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SPERMATOZOA OF THE DEEP-SEA CEPHALOPOD *VAMPYROTEUTHIS INFERNALIS* CHUN: ULTRASTRUCTURE AND POSSIBLE PHYLOGENETIC SIGNIFICANCE

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[Plates 1–6]

CONTENTS

Sperm ultrastructure in the rare deep-sea cephalopod *Vampyroteuthis infernalis* is described, based on formalin-fixed material held in the Australian Museum (Sydney). The species is the sole member of the coleoidean order Vampyromorpha, which represents a level of organization intermediate between that of the Sepioidea–Teuthoidea and the Octopoda. Spermatozoa of *Vampyroteuthis*, the simplest observed in any cephalopod, exhibit the following features: (1) a spheroidal acrosome lacking any complex substructure; (2) a short (8.5 μm) fusiform nucleus with a deep (2.2–2.5 μm) basal invagination (containing an extensive plug of dense material); (3) two triplet centrioles arranged parallel to the sperm longitudinal axis; (4) a short (1 μm) midpiece composed of a triangular cluster of mitochondria surrounding the centrioles; and (5) a tail (length 130–135 μm) that is continuous with one of the centrioles (here considered as a 'distal' centriole). An annulus and membranous skirt are absent, though the coarse fibres do fuse into a ring at the tail–midpiece junction). These cells show some resemblance to sperm or spermatids of sepioids and teuthoids (spheroidal acrosome, short nucleus) but are also remarkably similar to mid-spermatids of *Octopus* (with the exception of the uncondensed nucleus in *Octopus* spermatids). Sperm morphology supports the current assignment of *Vampyroteuthis* to

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a separate coleoidean order – Vampyromorpha – and also suggests that a close link exists between the Vampyromorpha and Octopoda.

1. Introduction

Vampyroteuthis infernalis Chun, 1903, is a rare, but widely distributed, deep-sea cephalopod of considerable systematic importance because it exhibits a mixture of teuthoidean and octopodan characteristics (Pickford 1940, 1957; Young 1977; Fioroni 1981; Boss 1982). The species was placed by Pickford (1939) into its own order, Vampyromorpha, thereby separating it from other (extant) dibranchiate cephalopods (subclass Coleoidea: orders Sepioidea, Teuthoidea, Octopoda; see Voss (1977) and Boss (1982) for current classification; sepiolids given ordinal status by Fioroni (1981)). The phylogenetic position of the Vampyromorpha within the Cephalopoda is unclear, but the group is believed to have arisen in the Lower Jurassic, or earlier, from phragmoteuthoids and been connected in some way with the origin of the Octopoda (Donovan 1977; Bandel & Leich 1986).

Relatively few cephalopod taxa have been studied for sperm structure and development, especially at the ultrastructural level (*Rossia*: Fields & Thompson (1976); *Eusepia*, *Loligo Alloteuthis*: Maxwell (1975); *Octopus*: Galangau & Tuzet (1968*a*, *b*), Longo & Anderson (1970); *Eledone*: Maxwell (1974); *Nautilus*: Arnold & Williams-Arnold (1978), Tsukahara (1985). The available data, however, suggest spermatozoa of decapods are distinct from those of octopods and the surviving tetrabranchiate *Nautilus*. Although some of the structural differences between cephalopod spermatozoa are possibly due to changes in fertilization environment (that is, where sperm are liberated from spermatophores (see Franzén 1967)), there seems little doubt that sperm morphology also reflects phylogenetic relationships within the Cephalopoda (Arnold 1984). Certainly in the molluscan class Gastropoda, sperm morphology correlates well with taxonomic units at and above the family level (see Nishiwaki 1964; Koike 1985; Healy 1983, 1986, 1988) and in the class Polyplacophora there are interesting, possibly class-specific sperm characteristics (see Russell-Pinto *et al.* 1983; Rousset-Galangau 1974; Sakker 1984). The present study describes spermatozoan ultrastructure in *Vampyroteuthis* and investigates the relationship between the Vampyromorpha and other coleoideans using sperm and spermatid features. To aid comparisons, reference will also be made to unpublished work by the author on other cephalopod spermatozoa, particularly those of *Octopus* spp., *Sepia rozella* and *Spirula spirula* (summarized in figure 8) and on spermiogenesis in *Octopus* (figures 4 and 5†) and *Vampyroteuthis* (preliminary notes and figure 6).

2. Materials and methods

Pieces of developing spermatophores (and testis tissue) from a formalin-fixed specimen of *Vampyroteuthis* held in the Australian Museum wet collection were processed for transmission electron microscopy (TEM). A mature spermatophore was available for study, but spermatozoa contained within it were not as well fixed as those from the spermatophoric gland. The specimen of *Vampyroteuthis* (registration number C. 154223) was captured by mid-water trawl at a depth of 640 m off Newcastle (33° 07′–01′S, 153° 11′–05′E), New South Wales, Australia, by the New South Wales Fisheries vessel *Kapala* during November 1979. Tissue pieces

† Figures 1–6 appear on plates 1–6.

(1–2 mm^3) were rinsed in seawater (1 h) then placed in a solution of osmium tetroxide (1 % by volume) (prepared in seawater) for 80 min, then rinsed again in seawater (30 min). Testis pieces of *Octopus* sp.† (obtained as fresh dead specimens from Sydney Fish Markets) were initially fixed in glutaraldehyde (3.5 % by volume) in 0.2 M buffer adjusted with sucrose (100 g l^{-1}), then osmicated and rinsed in buffer. All tissues were dehydrated using a graded ethanol series (20–100 %) and embedded in Spurr's epoxy resin. Ultrathin sections were cut by using an LKB IV ultramicrotome, collected on copper grids, stained with uranyl acetate and Reynold's lead citrate and viewed with a Philips 300 TEM (at 60 kV).

3. Results

(*a*) *Mature spermatozoa of Vampyroteuthis*

Spermatozoa of *Vampyroteuthis* consist of an acrosome and nucleus (collectively, the 'head' region), mitochondria and centrioles (collectively, the midpiece) and a single elongate tail (figure 1*a*, *b*, plate 1). The acrosomal vesicle is spheroidal, membrane-bound, approximately 1 μm in maximum diameter, and situated in a shallow depression at the apex of the nucleus (figure 1*a–e*). Contents of the vesicle are granular and moderately electron-dense with a slightly denser peripheral layer (0.08 μm thick) lining the posterior, internal surface of the vesicle (figure 1*c–e*). Membranous layers underlying and partly supporting the acrosomal vesicle, may represent either a remnant of nuclear membranes in this region of the sperm head or a true but imperfectly preserved subacrosomal deposit (figure 1*c–e*).

The sperm nucleus is approximately 8.5 μm long, straight and tapered both anteriorly and posteriorly, thereby giving it a fusiform profile in longitudinal section (figure 1*a*, *b*). Transverse sections of the nucleus are circular to ovoid in profile (figure 2*a*, *b*, plate 2). Irregular cavities present throughout the nucleus (figures 1*a–d* and 2*a*, *b*) are probably equivalent to those occurring in spermatozoa of numerous externally fertilizing invertebrates, though formalin fixation may have created some distorsion in the material studied. The basal invagination of the nucleus is 2.2–2.7 μm deep, circular to oval in transverse section and filled by an extensive plug of dense material (figures 1*a*, *b* and 2*b–e*). This plug contains numerous small cavities (round or oblong, diameter 0.02–0.03 μm) and basally, is attached to the centriolar pair (figures 1*a*, *b* and 2*d*, *e*). Figure 2*e* (white arrow) shows that the plug is penetrated by microtubules originating from the centrioles, indicating that the plug itself is some form of modified centriolar rootlet (also suggested by preliminary observations on spermatids), see figure 6. Fibres from the centrioles also attach to the periphery of the dense plug (figure 2*c*). The centrioles, arranged parallel to the longitudinal axis of the spermatozoon, each consist of nine triplets (embedded in a dense matrix) and a central rod-shaped granule (figures 1*a*, *b*, 2*d*, *e* and 3*a*, *b*, plate 3). The 'distal' centriole continues posteriorly for 0.3–0.4 μm as a hollow cylinder, after which it becomes continuous with the axoneme – coarse fibre complex (figures 1*b* and 2*d*, *e*).

Posterior to the nucleus, three or four spheroidal (sometimes irregularly shaped) mitochondria, each containing unmodified cristae, are arranged in a triangular cluster around the centrioles to form the sperm midpiece (figures 1*b*, 2*d*, *e* and 3*c*, *d*). Dense granules occurring

† Dr C. C. Lu (Museum of Victoria) (personal communication) advises that this species of *Octopus* is as yet un-named. A voucher specimen has been lodged with the Australian Museum (Sydney) (reference number C. 154739).

between mitochondria and centrioles (figures 2*c*, *e* and 3*c*) probably represent glycogen deposits and not a cytoplasmic remnant.

The sperm tail consists of a 9+2 axoneme, nine coarse fibres (one associated with each doublet), granular material (? glycogen) surrounding the axoneme – coarse fibre complex, and the enveloping plasma membrane (figures 2*d*, *e* and 3*e*, *f*). The central pair of axonemal microtubules originate from a dense granule positioned immediately below the distal centriole (figure 2*d*, black arrow). The coarse fibres seem to fuse into a ring (not the annulus) at the point of axoneme–tail origin (figure 3*d*, black arrow). Initially the coarse fibres are 0.03 μm thick (maximally), but decrease in diameter posteriorly and ultimately terminate, leaving the remainder of the tail to be composed of the axoneme and plasma membrane only (figure 3*f* inset). Figure 3*d* (white arrow) shows that at least in the initial portion of the sperm tail the axoneme – coarse fibre complex may be helically twisted. Fine granules, possibly a form of glycocalyx, line the plasma membrane of the tail immediately below the midpiece (figure 2*e*, black arrows) but could not be identified more distally. Some sections through the distal region of the tail indicate that the plasma membrane can be elaborated into wide flanges (figure 3*f*), but whether these are real or a product of fixation is not known. The length of the tail as determined by phase-contrast light microscopy is approximately 130–135 μm (see figure 3*d* inset). Of special interest is the absence of an annulus or a reflected membranous skirt at the point of tail emergence from the midpiece (see figure 2*e*): one or both of these structures are present in other cephalopod spermatozoa (see figure 8). Figure 7 summarizes in semi-diagrammatic form, the ultrastructural characteristics of *Vampyroteuthis* spermatozoa (excluding the tail flange structure, which may be an artefact of fixation).

(*b*) *Observations on spermiogenesis in Octopus sp.*

Spermatids of *Octopus* sp. show similarities to spermatozoa and spermatids of *Vampyroteuthis*, and to highlight this the following notes and figures 4, 5 and 6, plates 4–6 (preliminary results of a comparative study in progress) are included. Galangau & Tuzet (1968*a*, *b*) and Galangau (1969) present details of spermiogenesis in another octopod, *Octopus vulgaris*, which are very similar to data obtained for *Octopus* sp. The developing acrosomal vesicle of *Octopus* sp. is initially spheroidal with layered substructure appearing near the site of attachment to the nucleus (figures 4*a* and 5*a*). Subsequently the vesicle elongates, and the periodically banded substructure becomes clearly established (figure 4*b*). In addition to the periodic banding, mature acrosomes show a helical keel, the base of which can be seen in figure 4*c*. As the acrosomal vesicle undergoes its structural transformation, an invagination forms within the base of the spermatid nucleus (figures 4*d–e* and 5*a*, *b*). Initially this invagination is shallow and occupied by two almost parallel centrioles (figure 4*d*), but later it deepens considerably and is filled by an extensive plug of dense material, the developing 'extra-nuclear rod' (term of Longo & Anderson (1970) (figures 4*f* and 5*a*, *f*). The origin of the 'extra-nuclear rod' is uncertain.

Description of plate 1

Figure 1. *Vampyroteuthis infernalis*. (*a*, *b*) Longitudinal sections through sperm acrosome (a), nucleus (n), midpiece mitochondria (m), centrioles (c) and tail (t): note extensive dense plug (dp) within the basal invagination of the nucleus. (*c*, *d*) Longitudinal sections through acrosome (a) and apex of nucleus (n). Arrow indicates dense peripheral layer. (*e*) Transverse section through the base of the acrosomal vesicle: anterior rim of nucleus also visible. Arrow indicates dense peripheral layer. Scale bars: (*a*,*b*) 1 μm; (*c–e*) 0.5 μm.

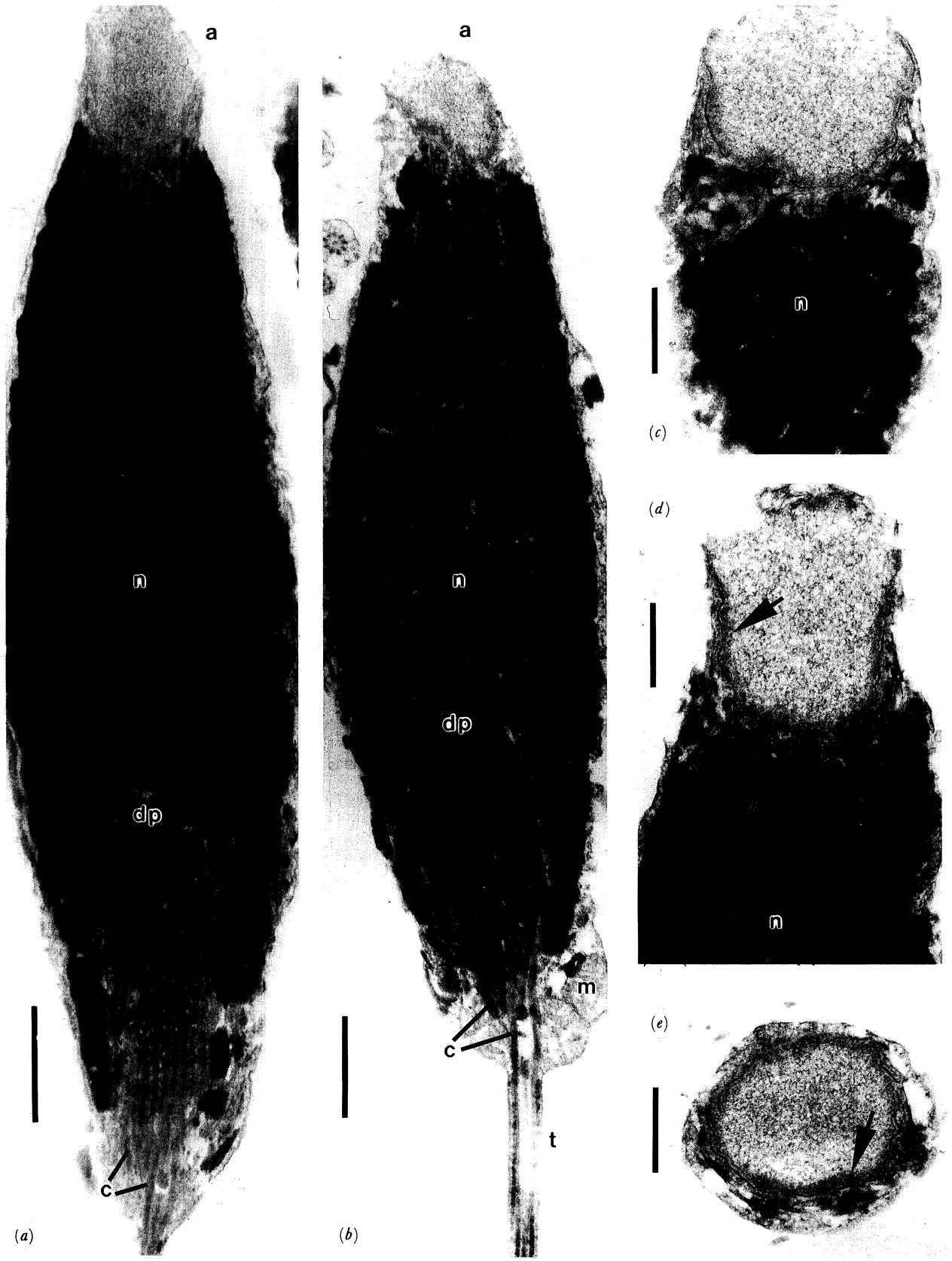

FIGURE 1. For description see opposite.

