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FINE STRUCTURE OF THE GILLS OF *JAERA NORDMANNI* (RATHKE) [CRUSTACEA, ISOPODA]

By A. BUBEL
Portsmouth Polytechnic, Marine Laboratory, Ferry Road, Hayling Island, Hants., England
AND M. B. JONES
Department of Marine Biology, University of Liverpool, Port Erin, Isle of Man

(Plates I–III, Text-fig. 1)

The fine structure of the gills of the euryhaline isopod *Jaera nordmanni* (Rathke) (Crustacea) have been studied using light and electron microscopy. The gills are the only permeable areas of the cuticle and each is covered by a thin cuticle, below which is an endocuticle and an epithelial layer. The cells of the latter all have the same basic ultrastructure and are characterized by a folded apical microvillous border, and a basal membrane which has many infoldings into the cell. The infoldings of the basal membrane are associated with numerous mitochondria. Other cell organelles are few. The gill haemolymph occurs below the epithelium and occasionally contains haemocytes. The haemocytes are described and their structure, together with the observed elements of the gill, are discussed in relation to the physiological functioning of the gill.

INTRODUCTION

The isopod genus *Jaera* Leach (Crustacea) is a common component of the fauna in estuaries (Green, 1968; Jones & Naylor, 1971; Naylor, 1972; Jones, 1974). The members of this genus are able to survive low salinity (Jones, 1972*a*; Harvey, Jones & Naylor, 1973) by active control of the osmotic concentration of the body fluids above that of the external environment (Jones, 1972*b*; Forbes, 1974). While it has been well established that the crustacean gill is the site of the transport system involved in osmoregulation (Koch, 1954; Shaw, 1960; Bielawski, 1964; Croghan, Curra & Lockwood, 1965; Quinn & Lane, 1966), there have been few studies on the fine structure of this organ (Copeland, 1968; Copeland & Fitzjarrell, 1968; Bielawski, 1971; Fisher, 1972; Talbot, Clark & Lawrence, 1972; Lockwood, Inman & Courtenay, 1973). The present paper describes the structure of the gills of *Jaera nordmanni* (Rathke) and relates the role of the various elements observed to the physiological functioning of the gill.

MATERIALS AND METHODS

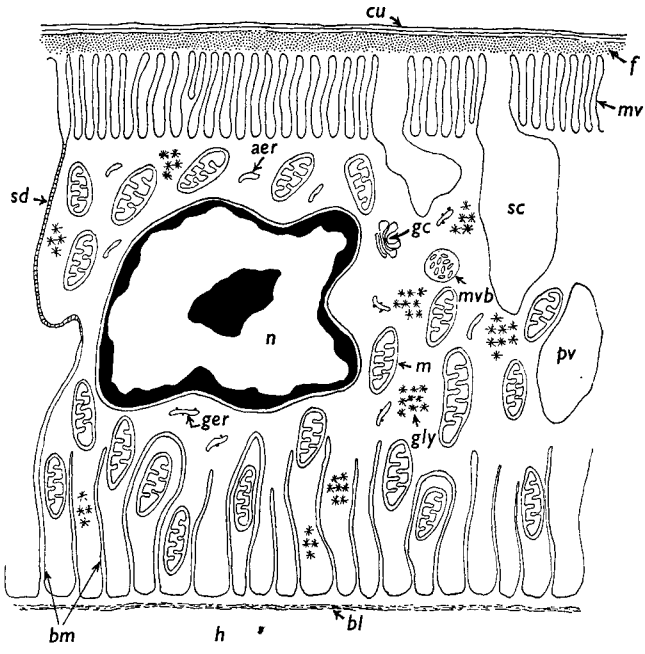
Specimens of *Jaera nordmanni* were collected from Castletown Estuary, Isle of Man (Jones, 1974) and transported live to Portsmouth on pieces of sponge soaked in habitat water. In the laboratory, the silver-staining technique outlined by McLusky (1968) was used to establish that the only permeable areas of the cuticle were the gills (pleopods). In *Jaera*, the functioning gills lie beneath an operculum which is formed by the fusion of the 1st pair of pleopods in the male (Jones & Fordy, 1971) and the 2nd pair of pleopods in the female (females lack the 1st pair) (Naylor, 1972). As the operculum completely covers the gills it was removed, prior to all further observations to allow good fixation. Whole animals were prepared for microscopic examination following the methods described by Bubel (1973). For light microscope work, thick sections (1–2 μm) were

stained with 0·5 % toluidine blue in 0·5 % borax, while ultra-thin sections (50–60 nm) were stained with uranyl acetate and lead citrate, and observed in a Philips 300 electron microscope operated at 60 kV.

