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Comparative ultrastructure of lipid storage sites in female *Euchaeta marina* and *Pleuromamma xiphias* (Copepoda: Calanoida)

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Abstract. Female *Euchaeta marina* (Prestandrea, 1833) have one large, thin-walled lipid sac, whereas female *Pleuromamma xiphias* (Giesbrecht, 1889) have two separate and morphologically distinct lipid storage sites. One lipid site in *P. xiphias* corresponds to the mesenteric tissue that surrounds the anterior region of the midgut. The morphology of these cells resembles that of mammalian brown adipocytes. The cytoplasm is filled with extensive smooth endoplasmic reticulum, numerous mitochondria and several deposits of intracellular lipid. The second lipid site of *P. xiphias* lies in the posterior region of the metasome and resembles the thin-walled lipid sac of *E. marina*. Both lie adjacent to, but are not contiguous with, the narrow mesenteric tissue surrounding the last region of the midgut. Both sacs contain a single, large deposit of intracellular lipid enclosed by a very thin rim of cytoplasm and resemble mammalian white adipocytes. The different habitats and reproductive processes of these two copepod species may relate to the observed variations in lipid cell morphology. The reserve lipid in *E. marina* plays a primary role in reproduction and is linked closely with the continuous cycle of oocytic maturation. The lipids synthesized and stored by *P. xiphias*, a strong vertical migrator, may be influenced by food availability, a function of their mesopelagic habitat. The primary role of the reserve lipids in this copepod may be to provide energy during migrations and between feeding periods, with relatively less lipid being allocated to reproduction.

Introduction

Lipids are carbon-rich, high-energy compounds that comprise an important biochemical component of numerous marine zooplankton, particularly calanoid copepods. The presence of lipids in copepods is recognized by the appearance of what has been termed an "oil sac" within the cephalothorax. The oil sac was described in several genera by Claus (1863), and later considered as a hydrostatic organ in *Calanus finmarchicus* by Lowe (1935). Considerably more information is available now with respect to biochemical aspects of lipids and changes in lipid content during the life cycle of various copepod species (see Sargent and Falk-Petersen 1988, and references therein). A review of the role of lipids in the biology of copepods has been written by Sargent and Henderson (1986).

In calanoid copepods, the reserve lipids consist of two major lipid classes, wax esters and triacyglycerides (TAG) (Bauermeister and Sargent 1979). The total lipid content and the relative proportions of these lipid classes vary among species (Lee et al. 1971, Lee and Hirota 1973). These variations are correlated with the habitat, food supply, and life-history strategy of the copepod (Sargent and Henderson 1986). Triacyglycerides are the more labile class of lipid, while wax esters constitute a long-term energy reserve. In times of low food concentration or starvation, TAGs are utilized by the copepod before the wax esters (Lee 1974, Lee et al. 1974, Lee and Barnes 1975, Hakanson 1984).

The wax esters are contained within the thin-walled oil sac (Benson et al. 1972), whereas smaller deposits of TAGs are dispersed throughout the body (Bauermeister and Sargent 1979). Nevertheless, little is known about morphological aspects of the cells associated with lipid metabolism and storage in calanoid copepods.

In this paper, the gross morphology and ultrastructure of lipid storage sites in adult females of two marine copepods, *Euchaeta marina* (Prestandrea, 1833) and *Pleuromamma xiphias* (Giesbrecht, 1889), are described and compared. These calanoid species differ from each other in their habitats and reproductive strategies. *E. marina* is a common member of the epiplanktonic community in tropical, subtropical, and temperate zones (Bradford 1974, Park 1975). A large, conspicuous oil sac is present in the posterior region of the cephalothorax. Female *E. marina* produce bright blue eggs that contain large lipid droplets (Blades-Eckelbarger unpublished observations). After spawning, the fertilized eggs are carried

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in a single egg sac attached to the female's genital area, where they are brooded until the first naupliar stage.

Pleuromamma xiphias is a relatively large (4.0 to 5.5 mm) mesopelagic calanoid found in most of the world's oceans. These copepods are strong vertical migrators (Haury 1988) and have been studied primarily for their bioluminescent capabilities (Buskey et al. 1989). An obvious, large oil sac is absent (Blades-Eckelbarger unpublished observations), but numerous smaller lipid deposits are present in anterior and posterior regions of the cephalothorax. In contrast to *Euchaeta marina*, the oocytes of *P. xiphias* contain numerous, small aggregations of lipid that can be resolved only at the electron microscopic level. The fertilized eggs are not brooded in an egg sac as in *E. marina*, but are spawned freely into the water (Blades-Eckelbarger unpublished observations).

Materials and methods

Adult female *Euchaeta marina* were sorted live from plankton samples taken in the Gulf Stream approximately 40 km due east of the Fort Pierce Inlet, Florida. Prior to fixation, adult females were grouped with respect to states of reproductive maturity as distinguished by the relative size and coloration of the developing oocytes residing in the oviducts. The following classification was used for *E. marina* (modified from Marshall and Orr 1955): State I, nearly transparent oocytes; State II, light blue oocytes; State III, dark blue oocytes (ready to be spawned).

Adult female *Pleuromamma xiphias* were sorted from oblique (500 m to surface) plankton tows taken during the night at various locations in Bahamian waters. Only two states of maturity were observed: State I, nearly transparent oocytes; State II, cream-colored ooctyes (ready to be spawned).

