Adv. Odonatol. 1: 105-115 *December 31, 1982*

FINE STRUCTURE AND TRACER PERMEABILITY OF THE CUTICLE AND CELL JUNCTIONS OF THE RECTAL CHLORIDE EPITHELIA OF *AESHNA CYANEA* LARVAE; A COMPARATIVE FREEZE-FRACTURE AND THIN-SECTION STUDY

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Three types of cell junctions are identified in the rectal chloride epithelia: Zonulae adhaerentes, pleated septate junctions and gap junctions. All three occur in characteristic arrangements between the apico-lateral cell membranes whereas only gap junctions are scattered between the baso-lateral cell membranes. The cuticle overlying the chloride epithelia differs from the cuticle of the adjacent respiratory epithelia by the occurrence of epicuticular depressions with a diameter of about 200 nm and a frequency of 2 to 3 per μm^2. Permeability studies with colloidal and ionic lanthanum ingested with the medium into the rectal lumen by the living animals showed that the tracer was able to penetrate exclusively the cuticle possessing epicuticular depressions over the chloride epithelia. Therefore the depressions seem to represent hydrophilic sites favoring cuticular permeability over the ion transporting epithelia. The tracer was also present in the pleated septate junctions, but always restricted to the apical three quarters of their length regardless of the duration of exposure (3 h to 8 d). This finding suggests that this type of cell junction may seal the intercellular channels.

INTRODUCTION

The chloride epithelia of *Aeshna cyanea* larvae located in opposing pairs at the bases of the rectal gill leaflets are the site of an active, osmoregulatory ion absorption of these freshwater insects (KOMNICK 1978). These transporting epithelia were fine-structurally described by GREVEN & RUDOLPH (1973) and WICHARD & KOMNICK (1974). Cell junctions play an important role in epithelial physiology, in particular in epithelial transport physiology. Desmosomes may occur as spot and belt desmosomes and serve as adhaesive

structures; gap junctions constitute the pathways for bilateral cell-to--cell communication. Sealing junctions preventing the uncontrolled paracellular movement of water and ions across epithelia are of special interest (BERRIDGE & OSCHMAN 1972). For vertebrate epithelia it is well established that this function is exerted by the tight junctions (STAEHLIN 1974). But in invertebrate epithelia tight junctions – with the exception of two findings (LANE 1979a; LANE & CHANDLER 1980) – have not been observed while desmosomes and gap junctions exist in both vertebrate and invertebrate epithelia. Ubiquitous in invertebrate epithelia are septate junctions, first observed by WOOD in 1959. Characteristic of these junctions is the "ladder"-like structure, which appears after conventional fixation when the junctions are cut across. This image is due to a series of thin septa that traverse the intercellular space between the opposed membranes. Recent lanthanum-staining and freeze-fracture studies on various arthropods lead to more detailed fine-structural information and to the recognition of two types of septate junctions: the smooth and pleated type; both types are functionally considered as adhaesive, sealing and communicative devices, (for review see NOIROT-TIMOTHÉE & NOIROT 1980; LANE & SKAER 1980). This paper will discuss the cellular junctions and the cuticle of the rectal chloride epithelia of *Aeshna cyanea* in fine structural and functional respect.

MATERIAL AND METHODS

For the examinations, larvae of *Aeshna cyanea* of nearly equal body lengths (2.5-3.0 cm) were selected. The fine structure was studied with 3 methods: (a) conventional fixation of the isolated rectum was carried out with either 2% OsO_4 or successively with 3% glutaraldehyde (GA) and 1% OsO_4. The fixative contained 100 mM Na-cacodylate buffer at pH 7.4/100 mM sucrose. Thin sections of the Araldite-embedded tissue were stained with uranyl-acetate and lead citrate. (b) Lanthanum staining of the isolated rectal tissue was achieved by the addition of either colloidal (1% lanthanum hydroxide according to REVEL & KARNOVSKY 1967) or ionic lanthanum (1% $LaCl_3$) to the fixatives and buffer washings. Thin sections of the Styrol-Methacrylate - or Araldite - embedded tissues were examined without further staining. (c) For freeze-fracturing isolated recta were fixed in 2.5% GA in 100 mM Na-cacodylate buffer. Tissue was briefly infiltrated with glycerol of 30% final concentration as cryoprotectant and shock-frozen in liquid propane cooled with liquid nitrogen. Freeze-fracture replicas were obtained with a Biotech 2005

apparatus (Leybold-Heraeus).