RESULTS

Light microscope observations

Each gill is approximately 370 μm long, 280 μm wide and 23 μm thick, and is covered by a thin cuticle (Pl. IA). Below the cuticle is an epithelium which on the dorsal surface of the pleopod is folded (Pl. IB) to enclose a series of haemolymph spaces. Distally the epithelium is tenuous where the haemolymph space is greatest (Pl. IA).



Text-fig. 1. Diagramatic representation of the ultrastructure of an epithelial cell.

Electron microscope observations

The cuticle, which covers the surface of the gill, is composed of two distinct layers (Text-fig. 1). An outer, moderately electron-dense epicuticular layer (see Holdich & Ratcliffe, 1970) is separated from a granular, electron-dense inner layer by an electron-light zone (Pl. IIA). Below the cuticle is a layer of amorphous, flocculent material which varies in thickness and compactness (Pl. IIA, B), and which is probably an endocuticle.

The epithelial cells are seated on a distinct, compact basal lamina, and are separated by long septate desmosomes (Text-fig. 1, Pl. IC, D). Beneath the desmosomes there are folded lateral membranes which have their intracellular spaces periodically obliterated by, what appear to be, tight junctions. Beneath the basal lamina is the haemolymph (Pl. IIB).

Apart from the differences in size, all the cells of the epithelium possess the same basic

ultrastructure. The most prominent features are the apical microvillous border and the extensive infoldings of the basal membrane (Text-fig. 1). The latter configurations are closely associated with large numbers of mitochondria (Pl. IIIA). The microvilli, which form the microvillous border, may be branched, have variable widths, and contain electron-dense material at their tips (Pl. IC, D, IIA). They are regularly orientated and closely apposed (Text-fig. 1).

In most sections the apical plasma membrane is deeply indented by invaginations which penetrate for variable distances into the cell cytoplasm and give rise to sub-cuticular spaces (Pl. IC). Occasionally, the invaginations reach the basal region of the cell (Pl. IIIA). Several pinocytotic vesicles also appear to be budded off from the invaginations and these may fuse to form the large subcuticular spaces which are frequently observed (Pl. IC). The extent of these subcuticular spaces varies from cell to cell, and in some instances they may occupy the bulk of the cell volume. They are generally empty, but in some sections contain flocculent material similar to that in the extracellular space (Pl. IIB).

The basal plasma membrane is usually folded up into the interior of the cell and although some of the invaginations almost reach the base of the microvilli, no connexion between the two has been observed (Pl. IIIA).

Elements of Golgi apparatus are occasionally observed in the apical region of the epithelial cells (Pl. IIA). They consist of 5 parallel rows of cisternae with expanded tips, and, characteristically lack contents (electron-light). Occasionally associated with the Golgi apparatus is a spherical multivesicular body which is membrane bound and contains numerous ellipsoidal vesicles embedded in a light granular matrix (Pl. IIA). Endoplasmic reticulum elements are poorly developed in the cells, and consist of short, elongate, agranular and granular profiles. Agranular endoplasmic reticulum is not so widespread as the granular elements, and are most frequently observed in the apical region. The ground substance of the cell cytoplasm contains particles similar in size and appearance to membrane associated ribosomes. Such loose particles are present in most regions of the cell and may be ribosomes or primary β-glycogen particles. Within the cytoplasm mitochondria are present in large numbers and occupy most of the cell volume (Pl. IC). They usually lie with their long axes parallel to the infoldings of the basal membranes and this orientation is generally retained in other regions of the cell (Pls. IC, IIIA). The mitochondria contain a very large number of densely packed tubular transversely orientated cristae and a matrix of high electron density (Pl. IIA). Microtubules are present in the cells usually in the form of longitudinal bundles which traverse most of the cell (Pl. IIC). Nuclei are observed in some sections, at intervals along the epithelium, and are lobed and include large areas of electron-dense chromatin. This material was absent from the region of nuclear pores. The outer nuclear membrane has occasionally been observed to carry ribosomes. Glycogen is noted in the cells to varying degrees, usually in the form of glycogen rosettes (Pl. IIC) which can occupy large areas of the cytoplasm. Glycogen particles are noticeably absent from cells which contain flocculent material.

