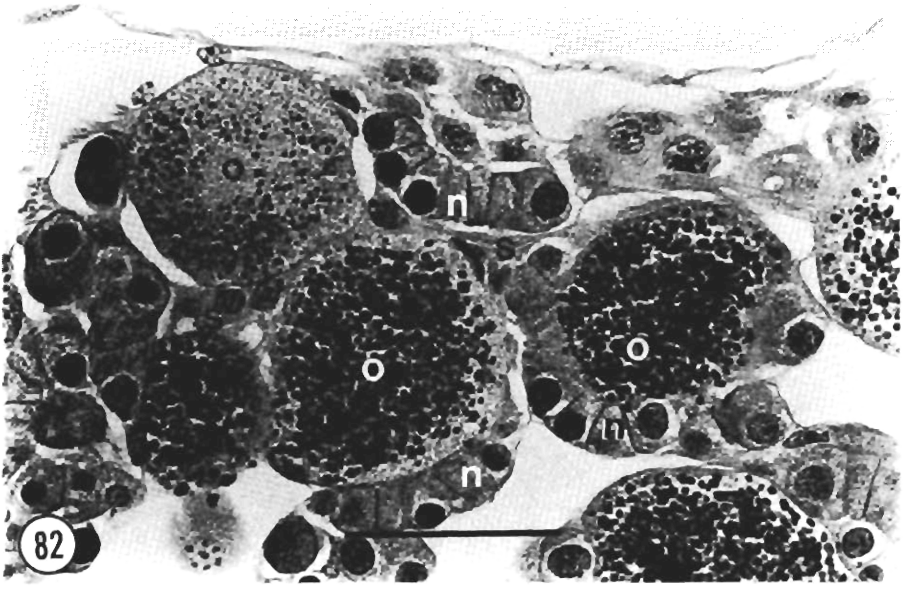

Fig. 82. Longitudinal section through the ovary of *Artemia* during vitellogenesis. (After Criel, 1991a.) o, maturing oocytes; n, nurse cells; s, somatic cells. Scale bar = 50 μm.

transport, as indicated by their well-developed network of indented plasma membranes (Criel, 1991a). The nurse cells eventually become polyploid, degenerate, and are phagocytosed by the surrounding somatic cells (Criel, 1991a). Engulfing of the nurse cells by developing oocytes, described earlier by Fautrez-Firlefyn (1951) using light microscopy, has not been confirmed or supported by more recent transmission electron microscopical (TEM) studies (Criel, 1991a).

The oviducts of *Artemia* depart from the ovaries in the region of the third abdominal (postgenital) somite and extend ventrally and anteriorly to open into the anterolateral border of the ovisac. Each oviduct is lined with secretory epithelium consisting of two cell types, with and without secretory granules (Fig. 85) that undergo secretory cycles (Criel, 1980a,b). The oviducts are surrounded by circular and longitudinal muscle fibers (Criel, 1991a). Laterally, the oviducts may become distended and are sometimes termed lateral pouches; apparently these lateral pouches are separated from the median and frontal parts of the ovisac by a "shutter" mechanism (Fig. 86) that is partly cellular in composition and partly fibrous (Criel, 1980a, 1991a).

Developing eggs pass down the oviduct into the lateral pouches and finally into the median ovisac, known only in anostracans. The median and frontal parts of the ovisac retain sperm and thereby function as a sort of seminal receptacle between copulation and fertilization. Sperm stored in these areas of the ovisac continue to mature and may acquire peripheral arms or may disintegrate (Criel, 1980a, 1991a). Specialized shell glands (Figs. 81A–C, 87) responsible for secretion of the final egg coating and opening into the ovisac also are unique to the Anostraca. These glands consist of clusters of two or four cells that vary in color depending upon the stage of the reproductive cycle. From each cluster there is a short duct, the lumen of which is lined with a chitinous membrane. The presence of a chitinous lining, plus the similarity of the gland's organization to integumental