(*Facing p.* 592)

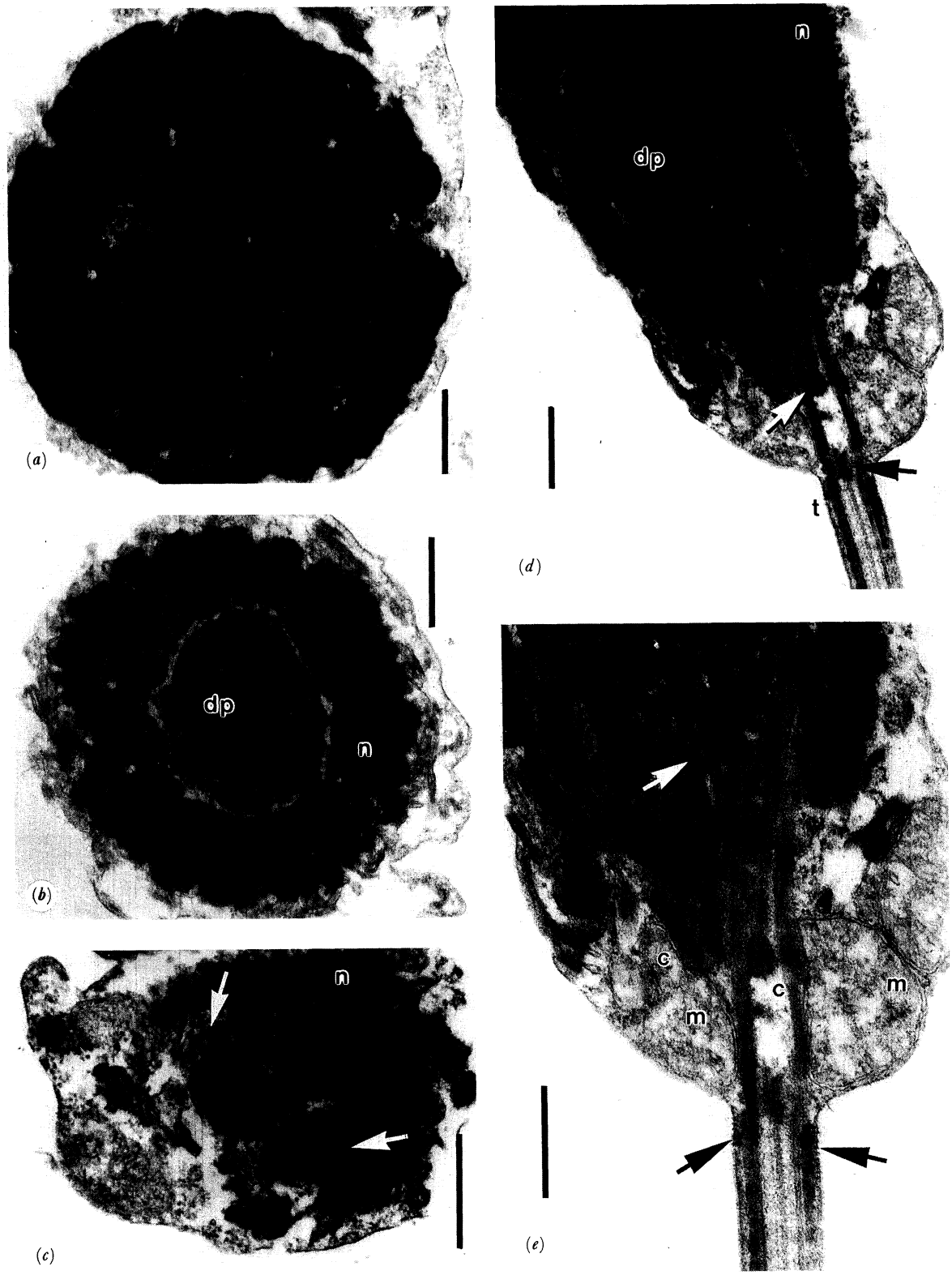

FIGURE 2. For description see facing plate 4.

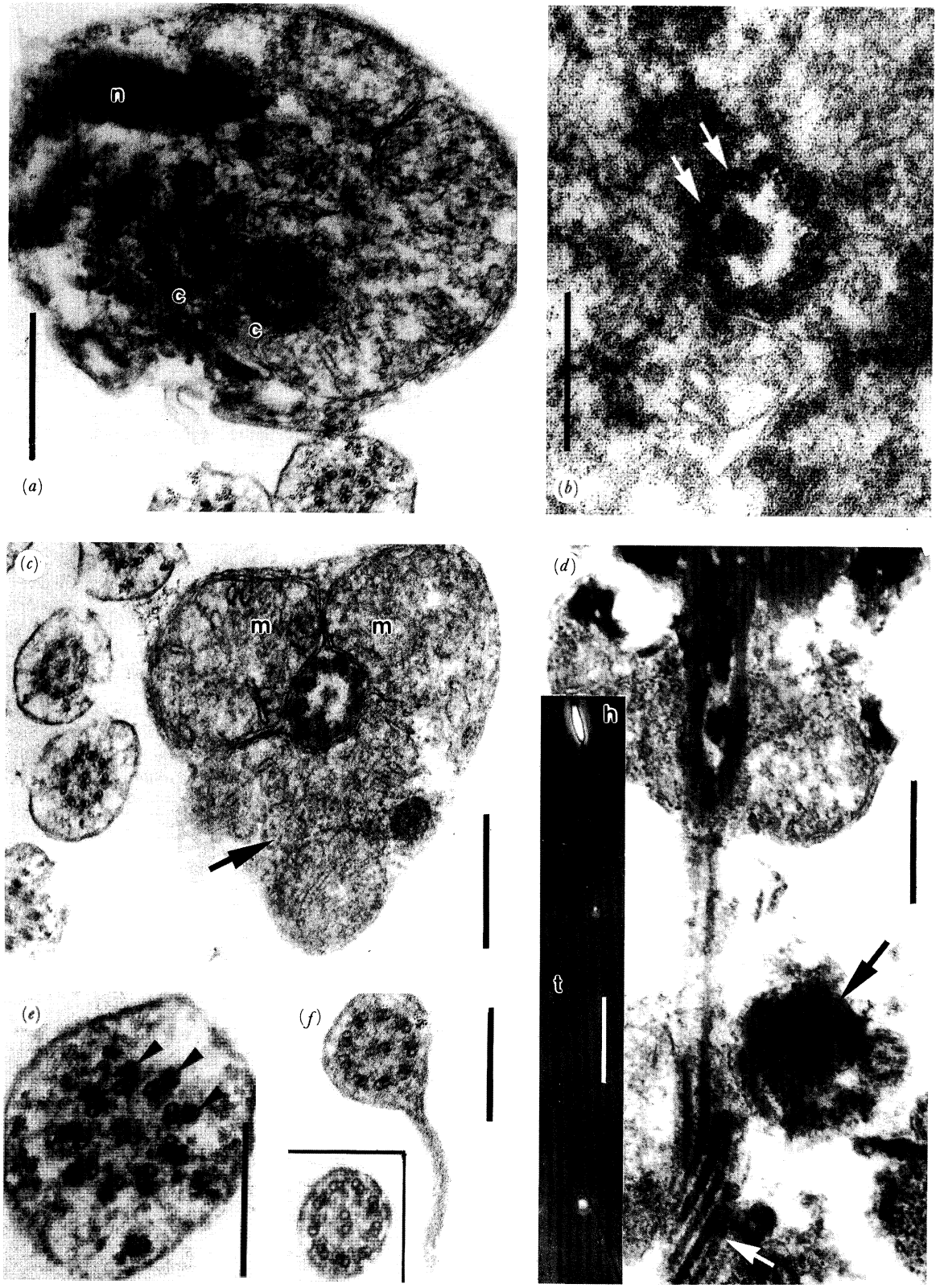

FIGURE 3. For description see overleaf.

Description of plate 2

Figure 2. *Vampyroteuthis infernalis*. (*a*) Transverse section through middle region of the sperm nucleus showing internal cavities and circular profile. (*b*) Transverse section through basal invagination of nucleus (n) showing the extensive plug of dense material (dp: note scattered cavities). (*c*) Transverse section through posterior extremity of the nucleus (n) and the dense plug. White arrows indicate fibres from centrioles attaching to the periphery of the plug. (*d*) Longitudinal section showing: the dense plug (dp) within the nuclear (n) invagination; two centrioles, one with its internal granule visible (white arrow); mitochondria surrounding the centrioles; tail (t) continuous with the 'distal' centriole. Black arrow indicates dense granule from which arise the central microtubules of the axoneme. (*e*) Detail of previous figure: note centrioles (c), midpiece mitochondria (m), ?glycocalyx (black arrows), microtubules penetrating the plug (white arrow: these originating from the centrioles). Scale bar 0.5 μm.

Description of plate 3

Figure 3. *Vampyroteuthis infernalis*. (*a*) Transverse section through both centrioles (c) at the nucleus-midpiece junction. (*b*) Detail of a centriole showing its constituent triplets (arrows). (*c*) Transverse section of the midpiece showing the distal centriole surrounded by a triangular cluster of mitochondria (m) and dense (?glycogen) granules (arrow). (*d*) Slightly oblique longitudinal section of the midpiece and proximal portion of the tail, showing (white arrow) helical twisting of the coarse fibre – axoneme complex. Visible in transverse section (black arrow) is a ring formed presumably from the fusion of the nine coarse fibres (at the midpiece–tail junction). Inset: phase-contrast micrograph showing spermatozoan head (h) and tail (t). (*e*) Transverse section through the tail showing the axoneme – coarse fibre complex surrounded by dense granules (?glycogen). (*f*) Distal region of tail with flange structures (?artefact). Inset: tail without flange. Scale bars: (*d* inset) 20 μm; (*a*,*c*,*d*) 0.5 μm; (*b*,*e*,*f*) 0.25 μm.

Description of plate 4

Figure 4. Spermiogenesis in *Octopus* sp. (*a–c*) Stages in development of the acrosomal vesicle from: spherioidal stage (*a*, showing initial signs of internal banding); to (*b*) elongating phase with bands obvious; to (*c*) mature acrosome with its banding and helical keel (k). Note also various stages of nuclear (n) condensation. (*d–f*) Initial stages in the formation of the 'extra-nuclear rod' (*d*), two centrioles occupy the nuclear invagination; (*e*), detail of proximal centriole; (*f*), persistence of distal centriole (c), possible involvement of proximal centriole in rod enr development). Nucleus (n) is still uncondensed. Scale bar 0.5 μm.

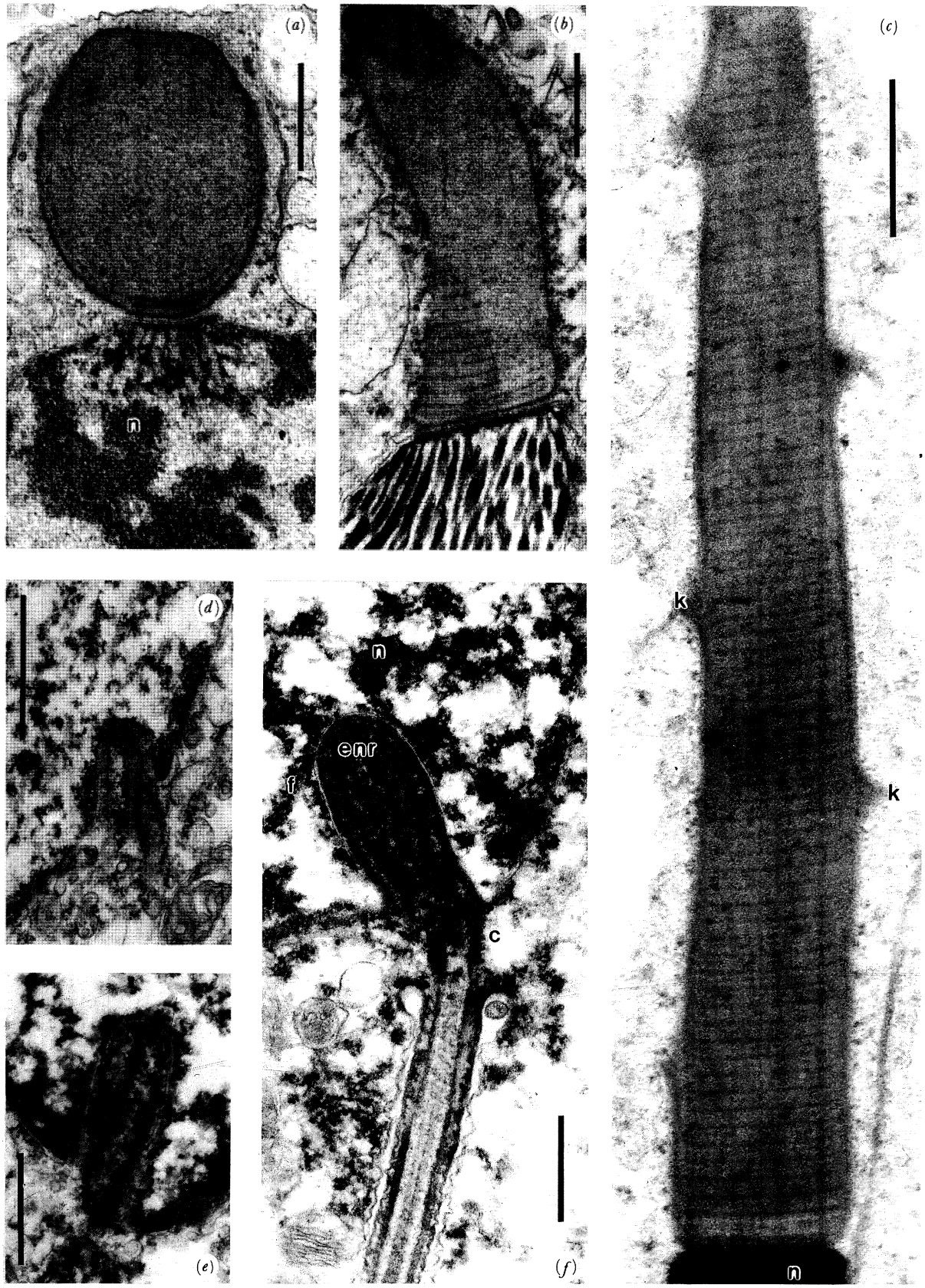

FIGURE 4. For description see opposite.

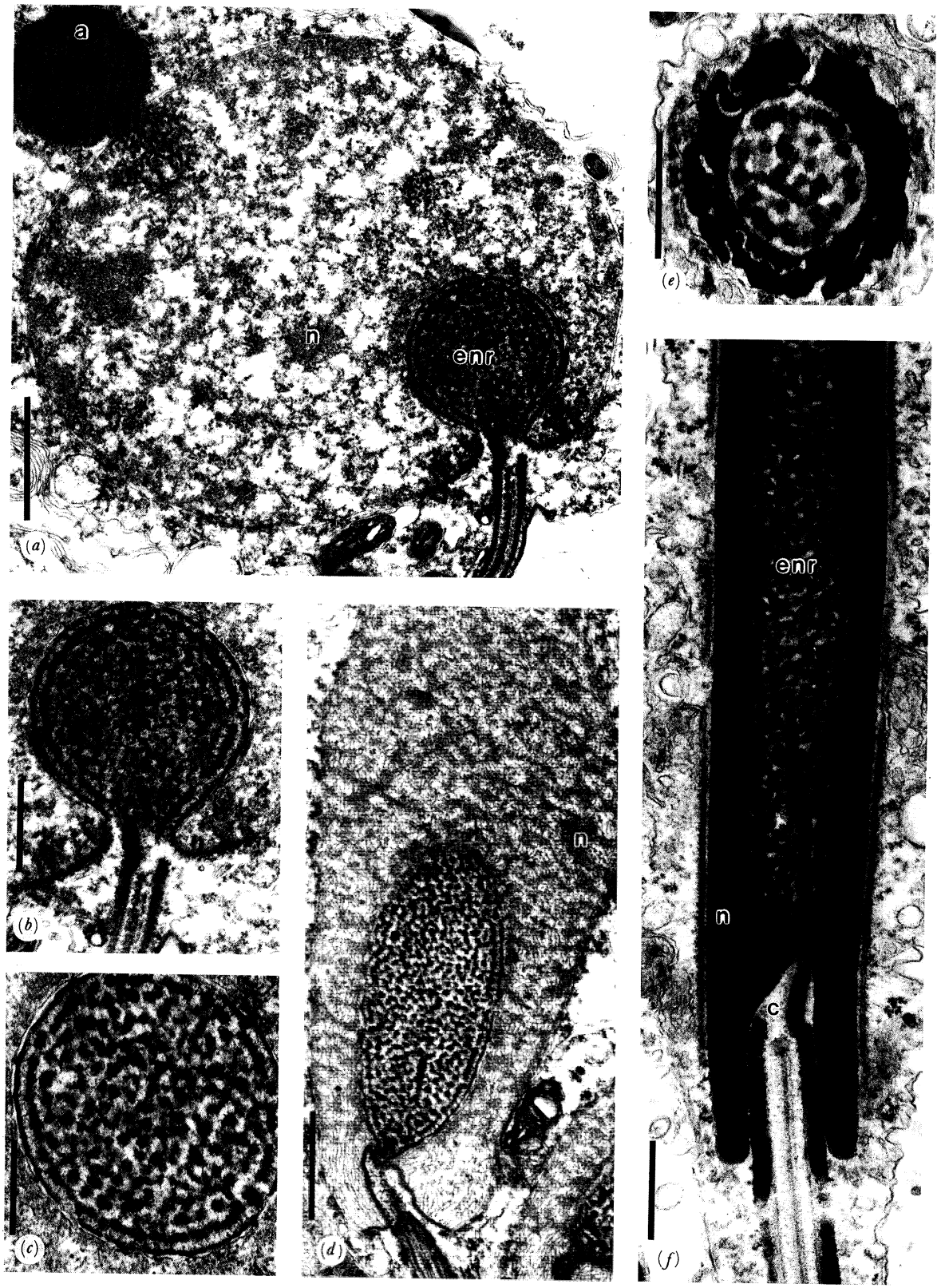

FIGURE 5. For description see facing page 593.