Haemocytes are occasionally observed in the haemolymph beneath the epithelial cells. They are normally ovoid (Pl. IIIB), although some with a lobed profile have been

observed. Each is characterized by a spherical nucleus which is usually eccentrically placed in the cell, and cytoplasm which is packed with numerous electron-dense granules and the occasional mitochondrion (Pl. IIIB). Small aggregations of glycogen, both as rosettes and individual β granules, occur in the peripheral cytoplasm. Endoplasmic reticulum, mainly of the granular type, occurs as short, elongate profiles scattered between the granules, and in the vicinity of the nucleus. In some haemocytes a Golgi complex has been observed in the vicinity of the nucleus.

DISCUSSION

The ultrastructure of the gill of *Jaera* is very similar to that of other crustaceans (Copeland, 1968; Bielawski, 1971; Fisher, 1972; Talbot *et al.* 1972; Lockwood *et al.* 1973). Common features in all the species studied are, a gill epithelium which has a large surface area, numerous mitochondria associated with the infoldings of the basal membrane, and flocculent material of the presumed endocuticle which separates the microvillous border from the overlying cuticle.

The large surface area of the gill is caused by the infolding of the apical microvillous border and of the basal membrane of the epithelial cells. These membranes are thought to be involved in osmoregulation by acting as salt pumps. It is well established that the sodium pump mechanism is of prime importance in osmoregulation in crustaceans (Bryan, 1960*a*, *b*; Shaw, 1960; Harris, 1970, 1972), and that sodium ions are usually taken into the animal's body with chloride ions.

Croghan *et al.* (1965) postulated that the outer membrane of the gill of the crayfish, *Austropotamobius*, pumped chloride ions, and the inner haemolymph membrane was responsible for the sodium ions. To account for active salt uptake in the crayfish, *Astacus*, Fisher (1972) proposed that in a low external salt concentration the microvilli of the apical membrane expand to give rise to subcuticular spaces which increase the gills surface area which consequently also increases the availability of ions. The same author postulated that chloride exchange occurred in the microvilli, and sodium ions were actively absorbed from the intracellular fluid, in the subcuticular spaces, and transported into the intracellular channels where osmotic gradients were set up. In high external salt concentrations, gill permeability could be reduced by closing or flattening the microvilli and this would suppress ion uptake (Fisher, 1972). Under these conditions regulation of the blood ion concentration would depend on the activity of the excretory system. The presence of specialized gill epithelial cells with large subcuticular spaces and tightly packed microvilli suggests that a mechanism of salt uptake similar to that proposed for *Astacus* (Fisher, 1972) occurs in *Jaera*.

In reaching the microvilli by this proposed route the salt would have to traverse the cuticle and endocuticle. The former has been shown to be permeable in *Jaera* with a silver-staining technique (McLusky, 1968) and the latter has also been implicated in the osmoregulatory process (Copeland & Fitzjarrell, 1968) as a possible site for the binding of salt. This model postulates that bound salt is ingested by pinocytosis in the microvillous layer, released in the cytoplasmic vicinity of the mitochondria, and transported to haemolymph-connected spaces by the metabolically active membranes. This

is supported by Holdich and Ratcliffe (1970) who also suggested that the flocculent material of the hind gut cells of the isopod *Dynamene bidentata* (Adams) could selectively bind ions before they were engulfed by pinocytosis. However, Bielawski (1971) reported that pinocytosis could not play an important part in the mechanism of salt uptake. The author based this on the lack of any clear pinocytotic vesicles on the apical side of the gill of *Astacus*, and the fact that pinocytosis would involve the uptake of too much water which would be physiologically damaging to the cells.