Permeability studies were carried out on the living animals with the use of 1.5% aqueous solutions of lanthanum hydroxide, lanthanum nitrate and lanthanum chloride. In order to ensure that only the luminal sides of the chloride epithelia were exposed to the lanthanum label, the larvae were dipped with their anal opening up to 8 days into the experimental solutions as shown in Figure 1. After exposure and prior to fixation the recta were thoroughly washed by causing the larvae to swim in lanthanum free water for about 10 sec. Further treatment was the same as described in section (a).

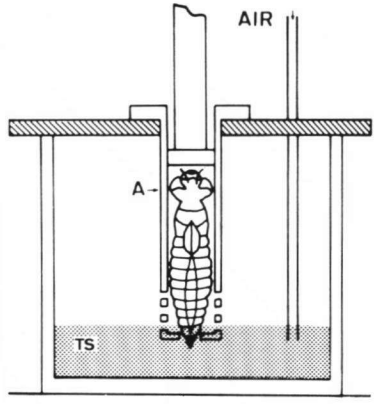

Fig. 1. Diagram illustrating the experimental arrangement for permeability studies with living larvae. A perforated plastic syringe as a device to immobilize the larvae; TS tracer solution.

RESULTS AND CONCLUSIONS

After conventional fixation the apico-lateral cell borders appear as meanders of parallel cell membranes (Fig. 2a). Higher magnification reveals in cross sections the characteristic "ladder"-like appearance of septate junctions in this region (Fig. 3d). Both the cell membranes and the septa are relatively electron-dense. The spacing of the junctional cell membranes is about 15 nm, the septa being about 8 nm wide and occurring in center-to-center distances of about 16 nm.

The addition of both colloidal and ionic lanthanum to the fixative leads to heavy staining of the junctional area and allows the clear discrimination of septate and gap junctions. Due to lanthanum staining the "ladder"-like structure of the septate junctions appears

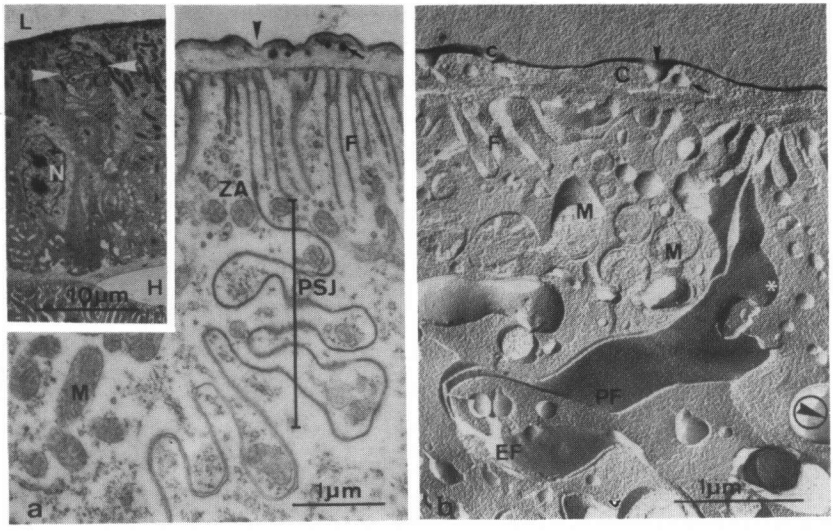

Fig. 2. Junctional complex of rectal chloride epithelia in thin sections and after freeze--fracturing: (a) Thin section after conventional Os-fixation. Arrow head: epicuticular depression; arrow: Osmiophilic granule; F apical membrane foldings; PSJ extension of pleated septate junction with interspersed gap junctions; M mitochondrium. Inset: Low magnification of a part of a chloride epithelium after GA/Os-fixation shown for topographic orientation of the junctional complex (white arrow heads).L luminal side; N nucleus; H haemocoel side;– (b) Freeze-fracture replica, C cross-fractured cuticle; c small fringe of cuticle surface; arrow head: epicuticular depression; arrow: cuticular granule; F apical membrane foldings; M mitochondria. Note the nearly particle free zone above the septate junction membrane (white asterisk).

in reverse contrast (Fig. 3a) when compared to the conventionally fixed preparations (Fig. 3d). As seen in Figure 3a the septa are inserted into the plasma membranes partly at about 45° and partly at 90°. In sections parallel to the junctional plasma membrane the septa are depicted as zig-zag lines (Figs 3b and c). Accordingly, these junctions are classified as pleated septate junctions. The septa are more or less parallel to each other, follow a partly straight and partly curved course, and show clear terminales at places (Fig. 3c).

