Adv. Odonatol. 1: 69-91 *December 31, 1982*

THE RECTUM OF LARVAL DRAGONFLIES AS JET-ENGINE, RESPIRATOR, FUEL DEPOT AND ION PUMP

H. KOMNICK

Institute of Cytology, University of Bonn, Ulrich-Haberland-Str. 61a,

5300 Bonn 1, Federal Republic of Germany

The rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. All of them are more or less causally connected with rectal ventilation. For each function specialized cells are present and the morphology and physiology of these are discussed.

INTRODUCTION

The unique larval rectum of anisopterous dragonflies has fascinated numerous scientists, and research on its structure and functions can be traced for 3 centuries (e.g. SWAMMERDAM 1680; POUPART 1702; REAUMUR 1742; CUVIER 1798; SUCKOW 1828; DUFOUR 1852; OUSTALET 1869; CHUN 1876; HAGEN 1880; FAUSSEK 1887; SADONES 1896; BABAK & FOUSTKA 1907; OGUMA 1913; TILLYARD 1916; RICH 1918; TONNER 1936; KOCH 1934, 1936).

According to current knowledge the rectum serves 4 major functions: locomotion, respiration, storage of reserve substances, and osmoregulatory ion uptake. The recognition of these functions is spread over two and a half centuries and was completed 5 decades ago, when KOCH (1934) correctly interpreted the vital argentaffinity of the rectal pads as indicative of chloride absorption. It is primarily the latter function that has attracted new interest during the past decade, when the rectum was studied with various modern techniques mostly as a model of transepithelial ion transport. This brief review will deal with the 4 main functions of the rectum on a more

cellular basis according to the recent trends of research in this field.

Three of the 4 functions, namely locomotion, respiration and ion absorption are closely interrelated in as much as they directly depend on rectal ventilation. There are also interrelations with the storage function because the rectal fat body cells lying near the sites of respiratory oxygen uptake are favourably placed for oxidative metabolism and for supplying the energy-consuming muscle and transport cells of the rectum.

Figures 1a and b, showing the posterior parts of the *Aeshna*–type larval intestine, may serve as an introduction to the anatomy. Cross sections through the branchial chamber of *Libellula* show the structural elements which perform the rectal functions (Fig. 1c).

Studies in the author's laboratory were mostly carried out on *Aeshna cyanea*, partly on *Libellula depressa* and for cytological comparison also on *Progomphus obscurus* collected from the Black Water River, Florida, after the 4th International Symposium of Odonatology in Gainesville.

LOCOMOTORY FUNCTION

Anisopteran larvae swim by jet propulsion resulting from vigorous expiration. It is unknown to whom the detection of this evident function can be ascribed. According to TONNER (1936) inspiration and expiration movements for breathing and swimming are fundamentally similar; they are merely faster and more vigorous in the case of swimming.

The ventilation movements result from the cooperation of various sets of body and rectal muscles. The rectal gill chamber lacks dilating muscles and filling of the chamber is achieved by the contraction of transverse muscles dilating the abdomen and by the pumping activity of the rectal ampulla. This pumping activity consists of alternate openings and closings of pre- and postampullar valves coordinated with alternate contractions of the dilators and constrictors of the ampulla (TONNER 1936).

Expiration on the other hand is caused by compression of the abdomen brought about by contractions of longitudinal, dorso-ventral, and oblique body muscles as well as by the contraction of the muscle fibres of the gill chamber (TONNER 1936). Repeated ejections of water through the anal opening as a result of vigorous expiratory movements propel the larva jerkily forward. When the anal opening of larval *Aeshna* is incidentally lifted into the air, the water droplets may be shot for about half a metre.

