AN ABSTRACT OF THE THESIS OF

SETH R. HOOTMAN (Name) for the MASTER OF SCIENCE (Degree)

in ZOOLOGY (Major) presented on June 25, 1973 (Date)

Title: FINE STRUCTURE AND FUNCTION OF THE NECK ORGAN IN THE BRINE SHRIMP ARTEMIA SALINA NAUPLIUS

Abstract approved: Redacted for Privacy
Patricia Harris Noyes

The morphology of the dorsal neck organ of the brine shrimp Artemia salina nauplius was investigated to determine its role(s) during larval development.

Light and scanning electron microscopic studies show that the neck organ is present in the pre-nauplius larval forms as early as 12 hours after hydration of the desiccated cysts. It reaches its maximum development by the second or third instar but dedifferentiates in later stages as the phyllopodia mature.

Areas of chloride ion accumulation in the E-2 and nauplius were localized by silver nitrate staining. The neck organ was the only portion of the larvae that stained to an appreciable degree, suggesting that it is the major site of sodium chloride secretion in the larval forms.

Ultrastructural study of the neck organ in the early nauplius

indicates that the organ serves the larva as a salt-secretory gland. The cells of the neck organ exhibit fine structural specializations commonly associated with epithelia involved in ion transport. The apical surfaces of the cells are extensively convoluted and the cell cytoplasm is divided into a web-like labyrinth which contains numerous mitochondria.

Electron microscopic examination of the neck organ also reveals that it serves as a stable point of attachment for the dorsal antennal musculature, thus compensating for the non-rigid nature of the larval cuticle. It is suggested that the unusual architecture of the neck organ in the nauplius of Artemia may be an adaptation related to efficiency in both salt secretion and locomotion.

Fine Structure and Function of the Neck Organ in the Brine Shrimp *Artemia salina* Nauplius

by

Seth R. Hootman

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed June 1973

Commencement June 1974

APPROVED:

Redacted for Privacy

Professor of Zoology
in charge of major

Redacted for Privacy

Associate Professor of Zoology
in charge of major

Redacted for Privacy

Chairman of Department of Zoology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented June 25, 1973

Typed by Mary Jo Stratton for Seth R. Hootman

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. Patricia J. Harris Noyes and Dr. Frank P. Conte for their guidance and support during the course of this study. I would also like to thank Dr. Richard D. Ewing for his help in preparation of the manuscript and for his enthusiasm in all matters pertaining to Artemia.

The scanning electron micrographs in this thesis were taken at Electros Analytics in Tigard, Oregon with the assistance of Dr. Franco Rossi and Mr. and Mrs. Charles Dwire. Their help is gratefully acknowledged.

I would like to offer special thanks to my wife, April, for her patience and encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

FINE STRUCTURE AND FUNCTION OF THE NECK ORGAN IN THE BRINE SHRIMP ARTEMIA SALINA NAUPLIUS

I. INTRODUCTION

Halobionts are organisms that have evolved special physiological mechanisms allowing them to flourish in saline habitats. The list of species that can truly be classed as halophilic or "salt-loving" is rather short, attesting to the harshness of excessively saline environments. Communities are therefore much simplified, since only limited numbers of life forms are able to cope with the problems presented by extreme salinity. Lower forms of life are most numerous, the prokaryotes being represented by several species of halobacteria which provide food for a few species of protozoa and an even smaller variety of higher animals. Of these last, only a few are halobiotic during their entire life cycle. Prominent among them is the brine shrimp Artemia salina (Leach).

Artemia is a Branchiopod crustacean of the order Anostraca which inhabits salt lakes, salterns, and brine pools worldwide (Weldon, 1909). The wide distribution of these brine shrimp is due in part to their unique ability to withstand the effects of great salinity changes in their aquatic environments. Field observations of Artemia larvae and adults have established that they can exist in waters ranging from nearly saturated brine to those approaching the osmolarity of rainwater

(Carpelan, 1957; Evans, 1913; Lochhead, 1941). Laboratory studies (Boone and Baas-Becking, 1931; Jennings and Whitaker, 1941) have confirmed these observations and indicated that salinities as high as 15% may be tolerated indefinitely by these hardy phyllopods.

Work by Croghan (1958a-d) and others has established the osmoregulatory abilities of the adults and the physiological basis for these abilities. A specialized system of salt-secretory cells appears to be responsible for the capacity of the adult brine shrimp to tolerate conditions of extreme salinity. This secretory epithelium lines the metapipodite segments of the branchiae. Copeland (1966, 1967) has studied the fine structure of these cells and has found them to possess characteristics common to salt-secretory epithelia from other animals, most notably the teleost gill (Doyle and Epstein, 1972; Philpott and Copeland, 1963) and the avian salt gland (Ernst and Ellis, 1969; Komnick, 1963). Staining of the animals with silver nitrate, a technique which localizes areas of chloride ion accumulation, provided additional evidence suggesting the metepipodites as the primary organs of salt secretion in the adults.

The early nauplii of Artemia do not develop branchiae with functional metepipodite segments until a week or more after emergence from the cysts, yet are nearly as tolerant to salinity extremes as the adults. Physiological studies (Conte, Hootman and Harris, 1972) have shown this tolerance to be due to an active regulation of the body

sodium levels. A rise in salinity of the external medium over the range 0.3% to 12% NaCl does not appear to cause more than a slight rise in the internal sodium levels of the early nauplii. Since the larval forms do not yet possess metepipodites, the question arises as to how this regulation of the internal salinity takes place in the nauplius.

In 1905, Nicolas Zograf described a ". . . calotte cervicale chez les nauplius de l'Artemia salina," a cap or shield covering the dorsal cephalothoracic region of the brine shrimp nauplius. This organ was round or oval in shape and appeared to be comprised of a sheet of epithelial tissue. Dejdar (1930) found, while studying the larval development of a variety of crustaceans, that this neck organ was an exclusively larval structure. It remained a prominent morphological feature of the nauplius and metanauplius for several days after hatching, but it degenerated in later stages. The adults showed no external vestiges of the organ.

Using nauplii, Dejdar repeated the silver nitrate staining procedure which had shown the metepipodites to be areas of chloride ion accumulation in the adults. He found that the neck organ stained intensely. Moreover, as the branchiae with functional metepipodites developed, the neck organ lost its staining ability. Based on these observations, Dejdar and others (Croghan, 1958c) have suggested that the osmoregulatory abilities of the nauplius might reside in this unusual organ. The neck organ could possibly serve as a salt-secretory gland in the larval brine shrimp.

The situation is somewhat complicated by the fact that the early nauplius also possesses paired antennal glands (Warren, 1938). These glands lie in the protopodites of the second antennae and appear to be functionally complete as early as the second instar stage. The regulation of various ionic species may, therefore, be more complex than first anticipated but may still involve the neck organ.

Another role for the neck organ was proposed by Paul Weisz in 1947. In his studies of the metameric development of the brine shrimp, he had shown that the neck organ was composed of a layer of simple, cuboidal-columnar epithelium. On the basis of histological studies, Weisz suggested that the neck organ might also play a supportive role during morphogenesis of the larvae, serving as a point of attachment for the powerful swimming muscles of the antennae.

The present work is an examination of the fine structure of the neck organ with regard to its proposed dual roles during development of the brine shrimp.

II. MATERIALS AND METHODS

Larvae of the brine shrimp were obtained in the form of desiccated cysts. These were purchased in vacuum packed cans from Longlife Fish Food Products (Harrison, N. J.) and stored, after the cans were initially opened, at -20°C. The dried cysts were routinely hydrated in a solution of Instant Ocean Sea Salts at 25°C in aerated Ehrlenmeyer flasks. Samples for observation and fixation were removed with wide-bore, disposable pipettes at timed intervals. No rigorous attempts were made to stage the larvae with regard to their molting cycles. They were simply timed from the beginning of hydration to the start of fixation and labelled according to the elapsed time.

Samples were removed from the culture flasks for four experimental procedures; A) whole mount histochemistry, B) scanning electron microscopy, C) light microscopy, and D) transmission electron microscopy.

Whole Mount Histochemistry

Larvae for use in histochemical localization of chloride ions were transferred from the culture flasks to small beakers and rinsed several times with distilled water to remove all of the NaCl from the surrounding medium. Most of the water was then drawn off by pipette and the larvae were covered with several volumes of 0.1 M $AgNO_3$.

After 20 to 30 minutes, the silver nitrate solution was decanted and the larvae were rinsed for 30 minutes with distilled water. During this rinse, specimens were exposed to a bright light after the photoreduction method of Ewer and Hattingh (1952). The rinsed larvae were then dehydrated in an ethanol series beginning with 40% and progressing in 20% steps to absolute ethanol. After six to eight hours in 100% ethanol, the larvae were transferred to a 1:1 v/v solution of ethanol and xylene where they remained for six hours. Upon completion of this stage, the solution was changed for 100% xylene. Following 24 hours in xylene, the specimens were immersed in a 1:3 v/v solution of xylene and neutral balsam. This solution was the final embedding medium. Larvae were mounted in groups of 10 to 15 on depression slides and photographed through a Zeiss light microscope.

Scanning Electron Microscopy

Specimens were prepared for scanning electron microscopy by two procedures, critical point substitution with fluorocarbons and freeze-dry lyophilization.

For the former, larvae were fixed in 5% glutaraldehyde at pH 7.2 overnight and dehydrated in an acetone series. The acetone was then replaced stepwise by Freon 113, a fluorocarbon. The Freon 113 was subsequently evaporated from the specimens with a

Bomar SPC-900 critical point sample drying apparatus following the procedure of Cohen, Marlow and Garner (1968).

Nauplii to be freeze-dried were fixed for six to eight hours in 1% osmium tetroxide at pH 7.2 and rinsed with several changes of distilled water in small culture tubes. All but a few drops of the water in each tube was drawn off and the tubes were lowered into a mixture of dry ice and acetone. The water and suspended nauplii were thus rapidly frozen. The tubes were then quickly placed in a vacuum flask and attached to a lyophilizer for eight hours.

The dried specimens from both procedures were mounted on metal pegs and coated with a thin layer of gold, after which they were photographed with a Cambridge Stereoscan electron microscope.

Light Microscopy

For thick sectioning, nauplii were fixed with 5% glutaraldehyde in 0.2 M sodium phosphate buffer at pH 7.2. First attempts at fixation of the larvae were unsuccessful, since they remained viable after up to 12 hours in the fixative at room temperature. The fixation procedure was subsequently modified as follows so that the nauplii would be rapidly penetrated by the fixative.

Nauplii from a culture flask were removed with a Pasteur pipette and drops containing the larvae were placed on a large rubber stopper under a dissecting microscope. A fine glass or metal needle

was used to remove the abdomen of each nauplius just posterior to the margin of the thoracic swelling. The animals were then quickly transferred by pipette into a vial containing 5% glutaraldehyde in 0.2 M sodium phosphate buffer (pH 7.2) at 4°C. The osmolarity of this primary fixative was adjusted by the addition of 15% sucrose. The fixative was allowed to come to room temperature (24°C) and left for six to eight hours. The specimens were then rinsed in 0.2 M sodium phosphate buffer adjusted to pH 7.2 plus 15% sucrose and postfixed in 1% osmium tetroxide in the same buffer. Dehydration was carried out in a series of ethanol solutions (50, 70, 95 and 100%). Propylene oxide was used as the transitional solvent in the manner recommended by Luft (1961) and the specimens were embedded in Epon 812 (Shell Chemical Corp., New York, N. Y.). Epon was used for the embedding medium for thick section light microscopy because sections of this plastic tended to wrinkle much less upon drying than did thick Araldite sections. Thick sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and mounted on glass slides. They were stained with methylene blue-azure II according to Richardson, Jarett and Finke (1960) and photographed through a Zeiss light microscope.

Transmission Electron Microscopy

Specimens were prepared in the manner described in the previous section. In addition, some samples were fixed in 1% osmium

tetroxide in 0.2 M sodium phosphate (pH 7.2) with 15% sucrose. Following dehydration in ethanol, the specimens were transferred through propylene oxide and infiltrated and embedded in Araldite 6005 (Ladd Research Industries, Burlington, Vt.) according to the method of Luft (1961). Thin sections (pale gold to silver-gray) were cut with glass knives on a Porter-Blum MT-1 ultramictorome and stained with uranyl magnesium acetate (Frasca and Parks, 1965) and lead citrate (Venable and Coggeshall, 1965). Grids were observed and photographed with an RCA EMU 3H electron microscope.

III. RESULTS

Developmental Events

Hydration of the cysts of Artemia in artificial seawater causes a resumption of larval development previously arrested by desiccation. Encysted embryos have for the most part been found to be in the gastrula stage (Dutrieu, 1960; Fautrez-Firlefyn, 1951) although Nakanishi et al. (1962) reported encysted blastulae. Development after hydration is very rapid, with nauplii appearing in the culture in less than 24 hours at 25°C.

Hatching of the nauplius takes place in at least three distinct steps; splitting of the cyst, "emergence" of the larva within the hatching membrane, and "hatching" of the nauplius from this membrane. The larval form at each step has been described and named. Nakanishi et al. (1962) have designated the two forms prior to the nauplius as the E-1 and E-2 stages. This terminology will be used in the following discussions.

While no rigorous attempts were made by the author to determine t_{50}'s for the onset of each stage, observations during collection of samples indicated the following timetable of events when larvae were cultured as described in the preceding chapter. Emerging larvae (E-1's) were first noted at about eight hours after initial hydration. They could be easily differentiated from the unemerged

cysts by the protrusion of part of the body of the larva from a crack in the cyst. In some cases, the faintly pigmented median eye could be discerned through the enclosing hatching membrane, indicating that the protruding portion of the larva was of the cephalic region. The percentage of this stage within the larval population increased to a high point at 14 hours and then rapidly declined as the percentage of emerged larvae (E-2's) increased. The appearance, increase, and decline of the E-2's follows a pattern identical to that of the E-1's, trailing it by three to four hours. E-2's first begin to appear at about 11 hours and are most abundant at 17 to 18 hours, thereafter declining rapidly in numbers. This decline corresponds with the first appearance of free-swimming nauplii in the culture at the 18 hour mark. The percentage of larval population in the nauplius form increased from less than 10% to greater than 50% in the following six hours.

