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Design of the Labial Cuticle in *Cenocorixa bifida* Hung. (Hemiptera: Corixidae) with Reference to Ionic Transport

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ABSTRACT—The surface topography and ultrastructure of the labial cuticle of *Cenocorixa bifida* were examined by scanning and transmission electron microscopy. The dorsal wall of the labium consists of seven sclerotized transverse bars each displaying two rows of semicircular grooves and pores. The cuticle is about 20 μm thick and is composed of epicuticle and lamellate exocuticle and endocuticle, the latter separated from the underlying epidermis by subcuticle containing amorphous material. The epicuticle is subdivided into an electron-dense very thin outer epicuticle and a homogenous thick inner epicuticle, which is penetrated by grooves. The exocuticle is filled with electron-dense blocks of material, which may provide mechanical support to the labial wall. The elongate epidermal cells display extensive infoldings of the apical plasma membrane (facing the cuticle) and contain abundant mitochondria in the cytoplasm. The presence of deep epicuticular grooves and pores in the thin labial cuticle and extensive apical membrane infolding and abundant mitochondria in the epidermal cells suggest that the labium in *C. bifida* is the site of osmoregulatory ionic uptake.

Key words: transverse bars, epicuticular grooves, pores, apical membrane Infoldings, mitochondria

INTRODUCTION

Aquatic insects, like other animals living in fresh water, have special problems of protecting themselves against the loss of ions and influx of water through the integument (Krogh, 1939; Nemenz, 1976). To overcome this problem, aquatic insects have developed different mechanisms to maintain the consistency of their body fluids. Dipterous larvae such as *Aedes, Culiseta, Chironomus* and *Drosophila* while maintaining lower permeability of their integument to water, have developed anal papillae/organs which absorb ions into the haemolymph (Wigglesworth, 1933, 1938; Koch, 1938; Gloor and Chen, 1950; Copeland, 1964; Sohal and Copeland, 1966; Eichelberg *et al*., 1972; Meredith and Phillips, 1973; Garrett and Bradley, 1984; Jarial, 1987, 1995). Special structures composed of single cells or cell complexes of the so called “chloride cells” covered by thin, porous cuticular plates and involved in chloride uptake from the medium have been reported in the inlegument of mayfly and stonefly nymphs (Wichard and Komnick, 1971, 1973; Komnick and Abel, 1971; Komnick *et al*., 1972; Kapoor and Zachariah, 1973; Filshie and Campbell, 1984).

The insect cuticle is composed of an outer epicuticle, a middle exocuticle and an inner endocuticle overlying the epidermis. The epicuticle is nonchitinous and is waterproofed with lipids. The exocuticle and endocuticle form the bulk of the cuticle and consist of chitin, proteins and some lipids (Weis-Fogh, 1970; Wigglesworh, 1976; Neville, 1998). The epicuticle is further divided into four layers: cement, wax, outer epicuticle and inner epicuticle (Locke, 1964; Weis-Fogh, 1970). It has been reported that the outer epicuticle in *Calpodes* larvae is the first layer of the cuticle to be secreted by the underlying epidermal cells (Locke, 1964, 1966, 1976).

The mouth parts of corixid bugs are adapted to a fluid diet containing vegetable matter and ooze which has brought about a high degree of modification of these structures. The head capsule is compressed dorsoventrally, resulting in the fusion of labium with the clypeus. The labrum is highly reduced and is concealed by the base of the labium. (Qadri, 1951). The labium in Corixidae is more or less a triangular structure and dorsally bears a deep stylet groove through which the maxillary and mandibular stylets exit when they are in action. The stylet groove is flanked by a series of transverse sclerotized bars (Qadri, 1951; Parsons, 1966). These transverse sclerotized bars are separated from each other by weakly sclerotized membranous regions in which the sense organs are located (Benwitz, 1956; Lo and Acton, 1969). It has been shown by autoradiography that the labium of *Cenocorixa bifida* takes up ^{22}Na

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from the labeled media (Jarial *et al*., 1969), suggesting its role in ionic regulation.

The aim of the present study was to further elucidate the surface and ultrastructural features of the labium of *C. bifida* and to correlate them with their function of transporting ions from the external medium to the haemolymph.