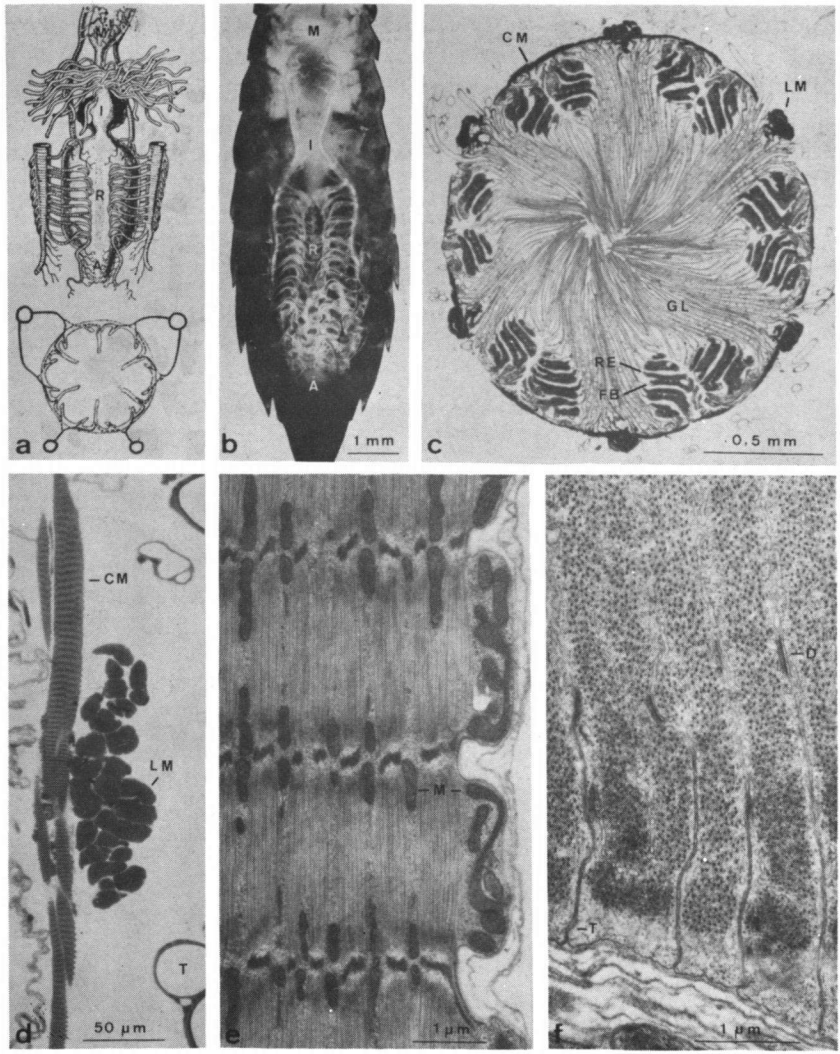

Fig. 1. (a) Posterior part of the intestinal tract of a larval dragonfly and diagrammatic cross section of the rectal gill chamber (adopted from IMMS, 1973); (b) Dissected abdomen of larval *Aeshna cyanea* showing posterior part of midgut (M), ileum (I), rectal gill chamber (R) and rectal ampulla (A), (photograph by H.-U. Herzog); (c) Cross section of the rectum of larval *Libellula depressa* showing gill lamellae (GL) in the middle, 6 double rows of chloride epithelia (RE) associated with layers of fat-body cells (FB) at the periphery, 6 bundles of longitudinal muscles (LM), and circular muscle-fibres (CM); (d) Cross section through the larval rectum of *A. cyanea* showing peripheral region with circular (CM) and longitudinal muscle fibres (LM); T trachea; (e) and (f) Electron micrographs of rectal muscle fibres of *A. cyanea* in (e) longitudinal and (f) cross section. T transverse system; D diad of transverse and sarcoplasmic reticulum systems, M mitochondria.

The gill chamber is equipped with circular muscle fibres and 6 bundles of longitudinal muscle fibres (Fig. 1c, d). The simultaneous contraction of both sets reduces the diameter and length, i.e. the luminal volume, of the gill chamber. The fine structure of these cross-striated mucsle fibres is characterized by numerous mitochondria around the fibre periphery and between the myofibrils at the Z and I level, and by a well developed T-system forming diads with the longitudinally-orientated sarcoplasmic reticulum, for excitation-contraction coupling (Fig. 1e, f). Thus, the rectal muscle fibres are similar in fine structure to the direct flight muscles of dragonflies (SMITH 1966), but appear less highly organized, especially less densely supplied with interfibrillar mitochondria.

RESPIRATORY FUNCTION

TILLYARD (1916) ascribed the recognition of this function to POUPART (1702). Many publications of the 19th and 20th centuries are devoted to the histology, tracheal supply and respiratory physiology of the branchial chamber. The mechanism and patterns of rectal ventilation, partly with respect to various oxygen tensions, were studied by BABAK & FOUSTKA (1907), TONNER (1936), and more recently by HUGHES & MILL (1966).