For transmission electron microscopy (TEM), the copepods were immersed individually in a paraformaldehyde + glutaraldehyde mixture (Karnovsky 1965) with 0.2 *M* phosphate buffer (pH 7.4) at room temperature for 15 to 30 min. After this time, the head and last urosomal segments were excised to facilitate rapid penetration of the fixative. The specimens were then transferred immediately into fresh fixative and held at room temperature for 2.5 to 6 h or stored in a refrigerator at 10 °C for 18 to 20 h. The latter time often resulted in better tissue fixation. This primary fixative was followed by three rinses in buffer (10 to 15 min each) and post-fixation for 1.5 to 2 h in 2% OsO_4 in 0.2 *M* phosphate buffer (pH 7.4), all at room temperature. The specimens were then dehydrated rapidly in ascending concentrations of ethanol, exchanged in propylene oxide, and embedded in Epon.

For light microscopy, 1 μm thick-sections were cut with glass knives on a Porter-Blum MT2-B ultramicrotome, stained with Richardson's stain (Richardson et al. 1960), and examined with a Zeiss WL compound microscope fitted with an Olympus PM-10AD 35 mm automatic camera system. Thin sections were cut with a diamond knife, stained with uranyl acetate followed by lead citrate, and examined with a Zeiss EM9-S2 transmission electron microscope.

Results

Euchaeta marina

The adult female *Euchaeta marina* stores excess lipids within a large, thin-walled sac referred to here as the lipid sac (Figs. 1a, b, 2b). Its length extends from the first to the last thoracic segment along the mid-axis of the metasome. The sac lies between the midgut and the ventral nerve cord (VNC) (Fig. 1a, b). Its dorsal wall borders upon a thin layer of mesenteric cells that surrounds the midgut (Fig. 2a). When filled with lipid and thus fully expanded, the lateral walls of the lipid sac touch the paired oviducts (Fig. 1a).

The proximal end of the lipid sac begins near the level of the maxillipeds where Zone II of the midgut merges with Zone III (terminology from Arnaud et al. 1978) (Fig. 1b). In this region, the wall of the sac is divided for a short distance, forming two narrow branches that extend laterally on either side of the VNC (Fig. 1c). Each branch borders upon an unidentified gland that lies between the VNC and the ventral body wall. The two extensions of the lipid sac are bathed on their inner and outer surfaces by hemolymph of the ventral perivisceral cavity (Fig. 1c).

At the ultrastructural level, the wall of the lipid sac appears as a single, narrow, multinucleate cell. Lateral cell membranes were never observed during extensive TEM examinations of serial sections from ten specimens. This apparent syncytium surrounds one large deposit of intracellular lipid that is not bounded by a membrane. The nuclei of the syncytium are elongate, roughly oval in shape, with large densely staining masses of heterochromatin (Fig. 2a).

The cytoplasm of the lipid cell is characterized by numerous, small lamellar and vesicular profiles of smooth endoplasmic reticulum (SER) (Fig. 2c). Round or oval mitochondria with parallel cristae are abundant and typically appear in close apposition to the lipid (Fig. 2c). In some specimens, a variable quantity of small, irregularly shaped, electron-dense masses were observed between the mitochondria and the surface of the lipid (Figs. 2b and 3a). Coated micropinocytotic pits and tubules were prevalent along the plasma membrane (Figs. 2c, 3b, c, d). Nearly spherical masses (ca. 250 to 300 nm diam.) of a moderately electron-dense granular

Fig. 1. *Euchaeta marina.* (a) Light micrograph, transverse section at level of midgut Zone III (G3), showing lipid sac filled with lipid (L), bordered by dorsal midgut (G3), oocytes (OC) in lateral oviducts, body musculature (MC) and ventral nerve cord (VNC); N: nucleus of oocyte (260×). (b) Light micrograph, sagittal section viewed from left side; H: dorsal heart; G2: midgut Zone II; G3: midgut Zone III; L: lipid in lipid sac surrounded by thin wall (arrowheads); OC: oocytes in part of oviduct; OV: ovary (115×). (c) Light micrograph, transverse section at level of maxillipeds, showing proximal end of lipid sac where it divides (arrowheads); branches extend from ventral edge of midgut (G), around ventral nerve cord (VNC) to border on unidentified gland (*); note small lipid inclusion (L) in right branch; HEM: hemolymph (660×)

Fig. 2. (See p. 52) *Euchatea marina.* (a) Transverse section showing thin wall of lipid sac (LSW) bordering on ventral mesentery (MES) of midgut Zone III (G3); arrowheads indicate dense masses along plasma membrane of lipid cell; L: lipid inclusion (extracted); MC: circular muscle of midgut; N1: nucleus of mesenteric cell; N2: nucleus of lipid cell (5900×). (b) Portion of lipid sac showing large lipid accumulation (L) enclosed by narrow cell (*); arrowheads indicate small, dense bodies along surface of lipid inclusion; note transverse section of nerve axon (NV) in close association with lipid cell (6100×). (c) Cytoplasm of lipid cell (LSW) contains vesicular endoplasmic reticulum and numerous mitochondria (M); arrowheads indicate endocytotic pit and tubule; HEM: hemolymph; L: lipid inclusion (28500×)

