Symbiontic bacteria in the gut of the blood-sucking Antarctic fish parasite *Gnathia calva* (Crustacea: Isopoda)

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Abstract

Structure and ultrastructure of the digestive tract of the ectoparasitic stages of the Antarctic isopod *Gnathia calva*, collected in the Weddell Sea and around the Antarctic Peninsula in the 1984–1985 season, are briefly described. There are only two digestive glands, with an ultrastructure similar to that of other isopods. The gut is divided into six regions: oesophagus, stomach, dilatable reservoir for sucked-in blood, sphincter, rectal vesicle with symbiontic bacteria, and rectum. The highly dilatable anterior hindgut (reservoir) takes part in the resorption of nutrients and stores lipids and glycogen. The rectal vesicle has an epithelium with all the features of an organ with high metabolism and the capacity for the transportation of small molecules. The surface is increased by irregular microvilli. A very thin intima and a basal labyrinth are present. The presence of symbiontic bacteria is discussed in correlation with the haematophagous nutrition of *G. calva*.

Introduction

Gnathia calva is a parasitic isopod frequent in the Weddell Sea and the area around the Antarctic Peninsula. The adult stages lead a cryptic benthic life, hidden within small hexactinellid sponges, while the juveniles, the so-called pranizae, are temporary ectoparasites of fishes (Wägele, 1987). The morphology of larvae, adult males and females differs greatly. Females and males have a broadened cephalothorax, the mouthparts are partly reduced, mandibles of adult males are enlarged and not used for feeding, and the setation of pleopods and tail-fan is simpler than in younger stages. Food is only ingested by pelagic, immature individuals, which pump their host's blood into their gut and then detach themselves from the fish to live from their reserves for a long time. These satiated individuals are usually found together with adult stages within small sponges.

While studying the life-cycle of this isopod, a comparison of the anatomy of the different postembryonal stages was prepared to get a more complete notion of the changes occurring during metamorphosis from larvae to adults. In the course of these studies a peculiar, bacteria-filled structure of the gut was discovered that is not known from other isopods and probably is an adaptation to the haematophagous way of life. In the following a first description of the digestive tract and especially of the symbionts of the praniza of *Gnathia calva* is presented.

Material and methods

The material was collected by means of an Agassiz-trawl during the cruises of RV "Polarstern" in the 1984–1985 season. *Gnathia calva* were caught in the Weddell Sea and around the Antarctic Peninsula (see Wägele, 1987). They were immediately sorted on deck and kept at $-1°$ to $0°C$ in aquaria, which were tansported to Germany in a temperature-controlled container. Praniza stages were sacrificed for electron microscopy on board and, after one year of culturing, in Oldenburg.

Electron microscopy. Specimens were cut into 2-mm-thick slices, fixed for 2 h in 3.5% phosphate buffered glutaraldehyde (pH 7.4, osmolarity adjusted to that of seawater with NaCl), repeatedly rinsed in sea-water, postfixed for 3 h in 2% OsO_4, dehydrated in ethanol, and embedded in Epon 812 after immersion in propylenoxyde. Thin sections were stained with uranyl acetate and lead citrate. The electron microscope was a Zeiss EM 109.