Since the work of KROGH (1920, 1941) and KOCH (1936) it is generally accepted that both oxygen uptake through the tracheal gills and oxygen transport within the tracheae from the absorptive to the consumptive site occurs passively by simple diffusion. Therefore Fick's diffusion law is applicable to describe the respiratory uptake of oxygen through the tracheal gills:

$$\frac{dm}{dt} = - K F \frac{dc}{dx}$$

According to this equation the net flux of oxygen dm/dt depends on the diffusion coefficient K, the respiratory surface area F, and the concentration gradient dc/dx where dc is the concentration difference and dx the diffusion distance, i.e. the thickness of the "respiratory membrane". F and dx are the only morphological parameters which determine the functional efficiency and hence the structure of gills. The larger F and the smaller dx, the greater is the oxygen uptake. The realization of these morphological conditions of Fick's diffusion law is clearly recognizable in the construction of the rectal tracheal gills.

The total respiratory surface area of the branchial chamber is difficult to estimate accurately because it is correlated with several parameters, such as the number, shape, size and density of tracheation of the gills. Furthermore, during larval growth the total respiratory surface area is enlarged by successive increases in the number and size of the gills to meet the progressive oxygen demands of increasing body size. For example in *Aeshna cyanea* the gill number increases from 84 at 5 mm body length to 240 at 25 mm body length. Thereafter, the gill number stays constant, but the gills continue to grow.

The number and shape of the rectal gills vary also with the species (TILLYARD 1916; RICH 1918). Despite the great variation in shape two basic forms of the gills may be distinguished, lamellar (Fig. 2a) and tubular (Fig. 4b).

As seen in Figure 1c the lumen of the branchial chamber of *Libellula depressa* is densely packed with gill lamellae. These are arranged in 6 double rows. The gill size is not uniform, being largest in the midregion of the branchial chamber and decreasing in the posterior and anterior directions. Whole-mount preparations of isolated gill lamellae (Fig. 2a) show the invasion of tracheae at the base and the dense supply with more or less parallel tracheoles in the middle and distal parts (Fig. 2b). In the basal part the larger tracheae are suspended in the haemolymph space of the gill lamella. They later branch into tracheoles which run closely underneath the thin epithelium. Tracheoles also invade the epithelial cells and even the cell nuclei (Fig. 2c, d), and finally form blind ends at the margin of the gill lamella. The tracheation of the epithelium on both sides of the gill lamellae (Fig. 2d, e) implies a doubling of the respiratory surface area.

In *Aeshna* gills, which were studied with the electron microscope by GREVEN & RUDOLPH (1973) and by WICHARD & KOMNICK (1974b), the tracheoles are almost exclusively inserted into the epithelium (Figs 2g, 7), whereas in *Libellula* they lie partly within and partly underneath the epithelium (Figs2e, f). This minor difference in the tracheation of the 2 species presumably compensates for the different thickness of the epithelia, so that in both species the diffusion distance is approximately the same (Figs 2e-g).

Electron micrographs show that the tracheoles are surrounded by a thin cytoplasmic sheath of their tracheoblasts (Figs 2e-g), regardless of whether they are located underneath the epithelium (Fig. 2f) or within it (Fig. 2g). The invasion of tracheoles into the epithelium occurs through deep infoldings of the basal plasma membrane and partly also through the intercellular clefts. Thus their location is