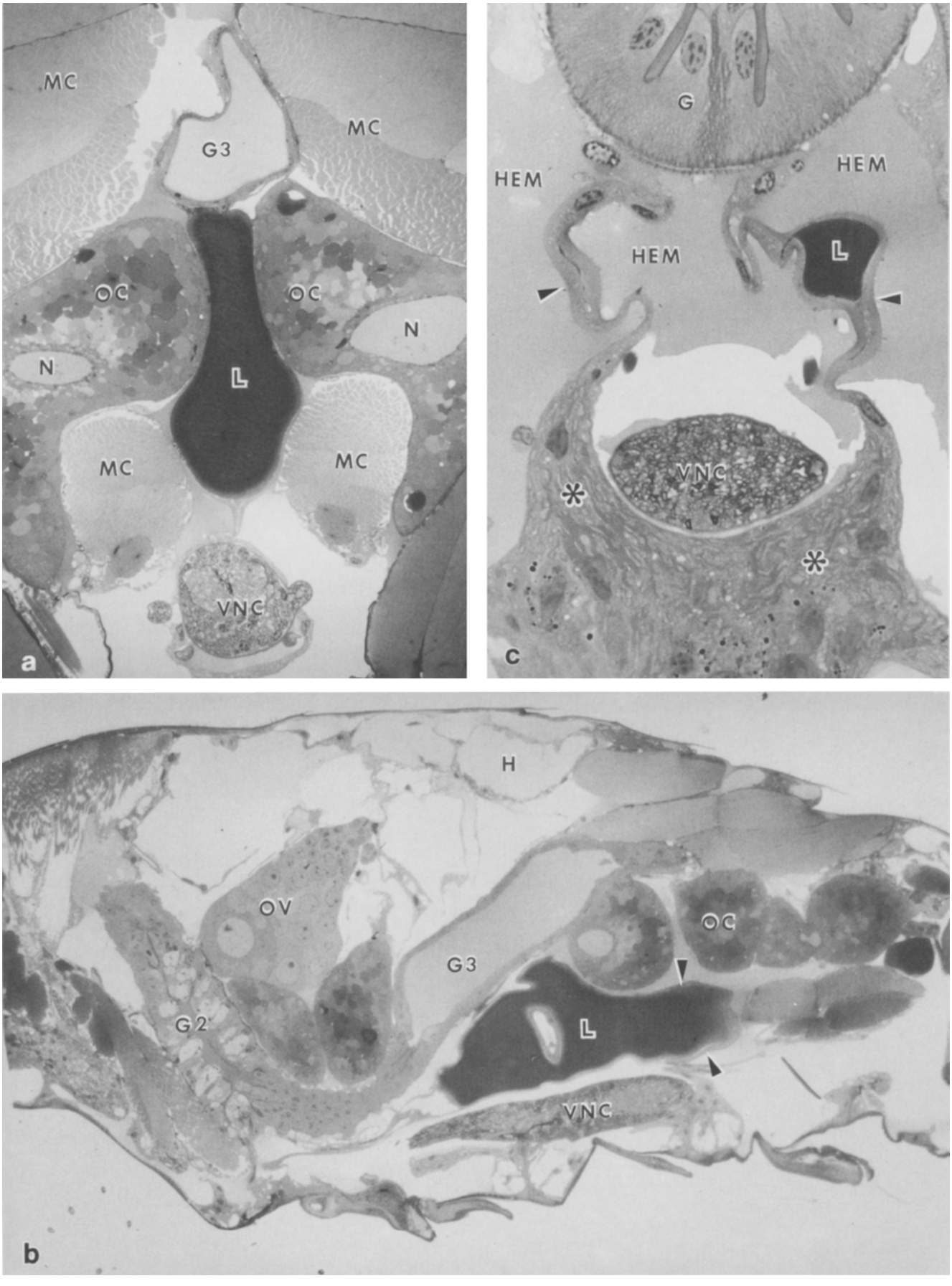
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Fig. 1