Histology. Semithin sections of formaline-fixed Epon-embedded, complete specimens were stained with toluidine blue. Drawings were prepared with the help of a camera lucida.
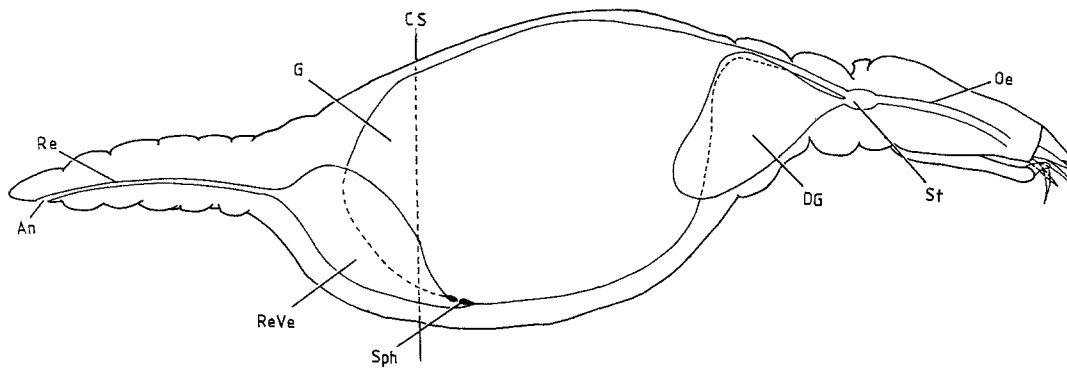
Fig. 1. *Gnathia calva.* Schematic drawing of the digestive tract (praniza stage). An: anus; CS: cross section (see Fig. 2); DG: digestive gland; G: swollen anterior hindgut ("reservoir"); Oe: oesophagus; Re: rectum; ReVe: rectal vesicle; Sph: sphincter; St: stomach
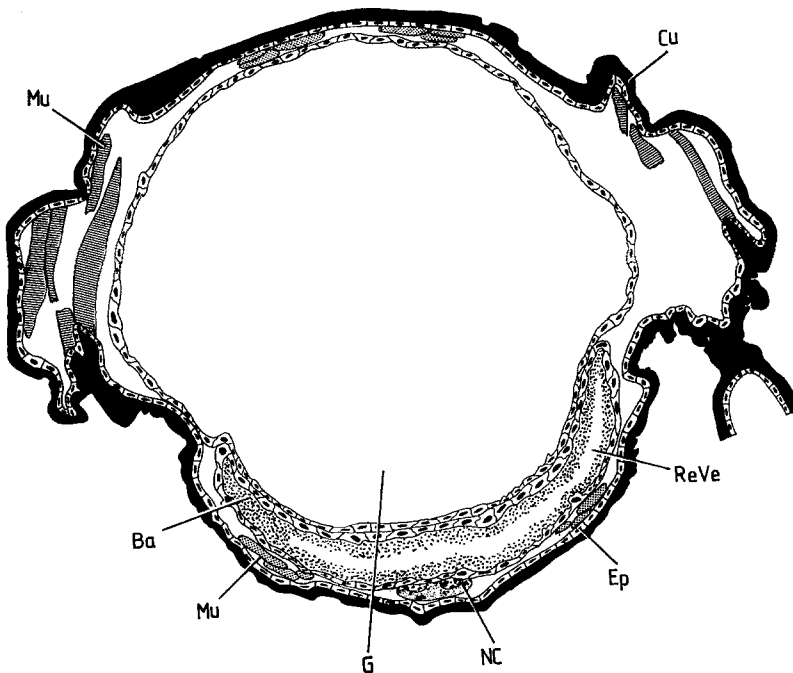
Fig. 2. *Gnathia calva.* Schematic cross section (CS in Fig. 1) through anterior hindgut and rectal vesicle (praniza stage). Ba: bacteria in rectal vesicle; Cu: cuticle; Ep: epidermis; G: anterior hindgut; Mu: muscle; NC: ventral nerve chord; ReVe: rectal vesicle. Cells of epithelia not shown in natural size

Results

General features of the digestive tract (Fig. 1)