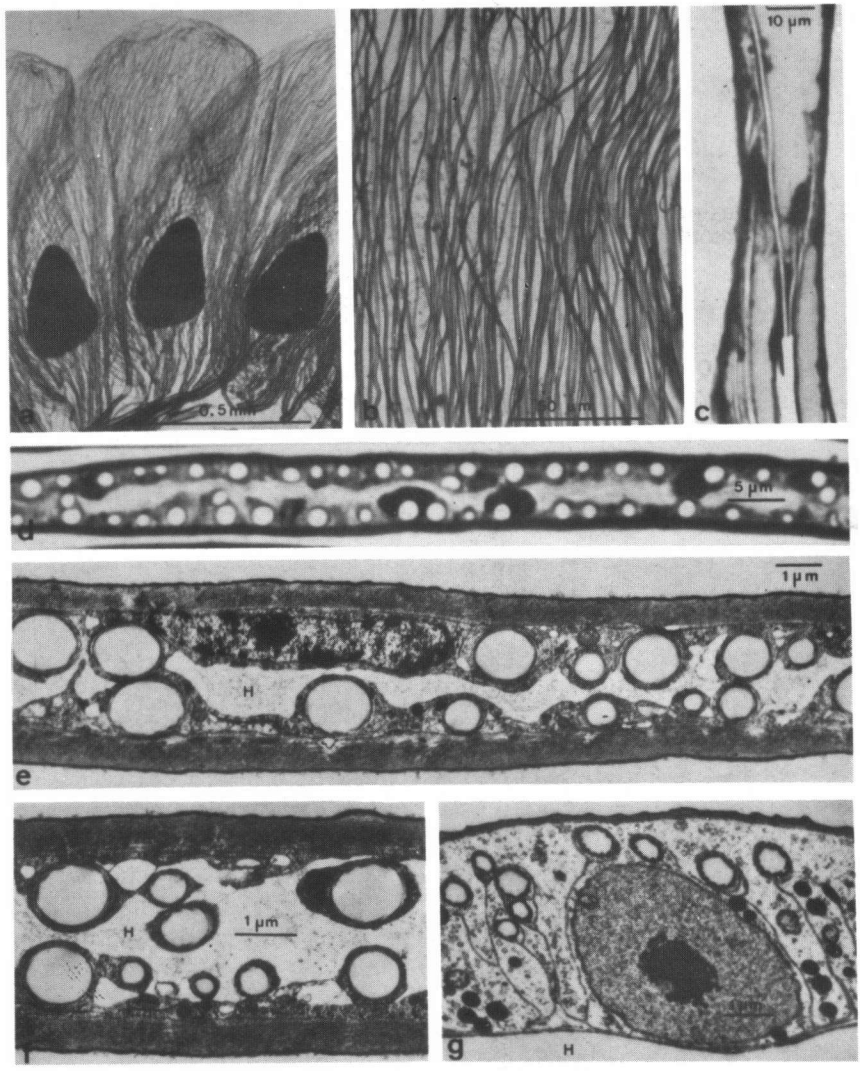

Fig. 2. Rectal gill lamellae of *Libellula depressa* (a-f) and *Aeshna cyanea* (g): (a) whole-mount preparation showing three isolated gill lamellae in side view with tracheae and chloride epithelia, the latter stained dark by the histochemical silver chloride reaction; (b) enlarged distal part of a gill lamella showing the dense and parallel arrangement of tracheoles in the respiratory epithelium; (c-d) sections through the respiratory epithelium showing tracheoles cut lengthwise (c) and across (d); (e-g) electron micrographs of cross sections through the respiratory epithelium. In (e) the tracheoles are partly with and partly underneath the epithelium, in (f) exclusively underneath the epithelium in the haemocoel (H), and in (g) exclusively within the epithelium.