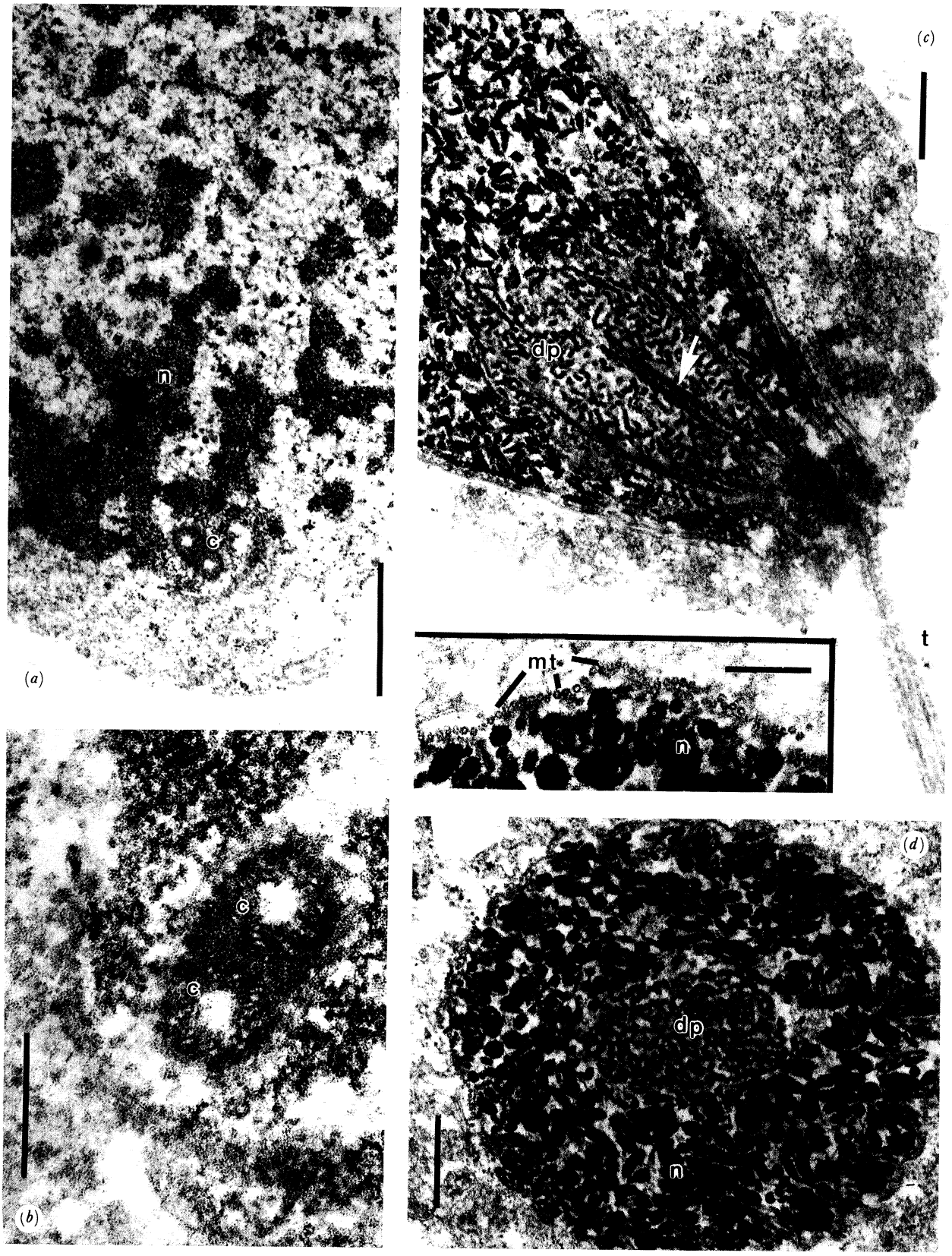

FIGURE 6. For description see overleaf.

Description of plate 5

Figure 5. Spermiogenesis in *Octopus* sp. (*a*) Relatively early stage spermatid showing spheroidal acrosome (a), developing 'extra-nuclear rod' (enr), condensing nucleus (n). (*b*) Detail of developing 'extra-nuclear rod' from previous figure. (*c*, *d*) Transverse (*c*) and longitudinal (*d*) sections through mid-spermatid showing the anastomosing fibres of the developing 'extra-nuclear rod' (compare with figure 6*c*, *d* of *Vampyroteuthis* spermatids). (*e*, *f*) Transverse (*e*) and longitudinal (*f*) sections through the 'extra-nuclear rod' of a late spermatid. The distal centriole (c) is continuous with the axoneme – coarse fibre complex. Scale bars: (*a*, *d*) 1 μm; (*b*, *c*, *e*, *f*) 0.5 μm.

Description of plate 6

Figure 6. Preliminary observations on spermiogenesis in *Vampyroteuthis*. (*a*, *b*) Presence of two parallel triplet centrioles (c) at the base of the nucleus (n) in a very early spermatid. (*c*, *d*) Longitudinal (*c*) and transverse (*d*) sections through the developing dense plug within the nuclear invagination. The fibrous structure of the plug is remarkably similar to the developing 'extra-nuclear rod' of *Octopus* (compare with figure 5*c*, *d*). (c inset) microtubules (mt) adjoining surface of condensing nucleus (n). Note in (*c*) penetration of the plug by microtubules (white arrow) originating from the centrioles (obscured in figure). Scale bars: (*a*) 1 μm; (*b*, *c* inset) 0.25 μm; (*c*, *d*) 0.5 μm.

Disappearance of one of the two centrioles during spermiogenesis lead Galangau (1969) to postulate that this body was produced through direct transformation of the proximal centriole. Although some stages suggest that this could be true (for example figure 4*f*), several factors such as the sheer volume of the developing rod, and the production of a strikingly similar (and almost certainly homologous) structure in *Vampyroteuthis* spermatids without loss of a centriole (see figure 6), indicate that the 'extra-nuclear rod' in *Octopus* spermatids and spermatozoa is probably some form of centriolar rootlet. Figures 4*d–f* and 5*a–f* illustrate the structural changes occurring during formation of the 'extra-nuclear rod' in *Octopus* sp. from early spermatids (figures 4*d–f* and 5*a*, *b*) to mid-spermatids (figure 5*c*, *d*: developing rod extensive) and very late spermatids (see figure 5*e*, *f*, note decrease in diameter and increase in length of the rod). Figure 8*b*, based on data presented herein and by Galangau (1969) shows the transient resemblance of a mid-spermatid of *Octopus* spp. to spermatozoa and spermatids of *Vampyroteuthis*.

(*c*) *Preliminary observations on spermiogenesis in Vampyroteuthis*

The process of spermiogenesis in *Vampyroteuthis*, currently under investigation by the author, will be detailed in a future paper. However, for comparison with *Octopus*, it is necessary to present some of these results herein (figure 6). Two triplet centrioles, arranged parallel to each other and embedded in a granular matrix (see figure 6*a*, *b*) occur in early spermatids (and persist into mature spermatozoa). The origin of the dense plug that occupies the nuclear invagination of later spermatids and mature spermatozoa is unclear, but its production does not involve transformation of a centriole. In advanced spermatids (figure 6*c*, *d*) the developing plug shows the same substructure of anastomosing fibres as the developing 'extra-nuclear rod' of *Octopus* spermatids, strongly suggesting that these two features are homologous. In *Vampyroteuthis*, microtubules continuous with the centrioles penetrate deeply into the plug (see figure 6*c*), and these persist into mature spermatozoa (figure 2*e*). Microtubules also envelope the condensing nucleus, but in this case are transient features (figure 6*c* inset).

4. Discussion

(*a*) *Comparison with other cephalopods*

Spermatozoa of *Vampyroteuthis infernalis* (figure 7) show some similarities to other cephalopod sperm as well as features that may prove to be unique to the species. Figure 8*a–f*, based on unpublished observations (J. M. Healy) and the work of other authors, summarizes much of the following discussion on comparative sperm morphology in the Cephalopoda.

Acrosome

The spheroidal acrosome of *Vampyroteuthis* spermatozoa closely resembles immature acrosomes of *Loligo forbesi* and *Eusepia officinalis* (see Maxwell 1975) and to a lesser extent developing acrosomes of *Spirula spirula* (J. M. Healy, unpublished data) and octopods (*Octopus* spp.: figures 4*a* and 8*b*; Galangau & Tuzet (1968*a*); Galangau (1969); *Eledone cirrhosa*: Maxwell (1974). At maturity, however, the acrosomes of these cephalopods and *Nautilus pompilius* (see Arnold & Williams-Arnold 1978; Tsukahara 1985) differ significantly from those observed in spermatozoa of *Vampyroteuthis* (figures 7 and 8*a*). In sepiids and *Loligo forbesi*, the acrosome is sac-shaped (see figure 8*d*) with dense rods lining the internal face of the vesicle

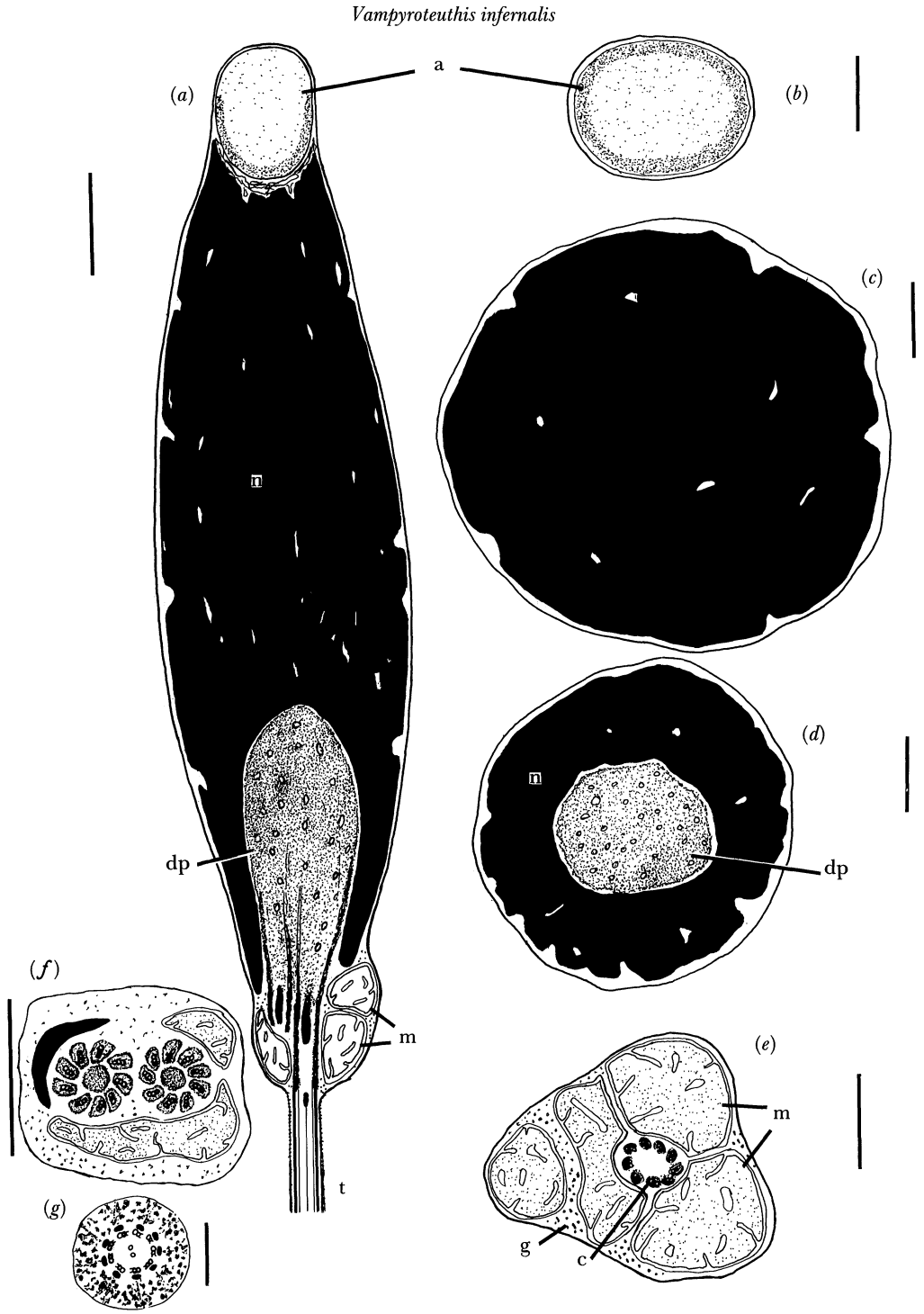

FIGURE 7. Semi-diagrammatic summary of *Vampyroteuthis* spermatozoa. (*a*) Longitudinal section through acrosome (a), nucleus (n), dense plug (dp), midpiece (mitochondria (m) plus centrioles (c)) and tail (t) of a spermatozoon. (*b*) Transverse section through acrosome (a) showing dense peripheral layer. (*c*) Transverse section through middle region of the nucleus. (*d*) Transverse section through basal invagination of nucleus (n) and dense plug (dp). (*e*) Transverse section through midpiece (mitochondria (m) arranged as a triangular cluster around the distal centriole (c)). Dense granules (g) (?glycogen) are also present. (*f*) Transverse section through both centrioles at nucleus–midpiece junction. (*g*) Transverse section through tail showing axoneme + coarse fibre complex surrounded by dense (?glycogen) granules. Scale bars: (*a*) 1 μm; (*b–g*) 0.5 μm.

(Maxwell 1975; J. M. Healy, unpublished data). In the acrosome of the sepiolid *Rossia pacifica*, complex folded septa are present instead of rods (Fields & Thompson 1976). Both *Rossia* and *Spirula* have slightly elongate acrosomes, each with a basal invagination and a differentiated apical zone (see figure 8*e*,*f*; see also Fields & Thompson (1976)). Franzén's (1955) light microscopical observations suggest that the same form of acrosome as *Rossia* and *Spirula* exists in the sepiolid *Sepietta oweniana*. In *Octopus* spp., the initially spherical acrosomal vesicle becomes transformed into an elongate, spirally keeled acrosome containing banded substructure (see figures 4*a–c* and 8*b*, *c* (Galangau & Tuzet 1968*a*; Galangau 1969); Leik 1970; Longo & Anderson 1970). Mature acrosomes of another octopod genus, *Eledone*, are helically coiled but according to Maxwell (1974) those of *E. cirrhosa* lack substructure. The acrosome of *Nautilus* also differs noticeably from that of *Vampyroteuthis* and in fact all other cephalopods: it is flattened in transverse profile, with a dense rod (? = axial rod, perforatorium) and a deep basal invagination (Arnold & Williams-Arnold 1978; Tsukahara 1985).

Nucleus

The sperm nucleus of *Vampyroteuthis* is short as also observed in the Sepioidea and Teuthoidea (figure 8*d–f*) (Retzius 1904; Franzén 1955; Maxwell 1974; Fields & Thompson 1976) but lacks the curved shape of most sepioid and teuthoid sperm nuclei (see figure 8*d*, *e*) and possesses an extensive plug of dense material within the nuclear invagination (figures 7 and 8*a*). A similar plug of dense material, known as the 'extra-nuclear rod' (Longo & Anderson 1970) or 'corps central intranucleaire' (Galangau 1969), occurs in spermatids and spermatozoa of *Octopus* spp. (see figures 4, 5 and 8*b*). Galangau (1969) considers that one of the two centrioles lodged in the nuclear invagination of early spermatids of *O. vulgaris*, transforms directly into this fibrous body of material (mature spermatozoa possessing only one centriole). However, the large size of the developing–mature 'extra nuclear rod' suggests that it may instead be a form of centriolar rootlet organized by one or both centrioles (and presumably assimilating the proximal centriole). Extensive, sometimes fibrous centriolar rootlets (associated with the nucleus) occur in spermatids and spermatozoa of other invertebrates (see, for example, Atwood 1975; Franzén 1982; Healy *et al.* 1988), though these usually exhibit some degree of banded substructure. The large dense plug observed within the nuclear invagination of *Vampyroteuthis* spermatozoa is connected to both centrioles via microtubules (figures 2*e* and 7*a*). During spermiogenesis it is produced without loss of a centriole and closely resembles the developing 'extra-nuclear rod' of octopod spermatids. This plug is here considered to be a form of centriolar rootlet and homologous with the 'extra-nuclear rod' or 'corps central intranucleaire' of *Octopus* spermatids and spermatozoa. Mid-spermatids of *Octopus* (see figures 5*d* and 8*b*) in many respects approach the form of *Vampyroteuthis* spermatozoa and spermatids (initially spheroidal acrosomal vesicle, slightly fusiform nucleus, basal invagination filled with an extensive, dense plug). The possible phylogenetic and systematic implications of this resemblance will be dealt with in the last section of the discussion.