The large numbers of hatchings occurring during this time period allow one to readily observe the hatching event. The previously quiescent E-2 makes several vigorous movements within the transparent hatching membrane during a period of several seconds prior to the actual escape. The hatching membrane then ruptures over the cephalothorax and the nauplius emerges, swimming immediately by means of lateral movements of the powerful second antennae. These appendages remain the chief organs of locomotion for over two weeks, during which time the nauplius (and later, metanauplius) undergoes a

series of molt cycles and the phyllopodia become progressively more functional as swimming appendages (see also Heath, 1924). Sexual maturity is reached in four to six weeks as evidenced by observation of copulating pairs within the cultures.

Histochemistry

Immersion of animals in dilute, silver-containing solutions has been used by a number of investigators to determine areas of halide accumulation on both the gross and fine structural levels. Silver nitrate solutions have generally been used for gross localizations (Croghan, 1958c; Dejdar, 1930; Ewer and Hattingh, 1952; Morse, Harris and Dornfeld, 1970), while silver lactate (Komnick, 1962; Wichard, Komnick and Abel, 1972) or silver acetate (Philpott, 1965) coupled with osmium tetroxide appear to be more suitable for fine structural studies. When tissues known to be active in salt secretion are exposed to the above solutions for a suitable length of time, a dense, granular precipitate forms at the tissue surface. This reaction product is believed to be a silver halide, most probably silver chloride. Identification of chloride as the precipitated anion has been supported by autoradiography (Komnick, Rhees and Abel, 1972) and selected area electron diffraction (Komnick and Bierther, 1969; Philpott, 1965; Wichard and Komnick, 1971).

Brine shrimp were found to be very selectively stained by

silver nitrate as shown in Figures 1 through 3. Staining ability is evident as early as the E-1 stage. In some E-1's, the whole visible area of the animal was observed to be covered with reaction product, while in others only part of the visible area was darkened. In the latter case, the boundary of the dark area was sharply defined. This difference in staining may be ascribed to the slightly differing orientation of larvae within the cysts. The stained area is difficult to correlate with any morphological feature of the animal, since the portion of the larva that protrudes from the cyst at this stage is very small. However, the area over which reaction product precipitates is assumed to be in the cephalothoracic region, since this is the part of the animal which first emerges during the transition from E-1 to E-2.

In contrast to the preceding stage, the E-2 larva is visible in its entirety, thus allowing the stained area to be easily observed and readily defined. Silver halide precipitates cover a circular area of tissue approximately 150 μm in diameter which lies on the dorsal cephalothorax of the E-2 (Figure 1). This corresponds exactly with the neck organ in both position and dimensions. Reaction product is densely and uniformly precipitated within this area, while only sparsely precipitated or absent over all other areas of the animal. The boundary of the staining area is sharply defined, indicating an abrupt transition with regard to either the permeability of the cuticle or the nature of the underlying epidermis.

In the nauplius, staining of the neck organ is as intense as in the E-2, except for its central portion (Figure 2). The absence of reaction product from an area roughly 40 μm in diameter suggests a loss of secretory function for the cells in the center of the neck organ. This ring-like distribution of precipitate was uniformly observed in all nauplii exposed to silver nitrate.

Transition of the staining reaction from the neck organ to the metepipodites has been well documented by Dejdar (1930) and was not investigated in the present study. Sexually mature brine shrimp were stained, however, and the results supported Dejdar's observations. Figure 3 illustrates the typical pattern of silver halide precipitation in an adult male. Reaction product is limited exclusively to the metepipodite segments of the branchiae. No precipitation is observed in the cephalic region, the former location of the neck organ.

Scanning Electron Microscopy

In the ten years since the scanning electron microscope has been commercially available, it has been increasingly used as a valuable tool in studying the finer structures of organisms. The external features of many of the more minute arthropods may now be described in much finer detail than that afforded by light microscopic studies. The small size of the larvae of Artemia made scanning

electron microscopic observation especially useful in obtaining an accurate picture of the larval morphology.

Both the critical-point drying method (Figures 4 and 5) and freeze-dry lyophilization (Figures 6, 7 and 16) proved to be very effective in preserving the overall surface topography of the larvae. The slight shrinkage of the larval epidermis noticeable in Figures 6 and 7 may be due to the effects of osmium fixation rather than the dehydration.

The neck organ is already identifiable in micrographs of 14 hour E-2's (Figures 4 and 5). In Figure 4, the larvae lies incased within the hatching membrane which is attached to the cyst at a point behind the abdomen of the E-2. Most features of the larva are not visible due to the electron opacity of the hatching membrane. The neck organ can, however, be identified. It appears as an oval spot of different density than the surrounding tissue beneath the membranous covering and is overlaid with a scattering of a crystalline substance. These crystals have as yet not been identified but may be silver chloride, since this E-2 was one of a number of specimens that were stained with silver nitrate prior to preparation for scanning electron microscopy.

Removal of the hatching membrane reveals the compacted form of the E-2 (Figure 5). By 14 hours after hydration a number of features of the nauplius stage are discernible. Both the first and

second antennae can be identified, bearing well developed setae and lying tightly pressed against the body. The neck organ has by this time nearly reached the maximum size that it will attain and covers one-third of the dorsal area of the larva. It is distinguishable by its cap-like appearance and by the large diameter (15 to 20 μm) of its component cells. The cells in the anterior part of the organ are particularly large and well-defined. Individual cells in the posterior portion are more difficult to identify and may be less fully differentiated than the more anterior cells. Heath (1924) had previously noted this difference in the neck organ of the first instar nauplius and ascribed it to the existence of two cell types.

Although the organ has reached its maximum diameter (200 to 250 μm) by the late E-2 stage, its appearance continues to change with further larval development. The nauplii in Figures 6 and 7 are second instars of 28 to 30 hours hydration. The stubby, conical body of the E-2 and newly hatched nauplius has become much more elongate with the rapid development of the abdomen. The neck organ is now a prominent feature of the larval topography, rising like a dome above the cephalothorax. Around the base of the dome lies a thin transitional band of tissue, strikingly emphasizing the discrete nature of the neck organ (Figure 6). The component cells are visible upon removal of the thin cuticular layer (Figure 7) and are by now of approximately equal size and development. There appear to be 50 to 60 cells in the neck organ epithelium of the nauplius in Figure 7.

Removal of the organ was found to be quite easily accomplished by microdissection of lyophilized specimens (Hootman, Harris and Conte, 1972). The zone of fracture corresponded in each case to the transitional band visible in Figure 6. This dissection exposed the hemocoelic space of the larva and the structures that lie within. Figure 16 is a dorsal view of a specimen after removal of the neck organ. The gut and the dorsal antennal myoblasts which lie atop it and insert into the second antennae may be readily observed. Weisz had observed the spindle-like muscle cells in 1947 and had counted five pairs of fibers which originated at three points along the dorsal axis of the larva. In Figure 16, at least five pairs of fibers are visible and they appear to originate at three loci along the ventral surface of the neck organ. The correspondence between Figure 4 in Weisz's paper and Figure 16 in the present work serves to substantiate the former interpretation which was based upon observation of sectioned specimens. The manner in which the dorsal muscles attach to the neck organ, if indeed they do, is not readily discernible in Figure 16. The indicated points of origin were therefore observed in thin-sectioned nauplii and the resulting micrographs will be discussed later in this chapter.

Light Microscopy

Epon sections (Figures 8 and 9) were found to be useful in

obtaining an accurate overall picture of the neck organ. Careful measurement of frontal sections through the gland gave maximum diameters of 180 to 200 μm, values in close accord with those obtained from scanning electron micrographs and photographs of whole animals. Arrangement of cells within the organ imparts to it a roughly radial symmetry when viewed in frontal section. In both Figures 8 and 9, this symmetry is evident as cells appear to radiate laterally from a point at the apex of the organ.

The most conspicuous aspects of neck organ cells at this level of observation are the large nuclei with their prominent nucleoli. The nuclei are most often located in the basal portions of the cells where they are surrounded by abundant yolk platelets. Large numbers of these yolk storage droplets are observed in most of the cells of the nauplius at this stage and give the larva an orange color which fades as these lipid reserves are subsequently metabolized.

Figure 8 is a section through the most basal area of the organ and is notable for the portion of a muscle cell visible to the right of the center of the gland. The presence of a myoblast at this level indicates a point of attachment although a definite contact is not readily discernible.

Transmission Electron Microscopy

The question of the functional nature of the neck organ epithelium

can best be answered from a morphological point of view by electron microscopy. Figures 10 and 11 illustrate the typical appearance of a neck organ cell. Each cell is characterized by nuclear, cytoplasmic and surface specializations pertinent to its alleged role in the salt-secretory capabilities of the gland.

As mentioned previously, the nuclei of these cells are quite large and are situated near the ventral margins of the cells. Measurements from a number of micrographs have indicated a maximum diameter of 15 μm for the round to oval nuclei. Different fixatives alter the appearance of the nuclei as shown in Figures 10 and 11. Figure 10 is a micrograph of a neck organ cell from a glutaraldehyde-osmium fixed specimen. It clearly illustrates the typical appearance and position of nuclei within these cells. The nucleoplasm of cells fixed in this manner is surprisingly homogeneous with little indication of extensive heterochromatic regions. The nucleoli are, however, quite prominent and may number as many as five in any one nucleus. The usual number observed per nucleus is three. At low magnification the nucleoli appear to be compact and of uniform density, although at high magnification both granular and fibrillar regions may be readily identified. In many cells the central amorphous regions of the nucleoli contain one or more vacuoles.

Osmium fixation results in a clearly different nuclear morphology. While nucleoli are still observed, they appear to occupy a lesser

percentage of the nuclear volume. Heterochromatic regions, on the other hand, are much more conspicuous (Figure 11). They appear as darkly staining granular aggregates distributed throughout the nucleoplasm and along the nuclear periphery. It should be noted that this difference in appearance of the nuclei is just the opposite of what one would expect on the basis of previous studies of the effects of chemical fixatives upon nuclear morphology (see Fawcett, 1966; Wolfe, 1972). No explanation will be offered here for this difference.

The bulk of the neck organ cell is composed of a labyrinth of smooth endoplasmic reticulum which is strikingly similar to the smooth-surfaced tubular reticulum of the chloride cells of teleost gills (Conte, 1969). This elaborate system of labyrinthine sinusoids occupies the majority of the cellular volume (Figures 10 and 11) and provides open channels from the basal areas to just below the apical surfaces of the cells. Denser regions of cytoplasm are usually confined to a thin strip underlying the apical surface of the cell and the area immediately adjacent to the nucleus. It is in the perinuclear region that most of the lipid droplets and aggregations of glycogen are found.

The neck organ cells contain large populations of mitochondria, a characteristic of cells from ion-transporting epithelia (Berridge and Oschman, 1972). The mitochondria are of varying size and shape and possess numerous, irregular cristae. They are distributed

throughout the cell but are most numerous along the prominent sinusoidal spaces. Those within the labyrinth of tubular reticulum are enclosed in capsule-like extensions of the cell cytoplasm with the cytoplasmic membranes lying in close proximity (150 to 250 A) to the outer mitochondrial membrane (Figures 12 through 15). This apposition of mitochondrial and sinusoidal membranes gives these organelles a trilamellar appearance at high magnification (Figure 15). Where aggregations of mitochondria occur, they generally assume the form of regular stacks (Figures 12 and 13). Each mitochondrion in the stack is separated from those adjacent to it by the apposed sinusoidal membranes which surround each and by a central canaliculus which appears to be a constricted part of the sinusoid (Figure 13). These complexes are nearly identical to the "mitochondrial pumps" (Copeland, 1967) of the branchial metepipodite epithelium of the adults. The primary difference is one of complexity. The number of mitochondria in an individual stack from a neck organ cell is generally much less, averaging about four and is never more than eight or nine. End-to-end alignments of mitochondria are also present in these cells, often lining the channels which connect the sinusoids with the hemocoelic space and constricting the channel into a number of smaller canaliculi.

Most other cytoplasmic organelles are poorly represented with the exception of rough endoplasmic reticulum cisternae which are

scattered throughout the cell. Golgi complexes, visible only in osmium fixed specimens, are small and occur mainly in the area immediately beneath the apical cell surface.

The surfaces of the neck organ cell also appear to be specialized in such a manner as to facilitate the transport of fluids and possibly of solutes. The neck organ epithelium does not possess a basement lamina (Figure 10), in contrast to the underlying gut epithelium or the surrounding squamous epidermal tissue. This situation allows the neck organ cells to be in direct contact with the hemocoelic fluid at their ventral margins and may allow hemocoelic fluid to pass virtually unimpeded to points within a very few micrometers of the cuticular surface.

The lateral cell surfaces are virtually indistinguishable in neck organ cells of 30 to 36 hour nauplii. The sinusoidal areas make identification of cellular boundaries impossible due to their extreme complexity when viewed in thin section. Only near the apical surfaces are the boundaries of adjacent cells distinguishable due to the presence of septate desmosomes (Figures 10, 11 and 14) between the apposed plasma membranes. These specialized zones of cell contact vary from 1 to 4 μm in length and may form a continuous junctional complex around the perimeters of the cells. In most micrographs, the intermembrane space appears to be filled with a dense, amorphous material (Figure 14), although in others the distinct periodicity

characteristic of septate desmosomes is observed. The apical terminus of the septate desmosome is generally characterized by an inconspicuous adhering zonule.

The apical surface itself consists of four zones. The first of these is the exocuticle, a uniform layer approximately 200 A thick (Figure 14). It is presumably this layer that is shed during each ecdysis of the larva. Immediately beneath the exocuticle lies a region which varies from 0.15 to 0.25 μm in thickness and is composed of a moderately electron-dense material which may be cuticle precursor. This second layer overlies the most apical zone of the neck organ cells. This zone is approximately 1 μm thick and consists of highly folded invaginations of the apical plasma membranes (Figures 11, 14 and 15). These infoldings are extremely irregular with respect to their orientation but appear to be villous in longitudinal section and tubular in cross section. Their occurrence greatly increases the apical surface area of the neck organ cells. Below the zone of apical infolding and immediately above the cytoplasmic labyrinth lies the fourth apical zone. This is a thin layer of dense cytoplasm which contains clusters of mitochondria (Figure 14), rough endoplasmic reticulum cisternae, and a few Golgi complexes. It provides the only appreciable barrier to free fluid transport from the hemocoel to the apical cell surfaces.