MATERIAL AND METHODS

Adult *Cenocorixa bifida* Hungerford were collected from White Lake in the Cariboo region of British Columbia, Canada, during the summer, 1966. The insects were transported to the laboratory in gallon thermos jugs half filled with lake water. They were maintained in lake water in plexiglass dishes at 5°C in a constant temperature cabinet until needed. In all cases insects were studied within two weeks of capture.

Ten adult insects were used in this study. For ultrastructural study, the heads of insects were removed with sharp scissors and fixed for 15 min in the following mixture: 1 part 5% osmium tetroxide, 1 part 10% glutaraldehyde and two parts 0.2M phosphate buffer (pH 7.2). This fixative is isosmotic to the haemolymph of the insect and hence presumably isosmotic with the tissues (Jarial *et al*., 1969). The heads were washed in several changes of the phosphate buffer. The labium was cut into small pieces, dehydrated in

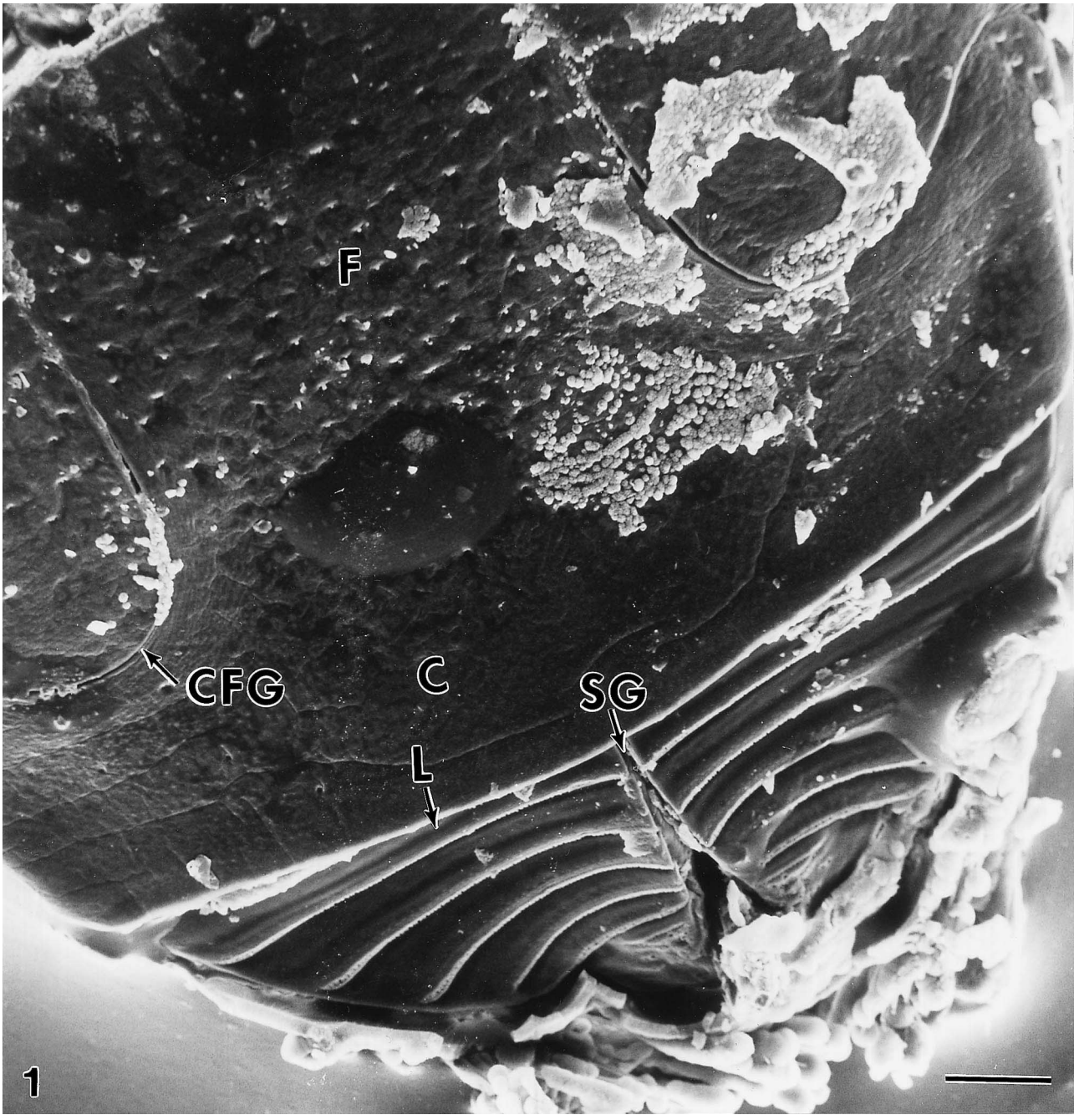

Fig. 1. Scanning electron micrograph of the frontal view of the head of *C. bifida* showing the labium (L) consisting of transverse bars flanking the stylet groove (SG). C, clypeus; CFG, clypeofrontal groove; F, frons. Scale bar, 0.1mm.

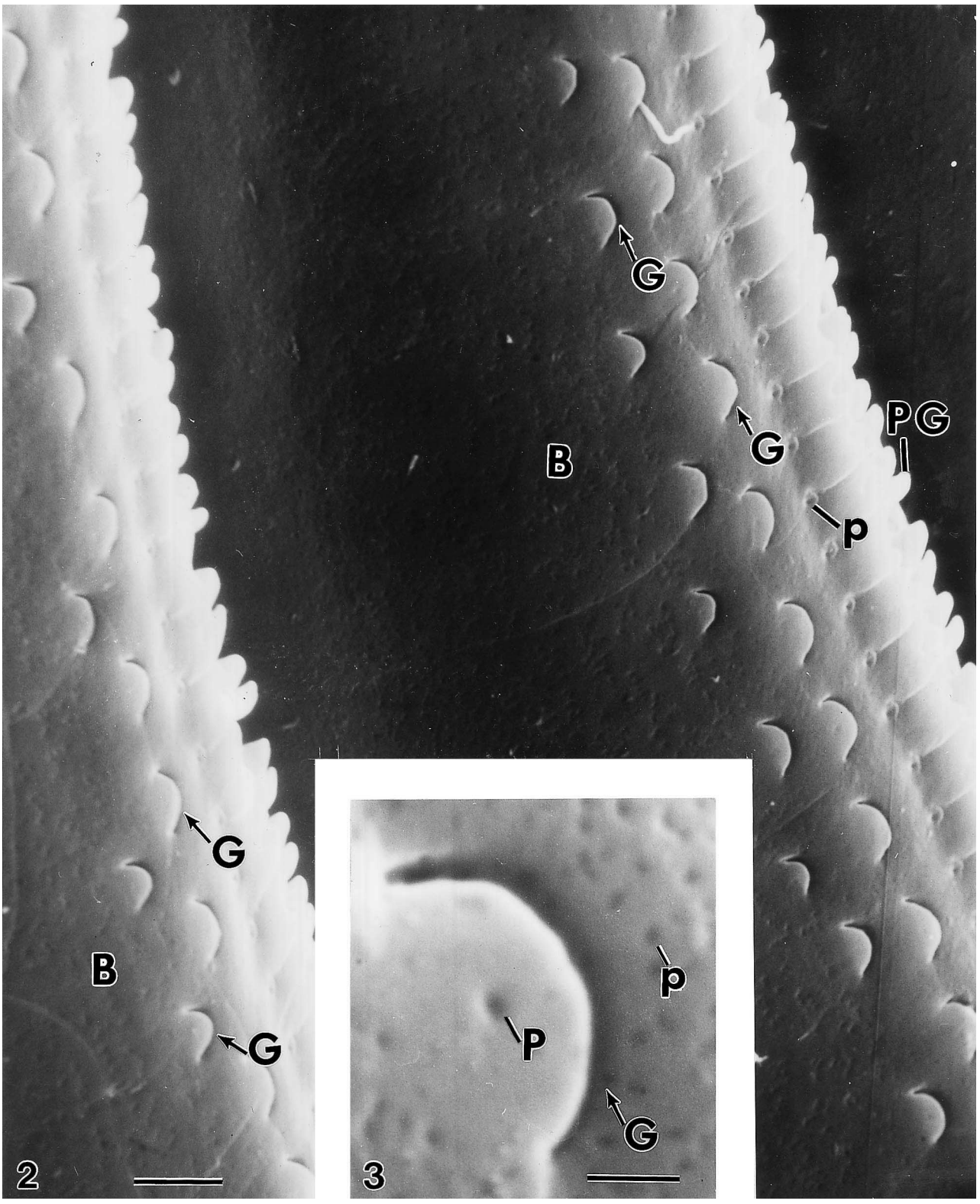

Fig. 2. SEM of the dorsal surface of two adjacent transverse bars (B) of the labium showing two rows of semicircular grooves (G). Also seen are the pegs (PG) and minute pores (p) of sensilla at the edge of each bar. Scale bar, 5 μm.
Fig. 3. Magnified image of a semicircular groove (G) and adjoining cuticle displaying a large central pore (P) and many small pores (p) on the surrounding cuticle. Scale bar, 1 μm.