The haemocytes of *Jaera* resemble those described in *Carcinus* (Johnstone, Elder & Davis, 1973) and *Astacus* (Stang-Voss, 1971). *Carcinus* haemocytes contain glycogen and an acid polysaccharide component, indicating that they are active in synthesizing carbohydrate (Johnstone *et al.* 1973). They also have a large intracellular pool of free amino acids which may play a role in isosmotic intracellular regulation (Evans, 1972). *Astacus* haemocytes are thought to be involved in haemocyanin synthesis (Stang-Voss, 1971). Since the epithelial cells of the gill of *Jaera* usually contain large quantities of glycogen, it is proposed that the haemocytes of *Jaera* may have a hepatic function (Johnstone *et al.* 1973), breaking down their glycogen stores and releasing the sugars for use in the epithelium.

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List of abbreviations used in Text-fig. 1 and Plates I–IV

aer	agranular endoplasmic reticulum	*h*	haemolymph
bl	basal lamina	*m*	mitochondria
bm	basal membrane	*mv*	microvilli
cu	cuticle	*mvb*	multivesicular body
de	dorsal epithelium	*n*	nucleus
f	flocculent material (endocuticle)	*pv*	pinocytotic vesicle
gc	Golgi complex	*sc*	sub-cuticular space
ger	granular endoplasmic reticulum	*sd*	septate desmosome
gly	glycogen	*ve*	ventral epithelium

EXPLANATION OF PLATES

PLATE I

A. Light micrograph of a transverse section through a *Jaera* gill. Scale line = 60 μm.

B. Detail of Pl. IA showing the regular arrangements of the haemolymph spaces (*h*). Scale line = 24 μm.

C. Electron micrograph of the gill epithelial tissue. Beneath the cuticle are numerous invaginations which give rise to subcuticular spaces and pinocytotic vesicles. The predominant feature of the tissue is the abundance of mitochondria. Scale line 2 μm.

D. Electron micrograph of the epithelial tissue at the tip of the gill. Note the reduction in cell size (cf. Pl. IC). Scale line 2 μm.

PLATE II

A. Electron micrograph of the apical region of an epithelial cell. Note the layering of the cuticle, outer cuticular layer (1), and inner cuticular layer (2) separated from the latter by electron-light zone (arrow). Scale line = 0·5 μm.

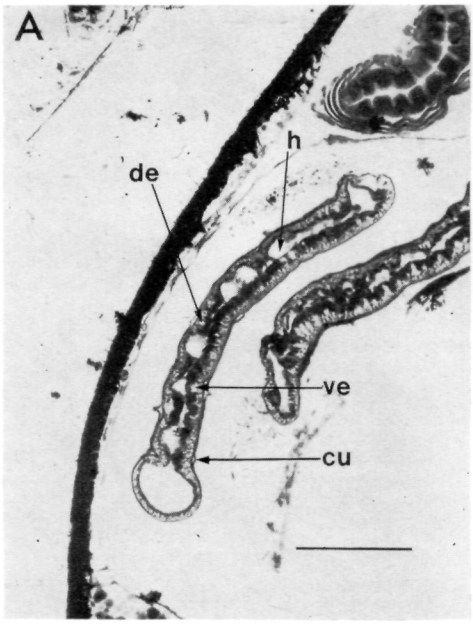
B. Electron micrograph showing the compact nature of the flocculent material below the cuticle. Scale line = 2 μm.

C. Electron micrograph showing the presence of microtubules (*mt*) in the cell which appear to terminate on the basal membrane. Scale line = 1 μm.

PLATE III

A. Electron micrograph showing the basal region of an epithelial cell. Note the highly infolded basal membrane with associated mitochondria and also the close association between infolded basal membrane and subcuticular space (arrows). Scale line = 1 μm.