In freeze-fracture replicas this type of septate junction shows rows of intramembranous particles (IMP) on PF[1], while EF appears relatively smooth, possessing only few particles (Fig. 2b). Small local shadowing angles reveal the presence of furrows on EF corresponding to the particle rows of PF (Fig. 3e). The PF particles measure about

[1]Nomenclature of fracture faces is performed according to BRANTON et al.(1975). The shadowing direction is indicated by the encircled arrow head at the right hand corner of the micrographs.

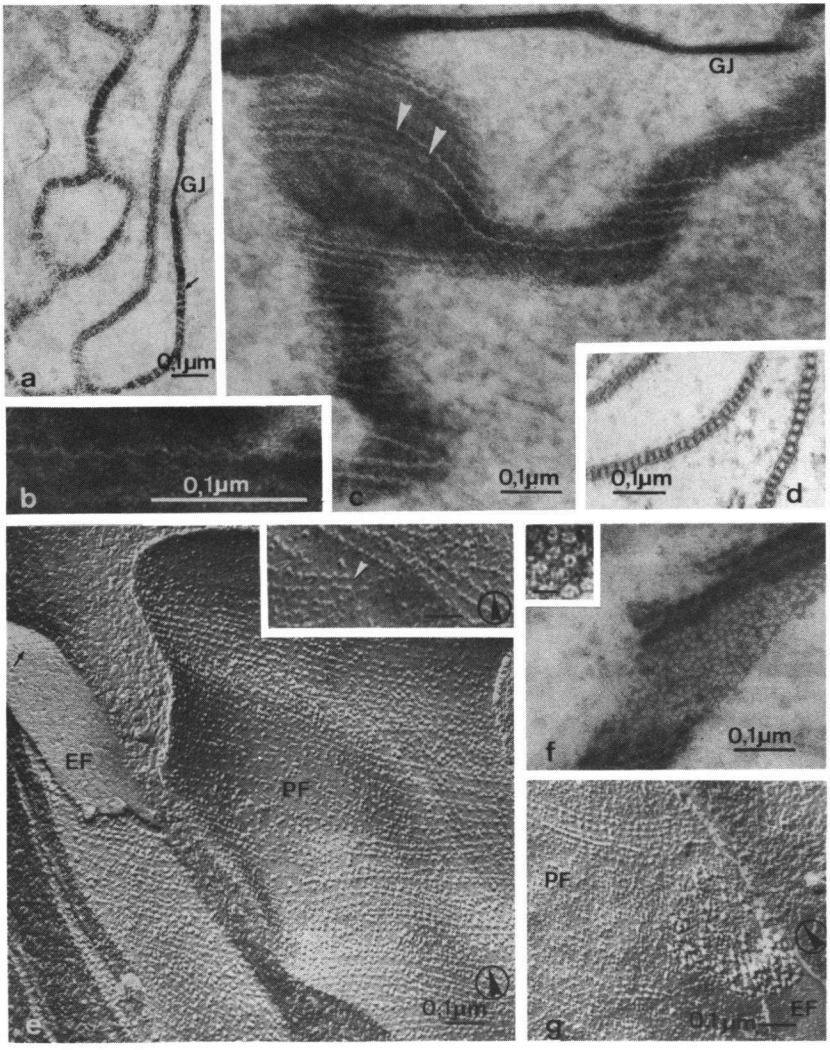

Fig. 3. Fine structural details of pleated septate (a to e) and gap junctions (f and g) of rectal chloride epithelia: (a) Cross section after lanthanum staining. Arrow: septa at various angles; GJ gap junction;– (b) High magnification of a longitudinal section through a septum parallel to the junctional membranes;– (c) Thin section after lanthum staining showing the arrangement of septa. The arrow heads point to terminating septa; GJ gap junction;– (d) Cross section of pleated septate junction after conventional GA/Os-fixation;– (e) freeze--fracture replica, arrow points to furrows on EF. Inset: terminating particle rows (arrowhead); – (f) Thin section showing a gap junction between the pleated septa. Inset: high magnification of some gap junction particles (bar: 25 nm);– (g) Freeze-fracture replica of a gap junction located between groups of strands of the septate junction.