In *Gnathia calva* the mouth of the praniza is found at the base of the mandibles, between hypopharynx and labrum. A short oesophagus ends in the stomach, which lies at the caudal part of the cephalothorax. As in other isopods (Scheloske, 1976; Wägele, 1981), the stomach is a pumping and filtering organ and connects the digestive glands (midgut glands) with the hindgut; a midgut is not present. Oesophagus, stomach and hindgut are lined by a cuticular intima (Fig. 3 A: Cu). There is only one pair of digestive glands (the "sacs entériques" of Monod, 1926; Mouchet, 1928), which originate from a common mesodermal tube connected to the posteroventral part of the stomach. The pair of sack-like mesodermal caeca, usually filled with brown, partly digested blood, are found dorsally at both sides of the hindgut and mostly end at the beginning of

the fourth pereonite; volume and length of the caeca depend on the stage of the digestive cycle of the individual (see also Monod, 1926: Fig. 76, p 188). The hindgut can be divided into four main regions: the highly dilatable blood-reservoir, which extends from the stomach to the seventh pereonite (= Monod's "intestin": Monod, 1926), the short sphincter, which closes the reservoir and controls the passage of nutrients from it to the rectum; then follows a less dilatable region of the hindgut, which forms a vesicle in the area between the sixth pereonite and the first pleonite. The anterior parts of this vesicle normally are flattened by the extended blood-reservoir. Flattened parts of the vesicle can be found ventrally of the reservoir (as in Fig. 2) or, in some individuals, also dorsally. The vesicle ends in a very thin, inconspicuous rectum; the anus lies ventrally on the pleotelson.

The thorax of resting, benthic pranizae is usually filled by the blood-containing "reservoir", whose contents can be seen shining through the integument. The vesicle, named by Monod (1926) "vesicule intestinale", contains no blood,

but is filled with a dense liquid (Monod, 1926: "liquide épais") and usually can only be detected by histological methods. While the functions of digestive glands and reservoir seem to be obvious (storage of food and reserves, production of enzymes, resorption), the rectal vesicle until now has been a neglected, obscure structure.

Ultrastructural and histological observations

Digestive glands. On cross sections, these glands have features similar to other carnivorous or haematophagous isopods (Jones et al., 1969; Wägele et al., 1981). The cells contain enormous lipid droplets, and the typical apical seam of luminal microvilli is present, though the villi are not very long (Fig. 3 B). The cytoplasm is rich in glycogen. Rough endoplasmic reticulum (ER) is present, in some cells as frequently as in the secreting cells of the digestive glands of omnivorous isopods (Smith et al., 1975; Hryniewiecka-Szyfter and Tyczewska, 1978). A basal labyrinth is developed, but with few and not very deep invaginations. The number of mitochondria varies. Some lysosome-like vesicles can be found, dictyosomes are rare, and few microtubuli of varying orientation are present. As in other isopods, a basement membrane and a network of muscle cells surround the gland basally.

The cells have all nearly the same appearance. At this stage of the digestive cycle, the glands seem to be mainly lipid- and glycogen-storing organs. The organelles for resorption of food and production of enzymes are present, but generally not very well developed.

Anterior hindgut (Reservoir). The highly dilatable anterior hindgut is filled with the blood of fishes that forms a dense, coagulated ball and still may contain some remains of blood cells (Fig. 3 C). The monolayered epithelium is very thin, due to the gut's voluminous contents often pressed against the epidermis, so that both epithelia (hindgut and epidermis) together may be thinner than the isopod's cuticle. The intima has the structure of an epicuticle, but procuticular elements are lacking (Fig. 3 A) and it is only about 360 Å thick. The organization of these cells is similar to that of other isopods (Holdich and Ratcliffe, 1970; Vernon et al., 1974; Holdich and Mayes, 1975; Wägele et al., 1981; Wägele, 1985), with a well developed basal labyrinth, numerous mitochondria and, an unusual feature, large lipid droplets in some areas, indicating that this part of the digestive tract is also involved in the resorption of food. Further organelles present in the anterior hindgut are lysosome-like bodies and multilamellated bodies. The nuclei seem to be comparatively larger than in other isopods and of irregular amoeboid outline. Fields of glycogen can also be found in many cells. Though the subcuticular microvilli are rarely seen, the folded basal membranes and the high number of mitochondria give these cells an aspect similar to that of the probably ion and water transporting areas of the hindgut of *Asellus aquaticus* (Hryniewiecka-Szyfter and

Tyczewska, 1980) or of *Armadillidium vulgare* (Palackel et al., 1984).