B. Electron micrograph showing haemocyte containing numerous cytoplasmic granules (*g*). Scale line = 1 μm.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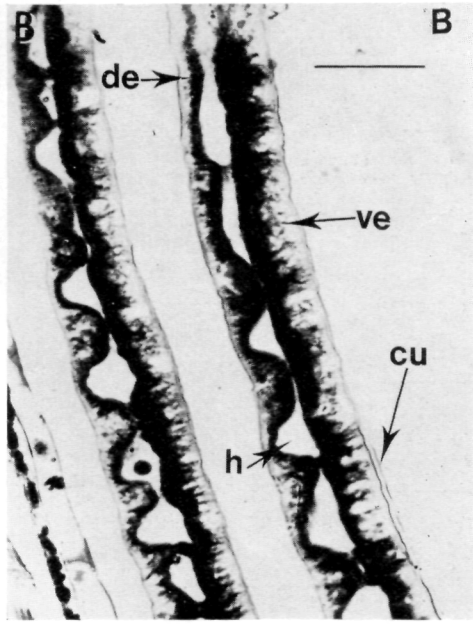
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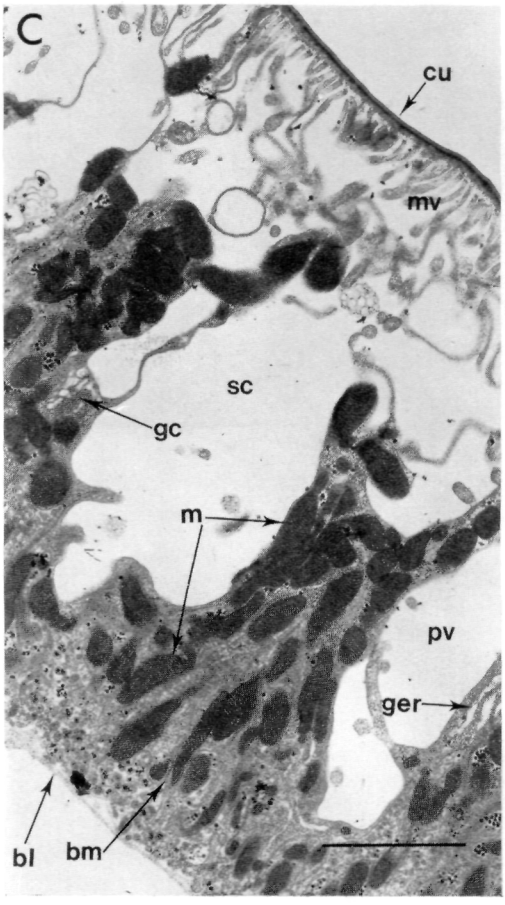
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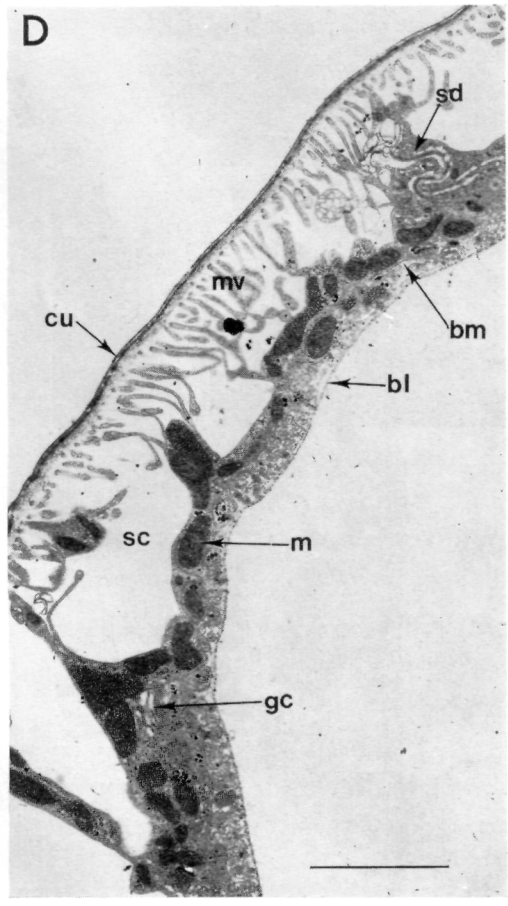
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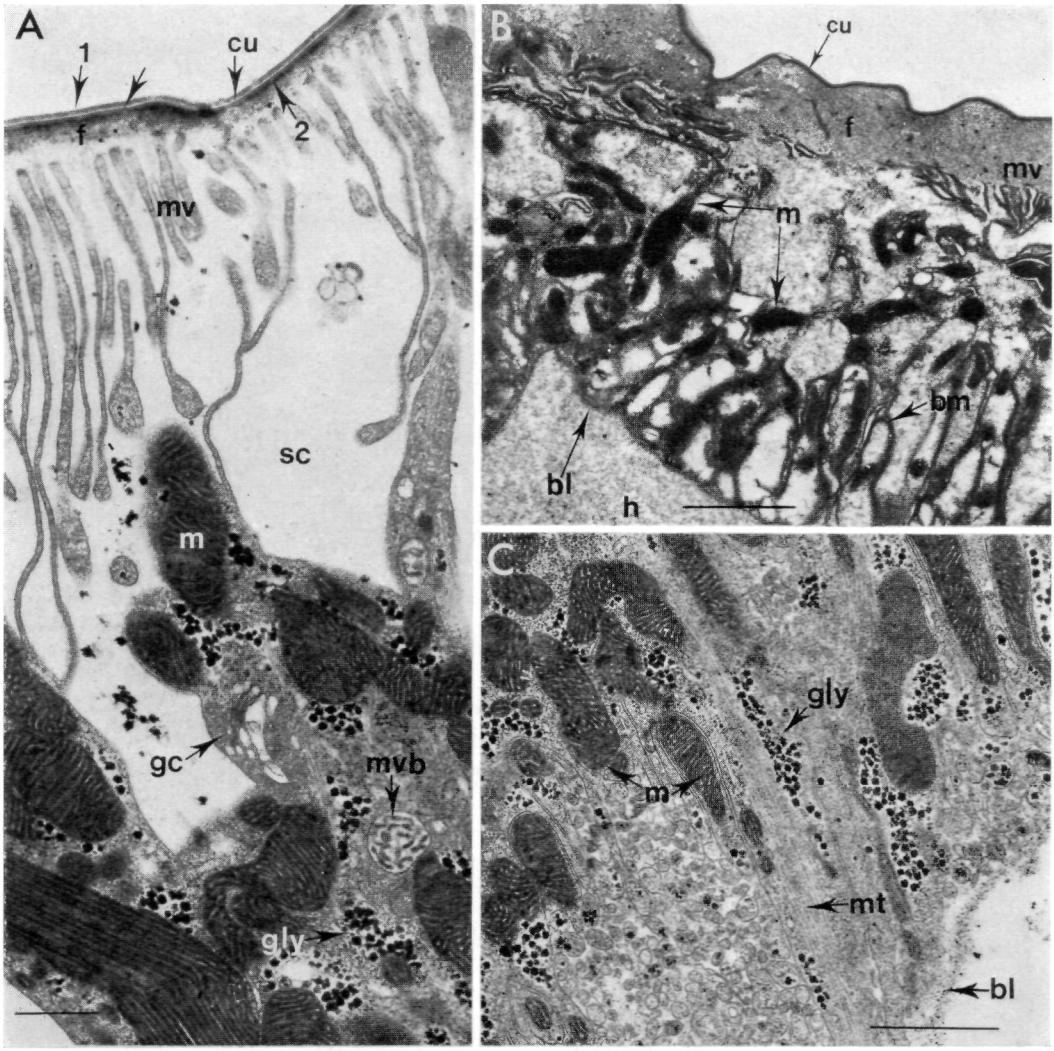