Midpiece

The arrangement of mitochondria in *Vampyroteuthis* spermatozoa – a triangular cluster of three or sometimes four mitochondria surrounding both centrioles – is distinct from the mitochondrial spur seen in most sepioids and teuthoids (compare figure 8*a*, *d*, *e*) (see also Franzén 1955; Maxwell 1975; Fields & Thompson 1976), the sleeve-like midpiece of *Spirula*

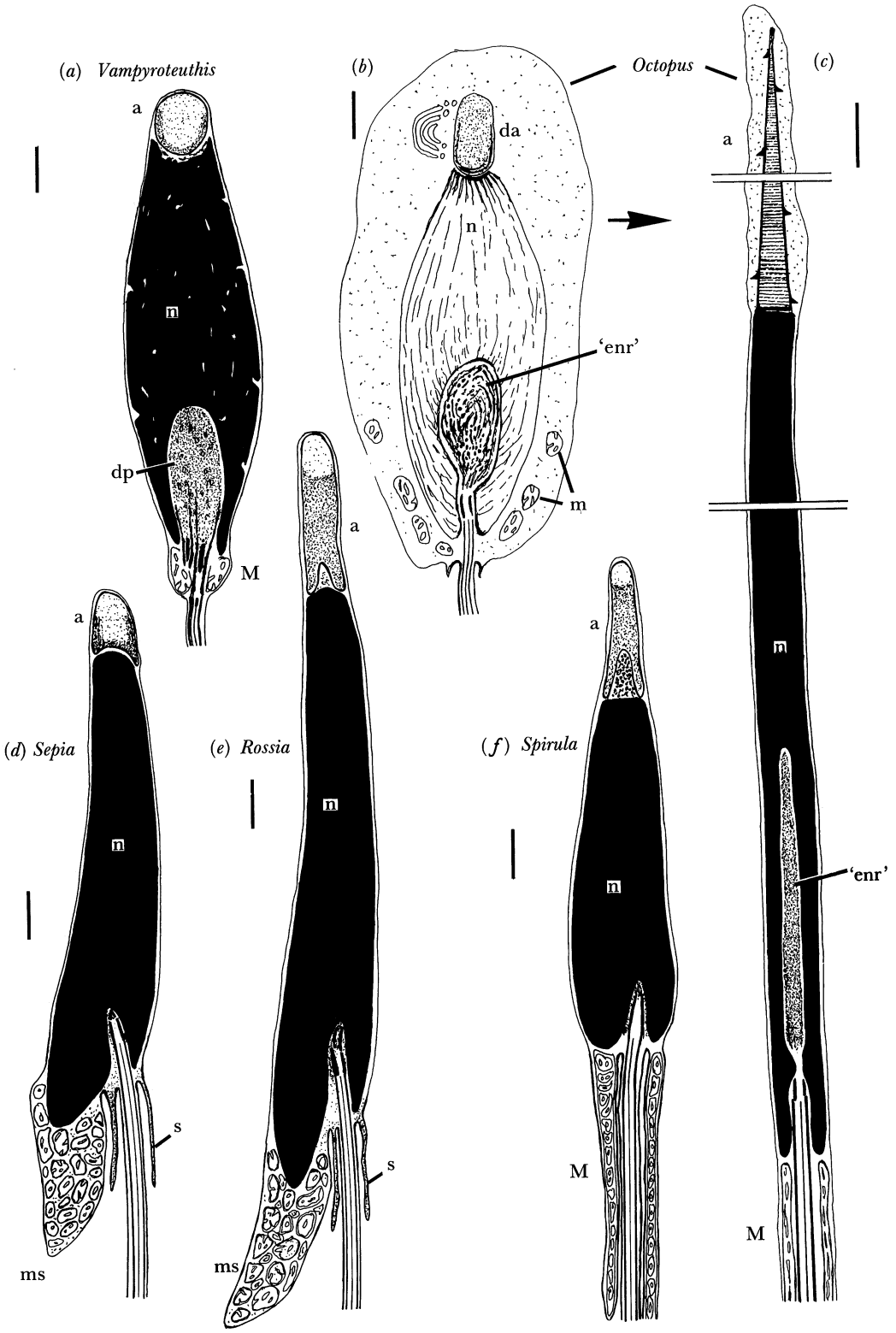

FIGURE 8. For description see opposite.

(figure 8*f*) and the slightly elongate midpieces of octopod spermatozoa (Galangau & Tuzet 1968*b*; Galangau 1969; Longo & Anderson 1970; Maxwell 1974; J. M. Healy, unpublished data). *Nautilus* spermatozoa have one groove on each side of the filiform (35–37 μm) nucleus into which fit elongate mitochondria (one per groove), an arrangement differing from all other cephalopods and probably unique within the Mollusca (see Arnold & Williams-Arnold 1978 and Tsukahara 1985) for descriptions and micrographs). In externally fertilizing molluscs such as bivalves and archaeogastropods (and many other invertebrates), the sperm mitochondria are clustered around the centrioles (Popham 1979; Koike 1985), suggesting that in *Vampyroteuthis* (which shows a similar positioning of sperm mitochondria) fertilization may take place externally and not in the oviduct or the ovary as in some octopods (see Franzén 1967; Mann 1984). The triangular cluster of mitochondria in *Vampyroteuthis* sperm cannot be considered a modification of the specialized 'mitochondrial spur' observed in spermatozoa of most sepioids and teuthoids: rather it may represent the original arrangement of sperm mitochondria in the Coleoidea. This pericentriolar positioning of sperm mitochondria has so far only been observed in *Vampyroteuthis*, but may eventually be found in the Sepioidea, Teuthoidea or in the unstudied octopod suborder Cirrata, a group sharing some anatomical features with *Vampyroteuthis*. The absence of an annulus or a reflected membrane skirt in *Vampyroteuthis* sperm may be peculiar to this species. Spermatozoa of other cephalopods, possibly with the exception of *Spirula*, possess an annulus, membrane skirt or both of these features (figure 8*d*, *e*) (Galangau & Tuzet 1968*b*; Galangau 1969; Longo & Anderson 1970; Maxwell 1975; Fields & Thompson 1976; Arnold & Williams-Arnold 1978; Tsukahara 1985).

Centrioles

Vampyroteuthis spermatozoa possess two triplet centrioles, arranged parallel to the sperm longitudinal axis and positioned essentially outside of the nuclear invagination (see figures 2*d*, *e*, 3*a* and 7). Two parallel centrioles also occur in *Nautilus*, but they are composed of doublets, not triplets and, as observed in other cephalopod sperm, are positioned within the nuclear invagination (Arnold & Williams-Arnold 1978; Tsukahara 1985). Fields & Thompson (1976) report two triplet centrioles within the nuclear invagination of the sepiolid *Rossia*, whereas one triplet centriole has been observed by Maxwell (1975) in spermatozoa of *Eusepia*, *Loligo* and *Alloteuthis*. Only one, possibly doublet, centriole occurs in *Spirula* (J. M. Healy, unpublished data) and octopod spermatozoa (Galangau 1969; Longo & Anderson 1970; J. M. Healy,

FIGURE 8. Semi-diagrammatic summary of sperm structure within the Coleoidea (*a*) *Vampyroteuthis infernalis* (note dense plug (dp), 'extranuclear rod' of *Octopus*). (*b*) Mid-spermatid of *Octopus* spp. Note resemblance to *V. infernalis* spermatozoon (da, developing acrosome; 'enr', developing 'extranuclear rod' (terminology of Longo & Anderson (1970)), m, mitochondria, n, condensing nucleus. (*c*) Mature *Octopus* spp. spermatozoa. The acrosome (a) has assumed its periodically striated and helically keeled form. The 'extranuclear rod' ('enr') is now elongated. Only the proximal portion of the midpiece (M) is depicted. (*d*) Mature spermatozoon of *Sepia rozella* showing mitochondrial spur (ms), membrane skirt (s), dome-shaped acrosome (a) and curved nucleus (n). (*e*) Mature spermatozoon of the sepiolid *Rossia pacifica*. Note mitochondrial spur (ms), membrane skirt (s), curved nucleus (n) and the slightly elongate acrosome (a, with a basal invagination and differentiated apical region, septal structures in the wall of the acrosome not shown). (*f*) Mature spermatozoon of *Spirula spirula*, showing acrosome (a) (with basal invagination and apical zone), straight nucleus (n) and sheath-like midpiece (M). Part (*f*) based on J. M. Healy (unpublished observations); (*b*, *c*) based on observations presented herein and additional work by Galangau & Tuzet (1968*a*), Galangau (1969) and Longo & Anderson (1970); (*d*) based on J. M. Healy (unpublished observations) (work by Maxwell (1975) suggests that sperm of *Eusepia officinalis*, *Loligo forbesi* and *Alloteuthis subulata* are very similar to that here illustrated for *Sepia rozella*). Figure 8*e* based on micrographs and line drawings of Fields & Thompson (1976). Scale bar 1 μm.

unpublished data), though in early *Octopus* spermatids, two almost parallel centrioles initially occupy the developing basal invagination of the nucleus (figure 4*d*) (Galangau 1969). Unlike the centrioles of other cephalopod spermatozoa, those observed in *Vampyroteuthis* each contain a dense rod-shaped granule within the centriolar lumen (see figures 2*d* and 7*a*, *f*).

Coarse fibres

The presence of nine thickened coarse fibres in the axonemal complex of cephalopod sperm and aspects of their physical and chemical structure have been described by several workers (Galangau & Tuzet 1968*b*; Longo & Anderson 1970; Maxwell 1974, 1975; Fields & Thompson 1976; Baccetti *et al.* 1975, 1976; Arnold & Williams-Arnold 1978). Coarse fibres of *Vampyroteuthis* spermatozoa are not as developed as those of most other cephalopods, though some micrographs suggest that they fuse into a ring near the base of the distal centriole, as has been reported in octopods (see Galangau & Tuzet 1968*b*; Galangau 1969; Longo & Anderson 1970; Maxwell 1974).

'*Glycogen*'

In spermatozoa of *Vampyroteuthis*, dense granules – possibly glycogen deposits – occur between mitochondria of the midpiece and around the axoneme – coarse fibre complex of the tail. Glycogen in spermatozoa of other cephalopods is distributed in varying ways: surrounding the nucleus (and its embedded mitochondria) in *Nautilus* (Arnold & Williams-Arnold 1978); between mitochondria in *Rossia* and probably other sepioids and teuthoids (Fields & Thompson 1976); around the axoneme – coarse fibre complex in the glycogen piece (post-midpiece region of the spermatozoan) of octopods (see Galangau & Tuzet 1968*b*; Galangau 1969; Longo & Anderson 1970; Maxwell 1974). The distribution of putative glycogen in *Vampyroteuthis* would seem to be intermediate between the arrangement seen in sepioids–teuthoids and that seen in octopods.

(*b*) *Relationship of Vampyroteuthis to other cephalopods as indicated by sperm morphology*

Although it is not the purpose of this paper to review cephalopod phylogeny (a topic treated in detail by Donovan 1964, 1977), Jeletzky (1966), Teichert (1967) and Bandel & Leich (1986), the information presented here on sperm morphology does invite some comment on the possible relationship of the Vampyromorpha (as exemplified by *Vampyroteuthis*) to other coleoidean cephalopods.

As previously noted in this discussion, spermatids and spermatozoa of *Vampyroteuthis* show some similarity to spermatids and spermatozoa of sepioids and teuthoids (nucleus short, acrosome simple and spheroidal as for example in sepiid spermatids), but significantly are also very similar to spermatids of *Octopus* spp. (early acrosomal vesicle spheroidal, short initially fusiform nucleus, basal invagination of nucleus extensive and filled with dense material: see figure 8*b*). Despite these resemblances, *Vampyroteuthis* spermatozoa remain distinct from spermatozoa of sepioids, teuthoids and octopods, and show features that could have occurred in spermatozoa of primitive coleoideans (for example, a spheroidal acrosome lacking any complex substructure, short straight nucleus, two triplet centrioles lying essentially outside the nuclear invagination, pericentriolar mitochondria). However, if the large plug of dense material occupying the nuclear invagination in *Vampyroteuthis* spermatids and spermatozoa can be accepted as homologous with the 'extra-nuclear rod' of *Octopus* spermatids and spermatozoa

(the view adopted herein), then the idea of a vampyromorphan origin for the Octopoda or that of a common origin for these two groups both seem feasible. No equivalent to this sperm structure has yet been found in mature or developing spermatozoa of any member of the Sepioidea or Teuthoidea. A study of spermiogenesis and spermatozoa in the most primitive group of octopods, the Cirrata, could reveal sperm types intermediate between those of *Vampyroteuthis* and *Octopus* spp., or at least help to qualify the Vampyromorpha–Octopoda connection suggested by general anatomy (see Young 1977), fossil evidence (see Donovan 1977; Bandel & Leich 1986) and sperm morphology. To this end the author is currently engaged in a survey of sperm structure within the Cephalopoda, some of the preliminary results of which have been included herein for comparison with *Vampyroteuthis*. For now it can be said that sperm morphology supports the current assignment of *Vampyroteuthis* to a separate coleoidean order, the Vampyromorpha.

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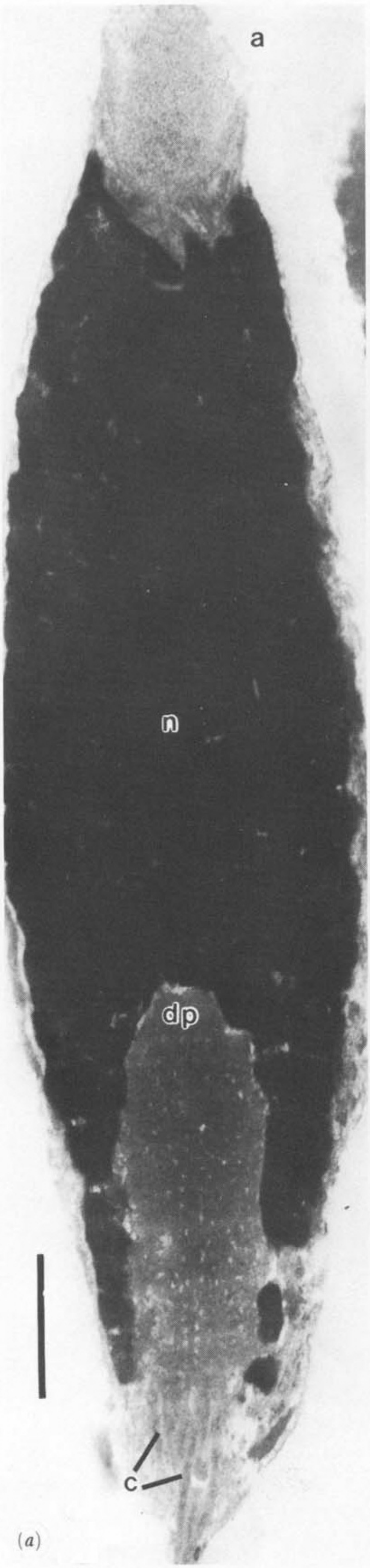