The preceding description of the neck organ bears mainly on its

possible function in salt transport. As mentioned in the introduction, another role for the neck organ has been suggested, that of a muscle attachment organ. In Figure 16, discussed previously, three points of attachment of the dorsal antennal muscles to the neck organ were indicated. Figures 17 and 18 illustrate the fine structure of these points. As the antennal muscles approach the dorsal midline of the larva, they bend upward slightly and converge to a small area at the base of the neck organ. The terminal Z lines of the myofibrils are modified into finger-like or knob-like processes which appear to complex with each other and form a small knot of tissue just ventral to a central neck organ cell. The extracellular space surrounding this muscle focus is filled with an irregular array of collagen-like fibrils 100 to 150 A in diameter (Figure 18). These fibrils are only observed in the proximity of the muscle foci and do not extend to an appreciable extent beneath surrounding neck organ cells. This fibrous mat appears to be the only connection between the dorsal antennal myoblasts and the neck organ cells, since no direct cell-to-cell contacts have been observed. It thus appears that this seemingly tenuous arrangement represents the mode of muscle attachment to the neck organ first observed by Weisz (1947).

IV. DISCUSSION

The dorsal or cephalic gland, otherwise known as the neck organ, is a common feature of the nauplius larvae from a variety of crustaceans (Dejdar, 1930), including both fresh- and saltwater species. In closely allied forms such as Branchinecta and Artemia (Branchiopoda, Anostraca), its external appearance is virtually identical. Furthermore, its existence in both species is transitory, since the organ degenerates during the late larval instar stages. This fact suggests that the neck organ serves an exclusively larval function that is either taken over by a separate organ in the adult or is dispensed with entirely. Previous studies had indicated that the organ in Artemia was involved in salt secretion and locomotion, functions performed by the phyllopodia in mature animals. The present work presents fine structural evidence which supports the above hypotheses.

Preliminary physiological work (Conte, Hootman and Harris, 1972) has shown that nauplii of Artemia are able to resist fluctuations in their body fluid sodium levels over a wide range of external salinities. Their ability to cope with the ionic and osmotic problems generated by extremes of salinity implies the existence of specialized mechanisms for the absorption or secretion of salts. In other animals, this mechanism takes the form of highly differentiated epithelial sheets or tubes composed of cells which exhibit characteristic

morphological and enzymatic specializations. The component cells of organs involved in salt and water transport adhere closely to a general organizational pattern, with only minor differences being noted among the various animal phyla. Typically, cells from salt-transporting epithelia possess numerous mitochondria and show extensive amplifications of apical, basal, and lateral plasma membranes. These epithelia also commonly contain high quantities of enzymes implicated in ion transport; such enzymes as the Na+K-activated ATPase first demonstrated by Skou in 1957 (see reviews by Bonting, 1971; Katz and Epstein, 1967; Skou, 1965).

The ultrastructural morphology of transporting epithelia in crustaceans has been the subject of a number of recent studies. Tissues of this type have been identified in the isopod hindgut (Holdich and Ratcliffe, 1970), the crab gill (Copeland, 1968), the caryfish gill (Bielawski, 1971; Fisher, 1972), the brown shrimp branchial chamber (Talbot, Clark and Lawrence, 1972a) and the antennal gland of the fiddler crab (Schmidt-Nielsen, Gertz and Davis, 1968).

Fine structural studies of osmoregulatory tissues in Artemia have previously been confined to the adult animals. The ultrastructure of both the branchial metepipodites (Copeland, 1967) and the maxillary glands (Tyson, 1968, 1969) have been investigated. Each, it appears, represents a discrete approach to the removal of salts from the

hemolymph. Since little physiological work aside from Croghan's studies (1958a-d) has been done concerning osmotic and ionic regulation in Artemia, the exact roles of the two organ systems are at present unclear. Croghan's work on the physiology of the branchiae (1958c) had shown that destruction of the branchial metepipodite epithelium causes a loss in the ability of the animals to osmoregulate. Specifically, they lost the ability to secrete or absorb NaCl against the concentration gradients at high and low external salinities. It appears, therefore, that the metepipodites are the sites of active NaCl secretion and absorption. The maxillary glands may also excrete NaCl, as is the case in other crustaceans (Riegel, 1963, 1965), but they appear to be limited in their regulatory capacity since they cannot compensate for loss of the metepipodites. These glands are functionally analogous to vertebrate nephrons and quite possibly play a more important role in the excretion of other ions and metabolic wastes (see review by Kirshner, 1967).

Warren (1938) has reported that the maxillary glands of Artemia are not fully differentiated until the sixth instar. Nor are the branchial metepipodites, products of metameric development, present in the early non-segmental nauplius. The absence of these two osmoregulatory organ systems does not, however, restrict the larvae to environments of intermediate salinity. The functions performed by the maxillary glands and the metepipodites in the adults may be carried

out by the antennal glands and the neck organ, respectively, in the larval forms. The antennal glands were not investigated during the present study, so their fine structural morphology must for the present remain in doubt. Light microscopic studies by Warren (1938) showed that the paired glands were located in the hemocoel at the bases of the second antennae and that they appeared to be functional only from the second through the tenth instar. Since nothing is known about the physiology of the antennal glands, predictions as to their function must be based on analogies drawn from studies on other crustaceans. Unfortunately, there is no physiological or ultrastructural study in the literature dealing with the antennal glands of larval branchiopods. It is probable, however, that the antennal glands function to a limited extent in the excretion of nitrogenous metabolic end products. Nauplii of the brine shrimp are exclusively ammonitelic (Bellini and DeVincentiis, 1960), and it may be that ammonia is the chief excretory product of the antennal glands.

The neck organ, on the other hand, appears to be functionally analogous to the metepipodites and probably serves as the major site of NaCl secretion. Specific precipitation of AgCl over the neck organ as early as the E-2 stage implies that determination of the neck organ epithlium is one of the earliest events in embryonic development of the brine shrimp. Since the larvae emerge, in most cases, into media of marked hypo- or hypertonicity, the ability to osmoregulate is a

prerequisite for survival. One would therefore expect the neck organ cells to have become functional to some extent by the time of emergence. This, indeed, appears to be the case.

These observations raise the question of when determination of those cells destined to comprise the neck organ occurs. It is possible that the neck organ cells are determined in the blastula stage. Two cell types have been reported in the desiccated cysts; small, diploid cells which comprise the majority of the cell mass and a much smaller number of very large, possibly polyploid cells (Nakanishi et al., 1962, 1963). The neck organ cells are the most likely candidates for polyploidy of all the cell types in the early nauplius, as evidenced by the characteristics of their interphase nuclei. In addition, Nakanishi et al. (1962) observed about 40 of the large-sized nuclei per cyst, a figure that agrees well with estimates of the number of cells in the neck organ. These observations indicate that development of the neck organ epithelium represents an unusually early commitment of embryonic resources to the problems of ionic and osmotic regulation.

Ultrastructural studies have lent ample support to the histochemical data which suggests that the neck organ is actively involved in ion transport. The ultrastructural specializations observed in neck organ cells are typical of solute transporting epithelia. Chief among these modifications is the amplification of the plasma membrane, particularly at the apical cell surface. These extensive infoldings of

the apical cell membrane occur in the osmoregulatory tissues of other arthropods (Bielawski, 1971; Copeland, 1967, 1968; Talbot, Clark and Lawrence, 1972a) at the cell-cuticle interface. Two roles for these infoldings have been suggested. Gupta and Berridge (1966) have postulated that the "apical leaflets" of the blowfly rectal papillae cells are modified as sites for transport enzyme systems. Copeland's studies of the brine shrimp metepipodite epithelium (1967), however, have led him to suggest a second function. He has proposed that the infoldings in those cells may be associated with secretion of the cuticle. It should be emphasized that the two roles presented here are not mutually exclusive in any cell. The apical infoldings of the neck organ cells may therefore serve both of the above functions, although the former is more likely since squamous epidermal cells covering the majority of the nauplius lack these apical specializations yet presumably also secrete cuticle.

Perhaps the most striking ultrastructural features of the neck organ cell are the cytoplasmic labyrinth and the mitochondrial complexes observed within it. The labyrinth itself is formed by ramification of basal and lateral plasma membrane infoldings into an extensive network of tubular reticulum similar to that observed in the teleost chloride cell (Kessel and Beams, 1962; Philpott and Copeland, 1963) and the labyrinth cell (Bertmar, 1972). So complex is this system that the lateral cell membranes are indistinguishable in

well-developed neck organs. The lack of any identifiable cell boundaries below the apical septate desmosomes suggests that maturation of the secretory epithelium involves a fusion of lateral plasma membranes between adjacent cells. An alternate explanation may be that the lateral labyrinthine areas are simply intercellular spaces which are filled with wildly interdigitating projections from adjacent cells. In either case, the presence of labyrinthine sinusoids reaching from the basal surface to within a few micrometers of the apical plasma membrane is significant with respect to the solute-transporting capabilities of the neck organ. As previously suggested, these channels allow fluid from the hemocoel to flow freely upward toward the cuticle until it reaches the narrow band of dense cytoplasm which underlies the zone of apical infolding. Thus the sinusoids may be described as dead-ended channels, albeit exceedingly complex ones, into which solute-laden hemolymph may be drawn. This sytem fits very well the "backward" standing gradient model developed by Diamond and Bossert (1968) to explain fluid transport in such diverse tissues as the elasmobranch rectal gland and the insect Malpighian tubule. Each of these secretory epithelia possesses backward inter- or intracellular channels opening in the direction from which fluid is being transported, and each is to some degree involved in NaCl secretion. By a combination of active solute transport out of the channels and the effects of bulk flow and osmotic filtration, solutes and

water may be drawn into the cytoplasm of the cell. The resulting hydrostatic and osmotic gradients favor secretion of a hypertonic fluid at the apical surface. The efficiency of this system depends upon the characteristics of the apical and basal plasma membranes, particularly with respect to forward pumping and backward diffusion of solutes. Application of Curran's double membrane model (Curran, 1965; Curran and MacIntosh, 1962) shows that a hypertonic fluid can be secreted if the channel membranes provide sites for solute pumps and are otherwise solute-impermeable. The apical membranes may be either porous and non-selective or equipped with solute pumps. These last two possibilities suggest that amplification of apical plasma membranes may be an adaptation which serves simply to increase the surface area available for either active or passive solute transport.

Passage of ionic solutes and water across the type of secretory epithelium outlined above necessitates active solute pumping at one or more cell surface. This, in turn, requires an abundance of metabolic energy for transport, generally thought to be supplied by generation of ATP during mitochondrial oxidative phosphorylation. It is significant in this respect that most transporting epithelia possess numerous mitocchondria in close association with one or the other (or both) cell surfaces. The neck organ cells are no exception and, in fact, possess a highly specialized mitochondrial complex previously

implicated in ion transport. This complex is a somewhat simpler version of the "mitochondrial pump" first described by Copeland (1964) in the anal papillae of the mosquito larvae. Similar structures have subsequently been described in the metepipodite epithelium of Artemia (Copeland, 1966, 1967) and the rectal papillae of the blowfly Calliphora (Gupta and Berridge, 1966). The most conspicuous feature of mitochondrial pumps is the compression of intracellular sinusoids to form narrow lamellae which are enclosed between adjacent, parallel mitochondria. The close association between mitochondrial and lamellar membranes is most probably a specialization related to the energetic demands of the ion-transporting mechanism. Intimate juxtaposition of the energy source (mitochondrial oxidative phosphorylation) and the pumping sites (membrane-bound transport enzymes) may allow a shorter, and thus more efficient, path for diffusion of ATP between the two loci. This, however, does not explain the peculiar stacking of mitochondria seen in these complexes, since juxtaposition of mitochondrial and sinusoidal membranes is a common occurrence throughout the cell. A satisfactory explanation for the existence of these unusual complexes has yet to be offered, although their occurrence in two arthropods (Culex and Artemia) that face extreme problems in ionic regulation suggests that their structure confers a particularly high degree of efficiency in ion transport.

Although histochemical and ultrastructural evidence gathered to

date supports the contention that the neck organ is a larval salt gland, one part of the picture is still largely missing. The ability of the neck organ cells to accumulate and secrete salts will inevitably depend both on the architecture of the cells and on the presence of membrane-linked solute pumps. Other tissues which exhibit the fine structural modifications seen in the neck organ generally have been found to be rich in Na+K-activated ATPase. Although the nauplius as a whole exhibits a high specific activity of this enzyme (Conte, Ewing and Peterson, in preparation), correlation of transport ATPase activity with the neck organ tissue has been hampered biochemically by the small size of the organ and histochemically by fixation difficulties. Histochemical localizations of Na+K-activated ATPase have, however, been successful in other tissues and analogies may be drawn. Localizations have been attempted on the rectal papillae of the blowfly (Berridge and Gupta, 1968), the salt glands of marine birds (Ernst, 1972a, b; Ernst and Philpott, 1970), the chloride cells of euryhaline fish (Ernst and Philpott, 1970; Mizuhira et al., 1970), and the sheep ruminal epithelium (Henrikson, 1971) among others. Although questions have been raised concerning the validity of the procedures used in some of the above studies, when taken together they indicate that Na+K-activated ATPase enzymes are located primarily within the amplified basal and lateral plasma membranes. If the neck organ epithelium does contain a substantial quantity of this enzyme, then we

may infer by analogy that the membranes of the tubular reticulum will be the sites of the sodium pumps.

It is worth noting with regard to the nature of the solute pumps that a small body of evidence is growing for the existence of a membrane-linked chloride pump in transporting epithelia (Huf, 1972; Kristensen, 1972; Motais and Garcia-Romeu, 1972). Two correlated pieces of evidence suggest that a chloride-transporting mechanism contributes to salt secretion in the neck organ. That the neck organ is able to accumulate chloride ions early in the E-2 stage has been demonstrated histochemically in this thesis. Other work, however, has shown that the Na+K-activated ATPase activity in the larvae is essentially zero until after hatching of the nauplius (Droukas, 1973). The theory that chloride ion is passively accumulated as a result of active accumulation of sodium cannot satisfactorily explain these observations, and thus suggests the possibility of active chloride pumping in the larval forms of the brine shrimp as an area for further investigation.

The structural modifications which set the neck organ apart from the rest of the larval epidermis may, in part, have resulted from its enlistment as a point of origin for the dorsal muscles of the antennae. If so, it represents a radical departure from the typical mode of myo-cuticular articulation observed in other arthropods. Although only a few ultrastructural studies have been carried out on

crustacean material (Bouligand, 1962; Koulish, 1973; Talbot, Clark and Lawrence, 1972b), the epidermal specializations observed are nearly identical to those from insects (Caveney, 1969; Lai-Fook, 1967) and arachnids (Smith, Järlfors and Russell, 1969). In each class, attachment of muscles to the cuticle is accomplished through the interpolation of highly differentiated epidermal cells. These cells appear to be functionally analogous to the tendons which connect skeletal muscle to bone in vertebrates and have therefore been called "tendon cells." Their role in the transferral of muscle tension to the exoskeleton is reflected in specializations both within the cytoplasm of the cells and at the cell surfaces. Chief among these are the conspicuous masses of oriented microtubules within the dense cytoplasm of the tendon cells and the extensive interdigitations between tendon cells and muscle fibers. The tendon cell-cuticle interface is characterized by hemidesmosomes on the cell plasma membrane which appose plaques of electron-dense material at the inner surface of the endocuticle (Smith, Järlfors and Russell, 1969).