8 nm in diameter and are stringed within a row at center-to-center spacings of about 20 nm. When occurring in parallel arrangement the particle rows run at distances of 15-20 nm and show random terminations (Fig. 3e inset). Figure 5 represents a diagrammatic synopsis of the results obtained from the pleated septate junction of the rectal chloride epithelia with the 3 techniques applied. Similar diagrams of pleated septate junctions of other invertebrate epithelia have been published by FLOWER (1971), NOIROT-TIMOTHÉE & NOIROT (1973), STAEHLIN (1974), CAVENEY & PODGORSKY (1975) and LANE & SKAER (1980). Accordingly the chloride epithelia in the unique rectum of larval dragonflies have nothing unique in this respect.

Like in other insect epithelia (e.g. GREEN et al. 1980; LANE 1979b) the utmost apical junction of the rectal chloride epithelia is represented by a zonula adhaerens (Fig. 2a). In freeze-fracture replicas this type of junction appears as a striking nearly particle-free zone on PF and EF between the apical cell membranes and the beginning of the pleated septate junction (Fig. 2b). Therefore the internal organization of the junctional membranes of the zonula adhaerens of the chloride epithelium is very similar to the zonula adhaerens of vertebrate epithelia (STAEHLIN 1974).

The third junctional structure in the apico-lateral region are the gap junctions, randomly interspersed between the pleated septate junctions. Additional gap junctions are present between the baso-lateral cell processes which interdigitate to form the labyrinthine intercellular spaces in the basal region of this epithelium. In tangential sections after lanthanum staining, the gap junctions show up as circumscribed areas studded with round electron-transparent particles (Fig. 3f). These subunits have an average diameter of 6 nm and possess a central electron-dense dot (Fig. 3f inset). The intercellular space measures about 15 nm in the adjacent pleated septate junction and is reduced to 6-7 nm at the site of the gap junctions (Figs 3a and c). These gap junctions are identified by their freeze-cleaving behaviour as "inverted gap junctions" according to the nomenclature proposed by FLOWER (1972). Cluster-shaped aggregates of about 10 nm particles are present on EF, while PF shows corresponding pits occurring in association with the particle rows of the septate junction (Fig. 3g). Unlike freeze-fracture replicas of vertebrate gap junctions where the particles prevail on PF, only few particles are retained on PF of the inverted type. The infiltration of the central channel with lanthanum of the particles justifies the assumption that they represent canalized connecting proteins, so-called con-

nexons (PERACCHIA 1980).

The presence of surface depressions in the cuticle of the rectal epithelia (Fig. 2a) was first observed by HERZOG (1979) in thin sections. In freeze-fracture replicas they appear as funnel-shaped depressions about 200 nm in diameter and the frequency being 2-3 per μm^2 (Figs 2b and 4a). The bottom of a depression shows an array of particles which are assumed to reflect a sieve-like substructure (Fig. 4f). The adjacent cuticle over the respiratory epithelium is lacking such depressions (Fig. 4a).

The permeability studies with both colloidal and ionic lanthanum established uniform results. Tracer particles are only present in the cuticle possessing epicuticular depressions, i.e. over the chloride epithelia (Fig. 4b). The label is absent from the cuticle of the respiratory epithelium and even from the cuticle over the intermediate cells (Fig. 4c), the latter being devoid of endocuticle (GREVEN & RUDOLPH 1973). Underneath the depressions a denser accumulation of lanthanum was frequently observed, indicating that the depression may offer the pathways for the tracer and thus presumably for other ions like Na^+ and Cl^- (Fig. 4e). So these and similar depressions (NOIROT & BAYON 1969; NOIROT & NOIROT-TIMOTHÉE 1969; FLOWER & WALKER 1979) may be functionally analogous to corresponding structures in the cuticle over various insect ion-transporting cells (KOMNICK & STOCKEM 1973; KOMNICK 1977).