Rectal vesicle and the symbionts. This part of the hindgut differs markedly from the preceding one. On cross sections, the light microscope reveals a very thin epithelium and a lumen filled with bacilliform microorganisms, which are seen in longitudinal and cross sections (Fig. 3 D). The epithelium contains some lipid droplets, but further details can only be detected with an electron microscope. The microorganisms are bacteria. The hindgut cells show an uncommon, fine structure: the cuticular intima is as thin as the cellular walls of the bacteria, consisting only of the epicuticle (around 25-nm-thick), and large subcuticular spaces separate the intima from the cell surface (Fig. 3 D; the actual spaces are often less wide than in Fig. 3 D). The apical cytoplasm has a very irregular appearance, with invaginations of the subcuticular space and long irregular, branched microvilli which often run parallel to the intima. The villi have a core of microfilaments and their length seems to vary greatly. The cells are very flat, a cross section shows only five to six cells and a nucleus is rarely found. Monod (1926) even thought that the epithelium was syncytical. Lateral cell membranes are present, and short septate junctions can be found at their apical zones. The granulated cytoplasm contains some lipid droplets (Fig. 3 D, 4 D), glycogen, rough ER in the basal half of the cell (Fig. 3 D, E) and a moderate basal labyrinth (Fig. 3 E, 4 D). The basement membrane is comparatively thin. Tubular mitochondria are present throughout the cell, and several subapical dictyosomes produce small vesicles, which accumulate under the cell membrane (Figs. 4 B, C).

The extracellular bacteria (Fig. 4 D) in the vesicle's lumen are at least ten times longer than they are wide. The diameter ranges from 0.6 to 1.1 µm. The light, central nucleoplasm is surrounded by a very dense cytoplasm, but invaginations of cell membranes are not present. The organization resembles that of *Escherichia coli*, with the cellular envelope (210 to 250 Å) consisting of a plasma membrane, a very similar outer membrane, and a thin periplasmic space, typical of gram-negative bacteria (Seltmann, 1982).

A peculiar feature is the frequent renewal of the outer membrane (Fig. 5 B), which detaches from the cell and is then found in the lumen of the rectal vesicle in the form of small rolls and vesicles (Figs. 4 E, 5 C). The bacterial wall may have two outer membranes, of which the innermost is probably younger. On some sections the bacteria are packed in a dense accumulation of rolls of old outer membranes.