In the generalized situation described above, muscles are inserted on schlerotized cuticular plates or on inwardly projecting apodemes. These provide a rigid point of origin for the muscle to work against. In the developing larvae of Artemia and other branchiopod crustaceans, however, the extreme thinness of the cuticle and its nonschlerotized nature render it less able to provide the rigidity

necessary for rapid movement. Contraction of the antennal muscles would cause significant deformation of the cuticle if the rigidity of the dorsal region depended solely upon the cuticle's mechanical resistance to stress. Clearly, some specialization was necessary to insure support for the locomotory musculature. The dome-like shape of the neck organ and the relative thickness of its component cells suggest that it is a particularly stable structure, well able to resist the pull of the dorsal muscles. The fine structure of the attachment points indicates that tension generated by muscle contraction is transferred to the apex of the neck organ which can offer the maximum resistance. But the fine structure also implies that the attachment complex is relatively weak and could be easily disrupted. This, in turn, suggests that the attachment points may not directly absorb the full force of muscle contractions. Since the dorsal muscle fibers are paired and contract and relax in synchrony, the majority of the contractile tension may be dissipated by their apposition. The attachment complexes would, then, serve mainly to stabilize the muscle origins and to take up any slack resulting from minor variations in contractile strength. The occurrence of a neck organ in the nauplius of many crustaceans can thus possibly be explained by its role in muscle support.

V. SUMMARY

Observations on the dorsal neck organ of the E-2 and nauplius larvae of the brine shrimp Artemia salina indicate that it plays an important role in both salt secretion and muscle support.

1. The neck organ is located on the dorsal cephalothorax of the larva and consists of 50 to 60 cells arranged as a simple, cuboidal epithelium. This epithelial sheet is present as a discrete organ as early as the E-2, and possibly the E-1 stage.
2. Uptake of silver ions is localized in the larval forms to the surface of the neck organ. The precipitation of silver implies that the cuticle over the neck organ is permeable and that the neck organ accumulates chloride ions.
3. The neck organ cells appear ultrastructurally to be modified for ionic and osmotic regulation. They are characterized by extensive infoldings of the apical plasma membranes and by the absence of a basal lamina. The cytoplasm of each cell is organized into a complex tubular reticulum which gives the cell a labyrinthine appearance. Open sinusoids within the labyrinth may allow hemolymph to permeate the cell to within 2 μm of the apical surface. Mitochondria are numerous within the cell and are enmeshed in the labyrinth in intimate association with the sinusoidal membranes. Parallel arrays of mitochondria in

juxtaposition with constricted sinusoidal canaliculi are frequently observed. These complexes may be intimately linked to the ion transport functions of the cells.

4. The neck organ also serves as a point of origin for the dorsal antennal musculature. These muscles originate at three points along the dorsal midline of the larva in attachment complexes of a type not previously observed in arthropods. The muscle fibers end in projections of the terminal Z bands that are embedded in mats of collagen-like fibrils at the bases of central neck organ cells. It is suggested that this method of muscle insertion is necessitated by the extreme thinness of the larval cuticle and that it may be the characteristic mode of insertion of the locomotory musculature for branchiopod nauplii in general.

BIBLIOGRAPHY

Bellini, L. and D. M. DeVincentiis. 1960. Observations on the end products of protein metabolism in diploid and tetraploid Artemia salina (Leach). Experimental Cell Research 21:239-241.

Berridge, M. J. and B. L. Gupta. 1968. Fine-structural localization of adenosine triphosphatase in the rectum of Calliphora. Journal of Cell Science 3:17-32.

Berridge, M. J. and J. L. Oschman. 1972. Transporting epithelia. New York: Academic Press.

Bertmar, G. 1972. Labyrinth cells, a new cell type in vertebrate olfactory organs. Zeitschrift für Zellforschung und Mikroskopische Anatomie 132:245-256.

Bielawski, J. 1971. Ultrastructure and ion transport in gill epithelium of the crayfish, Astacus leptodactylus Esch. Protoplasma 73:177-190.

Bonting, S. L. 1971. Sodium-potassium activated adenosine triphosphatase and cation transport. In: Membranes and ion transport, Vol. I, ed. E. E. Bittar. New York: John Wiley and Sons, p. 257.

Boone, E. and L. G. M. Baas-Becking. 1931. Salt effects on eggs and nauplii of Artemia salina L. Journal of General Physiology 14:753-763.

Bouligand, Y. 1962. Les ultrastructures du muscle strié et de ses attaches au squelette chez les cyclops (Crustacés, Copépodes). Journal de Microscopie 1:377-394.

Carpelan, L. 1957. Hydrobiology of the Alviso salt ponds. Ecology 38:375-390.

Caveney, S. 1969. Muscle attachment related to cuticle architecture in Apterygota. Journal of Cell Science 4:541-559.

Cohen, A., D. P. Marlow, and G. E. Garner. 1968. A rapid critical point method using fluorocarbons ("Freons") as intermediate and transitional fluids. Journal de Microscopie 7:331-342.

Conte, F. P. 1969. Salt secretion. In: Fish physiology, Vol. I, eds. W. S. Hoar and D. J. Randall. New York: Academic Press, pp. 241-292.

Conte, F. P., R. D. Ewing, and G. L. Peterson. Characterization and localization of Na+K-activated ATPase from Artemia salina nauplii. Manuscript in preparation.

Conte, F. P., S. R. Hootman, and P. J. Harris. 1972. Neck organ of Artemia salina nauplii (a larval salt gland). Journal of Comparative Physiology 80:239-246.

Copeland, E. 1964. A mitochondrial pump in the cells of the anal papillae of mosquito larvae. Journal of Cell Biology 23:253-263.

_____. 1966. Salt transport organelle in Artemia salenis (brine shrimp). Science 151:470-471.

_____. 1967. A study of salt-secreting cells in the brine shrimp (Artemia salina). Protoplasma 63:363-384.

_____. 1968. Fine structure of salt and water uptake in the land crab, Geocarcinus lateralis. American Zoologist 8:417-432.

Croghan, P. C. 1958a. The survival of Artemia salina (L.) in various media. Journal of Experimental Biology 35:213-218.

_____. 1958b. The osmotic and ionic regulation of Artemia salina (L.). Journal of Experimental Biology 35:219-233.

_____. 1958c. The mechanism of osmotic regulation in Artemia salina (L.): the physiology of the branchiae. Journal of Experimental Biology 35:234-242.

_____. 1958d. The mechanism of osmotic regulation in Artemia salina (L.): the physiology of the gut. Journal of Experimental Biology 35:243-249.

Curran, P. F. 1965. Ion transport in intestine and its coupling to other transport processes. Federation Proceedings 24:993-999.

Curran, P. F. and J. R. MacIntosh. 1962. A model system for biological water transport. Nature 193:347-348.

Dejdar, E. 1930. Die korrelationen zwischen kiemensacken und nackenschild bei phyllopoden (versuch einer analyse mit hilfe elektiver vitalfarbung). Zeitschrift für wissenschaftliche Zoologie 136:422-452.

Diamond, J. M. and W. H. Bossert. 1968. Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. Journal of Cell Biology 37:694-702.

Doyle, W. L. and F. H. Epstein. 1972. Effects of cortisol treatment and osmotic adaptation on the chloride cells of the eel, Anguilla rostrata. Cytobiologie 6:58-73.

Droukas, P. C. 1973. The role of glycerol in ion and osmoregulation during excystment in Artemia salina. M. S. thesis, Oregon State University, Corvallis.

Dutrieu, J. 1960. Observations biochemiques et physiologiques sur le développement d'Artemia salina Leach. Archives de zoologie experimentale et generale 99:1-134.

Ernst, S. A. 1972a. Transport adenosine triphosphatase cytochemistry. I. Biochemical characterization of a cytochemical medium for the ultrastructural localization of ouabain-sensitive, potassium-dependent phosphatase activity in the avian salt gland. Journal of Histochemistry and Cytochemistry 20:13-22.

_____. 1972b. Transport adenosine triphosphatase cytochemistry. II. Cytochemical localization of ouabain-sensitive, potassium-dependent phosphatase activity in the secretory epithelium of the avian salt gland. Journal of Histochemistry and Cytochemistry 20:23-38.

Ernst, S. A. and R. A. Ellis. 1969. The development of surface specialization in the secretory epithelium of the avian salt gland in response to osmotic stress. Journal of Cell Biology 40:305-321.

Ernst, S. A. and C. W. Philpott. 1970. Preservation of Na-K-activated and Mg-activated adenosine triphosphatase activities of avian salt gland and teleost gill with formaldehyde as fixative. Journal of Histochemistry and Cytochemistry 18:251-263.

Evans, T. J. 1913. Observations on Artemia salina. Nature 92:385-386.

Ewer, D. J. and I. Hattingh. 1952. Absorption of silver on the gills of a freshwater crab. Nature 169:460.

Fautrez, Firlefyn, N. 1951. Etude cytochemique des acides nucléiques au cours de la gametogénése et des premiers stades du developpement embryonnaire chez Artemia salina L. Archives de Biologie 62:391-438.

Fawcett, D. W. 1966. The cell: an atlas of fine structure. Philadelphia: W. B. Saunders Company.

Fisher, J. M. 1972. Fine-structural observations on the gill filaments of the freshwater crayfish, Astacus pallipes Lereboullet. Tissue and Cell 4:287-299.

Frasca, J. M. and V. R. Parks. 1965. A routine technique for double-staining ultrathin sections using uranyl and lead salts. Journal of Cell Biology 25:157-161.

Gupta, B. L. and M. J. Berridge. 1966. A coat of repeating subunits on the cytoplasmic surface of the plasma membrane in the rectal papillae of the blowfly, Calliphora erythrocephala (Meig.), studied in situ by electron microscopy. Journal of Cell Biology 29:376-382.

Heath, H. 1924. The external development of certain phyllopods. Journal of Morphology 38:453-483.

Henrikson, R. C. 1971. Mechanism of sodium transport across ruminal epithelium and histochemical localization of ATPase. Experimental Cell Research 68:456-458.

Holdich, D. M. and N. A. Ratcliffe. 1970. A light and electron microscopic study of the hindgut of the herbivorous isopod, Dynamene bidentata (Crustacea: Pericarida). Zeitschrift für Zellforschung und Mikroskopische Anatomie 111:209-227.

Hootman, S. R., P. J. Harris, and F. P. Conte. 1972. Surface specialization of the larval salt gland in Artemia salina nauplii. Journal of Comparative Physiology 79:97-104.

Huf, E. G. 1972. Role of Cl^- and other anions in active Na^+ transport in isolated frog skin. Acta Physiologica Scandinavia 84:366-381.

Jennings, R. H. and D. M. Whitaker. 1941. The effect of salinity upon the rate of excystment of Artemia. Biological Bulletin 80:194-201.

Katz, A. I. and F. H. Epstein. 1967. The physiological role of sodium-potassium activated adenosine triphosphatase in the active transport of cations across biological membranes. Israel Journal of Medical Science 3:155-166.

Kessel, R. G. and H. W. Beams. 1962. Electron microscope studies on the gill filaments of Fundulus heteroclitus from sea water and fresh water with special reference to the ultrastructural organization of the "chloride cell." Journal of Ultrastructure Research 6:77-87.

Kirschner, L. B. 1967. Comparative physiology: invertebrate excretory organs. Annual Review of Physiology 29:169-196.

Komnick, H. 1962. Elektronenmikroskopische lokalisation von Na^+ und Cl^- in zellen und geweben. Protoplasma 55:414-418.

_____. 1963. Elektronenmikroskopische untersuchungen zur funktionellen morphologie des iontransportes in der salzdrüse von Larus argentatus. I. Teil: bau und feinstruktur der salzdrüse. Protoplasma 56:274-314.

Komnick, H. and M. Bierther. 1969. On the histochemical localization of ions by electron microscopy, with special reference to the chloride reaction. Histochemie 18:337-362.

Komnick, H., R. W. Rhees, and J. H. Abel, Jr. 1972. The function of ephemerid chloride cells: histochemical, autoradiographic and physiological studies with radioactive chloride on Callibaetis. Cytobiologie 5:65-83.

Koulish, S. 1973. Microtubules and muscle attachment in the integument of the Balanidae. Journal of Morphology 140:1-14.

Kristensen, P. 1972. Chloride transport across isolated frog skin. Acta Physiologica Scandinavia 84:338-346.

Lai-Fook, J. 1967. The structure of developing muscle insertions in insects. Journal of Morphology 123:503-528.

Lochhead, J. H. 1941. Artemia, the "brine shrimp." Turtox News 19:41-45.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. Journal of Biophysical and Biochemical Cytology 9:409-414.

Mizuhira, V., T. Amakawa, S. Yamashina, N. Shirai, and S. Utida. 1970. Electron microscopic studies on the localization of sodium ions and sodium-potassium activated adenosine triphosphatase in chloride cells of eel gills. Experimental Cell Research 59:346-348.

Morse, H. C., P. J. Harris, and E. J. Dornfeld. 1970. Pacifastacus leniusculus: fine structure of arthrobranch with reference to active ion uptake. Transactions of the American Microscopical Society 89:12-27.

Motais, R. and F. Garcia-Romeu. 1972. Transport mechanisms in the teleostean gill and amphibian skin. Annual Review of Physiology 34:141-176.

Nakanishi, Y. H., T. Iwasaki, T. Okigaki, and H. Kato. 1962. Cytological studies of Artemia salina. I. Embryonic development without cell multiplication after the blastula stage in encysted dry eggs. Annotationes Zoologicae Japonenses 35:223-228.

Nakanishi, Y. H., T. Okigaki, H. Kato, and T. Iwasaki. 1963. Cytological studies of Artemia salina. II. Deoxyribonucleic acid (DNA) content and the chromosomes in encysted dry eggs and nauplii. Proceedings of the Japan Academy 39:306-309.

Philpott, C. W. 1965. Halide localization in the teleost chloride cell and its identification by selected area electron diffraction. Protoplasma 55:7-23.

Philpott, C. W. and D. E. Copeland. 1963. Fine structure of chloride cells from three species of Fundulus. Journal of Cell Biology 18:389-404.