an ethanol series to propylene oxide and embedded in Epon 812. Polymerization was carried out at 60°C overnight. Ultrathin sections were cut on a Porter-Blum MT1 microtome, stained with uranyl acetate, post-stained with lead citrate and examined with a Hitachi HU-11A transmission electron microscope (TEM). For Scanning electron microscopy, similarly fixed whole heads were dried using the liquid CO_2 critical point method, coated with gold/palladium and examined in an ETEC autoscan scanning electron microscope (SEM).

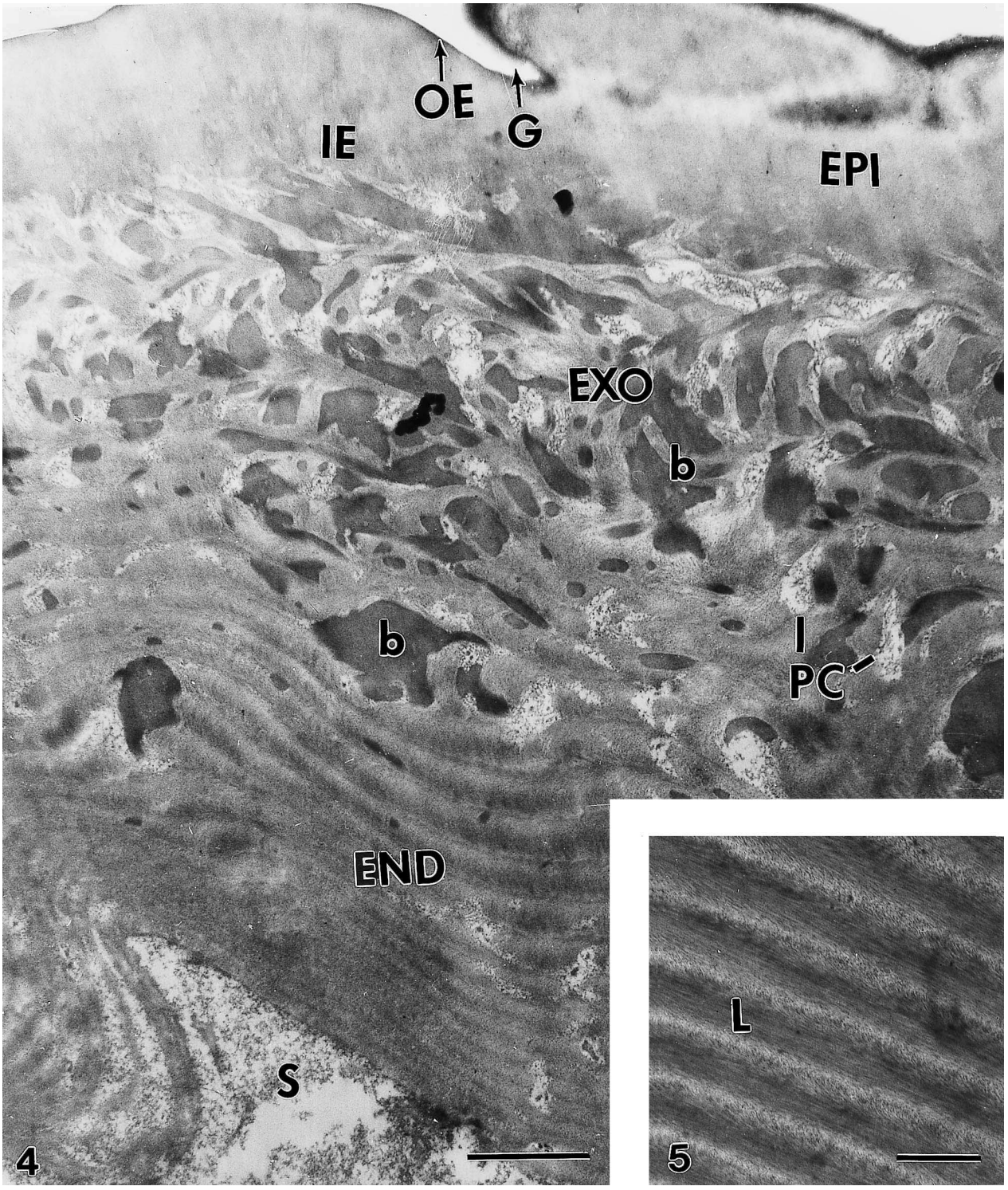

Fig. 4. Transmission electron micrograph of a section through the cuticle of a labial bar showing epicuticle (EPI) with its outer epicuticle (OE) and inner epicuticle (IE) layers, exocuticle (EXO) and endocuticle (END) containing many electron-dense blocks (b) and subcuticle (S). G, groove; PC, pore canals. Scale bar, 2 μm.

Fig. 5. TEM of endocuticle displaying parallel lamellae (L). Scale bar, 0.5 μm.

RESULTS

Scanning electron microscopic observations

In scanning micrographs the labium of *C. bifida* appears somewhat triangular in shape and greatly shortened, its width being four times its length. It is fused with the clypeus, which in turn is separated from the frons by a clypeofrontal suture. On the dorsal side it bears a deep stylet groove flanked by seven transverse sclerotized bars. The transverse bars are flattened, and they become progressively wider towards the lateral edges and shorter towards the tip of the labium (Fig.1). Near the inferior border each transverse bar displays two rows of semicircular grooves. Each semicircular groove is about 3 μm in diameter and 0.3 μm in width. The grooves are positioned such that those in the superior row alternate with those of the inferior row (Fig. 2). At higher magnification, the bar cuticle circumscribed by each groove exhibits a large centrally located pore about 0.3 μm in diameter. Many small pores (0.1 μm in diameter) are seen in the bar cuticle around the grooves (Fig. 3). A row of pegs and minute pores of sensilla are seen at the inferior edge of each transverse bar of the labium (Fig. 2).

Transmission electron microscopic observations

Transmission electron micrographs of the dorsal wall of the labium of *C. bifida* reveal that a thin cuticle (20 μm in