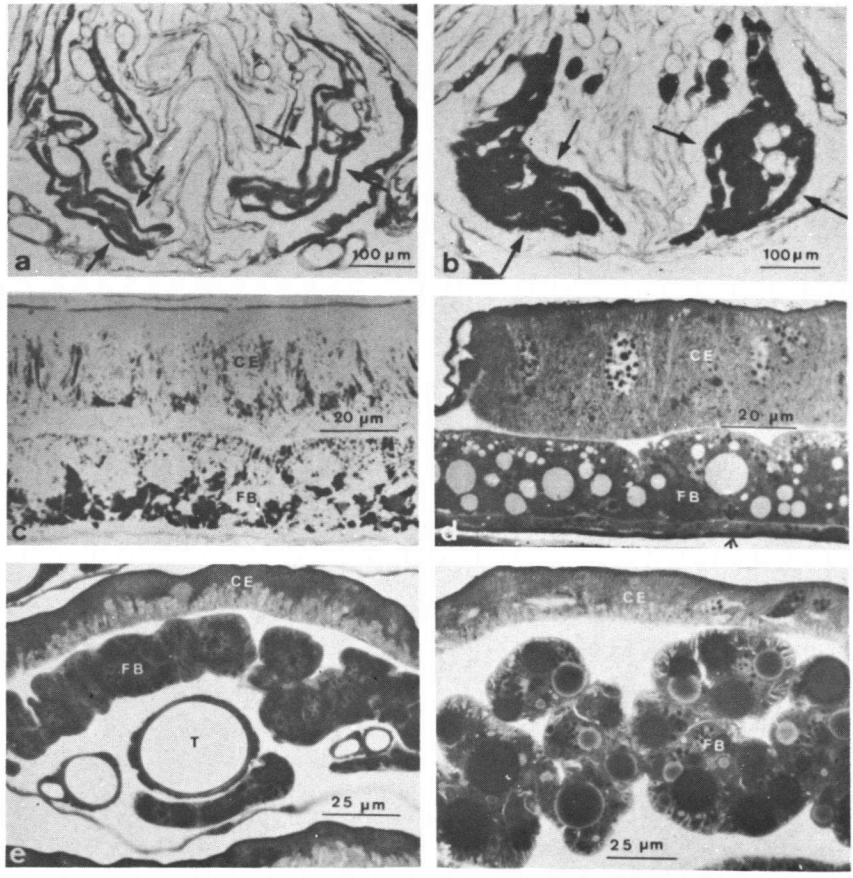

Fig. 3. Glycogen and lipid storage: (a, b) rectum of *Aeshna cyanea* fixed in Bouin's fluid (a) after 1 month of starvation and (b) after daily feeding with *Tubifex* for 1 month (paraffin sections; PAS staining for glycogen; the pairs of arrows point to the 2 opposing chloride epithelia with clusters of fat body cells in between);
(c, d) rectum of *Libellula depressa* fixed in glutaraldehyde/osmium after feeding with *Tubifex* for 1 month (arrowheads in (d)enclose the thin lining epithelium; 1 µm thick plastic sections); (c) PAS staining for glycogen; (d) Toluidin blue;
(e, f) rectum of *A. cyanea* fixed with glutaraldehyde/osmium, (e) after 1 month of starvation and (f) after 1 month of starvation followed by daily feeding with margarine for 2 weeks (toluidine blue staining, CE chloride epithelia; FB fat body cells; T trachea).