Richardson, K. C., L. Jarett, and E. H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technology 35:313-323.

Riegel, J. A. 1963. Micropuncture studies of chloride concentration and osmotic pressure in the crayfish antennal gland. Journal of Experimental Biology 40:487-492.

Riegel, J. A. 1965. Micropuncture studies of the concentrations of sodium, potassium and inulin in the crayfish antennal gland. Journal of Experimental Biology 42:379-384.

Schmidt-Nielsen, B., H. K. Gertz, and L. E. Davis. 1968. Excretion and ultrastructure of the antennal gland of the fiddler crab, Uca mordax. Journal of Morphology 125:473-496.

Skou, J. C. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochimica et Biophysica Acta 23:394-401.

_____. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. Physiological Reviews 45: 596-617.

Smith, D. S., U. Jarlfors, and F. E. Russell. 1969. The fine structure of muscle attachments in a spider (Latrodectus mactans, FABR.). Tissue and Cell 1:673-687.

Talbot, P. W., H. Clark, Jr., and A. L. Lawrence. 1972a. Light and electron microscopic studies on osmoregulatory tissue in the developing brown shrimp, Penaeus aztecus. Tissue and Cell 4:271-286.

_____. 1972b. Ultrastructural observations of the muscle insertion and modified branchiostegite epidermis in the larval brown shrimp, Penaeus aztecus. Tissue and Cell 4:613-628.

Tyson, G. E. 1968. The fine structure of the maxillary gland of the brine shrimp, Artemia salina: the end-sac. Zeitschrift für Zellforschung und Mikroskopische Anatomie 86:129-138.

_____. 1969. The fine structure of the maxillary gland of the brine shrimp, Artemia salina: the efferent duct. Zeitschrift für Zellforschung und Mikroskopische Anatomie 93:151-163.

Venable, J. H. and R. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. Journal of Cell Biology 25:407-408.

Warren, H. S. 1938. The segmental excretory glands of Artemia salina Linn. var principalis simon (the brine shrimp). Journal of Morphology 62:263-298.

Weisz, P. B. 1947. The histological pattern of metameric development in Artemia salina. Journal of Morphology 81:45-95.

Weldon, W. F. R. 1909. Crustacea. Cambridge Natural History, Vol. 4, Chap. II, pp. 18-54.

Wichard, W. and H. Komnick. 1971. Electron microscopical and histochemical evidence of chloride cells in tracheal gills of mayfly larvae. Cytobiologie 3:215-228.

Wichard, W., H. Komnick, and J. H. Abel, Jr. 1972. Typology of Ephemerid chloride cells. Zeitschrift für Zellforschung und Mikroskopische Anatomie 132:533-551.

Wolfe, S. L. 1972. Biology of the cell. Belmont, California: Wadsworth Publishing Company.

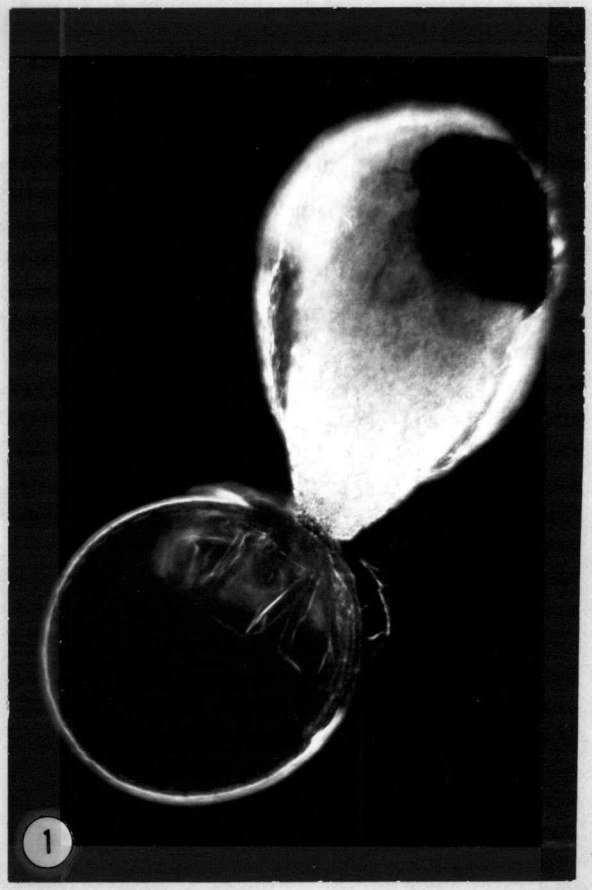
Zograf, N. 1905. La calotte cervicale chez les nauplius de l'Artemia salina. Comptes rendus hebdomadaires des seances de l'Academie des Sciences (Paris) 141:903-905.

APPENDIX

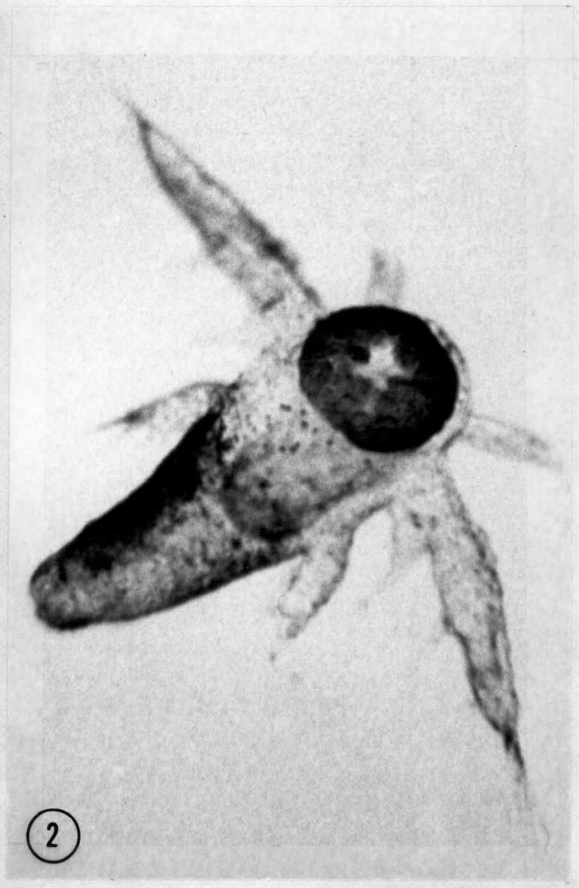
Figure 1. Twelve hour E-2 larva of A. salina stained with 0.1 M $AgNO_3$. Dense precipitates cover the area of the neck organ. 175X. 57 μm = 1 cm.

Figure 2. Newly hatched nauplius of A. salina stained with 0.1 M $AgNO_3$. Dense precipitates cover all but the central portion of the neck organ. 100X. 100 μm = 1 cm.

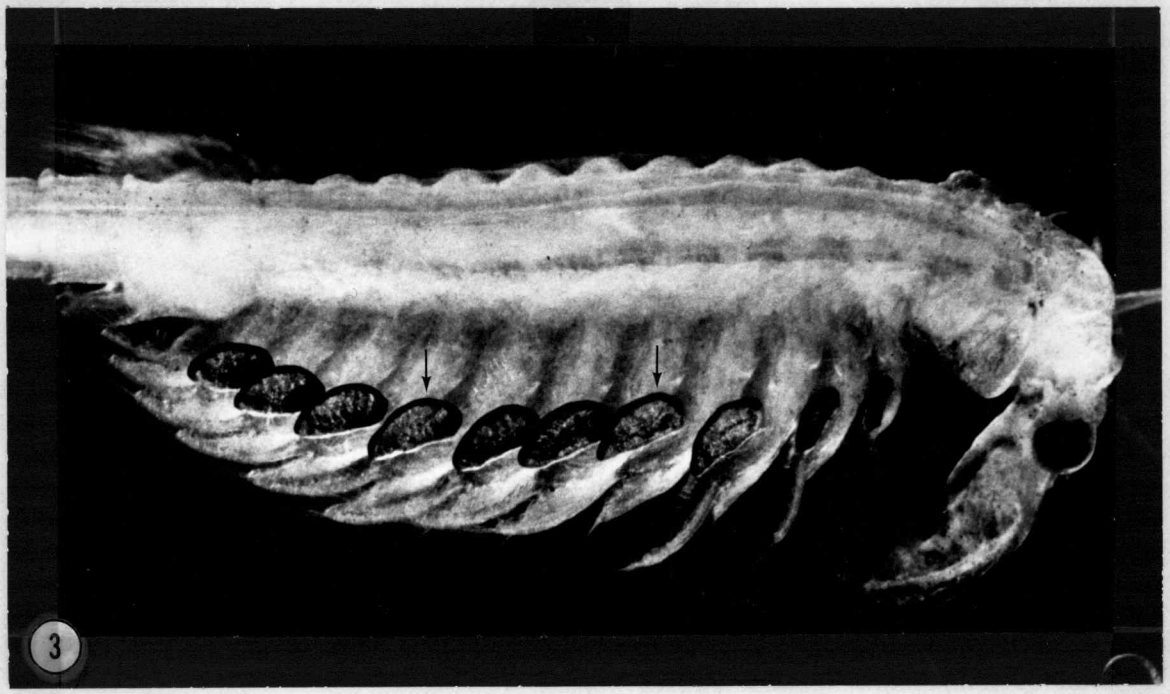
Figure 3. Adult male of A. salina stained with Ag NO_3. Dense precipitates cover the anterior ten pairs of branchial metepipodites (arrows). 20X. 500 μm = 1 cm.



1



2



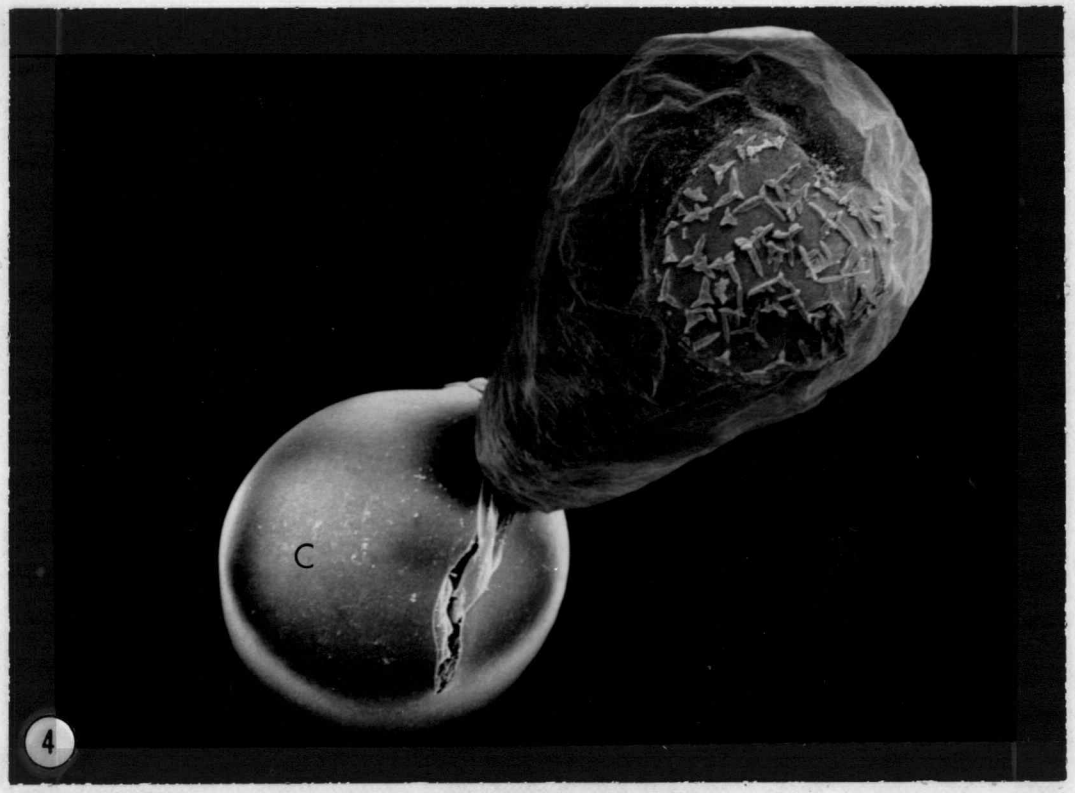
3

Figure 4. Fourteen hour E-2 larva of A. salina stained with 0.1 M $AgNO_3$. Unidentified crystals cover the area of the neck organ. Fixed in glutaraldehyde at pH 7.2 and dried by critical point substitution with Freon 113. 190X. 53 μm = 1 cm.

Figure 5. Fourteen hour E-2 larva of A. salina removed from the hatching membrane. Fixed in glutaraldehyde at pH 7.2 and dried by critical point substitution with Freon 113. 250X. 40 μm = 1 cm.