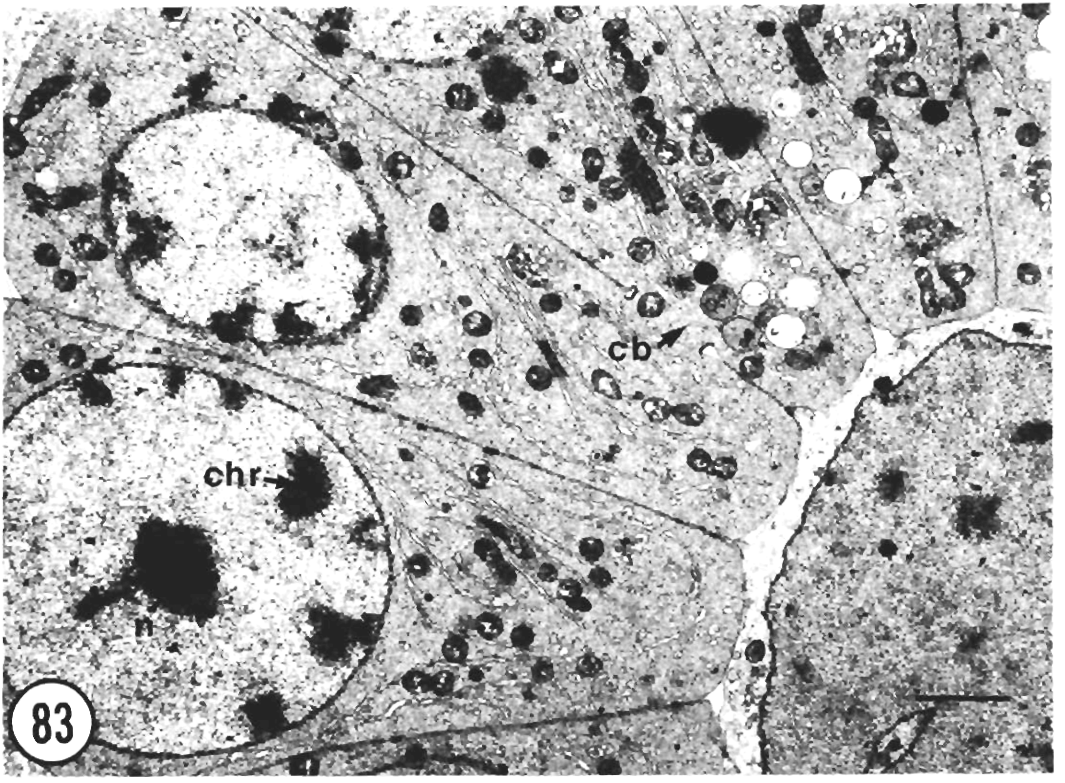

Fig. 83. TEM through a ribbon of developing oocytes in *Artemia*. (After Criel, 1980a.) cb, cytoplasmic bridge; chr, chromatin; n, nucleolus. Scale bar = 2 μm.

glands, led several workers (e.g., Benesch, 1969) to claim an ectodermal origin for both ovisac and shell glands. These glands may appear white or even colorless to a dark brown (Anderson et al., 1970; Fautrez and Fautrez-Firlefyn, 1971; De Maeyer-Criel, 1973), and it has been suggested that they play some role in the determination of oviparous vs. ovoviviparous development. However, the exact role remains unknown (Criel, 1980a, 1991a). Among white shell glands, at least two different types have been recognized by characteristics of the secretory granules. The two cell types are thought to be related to development of naupliar larvae vs. formation of the thin shell of embryos that will hatch just after oviposition (Criel, 1980a, 1991a; De Maeyer-Criel, 1973).

Notostracan oogenesis was the focus of several excellent papers by Trentini and Sabelli Scanabissi (1978, 1982), Sabelli Scanabissi and Trentini (1979), Tommasini and Scanabissi Sabelli (1989), and Tommasini et al. (1989). In *Triops,* oocytes are produced in blind tubules coming off the longitudinal oviduct (Fig. 81D,E). The germinal zones are located at the distal end of each tubule, and produce four cells that increase in size, eventually forming an ovarian follicle (Figs. 81D,E, 89). Each follicle bulges out into the surrounding hemocoel and is covered by a single thin layer of somatic follicular cells (Trentini and Sabelli Scanabissi, 1978). The four cells in the ovarian follicle are a true oocyte and three nurse cells. The quartet is produced by two mitoses of the original oogo-