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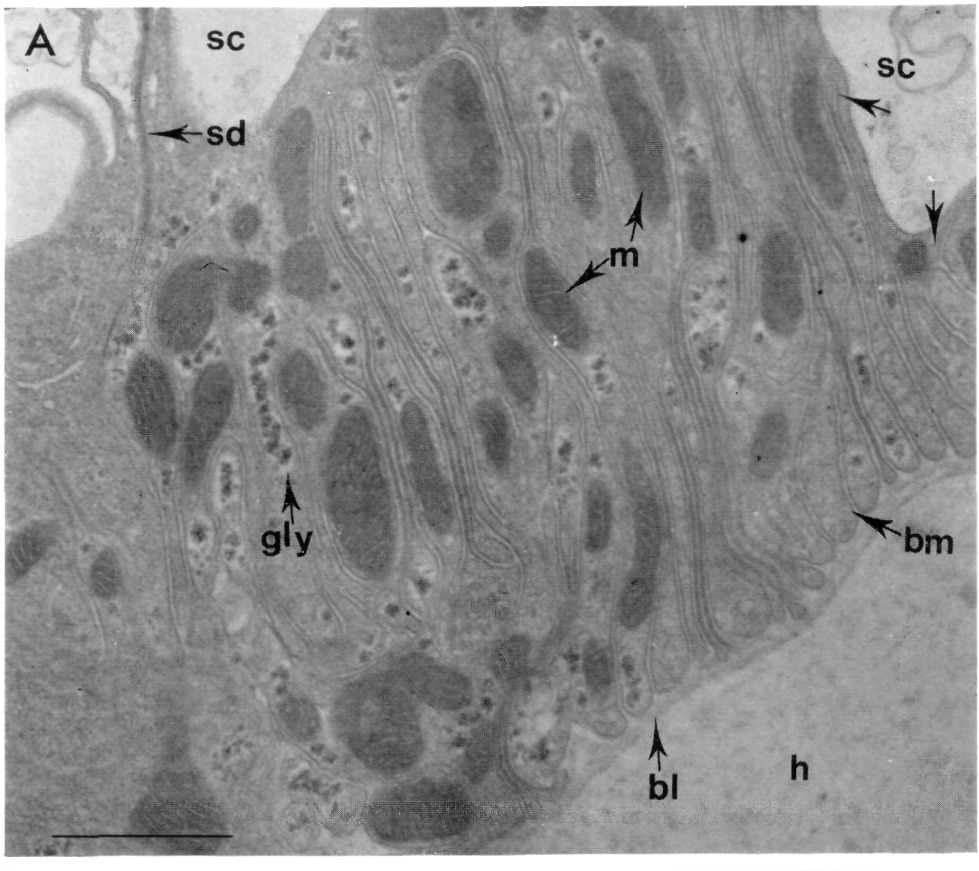
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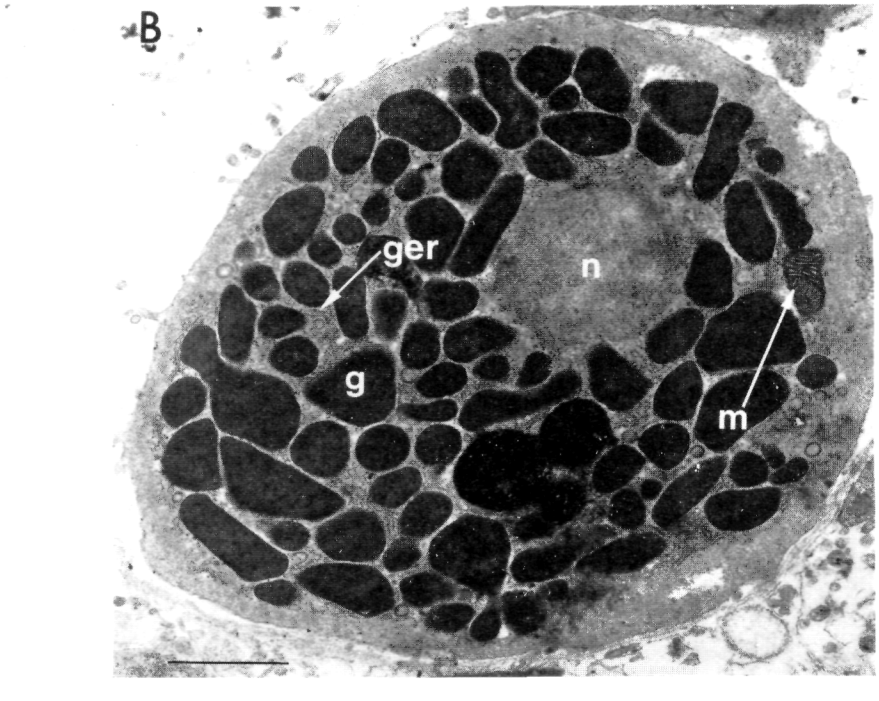
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