C - cyst

HM - hatching membrane


C
4

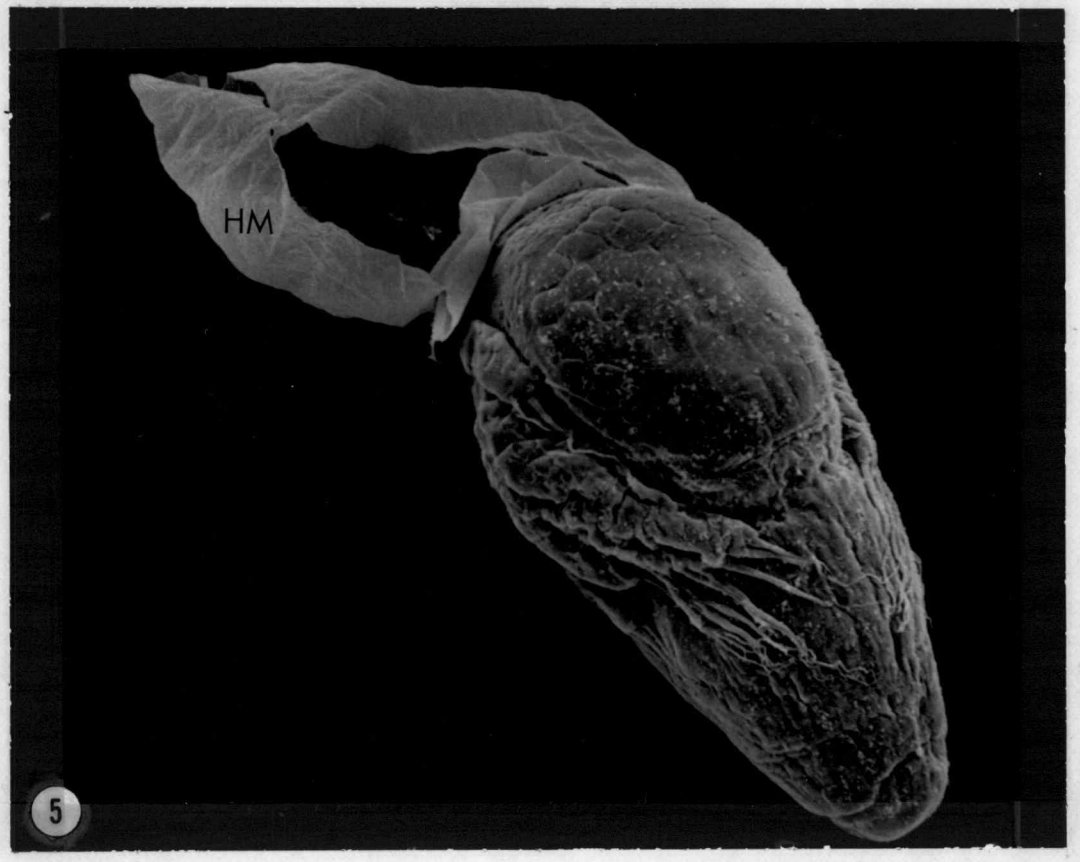
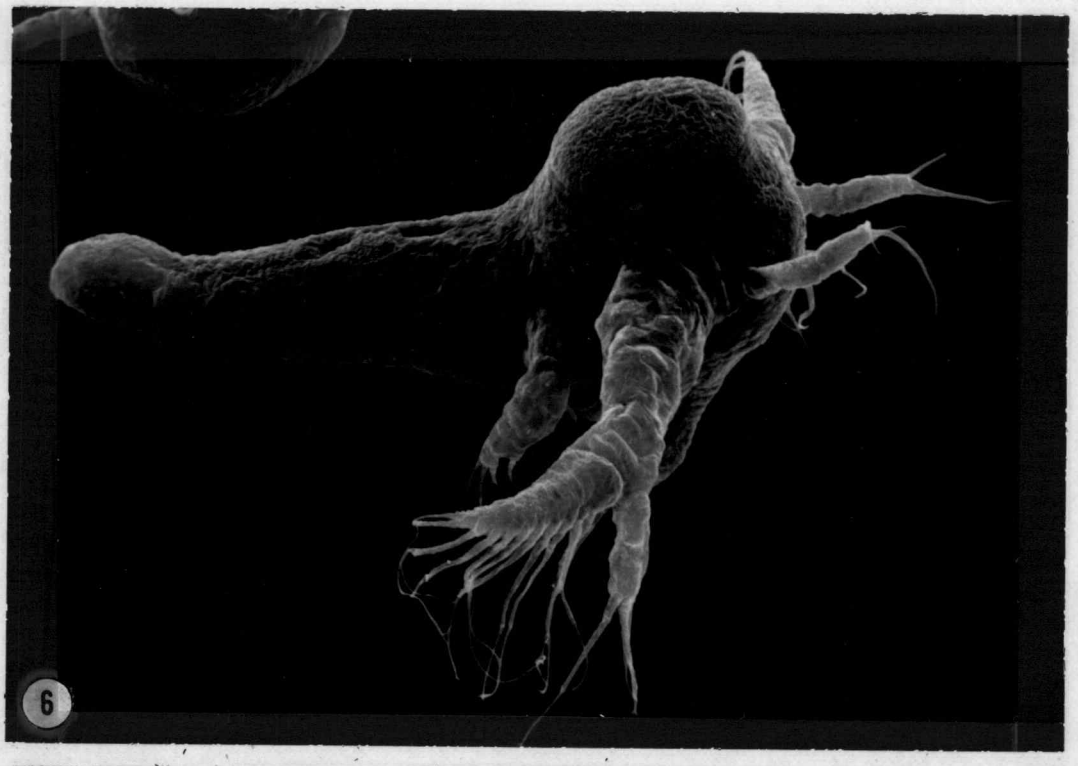
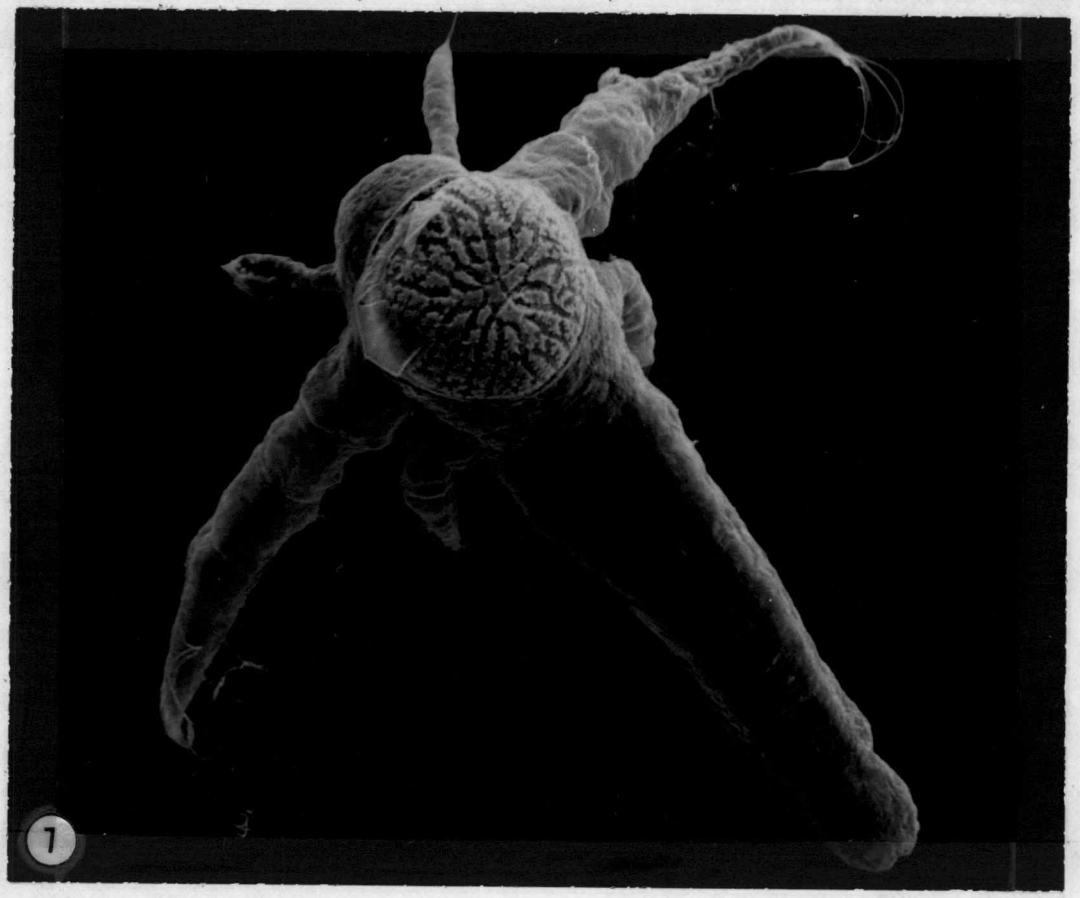

HM
5

Figure 6. Twenty-eight hour nauplius of A. salina. A distinct band of tissue separates the neck organ from the surrounding epidermis. Fixed in osmium at pH 7.2 and dried by lyophylization. 180X. 56 μm = 1 cm.

Figure 7. Thirty hour nauplius of A. salina from which the cuticle covering the neck organ has been removed. The apical surfaces of the cells which comprise the neck organ are clearly visible. Fixed in osmium at pH 7.2 and dried by lyophylization. 200X. 50 μm = 1 cm.



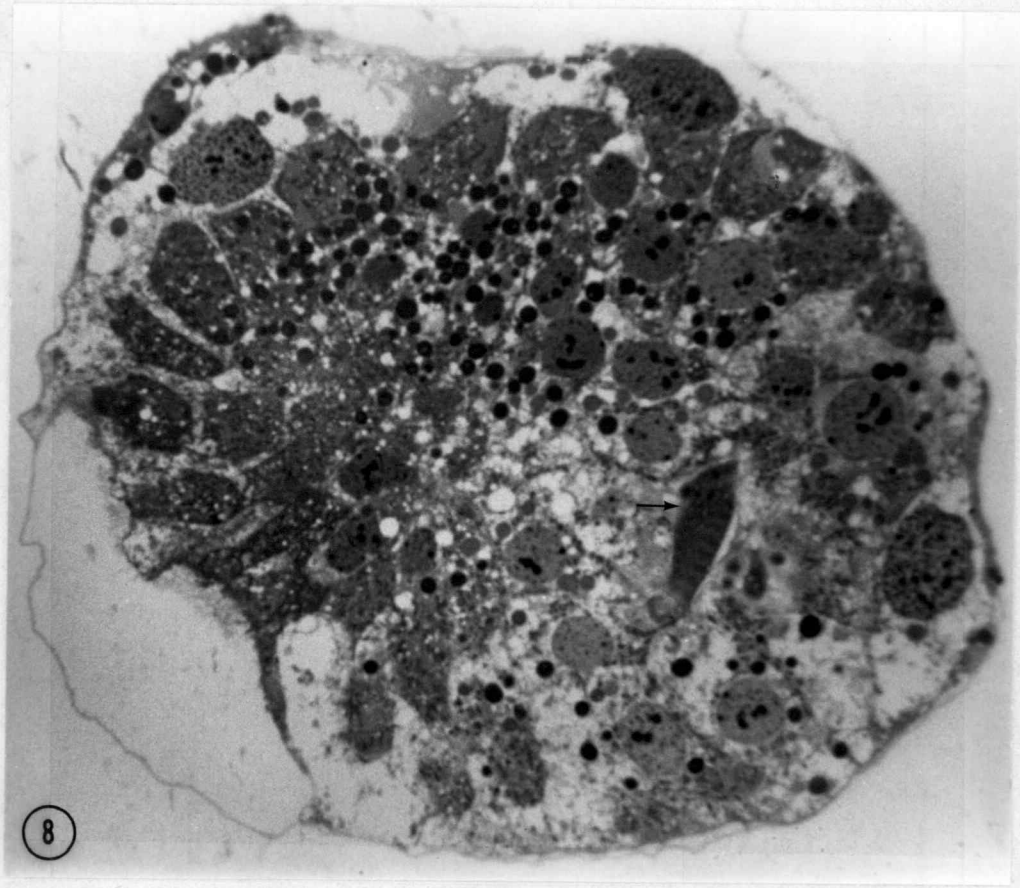
6



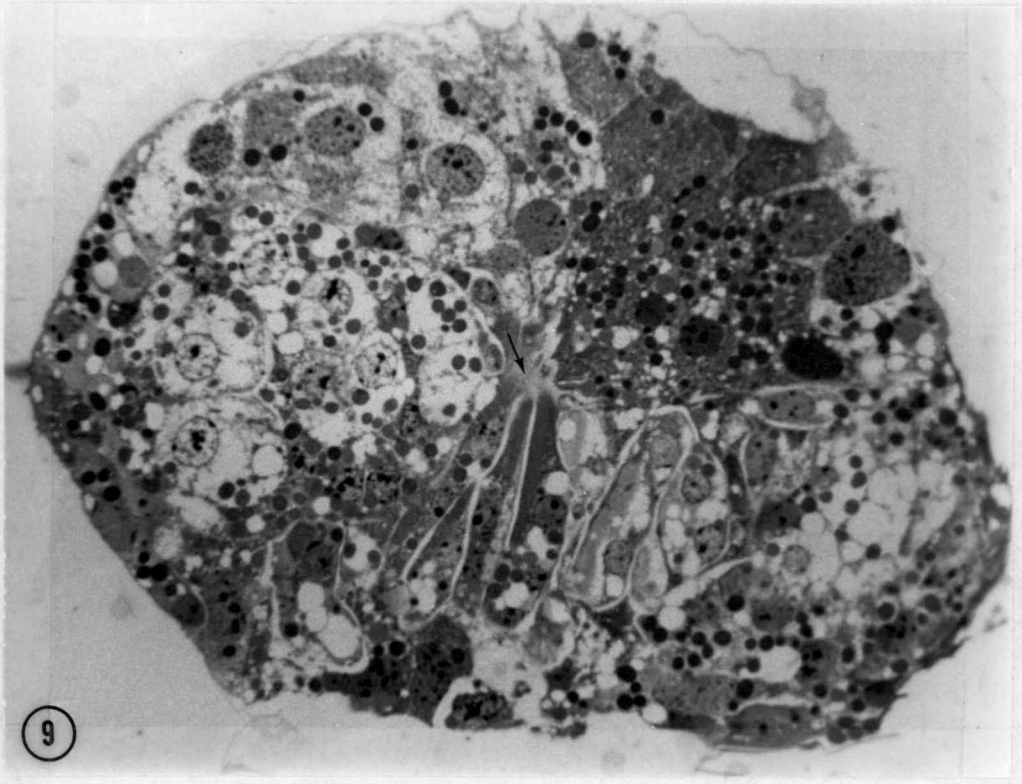
7

Figure 8. Frontal section through the basal area of the neck organ of a 36 hour nauplius. A portion of a muscle cell (arrow) is present. 520X. 19 μm = 1 cm.

Figure 9. Frontal section through the neck organ of a 30 hour nauplius. A central point of muscle attachment (arrow) is visible. 500X. 20 μm = 1 cm.



8



9

Figure 10. Neck organ cell from a 36 hour nauplius of A. salina. Cell boundaries are only discernible by the presence of septate desmosomes (arrows). Glutaraldehyde-osmium fixation at pH 7.2. 9,500X. 1.05 μm = 1 cm.