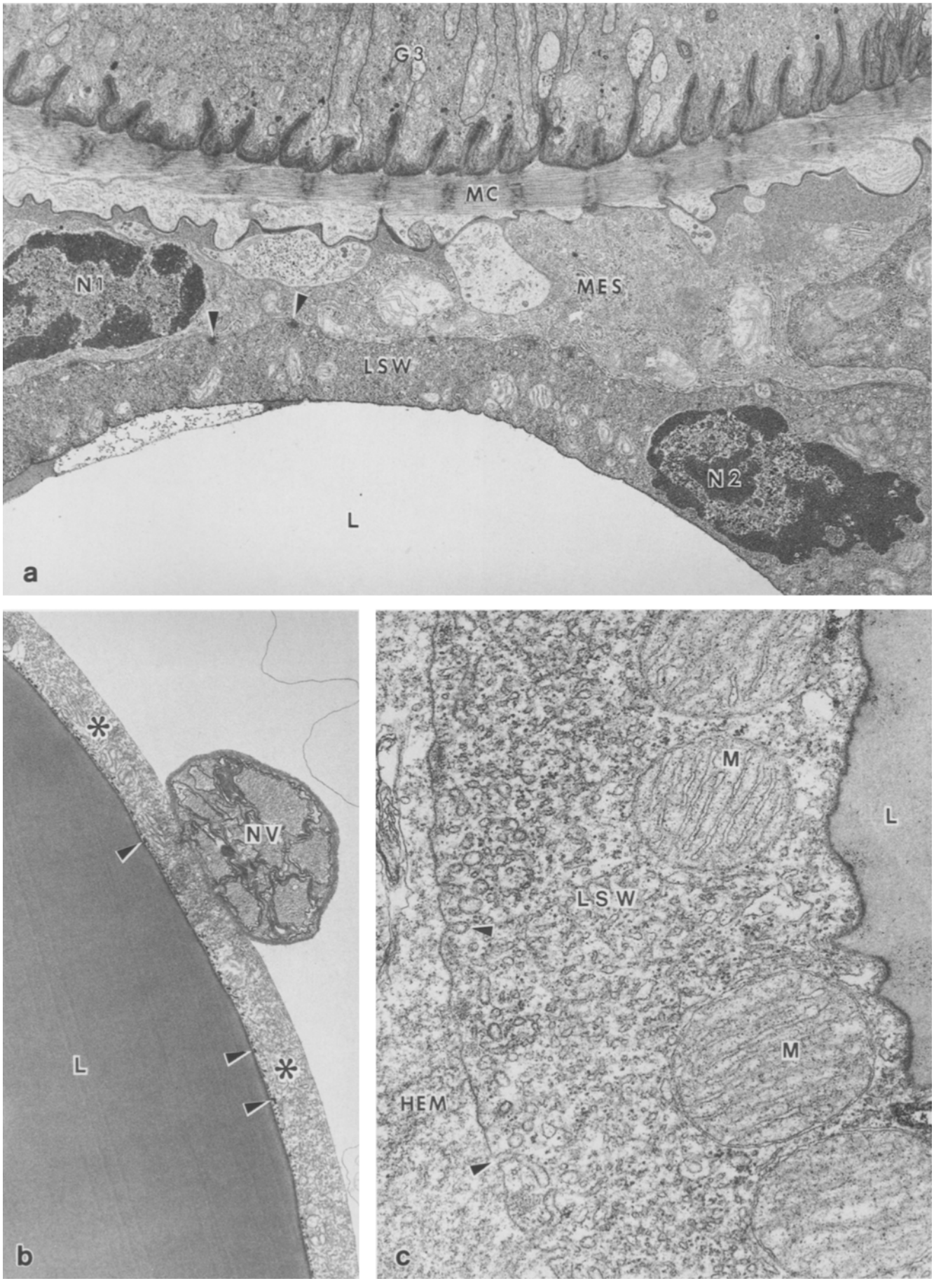

Fig. 2. (Legend on p. 50)

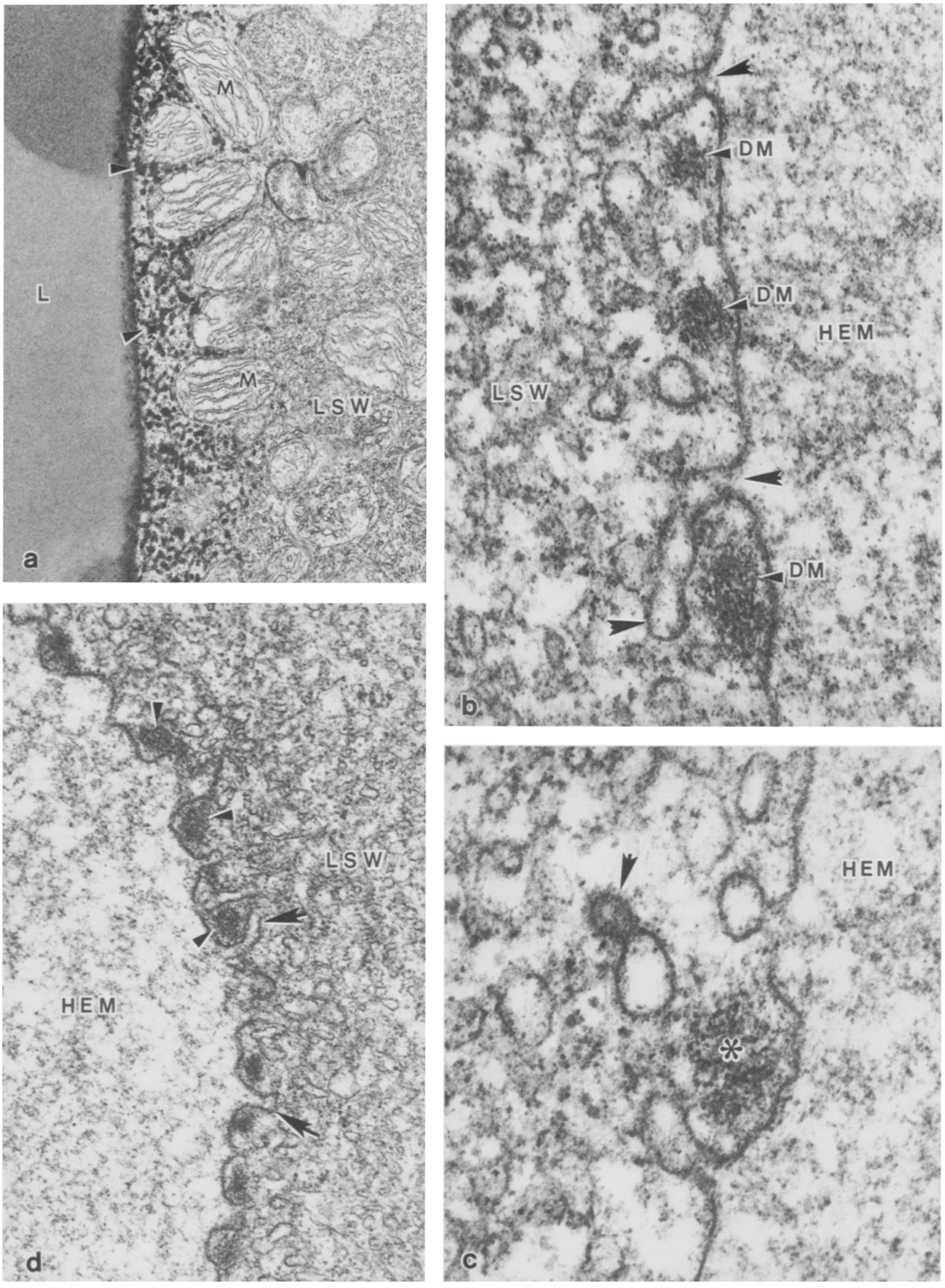

Fig. 3. (Legend overleaf)