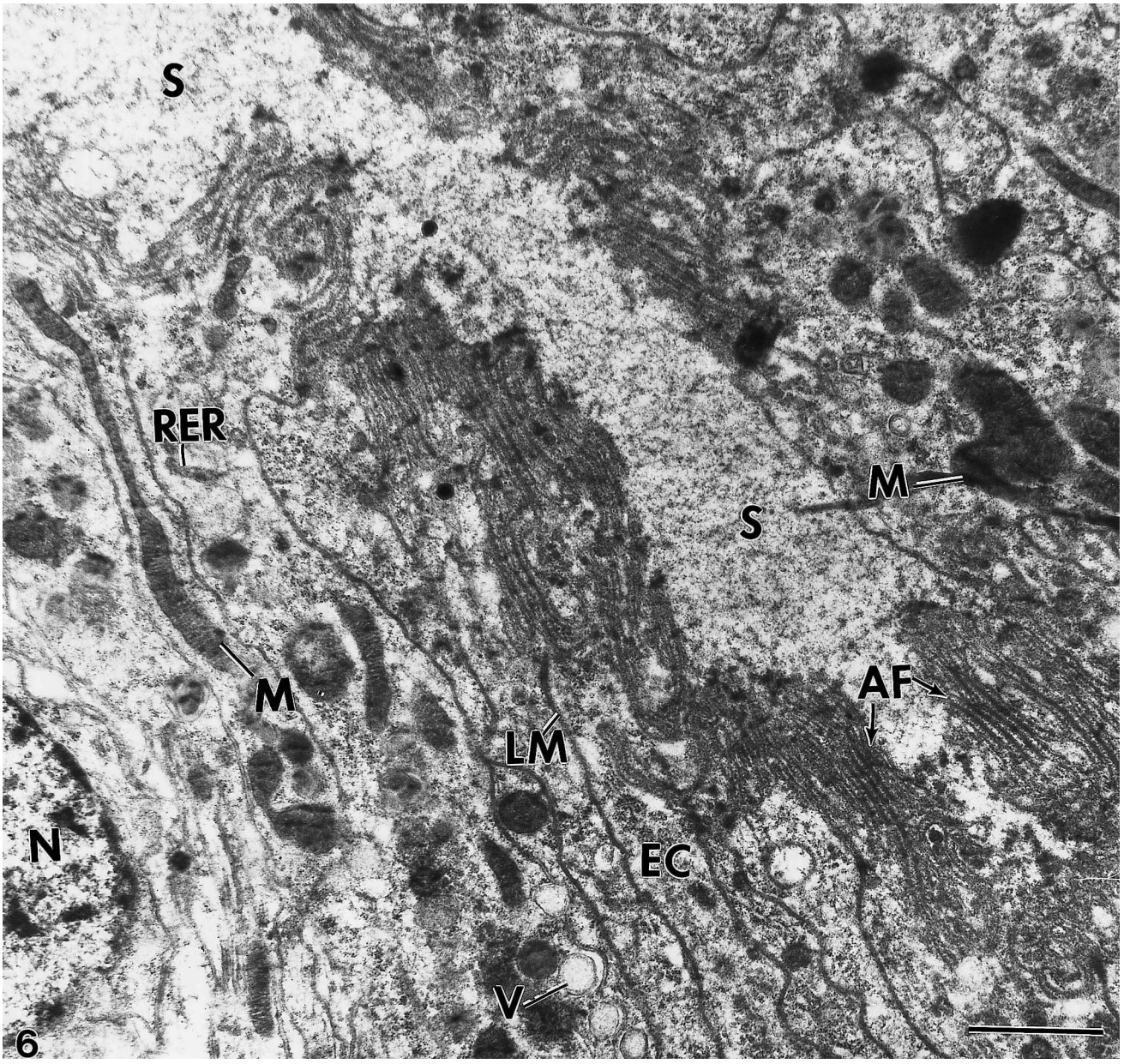

Fig. 6. TEM of a section through the epidermis showing subcuticle (S) extending to the elongate, narrow epidermal cells (EC) bounded by delicate lateral membranes (LM) and apical plasma membrane thrown into numerous infoldings (AF). M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; V, vesicles. Scale bar, 2 μm.

thickness) covers the epidermis. The cuticle consists of three layers: external epicuticle, middle exocuticle and inner endocuticle. The epicuticle is about 4 μm thick and is made up of an outer epicuticle which appears as a thin uniformly dark (about 10 nm thick) electron-dense line and a thicker homogeneous (4 μm thick) inner epicuticle but the cement and wax layers are absent (Fig. 4). The epicuticular grooves seen on the surface of the labial bars are lined by the outer epicuticle and penetrate almost half the thickness of inner epicuticle (Fig. 4). The exocuticle is about 6 μm thick and is filled with small and large electron-dense blocks of material, which tend to distort the lamellar arrangement in this layer (Fig. 4). The endocuticle, which is about 10 μm thick, displays regularly spaced horizontal 0.3 μm thick lamellae containing fibrils and a few large electron-dense blocks (Figs. 4,5). Pore canals containing many filaments traverse all three layers of the labial cuticle. A subcuticle region containing amorphous material lies between the endocuticle and epidermis (Figs. 4, 6).

Micrographs of the elongate epidermal cells show that their apical plasma membranes (facing the cuticle) are thrown into numerous closely-spaced infoldings. These infoldings on the cytoplasmic side are devoid of the particulate coat that has been reported in the anal papillae of salt-water larvae of *Aedes compestris* (Meredith and Phillips, 1973). The epidermal cells are bounded by delicate lateral membranes. The nuclei occupy the central zones of the cells. The cytoplasm contains abundant mitochondria some of which are fairly long, rough endoplasmic reticulum and vesicles. At places the subcuticle extends deep to come in contact with the epidermis (Fig. 6).

DISCUSSION

A physiological study of the body fluids of *C. bifida* has shown that this fresh-water insect is able to be hyperregulated by producing urine hyposmotic to the haemolymph (Scudder *et al*., 1972).