Cu - cuticle

G - glycogen

L - lipid droplet

m - mitochondrion

N - nucleus

Nu - nucleolus

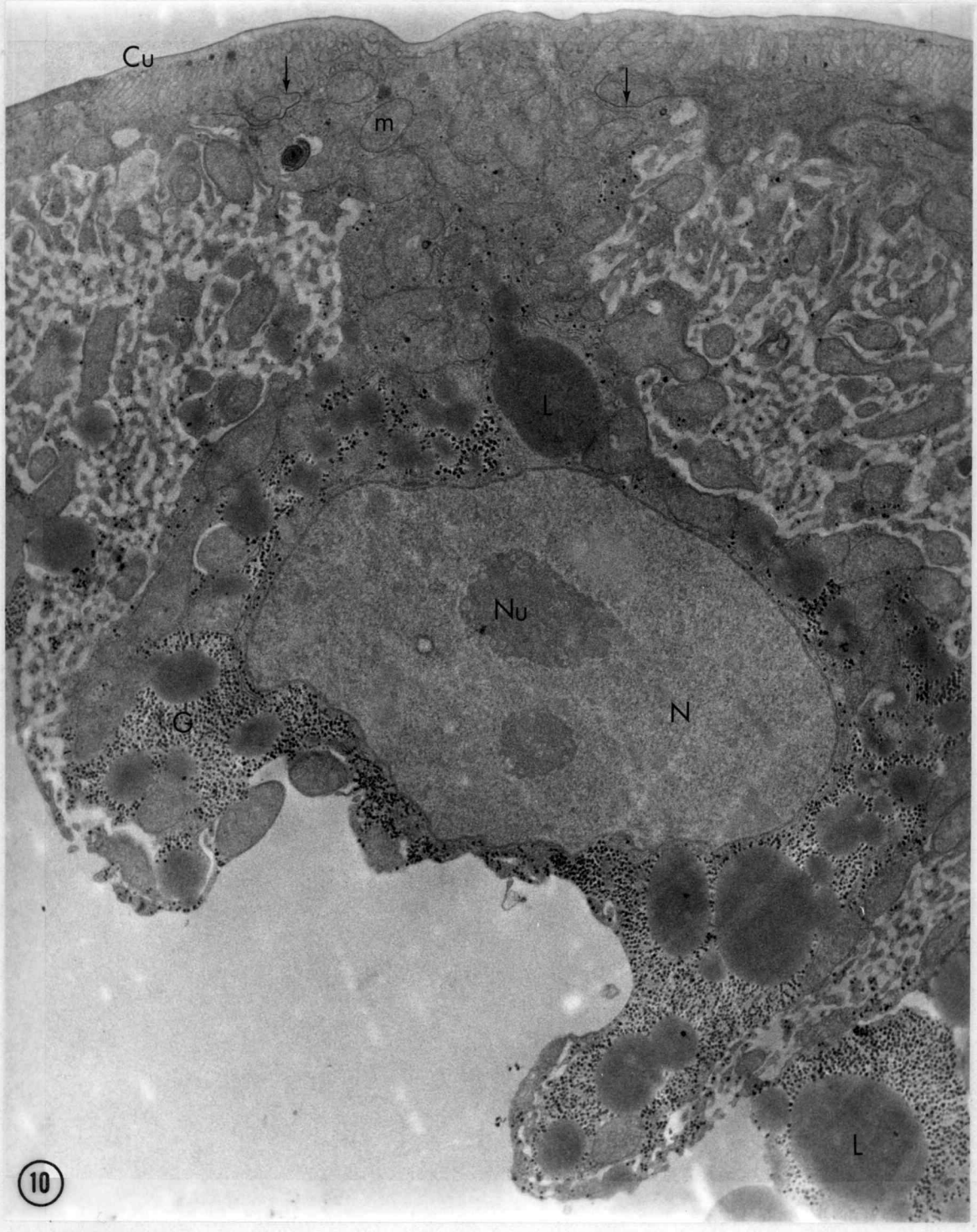

Cu
m
L
Nu
N
G
L
10

Figure 11. Neck organ cell from a 30 hour nauplius of A. salina fixed in osmium at pH 7.2. 14,200X. 0.7 μm = 1 cm.

ai - apical plasma membrane infoldings

Cu - cuticle

g - Golgi complex

m - mitochondrion

N - nucleus

sd - septate desmosome

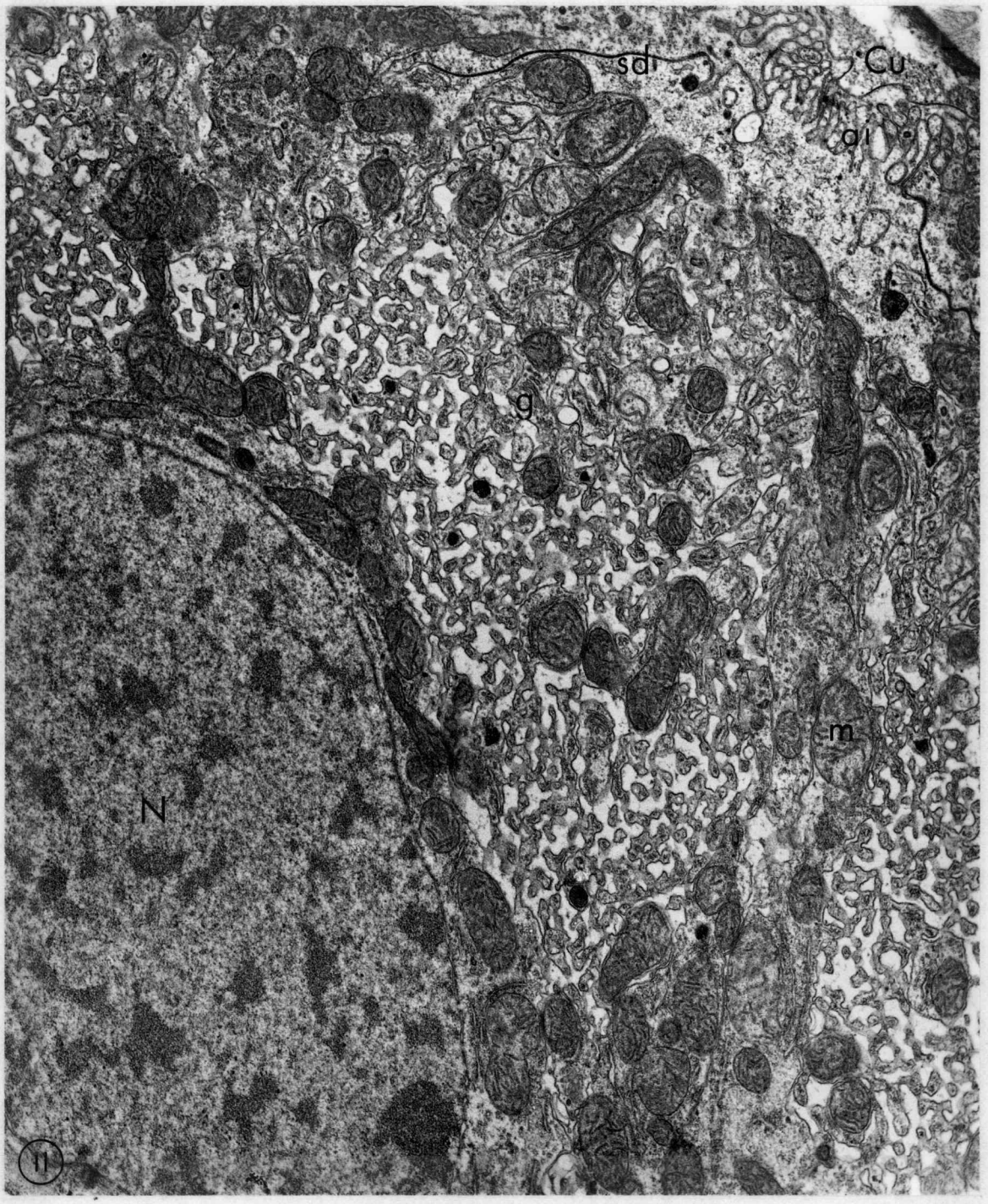

sd
Cu
al
g
m
N
11

Figure 12. A portion of the labyrinthine cytoplasm of a neck organ cell. Several mitochondrial "stacks" are visible. Osmium fixation at pH 7.2. 28,000X. 0.36 μm = 1 cm.

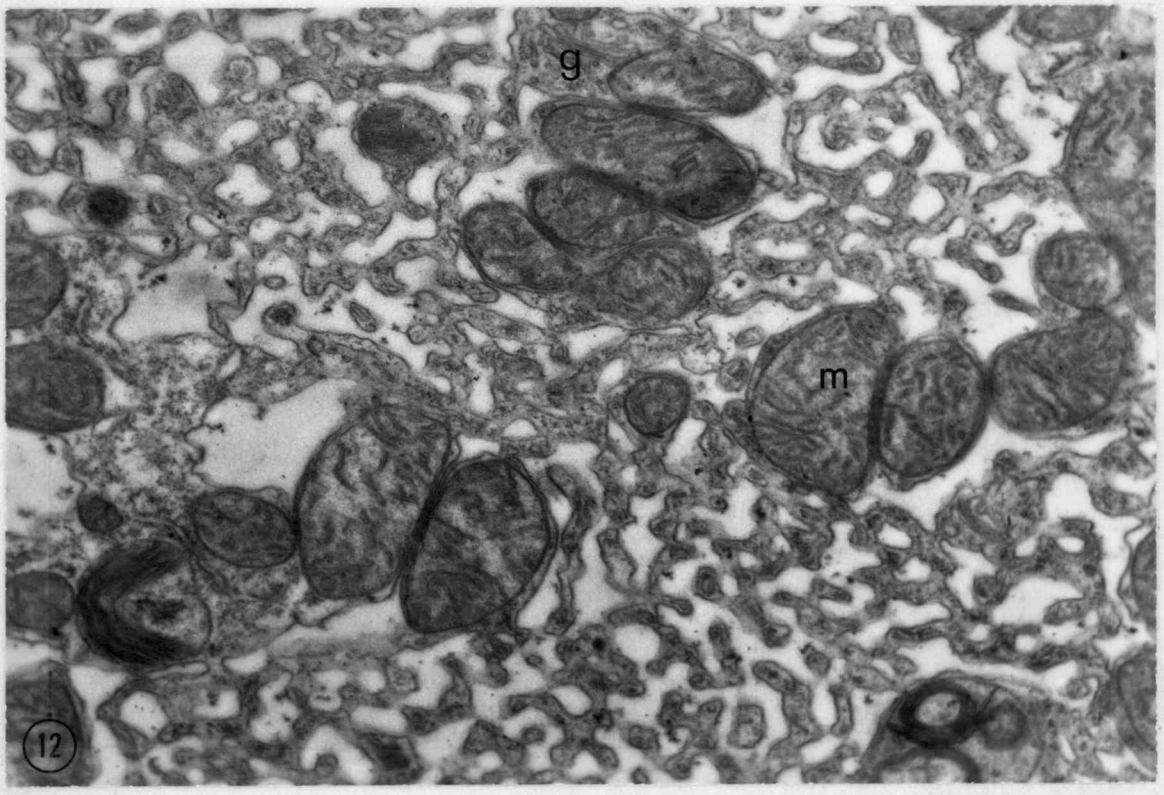
Figure 13. Mitochondrial stack in the perinuclear region. A central canaliculus (arrow) separating two mitochondria is evident. Glutaraldehyde-osmium fixation at pH 7.2. 50,400X. 0.2 μm = 1 cm.

g - Golgi complex

L - lipid droplet

m - mitochondrion

N - nucleus


g
m
12

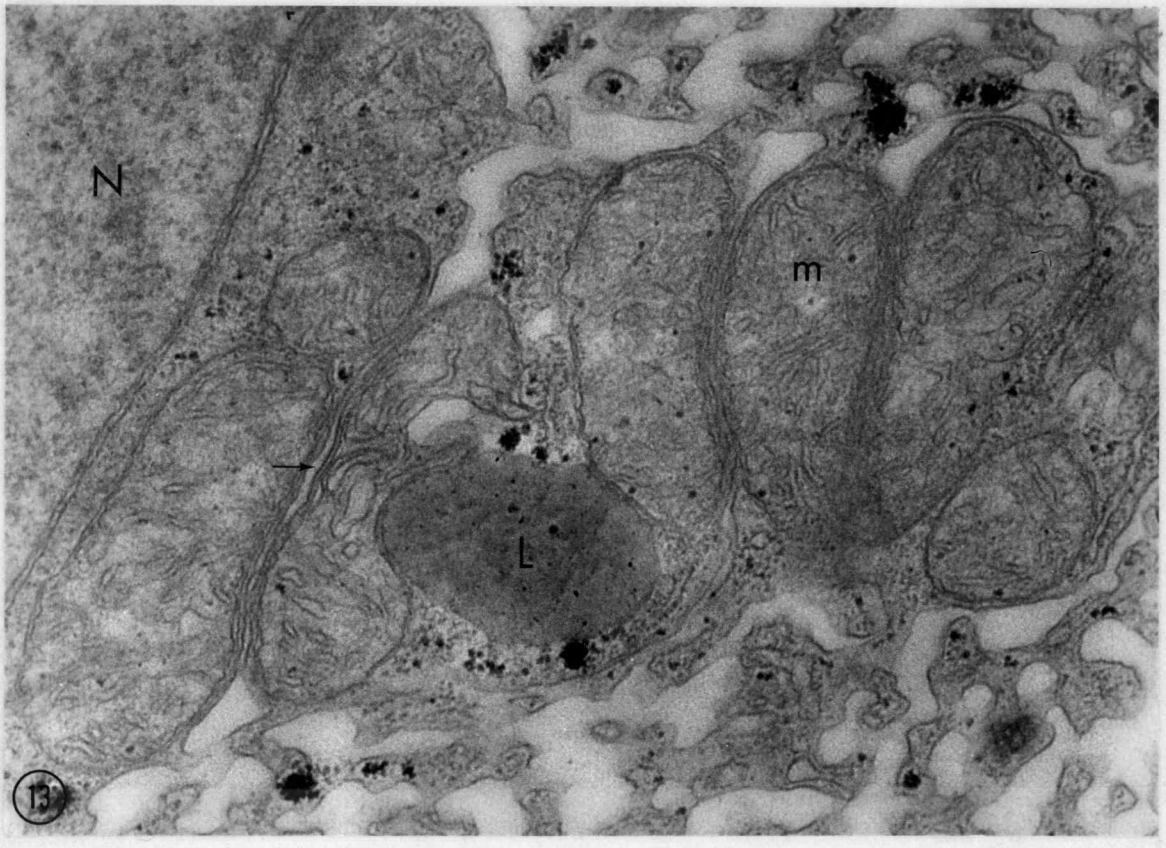

N
m
L
13

Figure 14. Apical surface of a neck organ cell. Glutaraldehyde-osmium fixation at pH 7.2. 35,000X. 0.29 μm = 1 cm.