substance were very numerous and closely apposed to the plasma membrane (Figs. 2c, 3b, c, d). These dense masses were most obvious in regions where the lipid cell bordered the hemolymph (Fig. 3d), but were observed also where the lipid cell apposed the gut mesentery (Fig. 2a).

The quantity of lipid enclosed by the sac appeared to correlate with the reproductive state of the adult female. The sac was filled with lipid in females with transparent oocytes representing the first phases of vitellogenesis. In females with dark blue oocytes, stages corresponding to advanced vitellogenesis and lipid elaboration (Blades-Eckelbarger unpublished observations), the lipid sac was partially or totally empty. Its surrounding wall remained intact but, due to expansion of the oviducts, its overall dimensions were reduced. In the advanced stages of oogenesis, the last region of the midgut (Zone III) was constricted greatly.

Pleuromamma xiphias

Two separate and morphologically distinct lipid storage sites are present in adult female *Pleuromamma xiphias*: (1) an anterior site characterized by several large lipid deposits scattered within the mesenteric tissue surrounding Zone I of the midgut (Fig. 4a); (2) a posterior site composed of a single lipid sac that lies dorsal to Zone III of the midgut in the third thoracic segment (Fig. 5a).

In the anterior site, large intracellular lipid deposits are present in wide epithelial cells that comprise the mesenteric tissue (Fig. 4a). In addition to the lipid inclusions, the cytoplasm of these cells contains extensive vesicular and lamellar SER, and numerous mitochondria of variable size (Fig. 4b–f). Smaller lipid droplets typically appeared in close contact with one or more mitochondria (Fig. 4c), and occasionally with the nucleus (Fig. 4d). The cisternae of the SER sometimes contained an electron-dense material (Fig. 4e). Where the cells border upon the hemolymph, invaginations of the plasma membrane were observed that appeared continuous with the SER (Fig. 4f).

The posterior lipid site is a single, thin-walled sac located in the third thoracic segment. It lies dorsal to the last zone of the midgut, in apposition with the thin layer of mesentery (Fig. 5a, b). The lipid sac is bordered proximally by the distal tip of the ovary, laterally by the dorsolateral body musculature and paired oviducts, dorsally and distally by hemolymph of the perivisceral cavity.

The ultrastructure of the posterior lipid sac is similar to that of *Euchaeta marina*. The narrow wall appears as a multinucleate cell that surrounds a single, intracellular lipid deposit. The cytoplasm contains small mitochondria and vesicular and lamellar SER (Fig. 5b, c). Where the plasma membrane borders the hemolymph, endocytotic pits and tubules often are very abundant. They contain a moderately electron-dense material similar in appearance to that of the hemolymph (Fig. 5c).

Discussion

The ultrastructural observations of female *Euchaeta marina* and *Pleuromamma xiphias* presented here illustrate that the morphology of the lipid-related tissues and their anatomical positions in calanoid copepods can be variable among species as well as within a given individual. Female *E. marina* have one thin-walled lipid sac, whereas female *P. xiphias* have two separate and morphologically distinct lipid-storage sites. The anterior lipid site in *P. xiphias* corresponds to the mesenteric cells surrounding the midgut. Conversely, the thin-walled, posterior lipid sac of *P. xiphias* and the lipid sac of *E. marina* are adjacent to, but not contiguous with the midgut mesentery.

The anatomical sites for the synthesis and catabolism of lipids in copepods have not been identified previously. The fine structure of the two lipid cell-types presented here suggests that lipid metabolism occurs in the anterior midgut mesentery in *Pleuromamma xiphias* and in the narrow cells composing the lipid sacs of both *Euchaeta marina* and *P. xiphias*. The mesenteric cells of the anterior site in *P. xiphias* contain abundant SER and mitochondria, organelles characteristic of lipid metabolism (Fawcett 1986). These cells share certain morphological features with mammalian brown adipocytes. The latter contain numerous small droplets of lipid and mitochondria are very abundant. Although endoplasmic reticulum is not a common feature in brown adipocytes, the presence of extensive SER has been reported in some animals (review: Slavin 1987).

In contrast, the cells composing the thin-walled lipid sacs in both *Euchaeta marina* and *Pleuromamma xiphias* resemble more closely the cells of mammalian white adipose tissue (Fawcett 1986). The white adipocyte consists of one large lipid inclusion surrounded by a thin rim of cytoplasm. The cytoplasm is characterized by an elongate nucleus, vesicular and lamellar profiles of agranular

Fig. 3. (See p. 53) *Euchaeta marina*. (a) Interface of lipid cell (LSW) and lipid inclusion (L); note abundant darkly staining masses (arrowed) and numerous mitochondria (M) (14 500 ×). (b) High magnification of lipid cell (LSW) showing endocytotic tubules on plasma membrane (large arrowheads) and masses of densely staining granular material (DM); HEM: hemolymph (81 450 ×) (c) High magnification of lipid cell plasma membrane showing fusion of coated vesicle with endoplasmic reticulum (arrowed) and dense granular material (*); HEM: hemolymph (95 200 ×). (d) Plasma membrane of lipid cell (LSW) with regularly spaced dense masses of granular material (small arrowheads) between endocytotic tubules (large arrowheads); HEM: hemolymph (21 700 ×)