Discussion and conclusions

The structure of the digestive tract of the praniza stage of *Gnathia calva* shows several adaptations to the haemotophagous way of life. There are only two midgut glands, which are sack-like and not tubuliform as in other isopods.
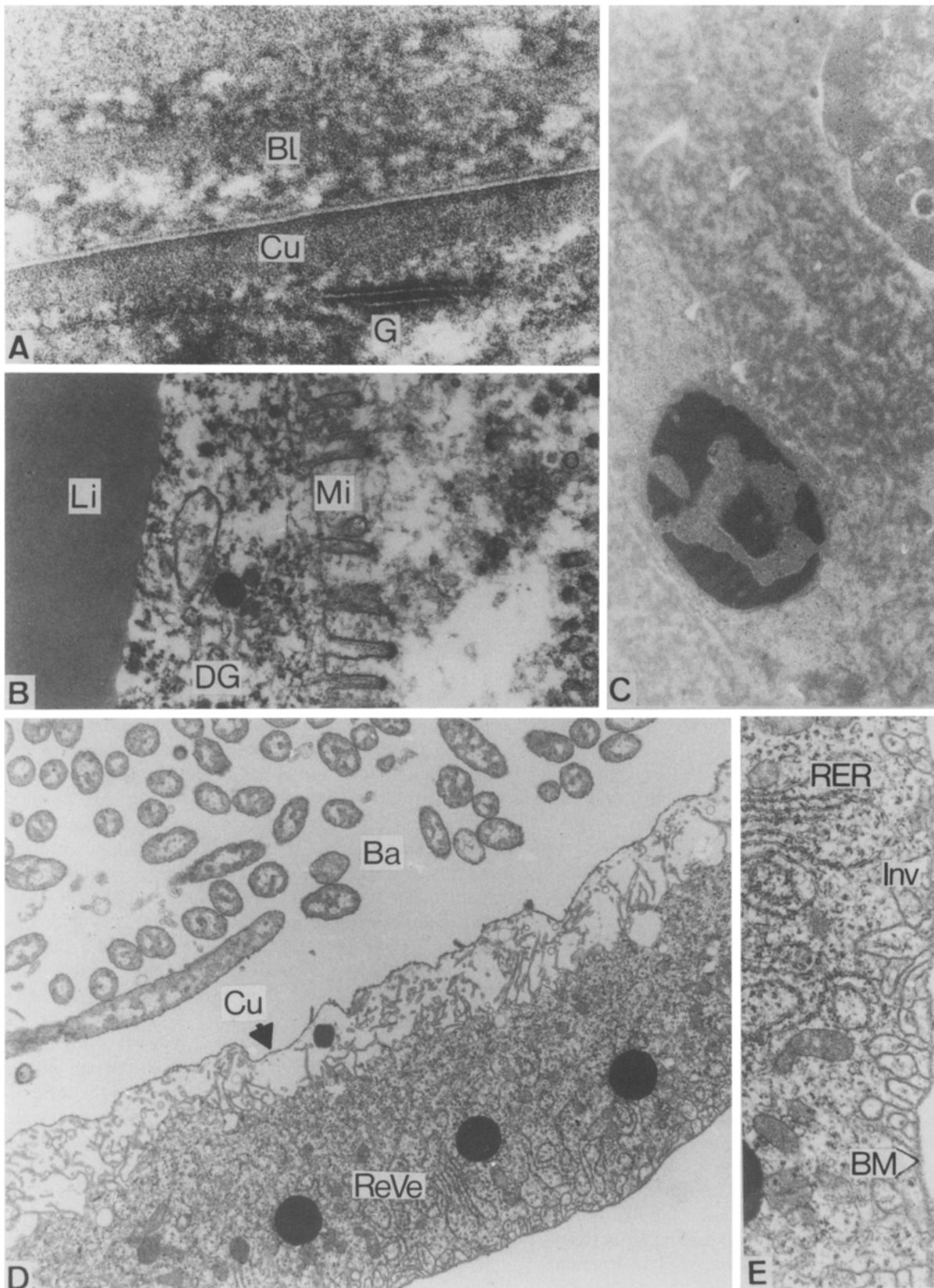
Fig. 3. *Gnathia calva.* Digestive tract (praniza stage). (A) Cuticle (Cu) of anterior hindgut ('reservoir'); Bl: coagulated fish blood, G: gut epithelium (280 000×). (B) Microvilli (Mi) of digestive glands (DG); Li lipid (22 000×). (C) Contents of the anterior hindgut: coagulated blood