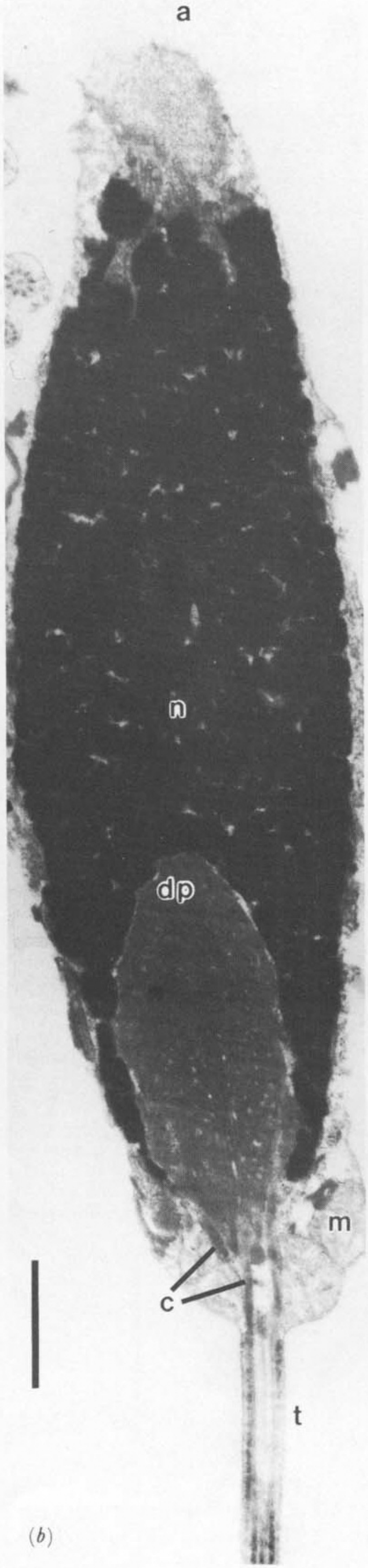

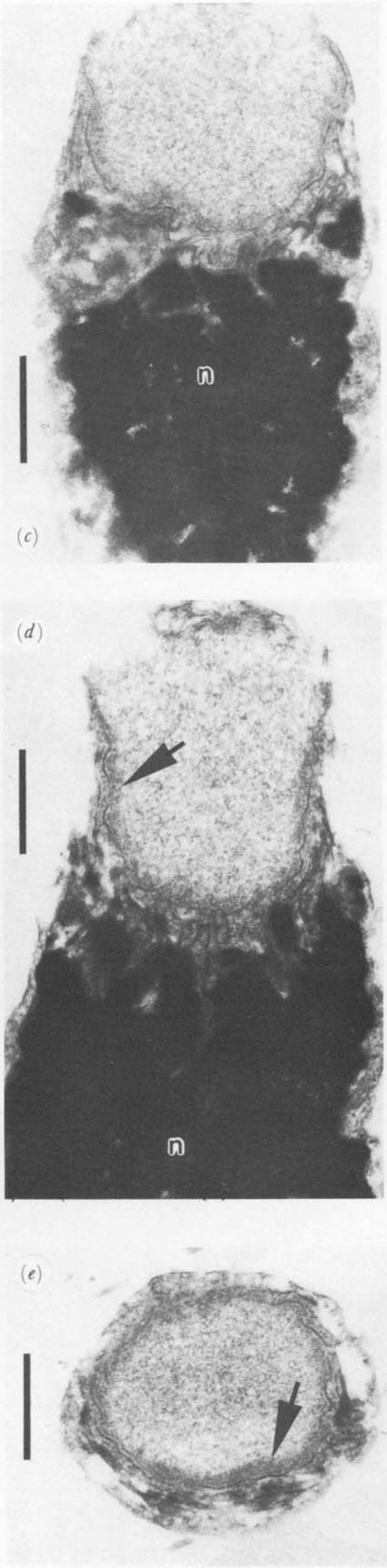

FIGURE 1. For description see opposite.

(a)

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(c)

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(d)

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(e)

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c

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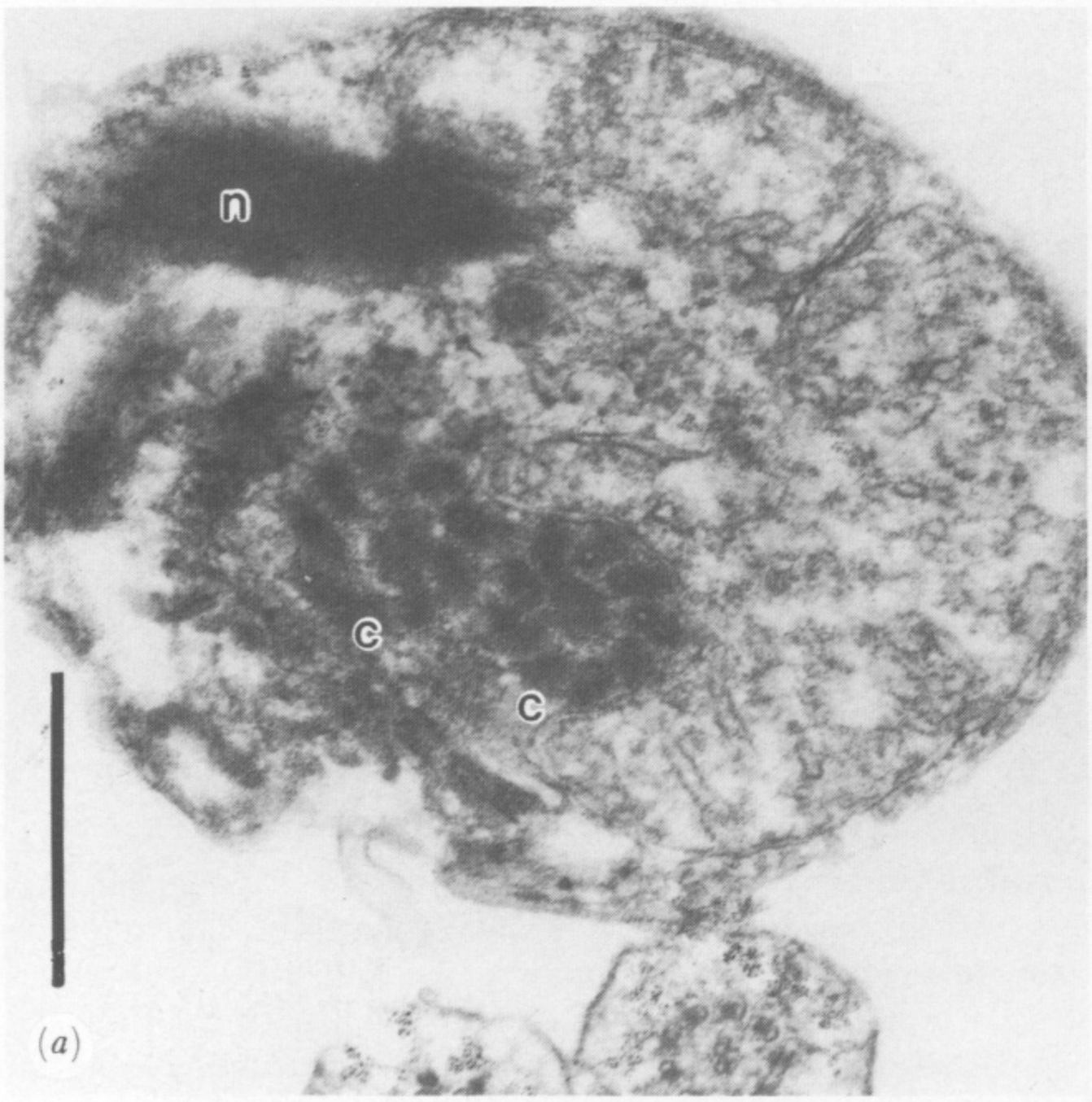
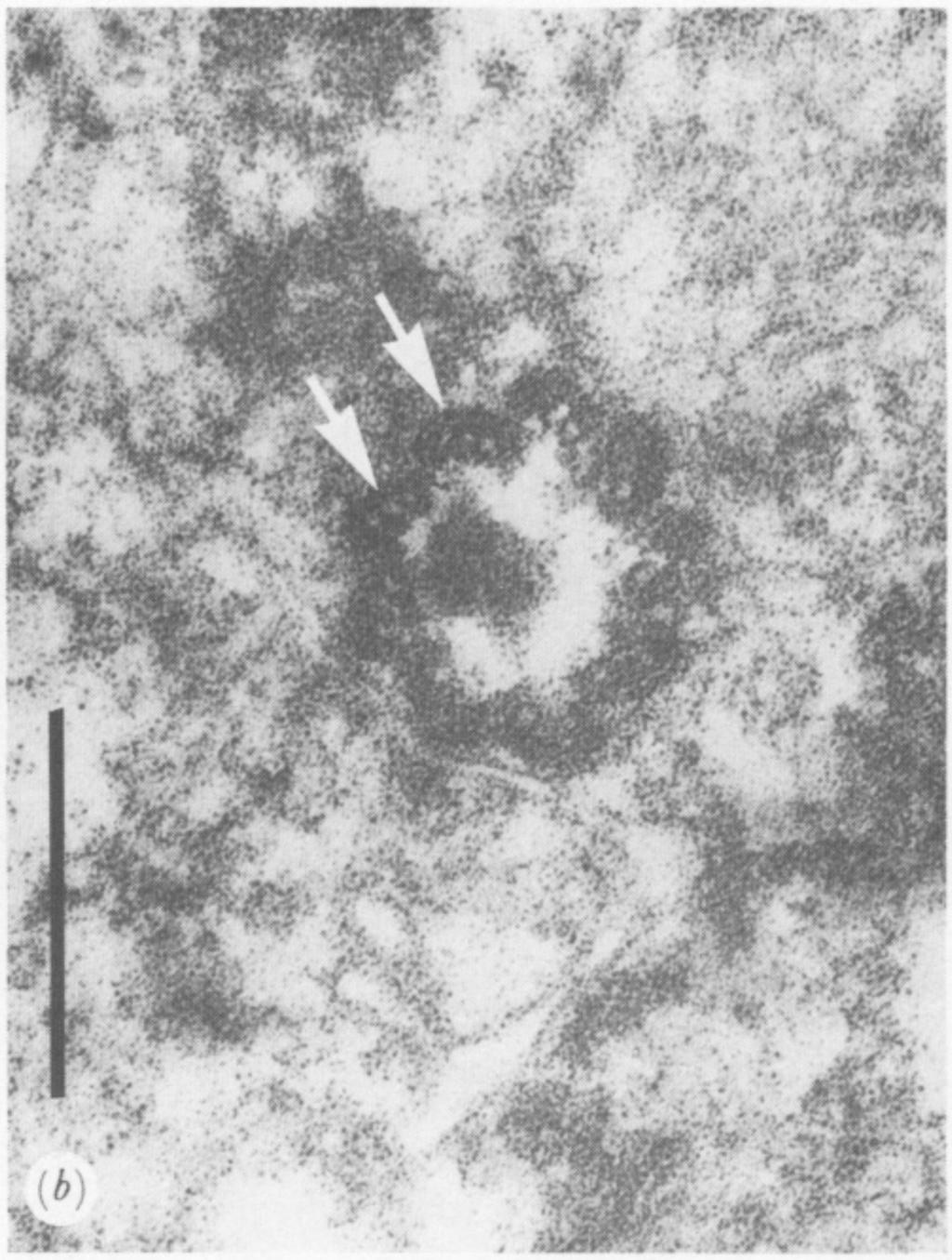
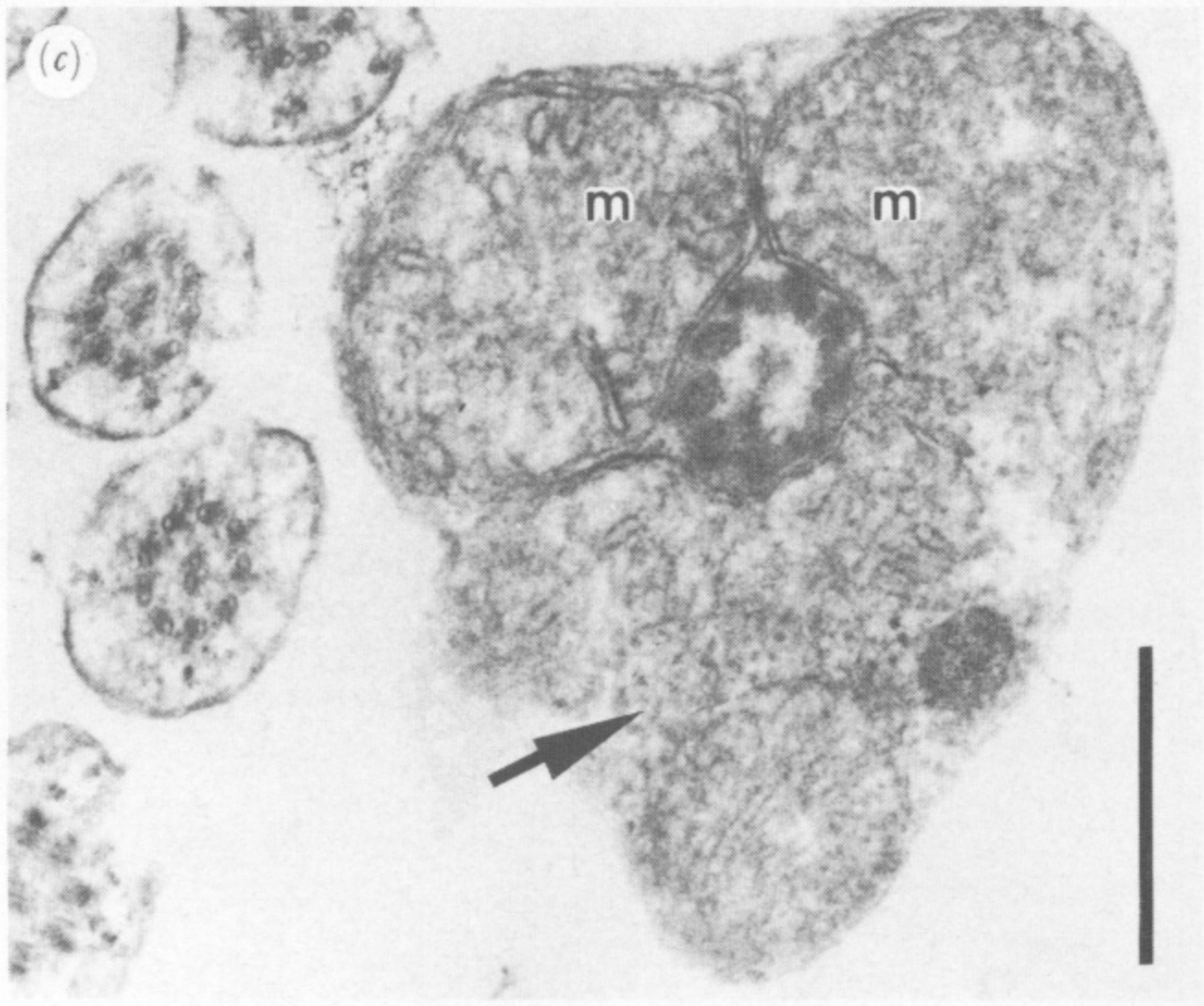
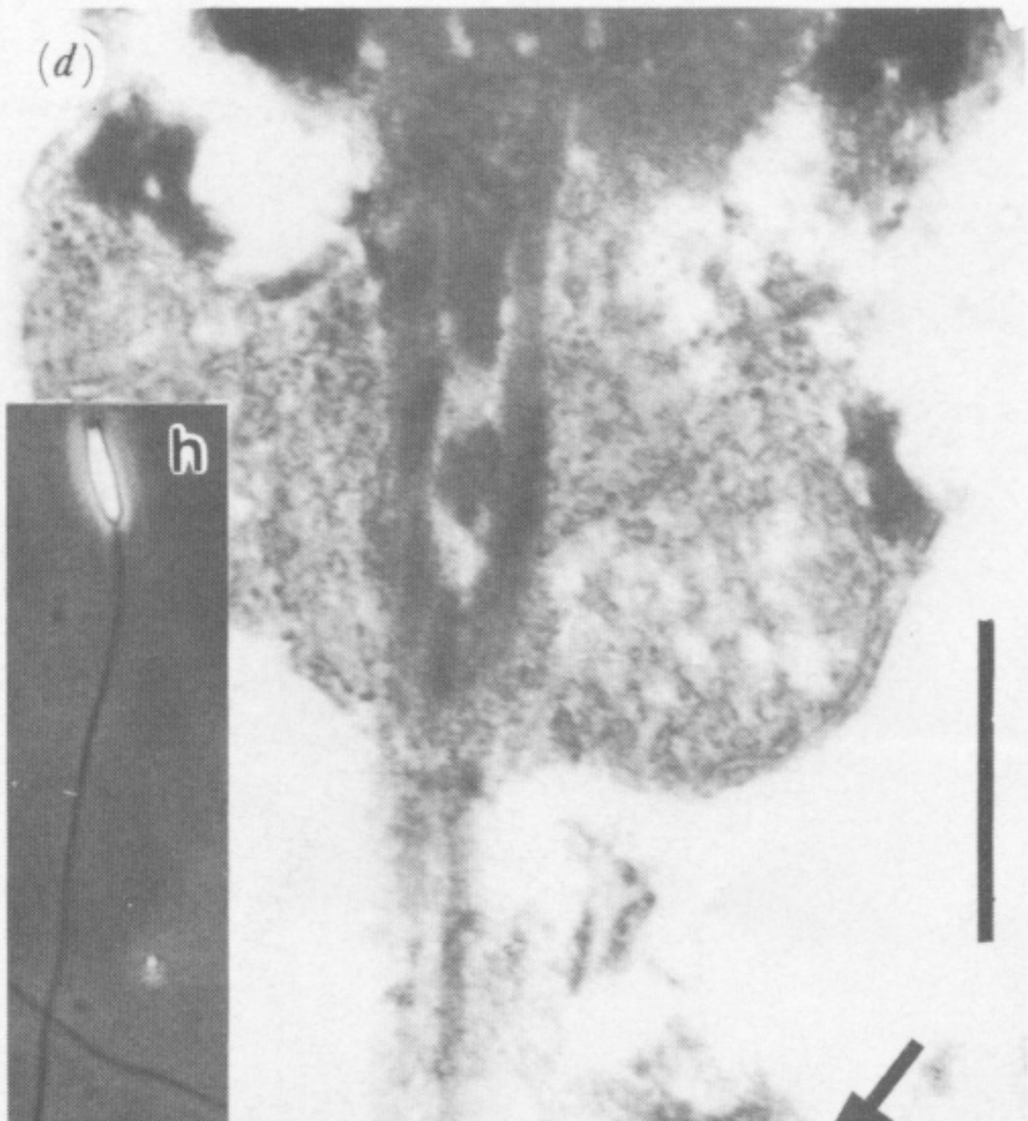
FIGURE 2. For description see facing plate 4.