Fig. 4. *Pleuromamma xiphias*, anterior lipid site. (a) Light micrograph, transverse section, illustrating three distinct lipid inclusions (L) in mesenteric cells (outlined by arrowheads) bordering on ventrolateral edge of midgut Zone I (G1); LU: lumen of midgut (295 ×). (b) Closer view of cytoplasm in mesenteric cell containing lipid (L), abundant smooth endoplasmic reticulum (SER) and numerous mitochondria (M) (5000 ×). (c) Higher magnification of lipid droplet (L) partially enclosed by mitochondrion (M) (15 000 ×). (d) Higher magnification of lipid droplet (L) in close association with nucleus (N) and mitochondria (M) (12 300). (e) SER of lipid cell containing dense material (7650 ×). (f) Higher magnification of plasma membrane showing invaginations (large arrowheads) and abundant SER (small arrowheads); HEM: hemolymph (47 500)

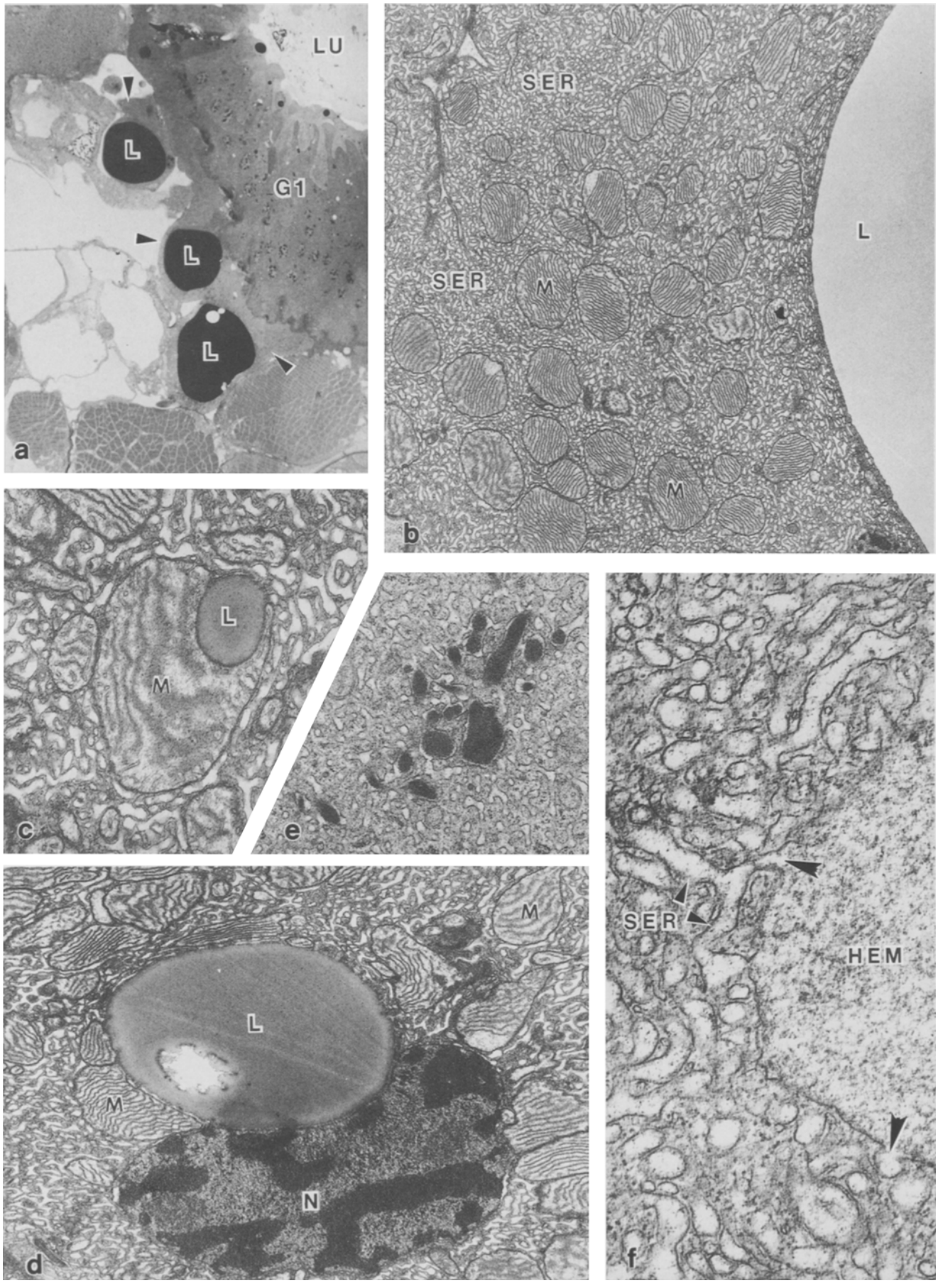

Fig. 4

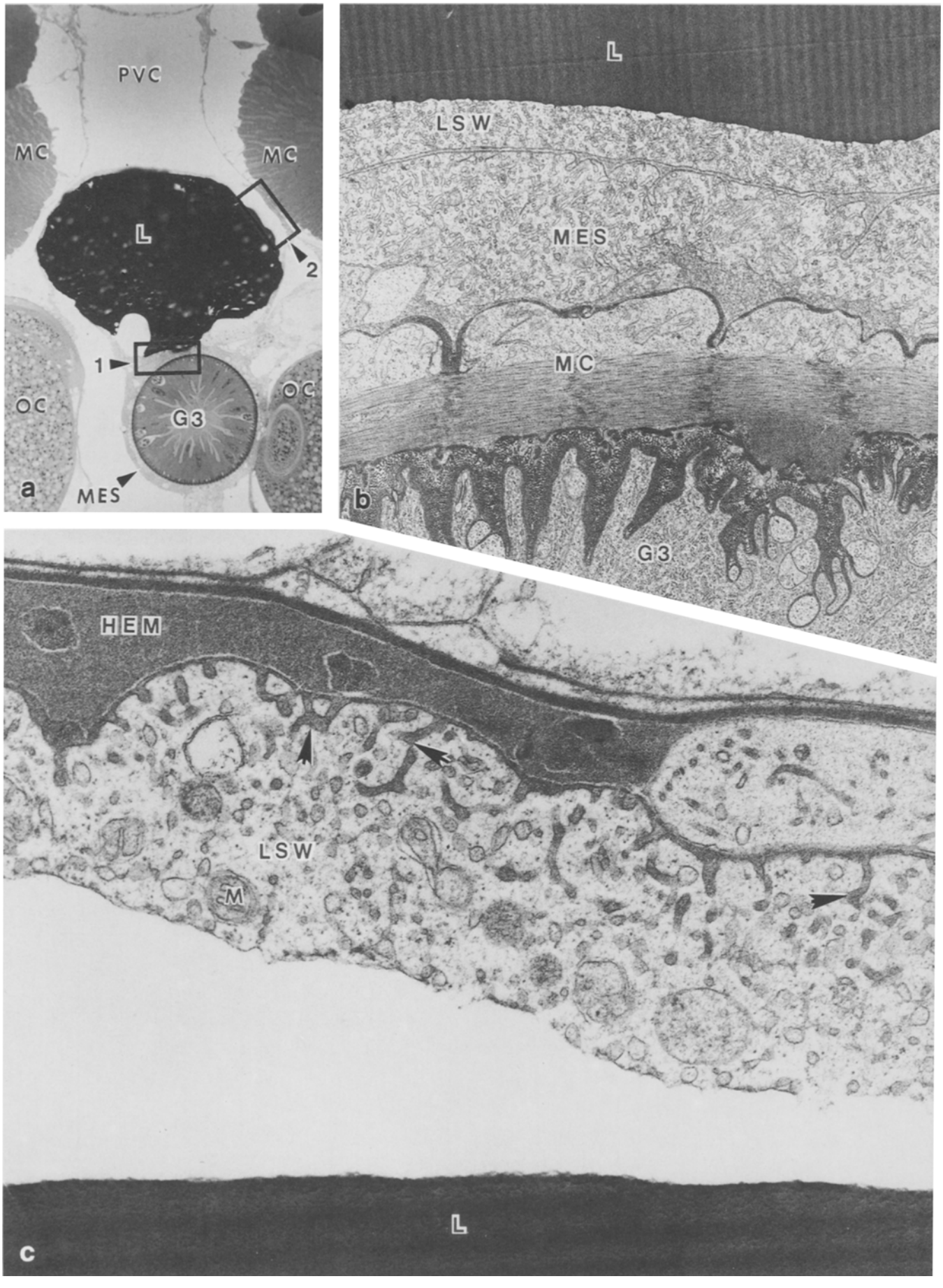

Fig. 5

endoplasmic reticulum, and numerous mitochondria with parallel cristae.

The presence of micropinocytotic activity and invaginations along the plasma membrane, in addition to observations of mitochondria in close contact with lipid inclusions, is further evidence that lipid metabolism occurs in both lipid cell-types of *Euchaeta marina* and *Pleuromamma xiphias*. Numerous invaginations along the plasma membrane have been noted also in mammalian white adipocytes. These may be involved in uptake of materials into the cell for lipid synthesis or in transport of materials from the lipid inclusion to the cell surface for release (Fawcett 1986). In *E. marina*, the dense, granular masses observed along the plasma membrane may represent either uptake of precursors for lipid synthesis, or material that is to be released into the hemolymph. Likewise, the endocytotic pits and tubules observed along the plasma membrane in the posterior lipid sac of *P. xiphias* contained electron-dense material similar in appearance to that of the hemolymph, implying transport into the cell. The small dense bodies observed between the mitochondria and the lipid inclusion in *E. marina* may represent active lipid formation or breakdown directly at the surface of the lipid droplet (Williamson 1964, Reger et al. 1989).