and nucleus of a blood cell (14 000×). (D) Aspect of the rectal vesicle with bacteria (Ba) and part of a flattened epithelial cell (ReVe); note lipids (black droplets); Cu: cuticular intima (6 000×). (E) Basal invaginations (Inv) of cell of rectal vesicle with rough endoplasmic reticulum (RER), mitochondria, basement membrane (BM) (8 000×)
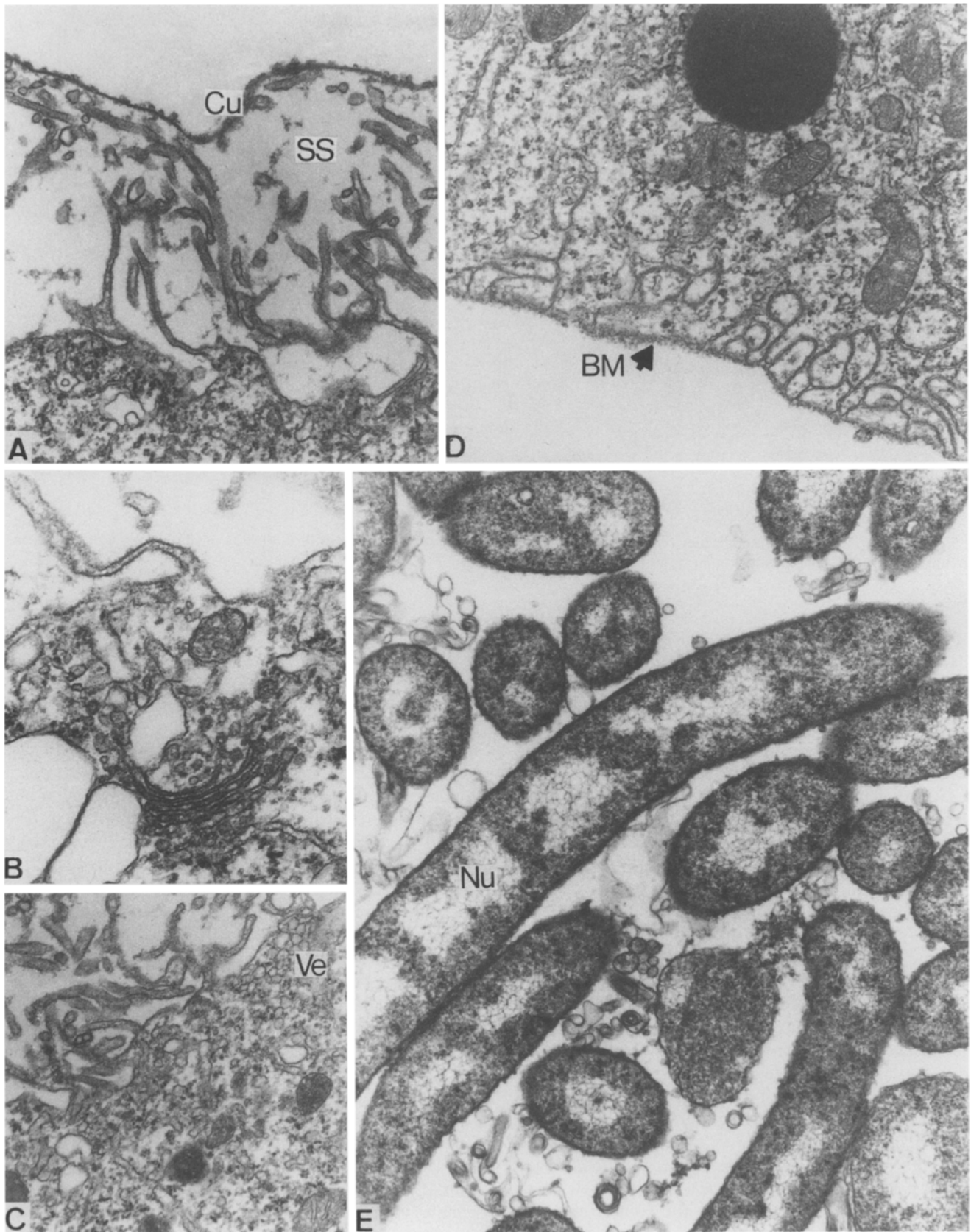
Fig. 4. *Gnathia calva.* Rectal vesicle (praniza stage). (A) Apical zone of epithelial cell with thin cuticular intima (Cu) and subcuticular spaces (SS) with irregular microvilli (22 000×). (B) Subapical