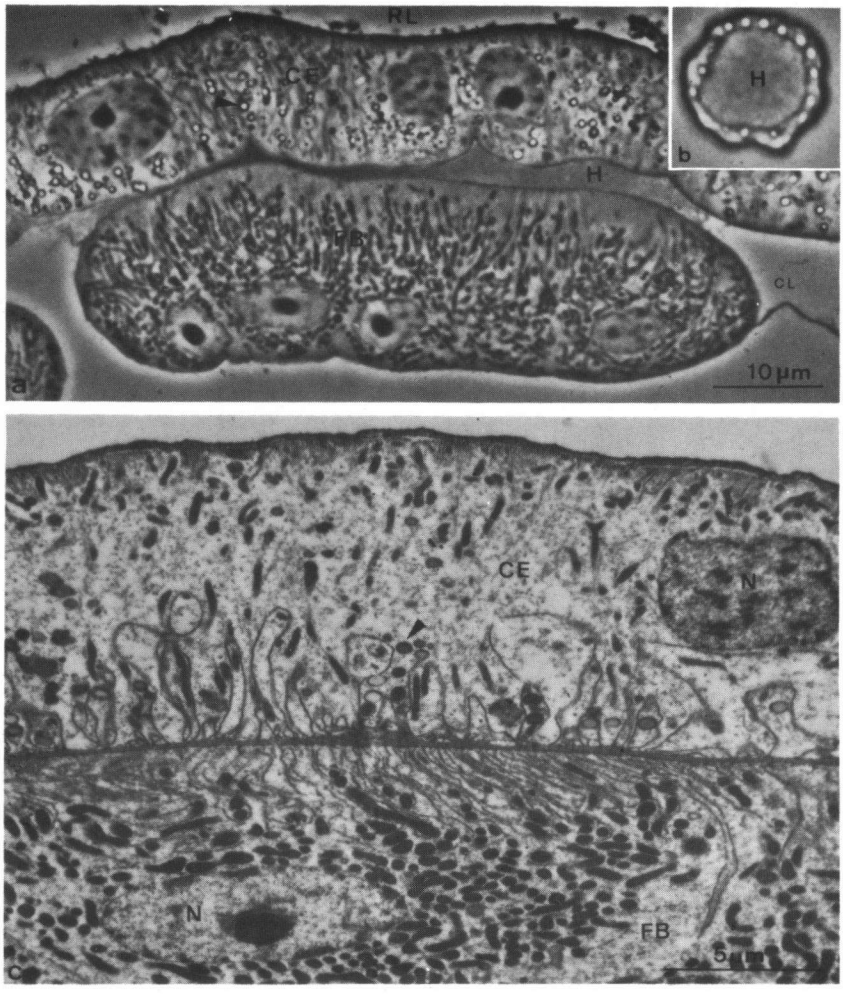

Fig. 4. *Progomphus obscurus*: (a) light- and (c) electron micrographs of cross sections through rectal chloride epithelia (CE) and fat body cells (FB) (arrowheads point to pigment granules occurring in the basal and midregion of the chloride epithelium); (b) light micrograph of a cross section through the distal end of a tracheal gill tubule. CL connective tissue lamella; H haemocoel; N nuclei; RL rectal lumen.

always extracellular, even in those cases where they appear to lie within the nucleus. This mode of tracheation leads to a reduction in the diffusion distance, bringing the oxygen receptors as close as possible to the external medium.The "respiratory membrane" consists of the delicate cuticle of the gill epithelium, 2 thin layers of cytoplasm of the epithelial cell and tracheoblast with the intercellular cleft in between, and the tracheolar cuticle. These amount to a total thickness of about 0.2 – 1 μm. The tracheation is so dense that the lateral distance between the tracheoles rarely exceeds twice their diameter. This profuse tracheation appears very effective in making full use of the diffusing oxygen (WICHARD & KOMNICK 1974a).

STORAGE FUNCTION

Clusters of fat-body cells are regularly present in the haemolymph space of the basal region of the tracheal gills. In *Aeshna* these cells typically occupy the space between the two contralateral chloride epithelia where they often surround the branches of the tracheae (Figs 3a, b, e, 7). They also accompany the tracheae into the main body cavity and are thus more or less continuous with the large clusters of fat-body cells associated with the tracheae around the branchial chamber. In *Libellula* gills the fat-body cells form an epithelium-like stratum underneath the chloride epithelium (Figs 1c, 3d). This stratum rests on a thin lining epithelium at the opposite side of the gill lamella (Fig. 3d). In *Progomphus* these fat-body cells are extremely rich in mitochondria and their plasma membrane is preferentially plicated at the side facing the chloride epithelium (Fig. 4a, c).

The function of these cells was already recognized by SADONES (1896) who described them as adipose cells. Later on, RICH (1918) also aware of their function used the term trophocytes, whereas TILLYARD (1916) was unable to detect any lipid inclusions. Therefore he denied that they were fat-body cells and proposed the neutral designation hypobranchial cells. More recently LEADER & GREEN (1978), apparently impressed by the rich supply with mitochondria and highly folded plasma membrane, concluded that these cells in *Uropetala* are modified fat-body cells. They also concluded from the failure of Amaranth infiltration that these cells were sealing an extracellular sinus underneath the chloride epithelium, and speculated that they might be involved in the ion-absorption process. This might hold true of *Uropetala*, but it certainly does not apply to *Aeshna*, *Libellula* and *Progomphus*. The occasional impression of an isolated