Figure 15. Mitochondria near the apical surface of a neck organ cell. Glutaraldehyde-osmium fixed at pH 7.2. 55,000X. 0.19 μm = 1 cm.

ai - apical plasma membrane infoldings

Cu - cuticle

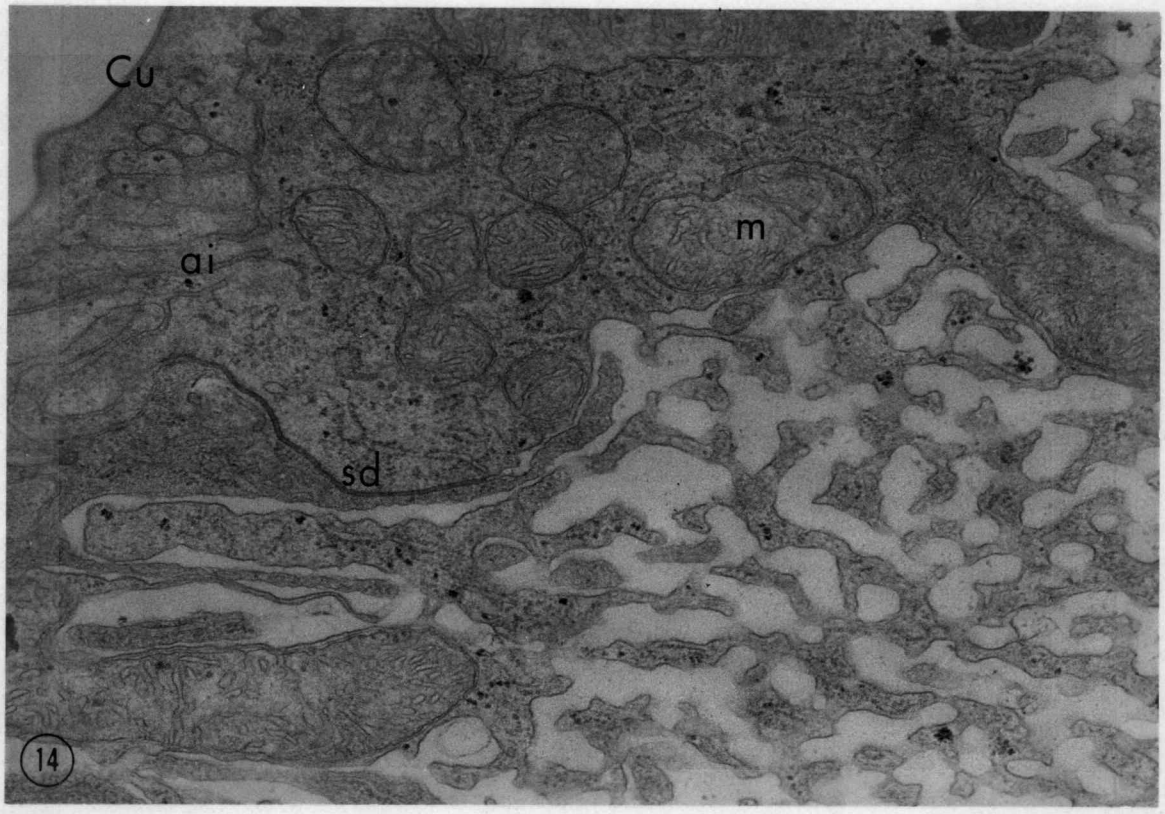
m - mitochondrion

sd - septate desmosome

1 - inner mitochondrial membrane

2 - outer mitochondrial membrane

3 - sinusoidal membrane


Cu
ai
m
sd
14

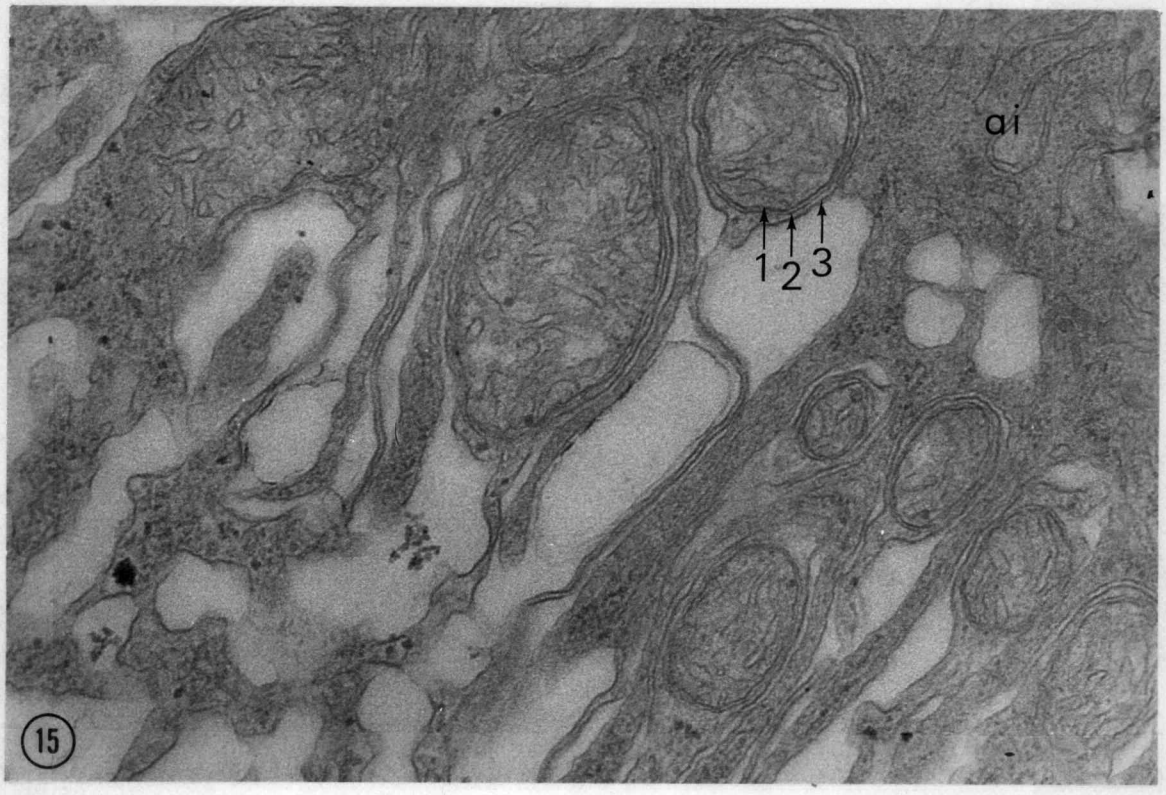

ai
1
2
3
15

Figure 16. Dorsal view of a 30 hour nauplius after removal of the neck organ. Three points of muscle attachment (arrows) are indicated along the dorsal midline of the larva. Fixed in osmium at pH 7.2 and dried by lyophylization. 400X. 25 μm = 1 cm.


16

Figure 17. Dorsal antennal muscles of a 30 hour nauplius converging to a point of origin at the ventral surface of the apex of the neck organ. Portions of four muscle cells and one neck organ cell are visible. Glutaraldehyde-osmium fixation at pH 7.2. 31,500X. 0.3 μm = 1 cm.

m - mitochondrion

mf - myofibril

z - Z band of a myofibril

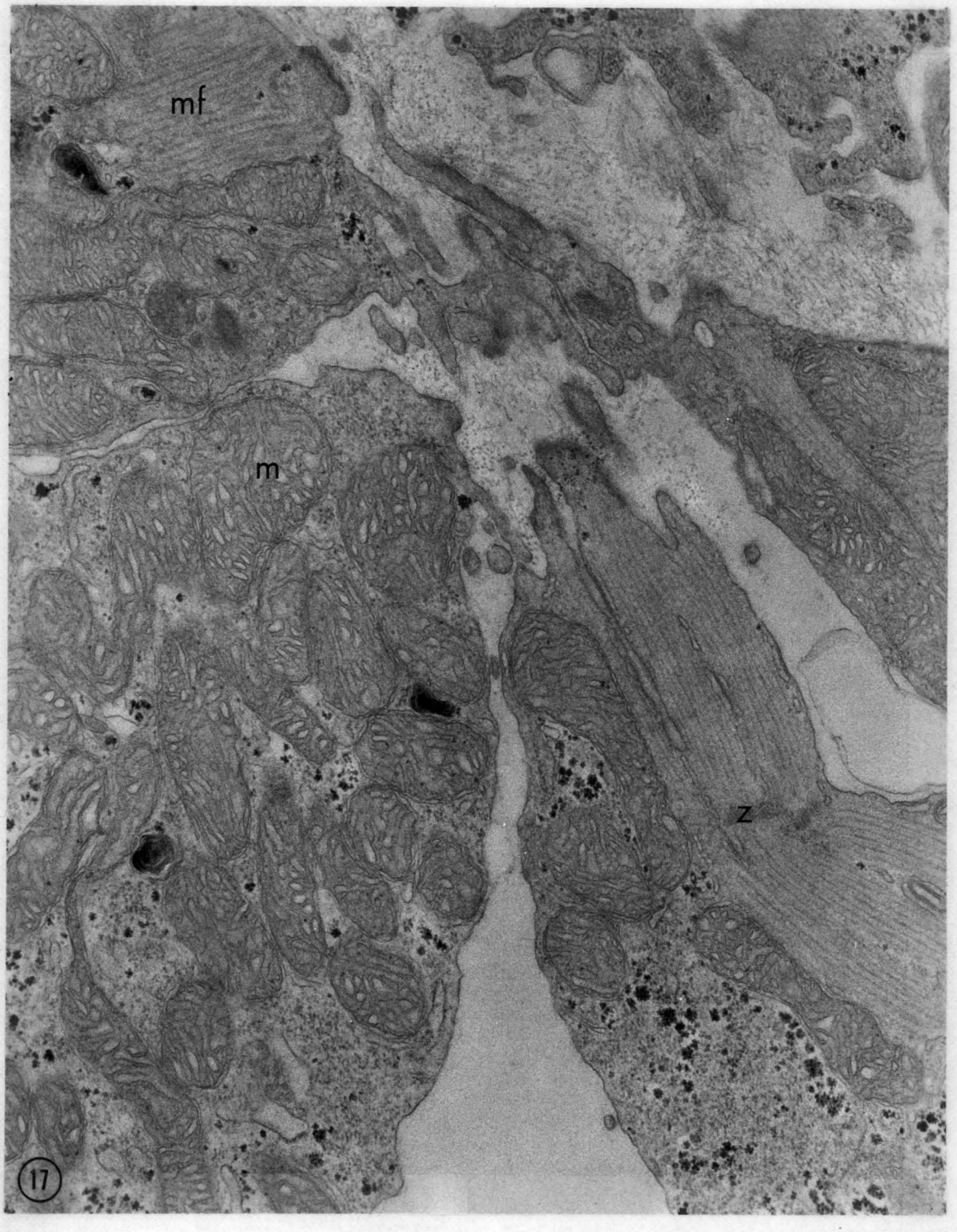
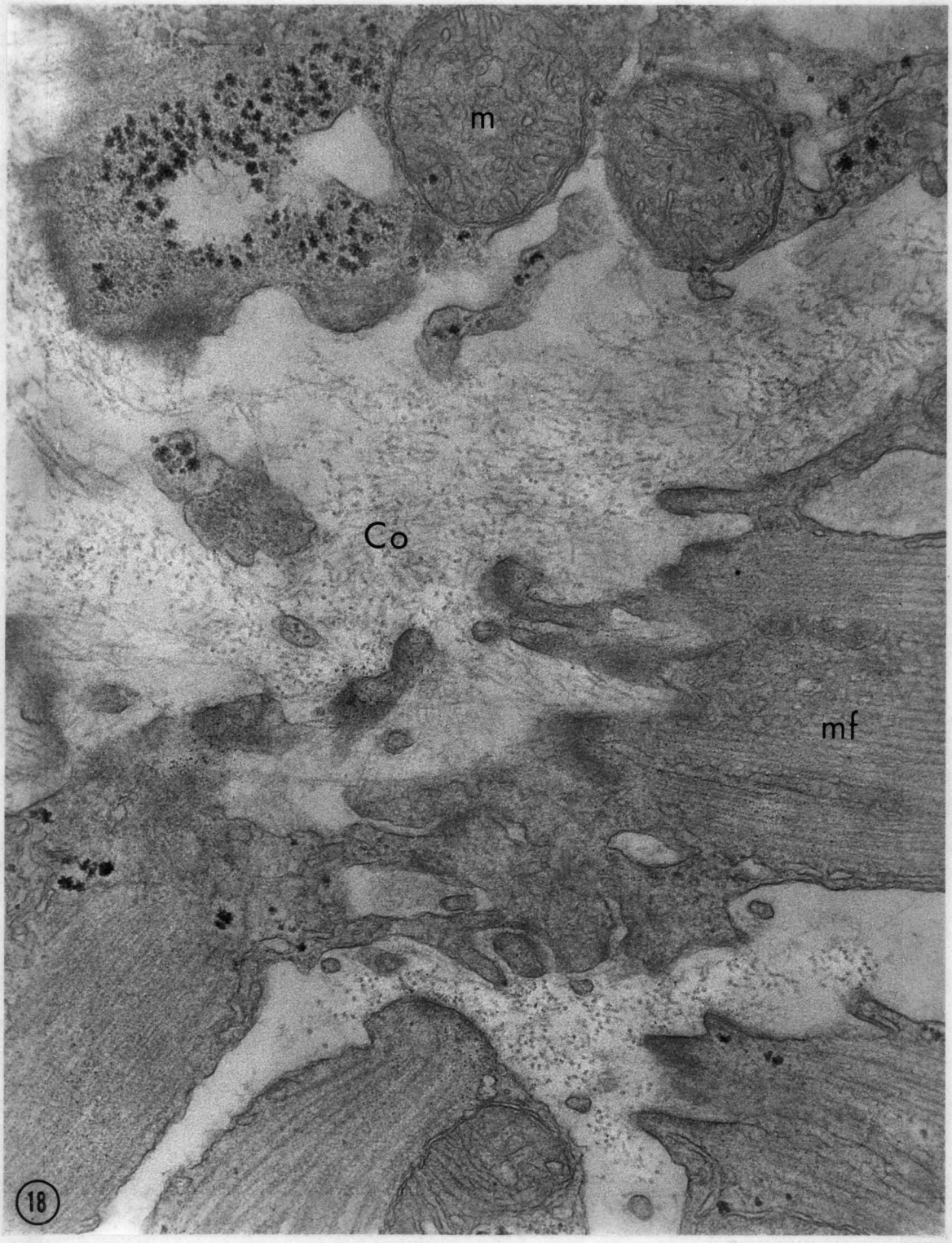

mf
m
z
17

Figure 18. Central origin of dorsal antennal myoblasts. The neck organ cell visible exhibits the characteristic apposition of mitochondrial and sinusoidal membranes. Fixed in glutaraldehyde-osmium at pH 7.2. 52,500X. 0.2 μm = 1 cm.

Co - collagen-like fibrils

m - mitochondrion

mf - myofibril


m
Co
mf
18