cytoplasm with dictyosome (40 000×). (C) Golgi vesicles (Ve) accumulated in subapical cytoplasm (22 000×). (D) Basal cytoplasm of epithelial cell with dark lipid droplet, numerous mitochondria, rough ER, basal invaginations; BM: basement membrane (22 000×). (E) Symbiontic bacteria; note extracellular rolls and vesicles of membranes (22 000×)
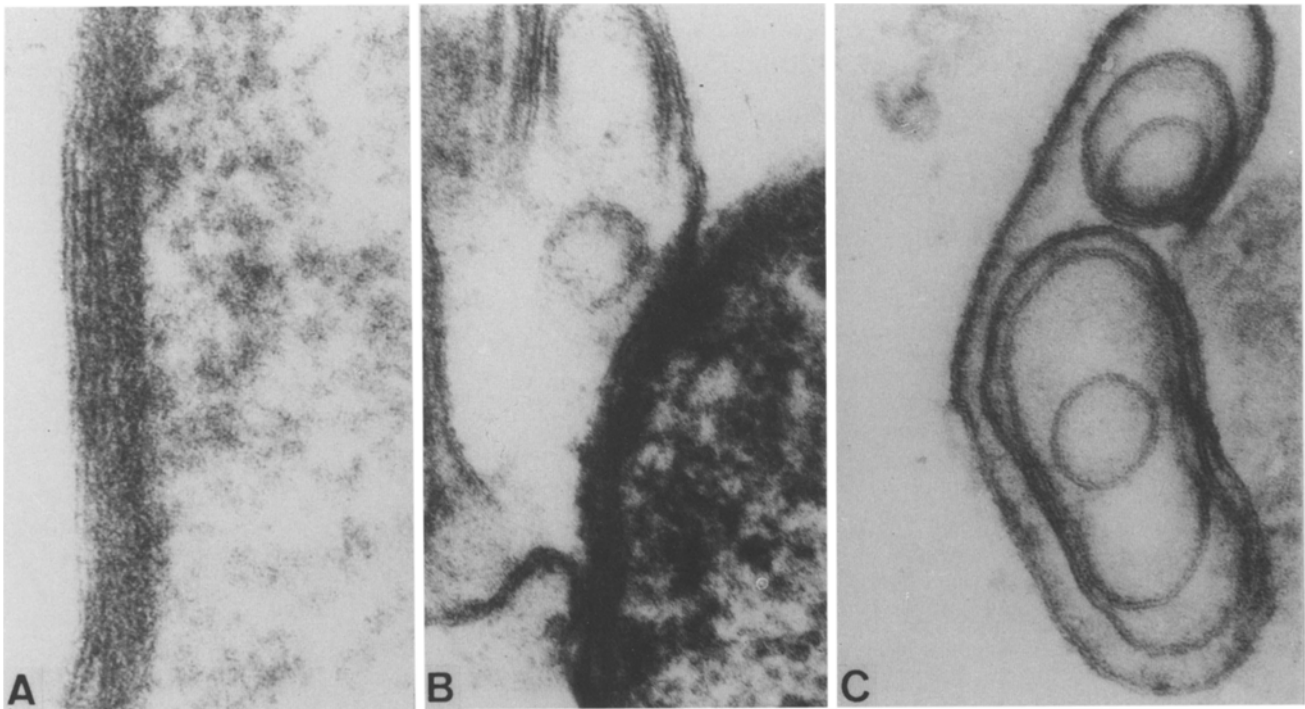
Fig. 5. *Gnathia calva.* Cellular membranes of symbiontic bacteria. (A) Layers of bacterial wall (280 000×). (B) Loosening outer membranes (170 000×). (C) Shed extracellular outer membranes (170 000×)