Colloidal and ionic lanthanum also invaded into the pleated septate junctions of the chloride epithelia. But the label never extended for more than the apical three quarters of their length regardless of the duration of tracer application (Fig. 4d). This finding is in contrast to the results of similar permeability experiments on other invertebrate epithelial tissues. These experiments, however, included the dissection of the animals prior to tracer application and lead to complete permeation of pleated septate junctions by the tracers applied (e.g. LANE & TREHERNE 1972; LESLIE 1975; RYDER & BOWEN 1977). Only SZOLLOSI & MARCAILLOU (1977) found a limited penetration of lanthanum into pleated septate junctions of an epithelial barrier in the locust. The present results are indicative of a sealing function, reflecting a more natural behaviour of this junction, because the rectum was exposed to lanthanum solutions under in-vivo conditions without any previous operative treatment. On the other hand, the complete permeation of the pleated septate junctions of the rectal transporting epithelia of cockroaches after the injection of lanthanum solutions into the rectum (LANE 1979b) seems to in-

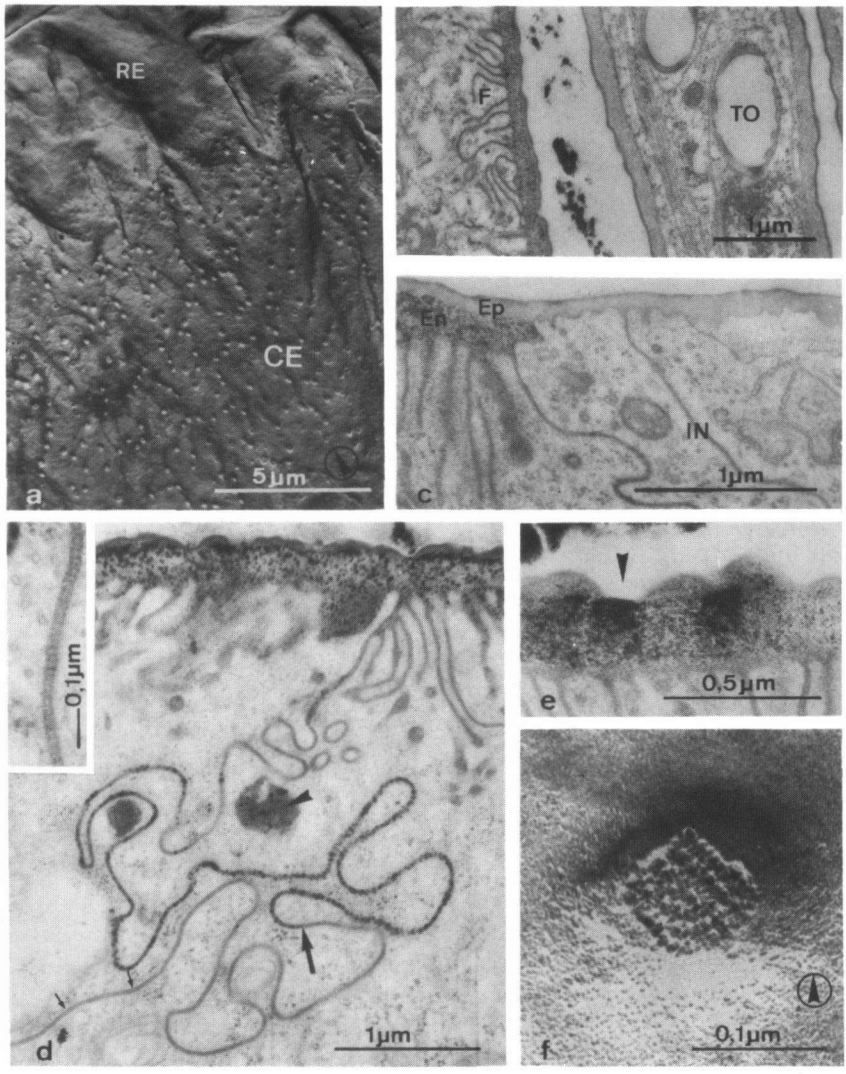

Fig. 4. The surface of the rectal cuticle revealed after freeze-fracturing (a and f) and thin sections after permeability experiments (b to e): (a) Freeze-fracture replica, note that the epicuticular depressions are restricted to the area of the chloride epithelium (CE), RE, adjacent respiratory epithelium;– (b) Thin section of a rectum fixed after exposure to lanthanum for 2 days. The tracer exclusively appears in the cuticle over the chloride epithelium (F apical foldings), while the opposed respiratory epithelium (TO tracheole) is not labelled; – (c) Cross section of the marginal region of a chloride epithelium (24 hours of in vivo lanthanum application), showing the sharp limitation of label to the cuticle of the chloride epithelium. IN, intermediate cells; EP, epicuticle; EN, endocuticle;– (d) Thin section through the apical region of a chloride epithelium (3 days of in vivo lanthanum application), showing