The labium in Heteroptera is the rostral part of the head, which is directly exposed to water and is adapted to a fluid diet. In Corixidae, the labium is more or less triangular, much shorter and dorsally bears a deep stylet groove flanked by a series of sclerotized transverse bars (Qadri, 1951; Parsons, 1966). These transverse bars are separated from each other by weakly sclerotized membranes in which the sense organs are located (Benewitz, 1956; Lo and Acton, 1969).

Jarial *et al*. (1969) found that the labium in $AgNO_3^-$ treated *C. bifida* becomes darkly stained and silver grains enters the labial cuticle. In addition, it was shown by autoradiography that the labium takes up ^{22}Na when the insects are placed in labeled media, suggesting that the labium is the site of ion absorption.

This study has demonstrated that the sclerotized transverse bars of the labium of *C. bifida* dorsally display two rows of semicircular grooves that penetrate almost half of the thickness of inner epicuticle. In addition, large pores are centrally located on the cuticle circumscribed by the semicircular grooves and numerous small pores on the cuticle around the grooves. Staddon (1964, 1966) has shown that the cuticle in *Corixa dentipes* plays an important role in the osmotic uptake of water. It is reasonable to assume that the grooves and pores found on the labial epicuticle of *C. bifida* play a role in the uptake of water as well as ions from the surrounding medium.

Transmission electron micrographs of the labium of *C. bifida* reveal that its cuticle is relatively thin measuring about 20 μm in thickness in contrast to the very thick cuticle of terrestrial insects which is up to 200 μm in thickness (Chapman, 1969). The cuticle is composed of three clearly distinguishable layers as in other insects: outer epicuticle, middle exocuticle and inner endocuticle. The epicuticle is further subdivided into a very thin electron-dense outer epicuticle and a thick homogeneous inner epicuticle but the cement and wax layers are absent. It resembles the epicuticular lining of trachea which is permeable to water (Locke, 1964, 1966, 1976) and the epicuticle of porous plates overlying the chloride cells in the integument of mayfly nymph that is permeable to colloidal lanthanum (Komnick and Abel, 1971; Filshie and Campbell, 1984). The outer epicuticle dips from the surface into the semicircular grooves and lines them as they traverse the inner epicuticle, presumably facilitating entry of ions into the underling epidermis.

Locke (1964) has proposed that a phase change of the lipids in the wax canals in the cuticulin (outer epicuticle) induced by water or high humidity in the environment may account for the permeability of the cuticle to water. Since the wax layer is not usually retained after dehydration in ethanol series for electron microscopy, Locke's hypothesis of permeability of the cuticle to water may also be applicable to labial cuticle of *C. bifida in vivo*.

The electron dense blocks of presumably protein rich material (Neville, 1998) in the lamellate exocuticle and endocuticle provide mechanical support to the somewhat flexible dorsal labial wall. The pore canals, which traverse all three layers of the cuticle, contain many filaments in sections. Since each pore canal usually contains a single filament, it appears that these relatively large pore canals are formed by fusion of smaller pore canals (Neville, 1998). The amorphous material in the subcuticle located between the endocuticle and the epidermis probably contributes to the formation of the endocuticle (Neville, 1998).

The predominant ultrastructural features of the epithelial cells of the labial epidermis are the extensive infoldings of the apical plasma membrane (facing the cuticle) and the presence of abundant mitochondria both of which are commonly exhibited by epithelia that specialize in salt and water absorption and transport (Berridge and Oschman, 1972). Such a close association of membranes extensively infolded and abundant mitochondria provide a large surface area and energy for active ionic transport. These ultrastructural features of the labial epithelial cells closely resemble the

ultrastructure of anal papillae/organs of dipterous larvae (Copeland, 1964; Sohal and Copeland, 1966; Jarial, 1987, 1995) and the abdominal chloride epithelia of caddis fly larvae (Wichard and Komnick, 1973). Two types of chloride cells that take up measurable amount of radioactive chloride from solution have been observed in the labium of *Corixa punctata* (Komnick and Schmitz, 1977). Chloride cells were not seen in the labium of *C. bifida*.

In conclusion, the structural features such as epicuticular grooves and pores, extensive apical membrane infoldings and abundant mitochondria in the epidermal cells, in conjunction with ^{22}Na uptake demonstrated earlier (Jarial *et al*., 1969), strongly suggest that the labium of *C. bifida* is engaged in the active transport of ions from the medium into the haemolymph.

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