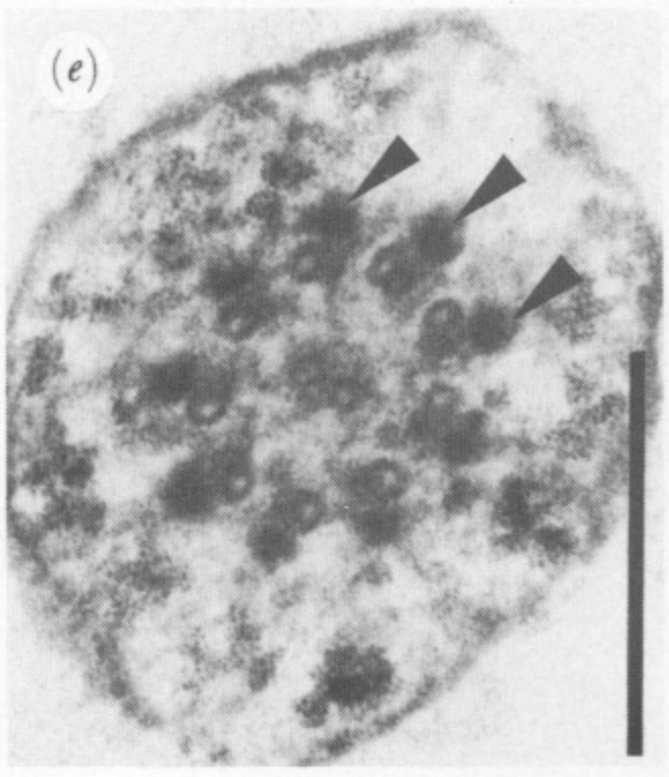
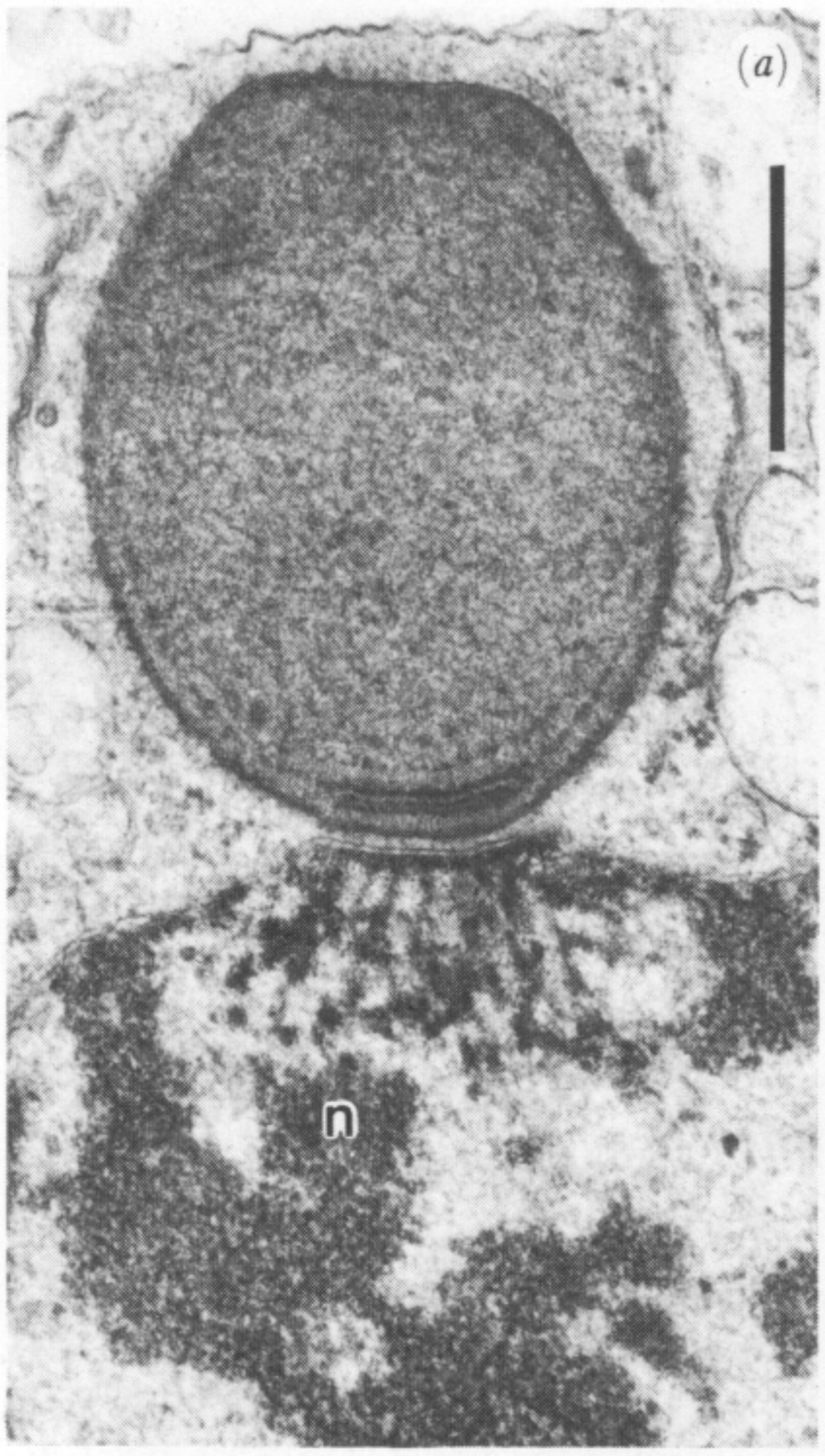
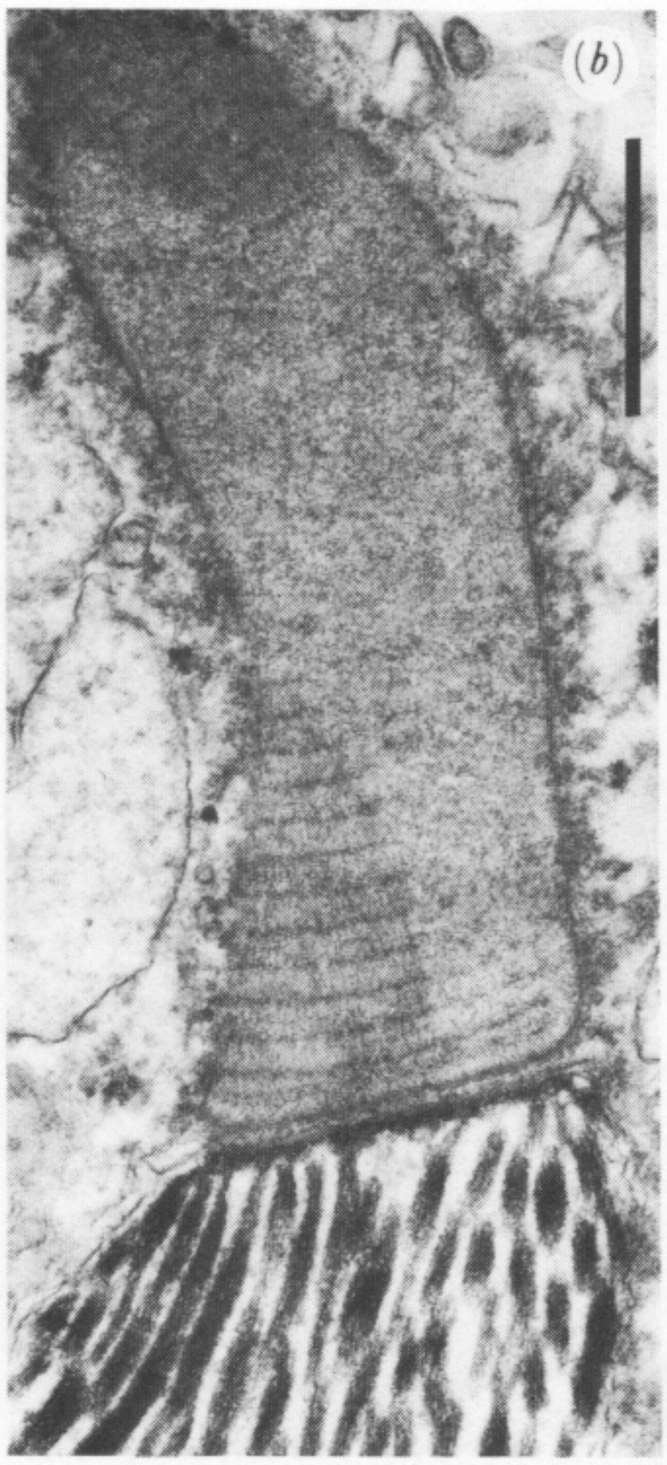
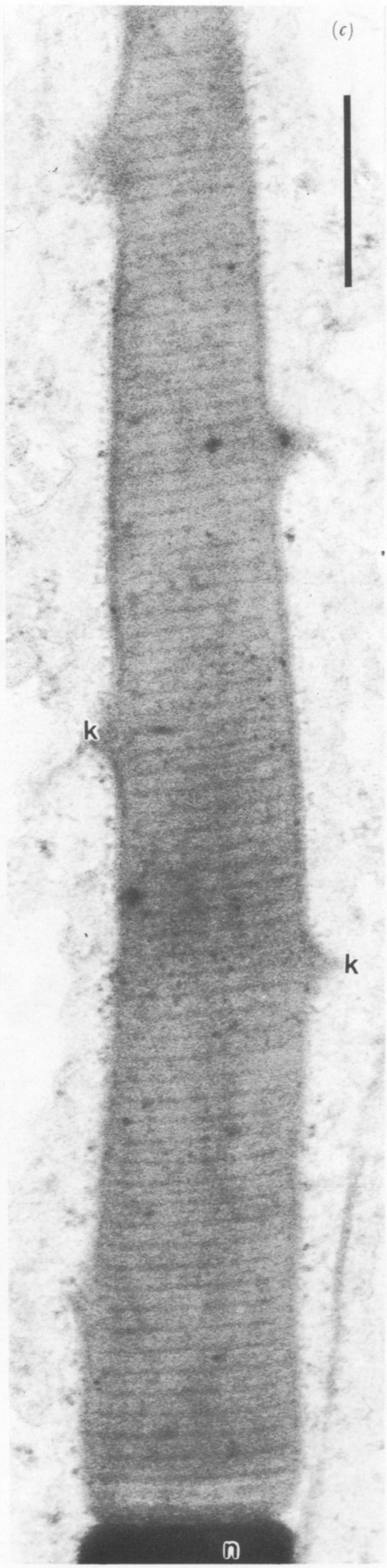
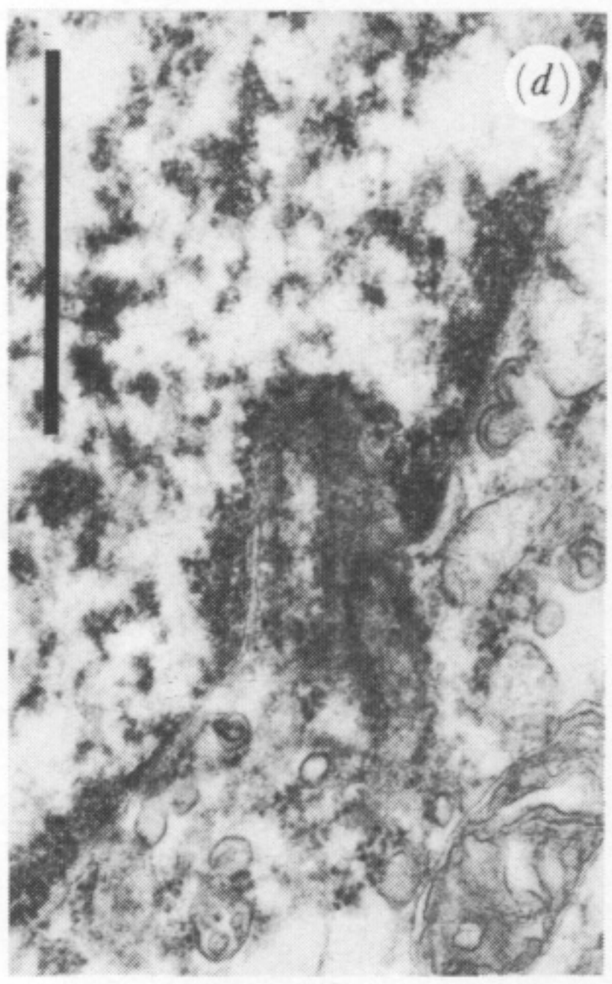


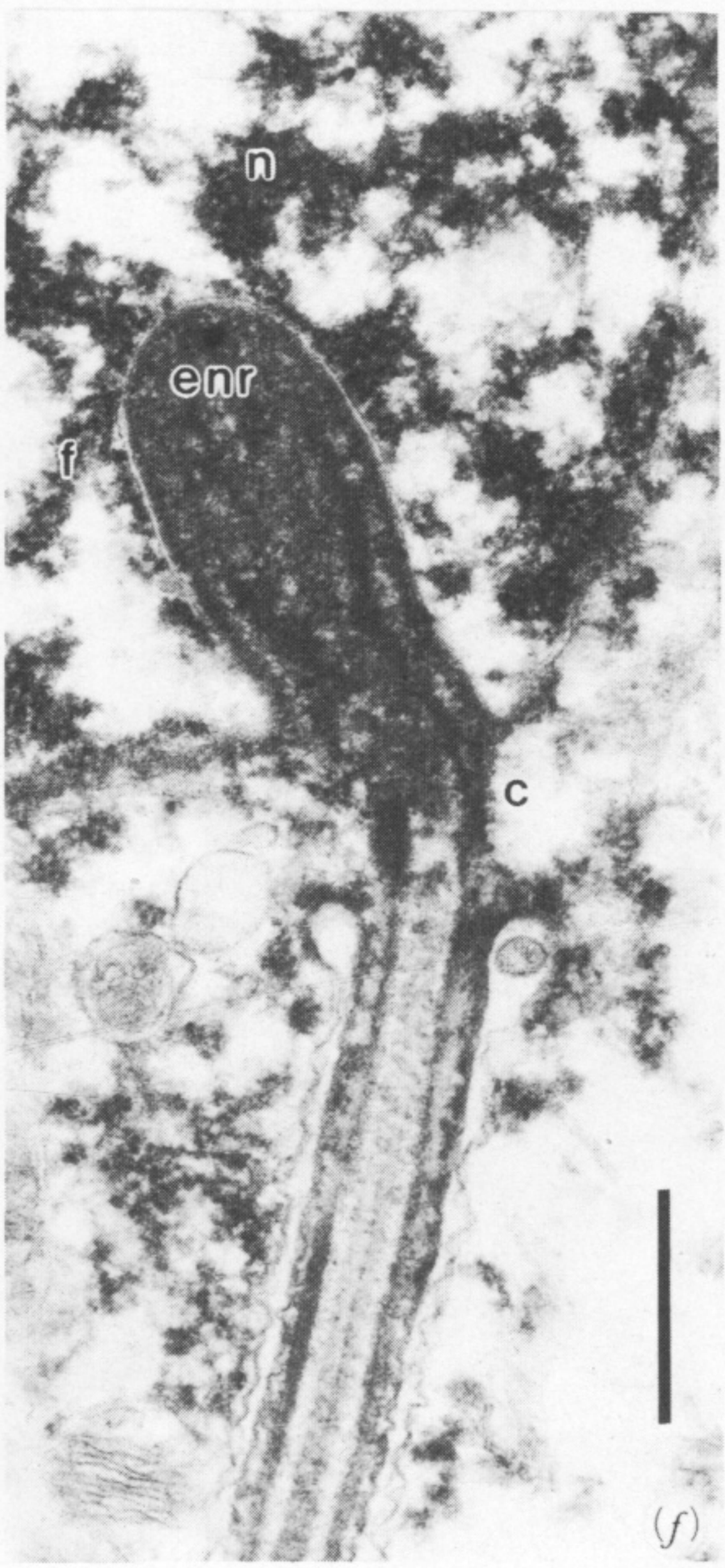
FIGURE 3. For description see overleaf.