dicate that pleated septate junctions in general may be "leaky" or "tight" in different epithelia and thus be analogous to the vertebrate tight junctions (CLAUDE & GOODENOUGH 1973). Nevertheless, one should always keep in mind that lanthanum is an unphysiological element and it remains to be elucidated whether the conclusions drawn from its experimental use are also valid for physiological materials.

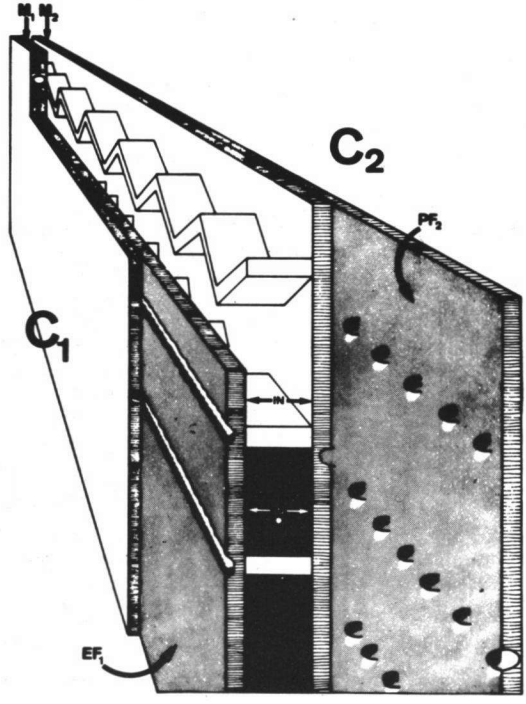

Fig. 5. Diagram showing the inter- and intra-membrane differentiations of pleated septate junction of rectal chloride epithelia as revealed by thin-sectioning and freeze-fracturing. M_1 and M_2: the two opposed junctional cell membranes of the neighbouring cells C_1 and C_2. The upper part of the intercellular space (IN) shows a pleated septum. The lower part (IN*) illustrates the black lanthanum staining of the interspaces between the septa. The rows of intramembranous particles on PF leaving furrows on EF are believed to run in register with the septa and serve for intramembranous anchorage of the intercellular septa.

(Fig. 4, continued)
label within the cuticle and the septate junction, but restricted to the point marked by the large arrow. A substantial, basally orientated part of the junction is unlabelled. Arrow head: cytoplasmic lanthanum aggregate. Inset: higher magnification of the unlabelled septate junction marked by the two small arrows;– (f) High magnification of an epicuticular depression in en face view after freeze-fracturing. Note the densely packed particles on its basis plate.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Deutsche Forschungsgemeinschaft to Prof. W. STOCKEM. The author is grateful to Prof. H. KOMNICK for constructive discussion and to Mrs. I. BAAS for helpful technical assistance.

REFERENCES

BERRIDGE, M.J. & J.L. OSCHMAN 1972. Transporting epithelia. Academic Press. New York and London.

BRANTON, D., S. BULLIVANT, N.B. GILULA, M.J. KARNOVSKY, H. MOOR, K. MUHLTHALER, D.H. NORTHCOTE, L. PACKER, B. SATIR, P. SATIR, V. SPETH, L.A. STAEHLIN, R.L. STEERE & R.S. WEINSTEIN 1975. Freeze-etching nomenclature. Science 190: 54-65.

CAVENEY, S. & C. PODGORSKY 1975. Intercellular communication in a positional field. Ultrastructural correlates and tracer analysis of communication between insect epidermal cells. Tissue Cell 7: 559-574.

CLAUDE, P. & D.A. GOODENOUGH 1973. Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia, J. Cell Biol. 58: 390-400.

FLOWER, N.E. 1971. Septate and gap junctions between the epithelial cells of an invertebrate, the mollusc Cominella maculosa. J. Ultrastr. Res. 37: 259-268.

FLOWER, N.E. 1972. A new junctional structure in the epithelia of insects of the order Dictyoptera. J. Cell Sci. 10: 638-691.