They may produce enzymes at the beginning of a digestive cycle for the treatment of the fresh blood that the isopod has sucked in from its fish host, but during the main resting phase the gland's secretion is less important. The cells are mainly involved in resorption of nutrients and the storage of lipids and glycogen. An important contribution to the secretion of anticoagulants is probably provided by the salivary glands (Wagner, 1866; Monod, 1926: Fig. 82), at least during the uptake of blood, as is known from some cymothoid fish parasites (Romestand and Trilles, 1976 a, b). The enzymes of the digestive glands (midgut glands) are responsible for the proper digestion.

The anterior hindgut is a receptacle for undigested food and stores it for a long time, as in cirolanid, cymothoid and anthurid isopods (Jones, 1968; Romestand and Trilles, 1976 b; Wägele, 1981), and for this purpose the epithelium is highly distensible and the reservoir is closed caudally by a sphincter. The very thin intima, which in other isopods usually shows some procuticular layers in addition to the epicuticle (e.g. Holdich and Ratcliffe, 1970; Hryniewiecka-Szyfter and Tyczewska, 1979, 1980; Wägele et al., 1981) and the ultrastructural indications for the transport of water and ions may play an important role during the phase of dehydration of the fresh food; water could be excreted from the isopod's body by well developed maxillary glands, which were also seen on the cross sections studied. The presence of lipids is unusual. Normally the isopod hindgut is not involved in resorption of nutrients or the accumulation of glycogen or lipid (Holdich and Ratcliffe, 1970; Wägele et al., 1981). This may be a consequence of the long storage of food in the reservoir.

The function of the symbiontic bacteria is not clear. As similar bacteria are not known from any other isopod, not even from other crustaceans, it seems possible that a physiological task correlated with haematophagy, which cannot be performed by the isopod, is accomplished by the bacteria. The symbionts may receive nutrients directly from the anterior hindgut through the sphincter, which controls the passage. However, no material similar to the blood of the reservoir was found in the rectal vesicle. The isopod can secrete nutrients as well, an indication being the Golgi-vesicles found in the apical cytoplasm of the hindgut cells. The (unknown) essential products of the bacteria are resorbed by the branched microvilli of the vesicle epithelium, with the very thin intima probably functioning as a peritrophic membrane.

The gut of many isopods seems to be completely free of bacteria (Boyle and Mitchell, 1978), even the specialized, wood-feeding species of *Limnoria* produce their own cellulase (Ray and Julian, 1952; Becker and Kampf, 1955; Ray, 1959). In *Hemioniscus balani*, an isopod parasitic of balanids, an "oval body" was described in the rectal area (Buchholz, 1866), which might be similar to that of *Gnathia calva*. In *Meinertia oestroides* (Isopoda: Cymothoidae) two "diverticules intestinaux" were discovered ventrally of the rectum, but their structure and content remain unknown (Romestand and Trilles, 1976 b). The liquid within these caeca has a haemolytic, but not an anticoagulant, effect.

Bacteria present in the gut of the terrestrial isopod *Porcellio scaber* produce a chitinase (Jeuniaux, 1956). Microorganisms that are taken up with food continue their

action in the hindgut of *Philoscia muscorum* and produce cellulase (Hassal and Jennings, 1975). Shrewsbury and Barson (1952) reported the occurrence of several *Streptococcus* spp., gram-negative and gram-positive bacteria in the guts of species of *Armadillidium* and *Porcellio*. Generally, in the digestive tract of crustaceans, only a few specific microorganisms are known (Dall and Moriarty, 1983), and specialized symbionts were unknown until now. The case of *Gnathia calva* reminds one of many other haematophagous invertebrates, which also need the help of symbiontic bacteria, either for the digestion of blood or for the supply of vitamins and essential amino acids. Intracellular bacteria in specialized cells, and extracellular bacteria in special pockets of the oesophagus or of the midgut are found in many insects, mites and hirudineans (Buchner, 1953). It is obvious that this type of symbiosis has often evolved independently.

Further studies are necessary to discover at which stage the bacteria appear for the first time and to find out how long the large population of symbionts is maintained. Possible ways of infection are per os during the marsupial period of the mancas, per ovum during the maturation of the oocytes in the maternal ovary, or directly from the environment. As no intracellular bacteria were seen on the sections, bacteria were also absent in other regions of the gut, the most probable way and time of infection is the first one mentioned.

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