The lipids present in calanoid copepods have been isolated as two major lipid classes, wax esters and triacylglycerides (TAG) (Bauermeister and Sargent 1979). The wax esters are stored in the thin-walled oil sac that is separate from the gut tissue, while the TAGs are stored in smaller reserves elsewhere throughout the body (Benson et al. 1972, Bauermeister and Sargent 1979, Henderson and Sargent 1980). Henderson and Sargent concluded from studies of *Euchaeta norvegica* that the synthetic site of wax ester is probably in the cells enclosing the oil sac. In the same study, isotopically labelled gut tissue isolated from *E. norvegica* was active in the biosynthesis of glycerol 3-phosphate, a precursor of TAGs. From these data, the authors proposed that TAG synthesis occurs in the gut cells themselves or in cells associated with the gut and thus separate from the cells of the oil sac. This biochemical information coupled with the present morphological observations of *Euchaeta marina* and *Pleuromamma xiphias*, suggest that the mesenteric cells of the anterior lipid site in *P. xiphias* are involved in TAG metabolism and that the cells composing the thin-walled lipid sac in both *P. xiphias* and *E. marina* are associated with the elaboration of wax esters. Analyses of lipid content in *E. marina* and *P. xiphias* further support this speculation (Lee and Hirota 1973). Wax esters are the major lipid class in adult female *E. marina*. In contrast, both wax esters and TAGs comprised the total lipid content of adult female *P. xiphias* with TAGs predominating. If such is the case, then female *P. xiphias* are able to simultaneously synthesize and store wax esters in the posterior thin-walled sac and TAGs in the gut mesentery (anterior site). Variations in morphology of the lipid-related tissues of copepods may provide an indication of the specific class of lipid that is stored.

An accepted hypothesis states that the degree of lipid synthesis in copepods is influenced primarily by the relative abundance and seasonality of the food supply. In those environments where short periods of abundant food are followed by long periods of food scarcity, e.g. the polar regions and deep sea, copepods synthesize more lipid for long-term storage (Lee et al. 1971, Lee and Hirota 1973). However, unlike copepods from high latitudes where lipid metabolism is governed by seasonal changes in food availability, the lipid cycle of *Pleuromamma xiphias*, a strong vertical migrator, appears to be based on a much shorter temporal scale, i.e., a diurnal period. Petipa (1964) reported that large quantities of lipid were used during the daily vertical migrations of *Calanus helgolandicus*. Gatten and Sargent (1973) suggested that *C. finmarchicus* dwelling near the surface at night are active in wax ester biosynthesis and have relatively low levels of lipid, whereas those from deeper depths are richer in lipid and less active in wax ester biosynthesis. They proposed that the increased rates of wax ester biosynthesis near the surface at night probably reflect a high rate of dietary input. *P. xiphias* dwell in mesopelagic depths during the day and migrate at night to the surface, where they feed on abundant plankton in the euphotic zone (Lee and Hirota 1973). It is suggested here that excess food consumed by the female during the few hours at night may be stored as TAGs in the anterior gut mesentery and constitute a short-term energy supply. Additional reserves would be retained in the posterior lipid sac in the form of wax esters. Both lipid deposits may be utilized by the female between daily feeding periods for migration, for general metabolic maintenance while dwelling at the mesopelagic depths, and for gonadal development. Relatively little lipid is elaborated by the oocytes of *P. xiphias* (Blades-Eckelbarger unpublished observations). Therefore, the lipid synthesized and stored in the females is probably used primarily for basic metabolism and migration.

In the female *Euchaeta marina* examined here, the lipid cycle throughout the adult stage appears to be closely attuned to oocyte maturation and episodes of spawning rather than to the food supply, which remains relatively constant in the subtropical epiplanktonic zone. These calanoids reproduce continuously (Blades-Eckelbarger unpublished observations). The spawned eggs are brooded in an externally attached sac where they develop to the first naupliar stage. Simultaneous with the spawning episode, early vitellogenic oocytes pass from the ovary into the oviducts, where they undergo a growth

Fig. 5. *Pleuromamma xiphias*, posterior lipid sac. (a) Light micrograph, transverse section; thin-walled lipid sac (L) lies between midgut Zone III (G3) and perivisceral cavity (PVC); Rectangle 1 is detailed in Fig. 5b, Retangle 2 in Fig. 5c; MC: body musculature; MES: narrow mesentery surrounding midgut; OC: oocytes in lateral oviducts (320 ×). (b) Higher magnification of Retangle 1 in Fig. 5a, showing thin-walled lipid sac (LSW) bordering on midgut mesentery (MES); G3: basal region of midgut cells; L: lipid inclusion; MC: circular muscle layer (8 700 ×). (c) Higher magnification of Rectangle 2 in Fig. 5a, showing narrow wall of lipid sac (LSW) adjacent to hemolymph (HEM); note numerous endocytotic tubules along plasma membrane (arrowed) containing material similar to hemolymph; L: lipid inclusion (pulled away from lipid cell): M: small mitochondrion (21 000 ×)

phase and final stages of maturation and yolk accumulation. This group of oocytes cannot be spawned until the first group of embryos hatch from the egg sac. At this time, the young oocytes are relatively small and the lipid sac is full. As vitellogenesis progresses, lipid droplets appear in the ooplasm and the oocytes increase in volume, concurrent with a reduction in the size and content of the lipid sac. Just prior to spawning, the mature oocytes fill the oviducts and the lipid sac appears collapsed and empty.

This cyclic change in the amount of lipid present in the lipid sac of *Euchaeta marina* appears to be a function not only of lipid utilization and allocation to the oocytes, but also of a physical restriction imposed by the increased volume of the maturing oocytes. Their expanded size fills the oviducts leaving little room in the body cavity for the lipid sac. It is unlikely that the female can feed efficiently at this time, since the last zone of the midgut is greatly constricted. As the spawned eggs move into the external egg sac, however, more space becomes available in the body cavity. The midgut expands, the female resumes active feeding, and lipids derived from her diet fill the lipid sac once again. Here, they are held in reserve until the elaboration of lipids commences in the subsequent group of developing oocytes. This cycle, probably under neuroendocrine or hormonal control, continues until the female's reproductive potential is exhausted.

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