FLOWER, N.E. & G.D. WALKER 1979. Rectal papillae in Musca domestica: The cuticle and the lateral membranes. J. Cell Sci. 39: 167-186.

GREVEN, H. & R. RUDOLPH 1973. Histologie und Feinstruktur der larvalen Kiemenkammer von Aeshna cyanea Müller (Odonata: Anisoptera). Z. Morph. Tiere 76: 209-226.

GREEN, L.F.B., P.R. BERQUIST & S. BULLIVANT 1980. The structure and function of the smooth septate junctions in a transporting epithelium: The malpighian tubules of the New Zealand glow-worm Arachnocampa luminosa. Tissue Cell 12: 365-381.

HERZOG, H.U. 1979. Einfluss verschiedener Aussenkonzentrationen auf die Haemolymphe und die rectalen Chloridepithelien von Libellenlarven. Dipl. Arbeit, Univ. Bonn.

KOMNICK, H. 1977. Chloride cells and chloride epithelia of aquatic insects. Int. Rev. Cytol. 49: 285-329.

KOMNICK, H. 1978. Osmoregulatory role and transport ATPases of the rectum of dragonfly larvae. Odonatologica 7: 247-262.

KOMNICK, H. & W. STOCKEM 1973. The porous plates of coniform chloride cells in mayfly larvae: High resolution analysis and demonstration of solute pathways. J. Cell Sci. 12: 665-681.

LANE, N.J. 1979a. Tight junctions in a fluid-transporting epithelium of an insect. Science 204: 91-93.

LANE, N.J. 1979b. Freeze-fracture and tracer studies on the intercellular junctions of insect rectal tissues. Tissue Cell 11: 481-506.

LANE, N.J. & H.J. CHANDLER 1980. Definitive evidence for the existence of tight junctions in Invertebrates. J. Cell Biol. 86: 765-774.

LANE, N.J. & H. leB. SKAER 1980. Intercellular junctions in insect tissues. Adv. Insect Physiol. 15: 36-213.

LANE, N.J. & J.E. TREHERNE 1972. Studies on perineural junctional complexes and the sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. Tissue Cell 4: 427-436.

LESLIE, R.A. 1975. The effects of ionic lanthanum and hypertonic physiological salines on the nervous system of larval and adult insects. J. Cell Sci. 18: 271-286.

NOIROT, Ch. & C. BAYON,1969. La cuticule proctodéale des insectes: mise en évidence de "dépressions épicuticulaires" par le microscope électronique à balayage. C. r. hebd. Séanc. Acad. Sci. Paris D 269: 996-999.

NOIROT, Ch. & C. NOIROT-TIMOTHÉE 1969. La cuticule proctodéale des insectes. I. Ultrastructure comparée. Z. Zellforsch. 101: 477-509.

NOIROT-TIMOTHÉE, C. & Ch. NOIROT 1973. Jonctions et contacts intercellulaires chez les insectes. I. Les jonctions septées. J. Ultrastruct. Res. 37: 119-137.

NOIROT-TIMOTHÉE, C. & Ch. NOIROT 1980. Septate and scalariform junctions in Arthropods. Int. Rev. Cytol. 63: 97-140.

PERACCHIA, C. 1980. Structural correlates of gap junctions permeation. Int. Rev. Cytol. 66: 81-146.

REVEL, J.P. & M.J. KARNOVSKY 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. 33: C7.

RYDER, T.A. & I.D. BOWEN 1977. Studies on transmembrane and paracellular phenomena in the foot of the slug Agriolimax reticulatus (Mü.). Cell Tiss. Res. 183: 143-152.

STAEHLIN, L.A. 1974. Structure and function of intercellular junctions. Int. Rev. Cytol. 39: 191-283.

SZOLLOSI, A. & C. MARCAILLOU 1977. Electron microscope study of the blood-testis barrier in an insect: Locusta migratoria. J. Ultrastruct. Res. 59: 158-172.

WICHARD, W. & H. KOMNICK 1974. Zur Feinstruktur der rectalen Tracheenkiemen von anistopteren Libellenlarven. II. Das rectale Chloridepithel. Odonatologica 3: 129-135.

WOOD, R.L. 1959. Intercellular attachment in the epithelium of Hydra as revealed by electron microscopy. J. biophys. biochem. Cytol. 6: 343-353.