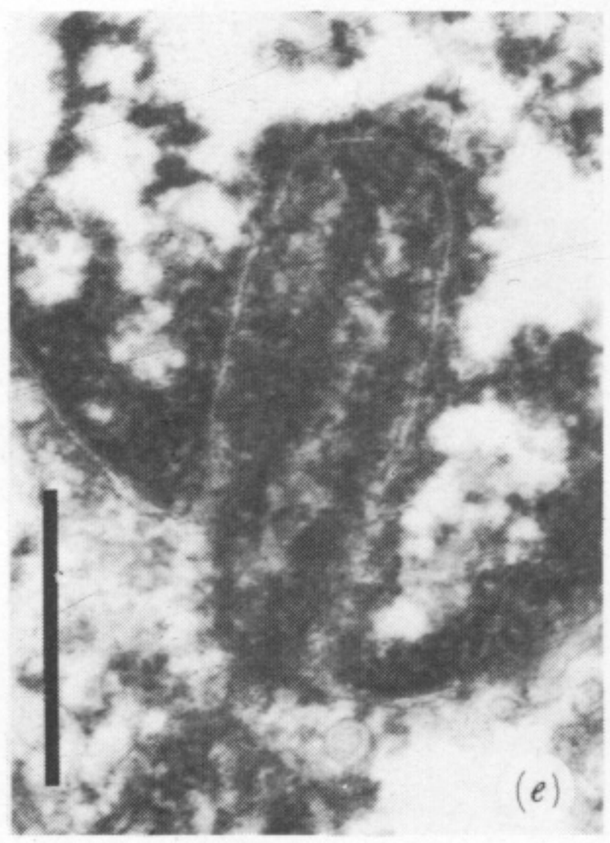
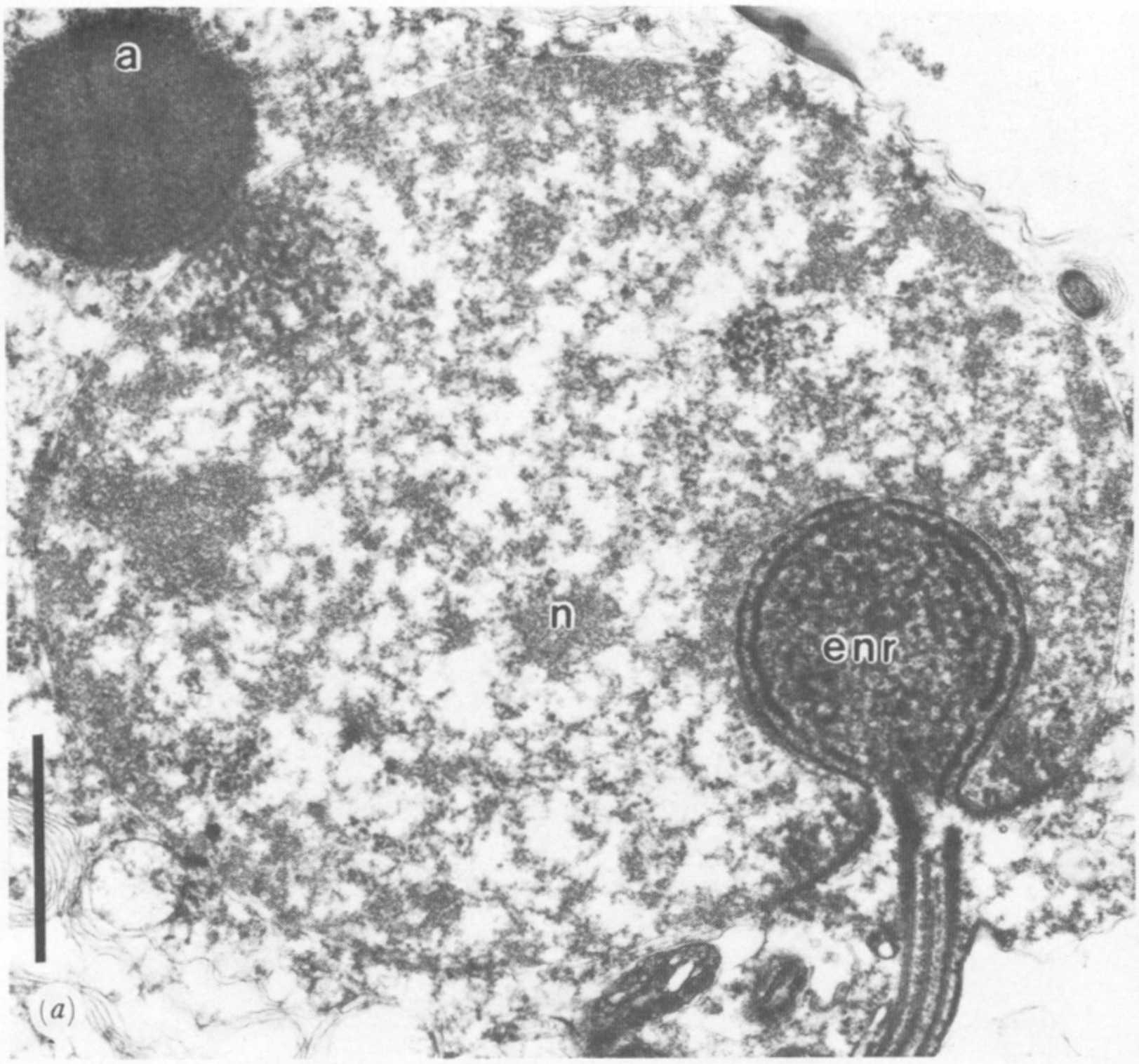
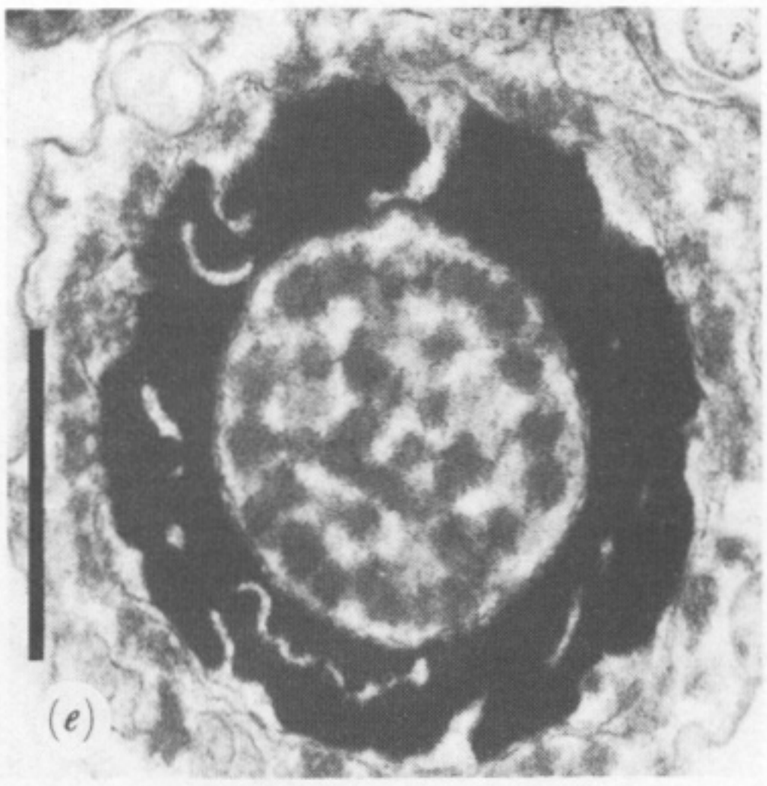
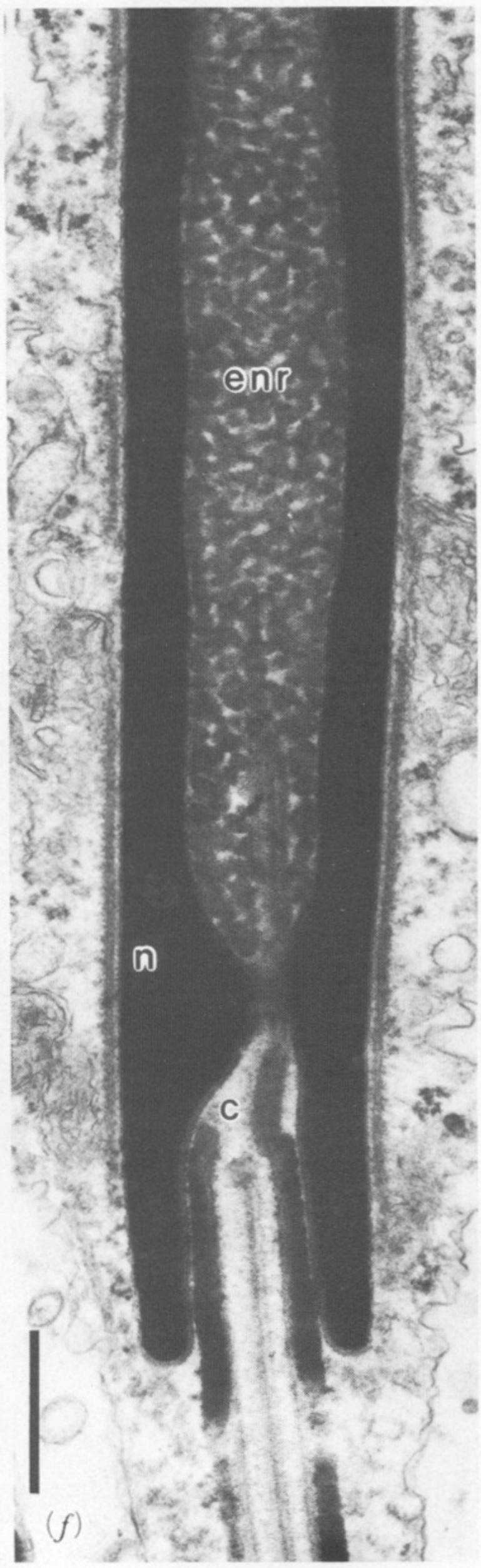
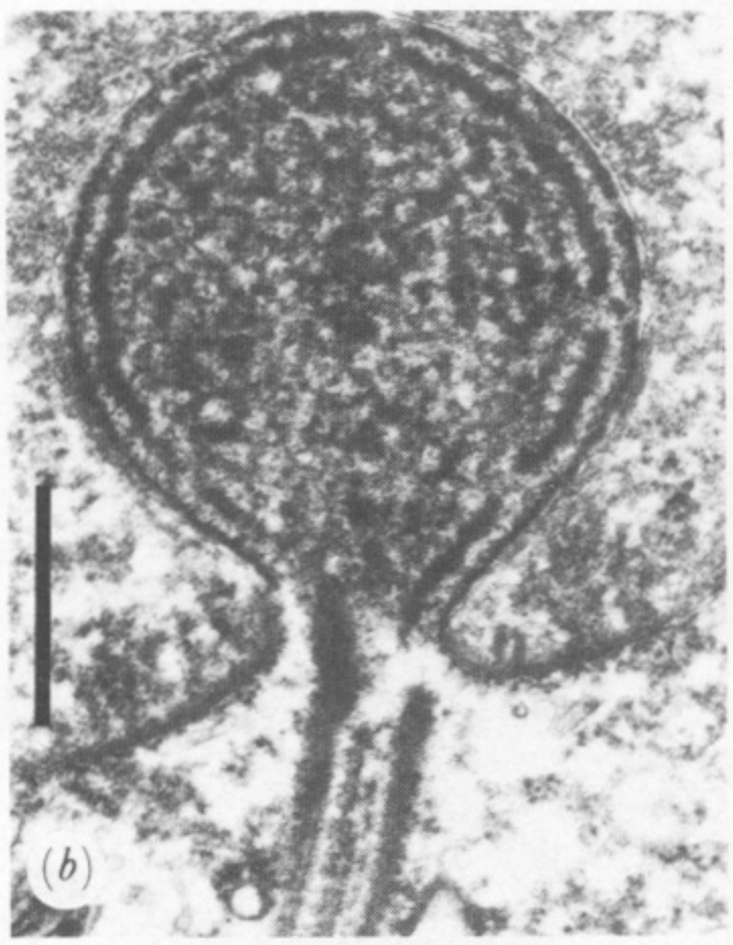


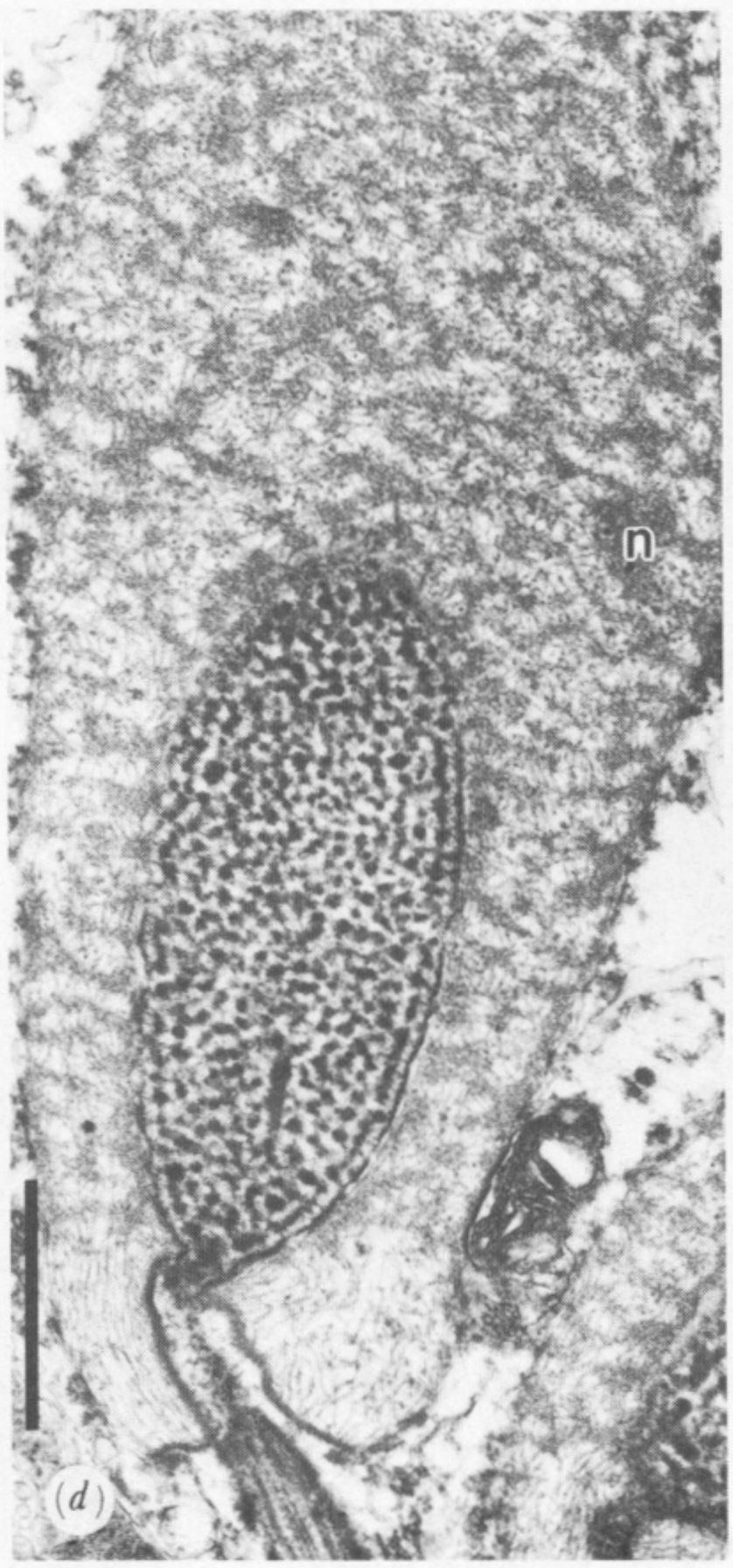
FIGURE 4. For description see opposite.











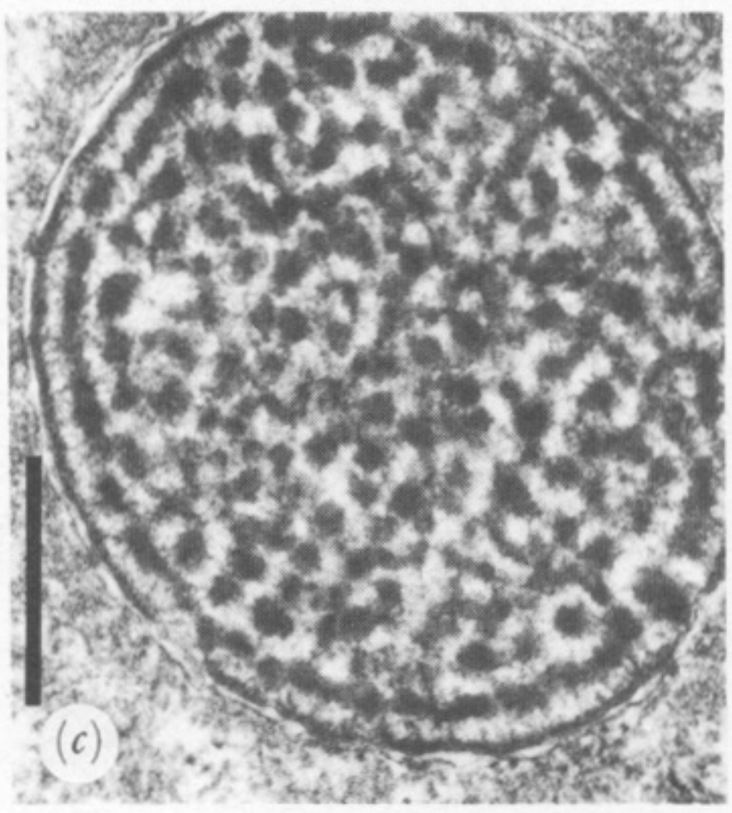
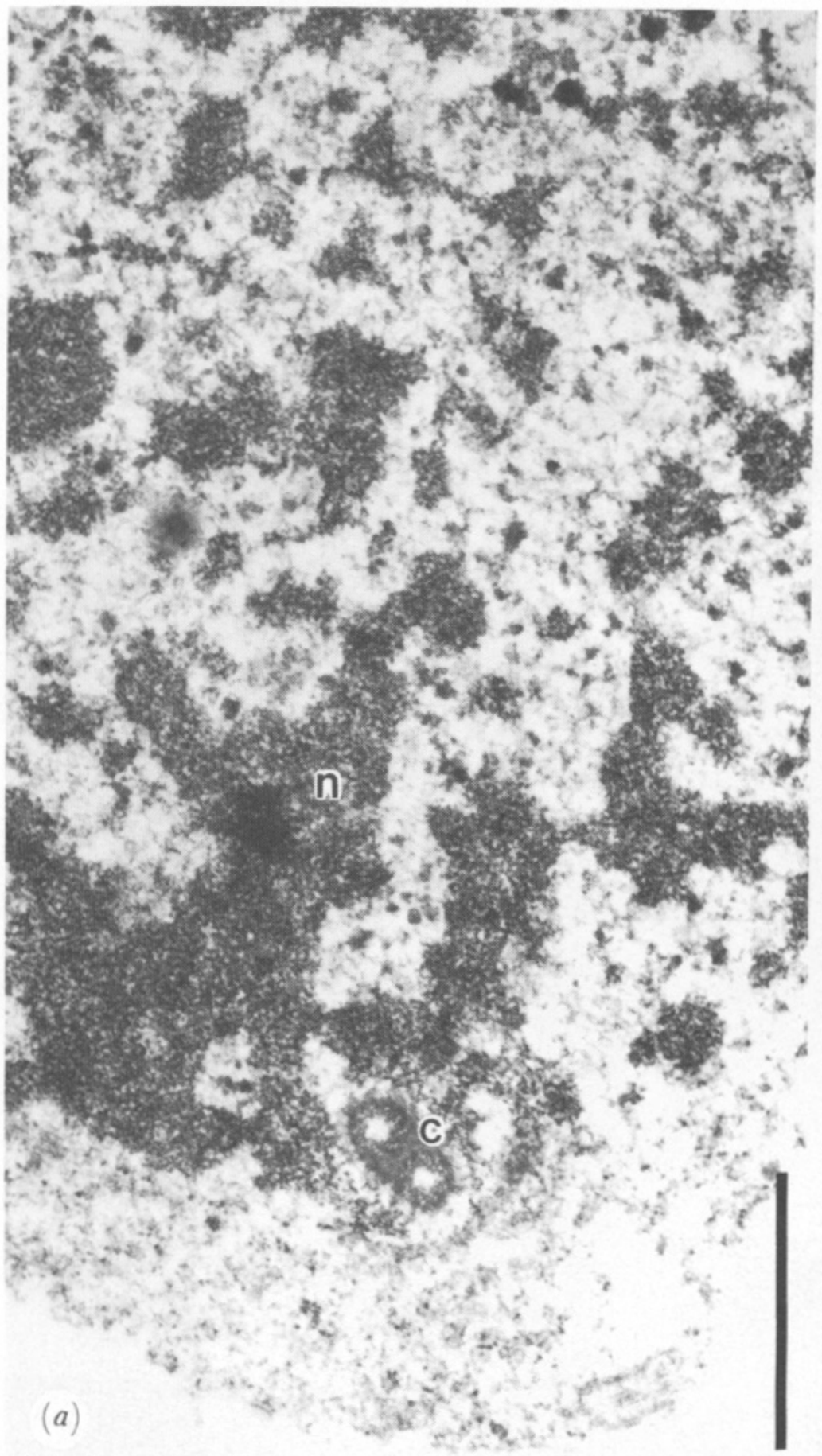
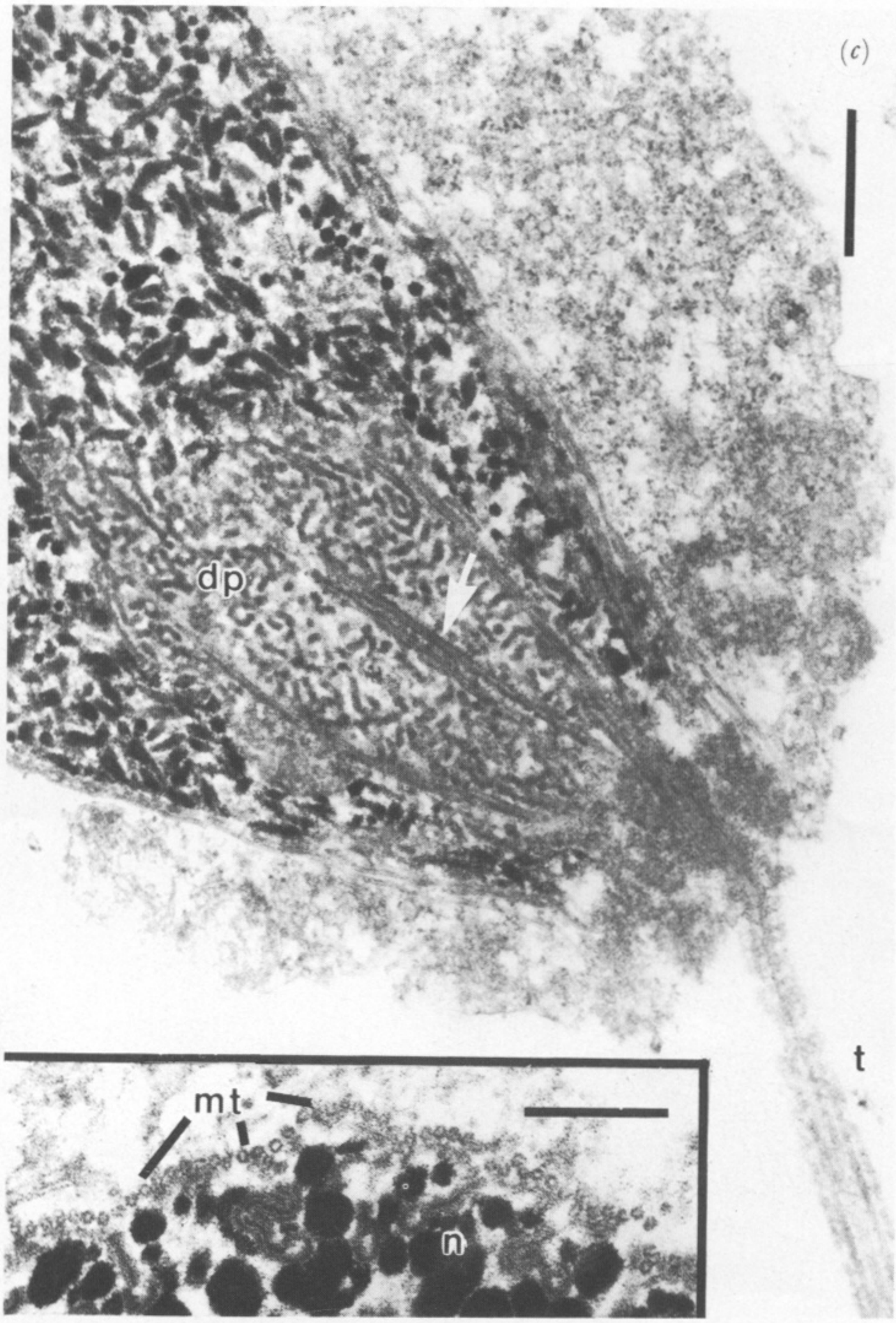
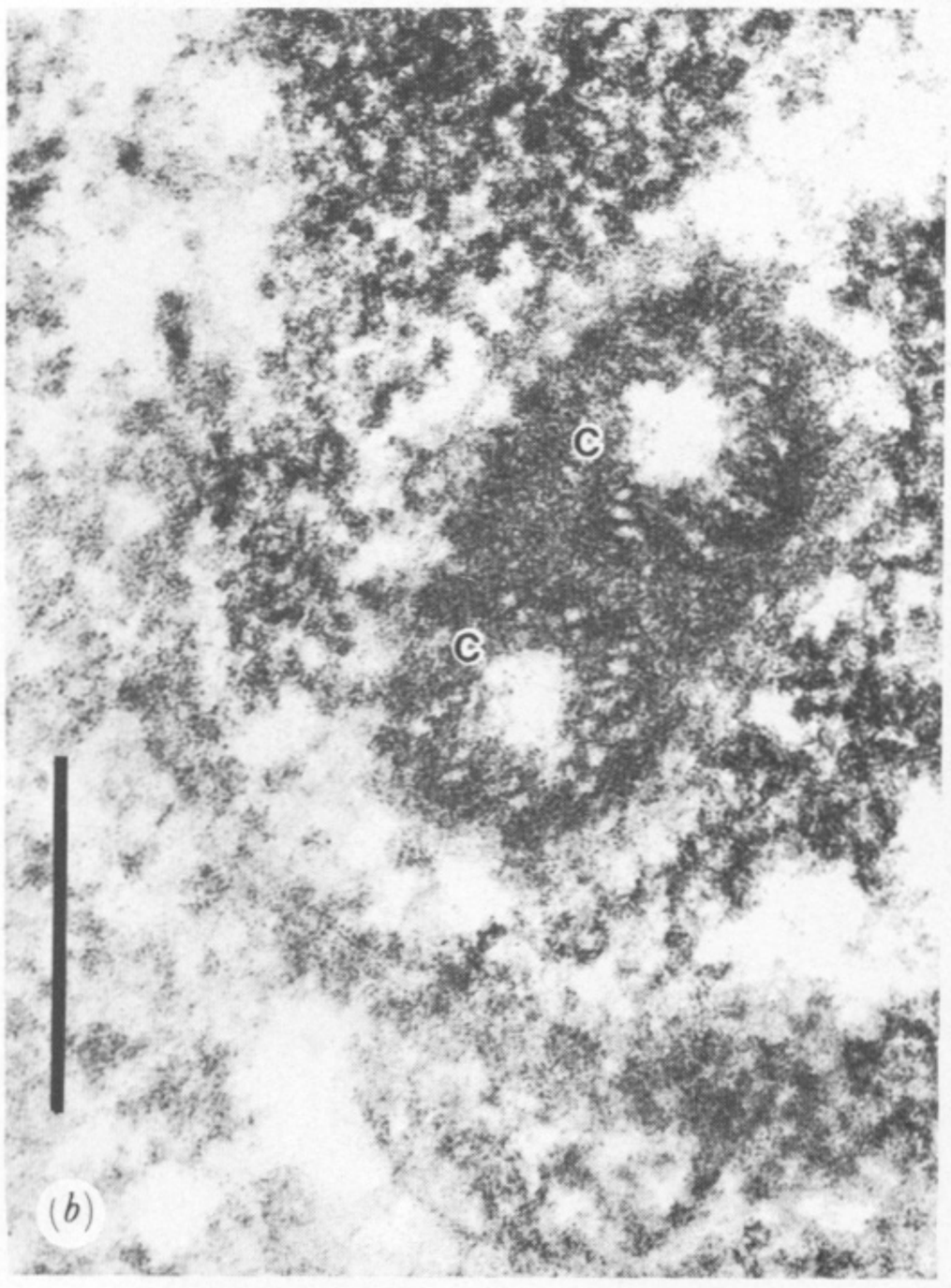


FIGURE 5. For description see facing page 593.







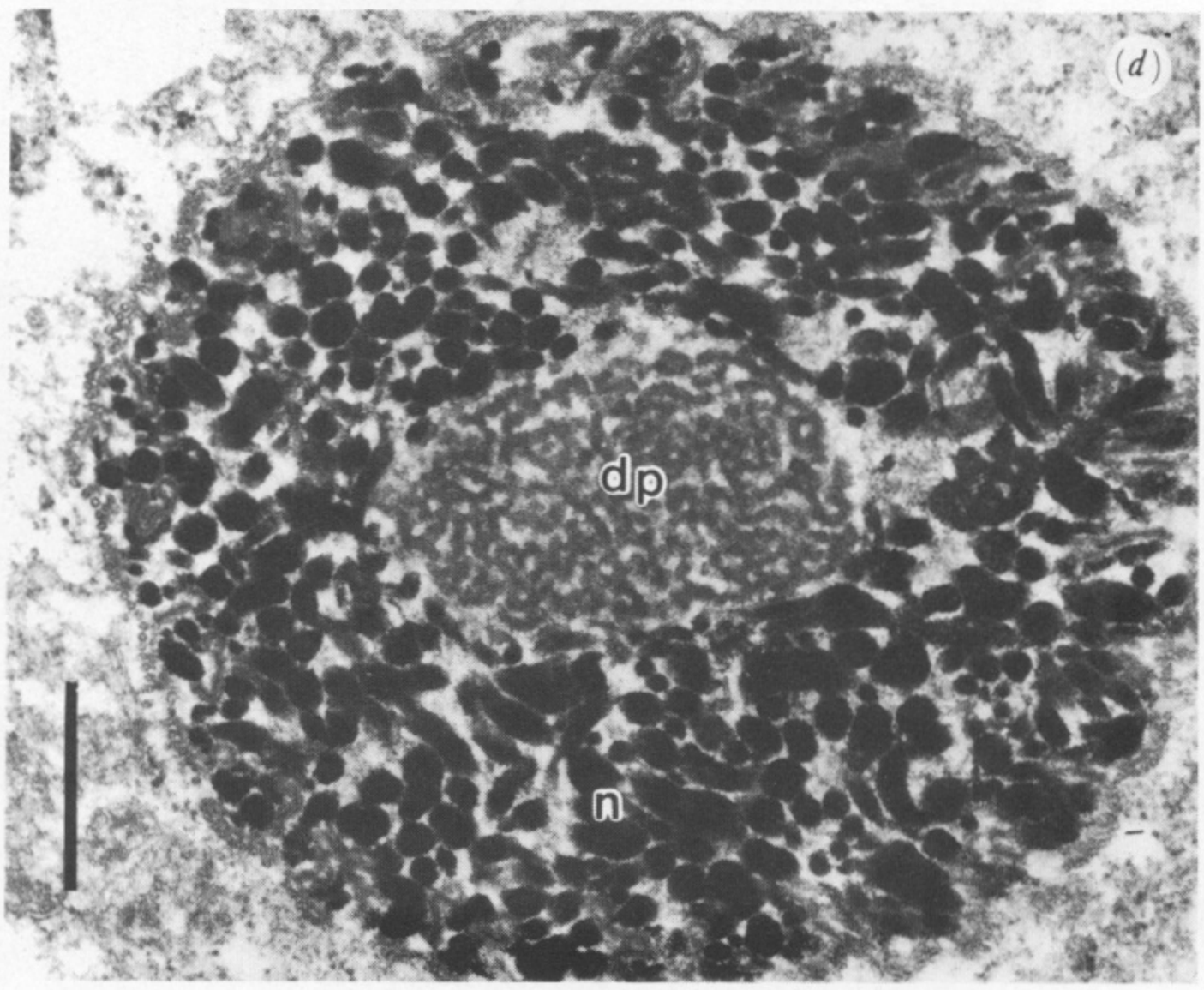


FIGURE 6. For description see overleaf.