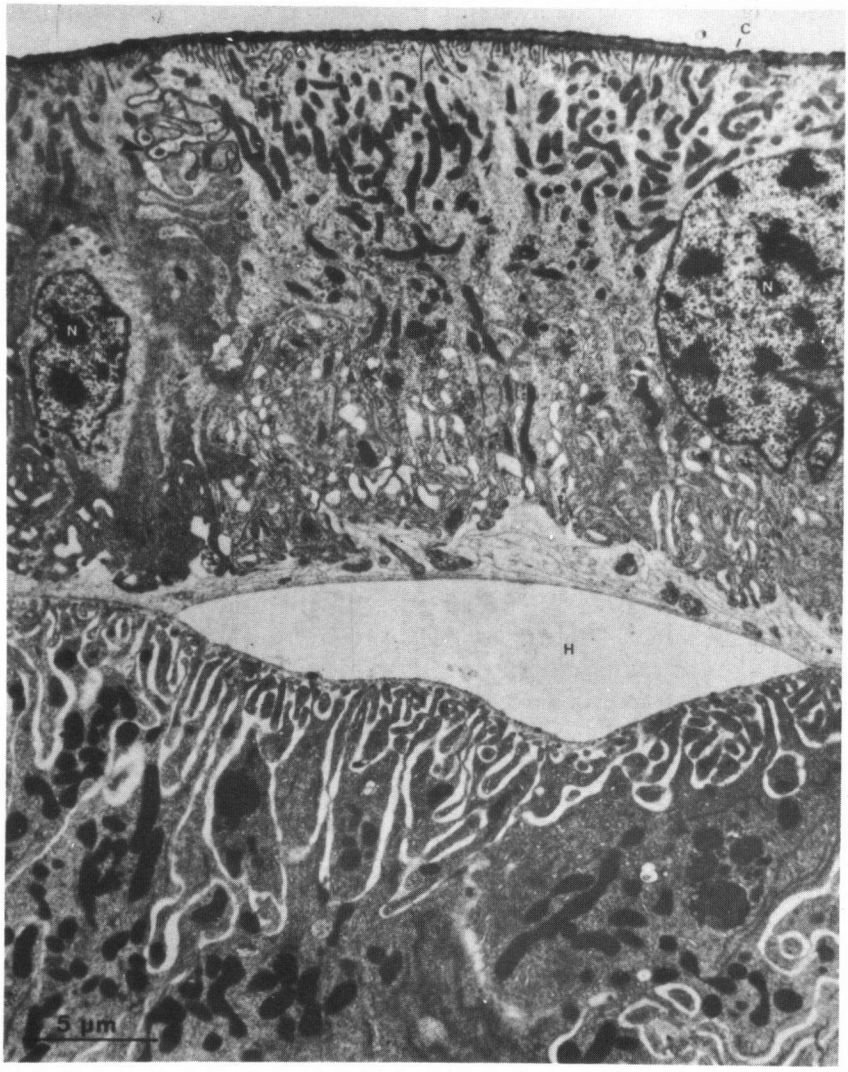

Fig. 5. Electron micrograph of rectal chloride epithelium (upper part) and fat-body cells (lower part) of *Aeshna cyanea*. C cuticle; H haemocoel; N nuclei. The arrowheads enclose the junctional complex in the apical region.

sinus between chloride epithelium and fat-body cells of *Aeshna* (Fig. 5) depends on the cutting direction. Such spaces are in open connection with the haemocoel elsewhere. In the case of *Libellula* the presence of the lining epithelium on the other side of the fat-body cells contradicts the two-step transport process assumed by LEADER & GREEN. The fine structure of the fat-body cells of *Progomphus* (Figs 4a, c) closely resembles the description given by LEADER & GREEN for these cells in *Uropetala*, but the spaces between chloride epithelium and fat-body cells are in open communication with the haemocoel. Therefore, any direct participation in the ion-absorption process can be excluded.

The prevailing structures of the fat-body cells in *Aeshna* are rough endoplasmic reticulum, lysosomes, mitochondria and foldings of the plasma membrane as a consequence of cellular interdigitation (Fig. 5). The author conforms to the interpretation of GREVEN & RUDOLPH (1973) that the structural features are indicative of a high metabolic activity of these cells.

Evidence for a storage function of these cells is presented in the results of starving and feeding experiments performed on *Aeshna* and *Libellula*. After starvation for 1 month the cells are almost completely deprived of glycogen and fat droplets (Figs 3a, e) whereas daily *ad libitum* feeding with *Tubifex* leads to a high content of glycogen and lipid droplets (Figs 3b, c, d). (No tests for protein storage were made). Regular feeding leads also to accumulation of glycogen in the cells of the chloride epithelia (Figs 3b, c). When 4 weeks of starvation which lead to lipid deprivation of the fat-body cells (Fig. 3e) are followed by 2 weeks of lipid feeding, the cells are refilled with small and large fat droplets (Fig. 3f). The largest droplets observed measured 25 μm in diameter. Lipid transport from the midgut to the rectal fat body cells is much faster than the time period of 2 weeks chosen in this experiment indicates. Three hours after the oral application of ^{14}C-oleic acid to starving *Aeshna* larvae a substantial fraction of the label is already released from the midgut epithelium into the haemolymph in the form of free fatty acid and diglyceride, taken up by the rectal fat-body cells, and incorporated into storage triglyceride (KOMNICK et al. 1982). These results justify the consideration that these rectal cells are rather normal fat-body cells which, according to WIGGLESWORTH (1965), do not only serve as lipid stores but are equally important as glycogen and protein stores. The presence and accumulation of these materials largely depends on the feeding conditions.

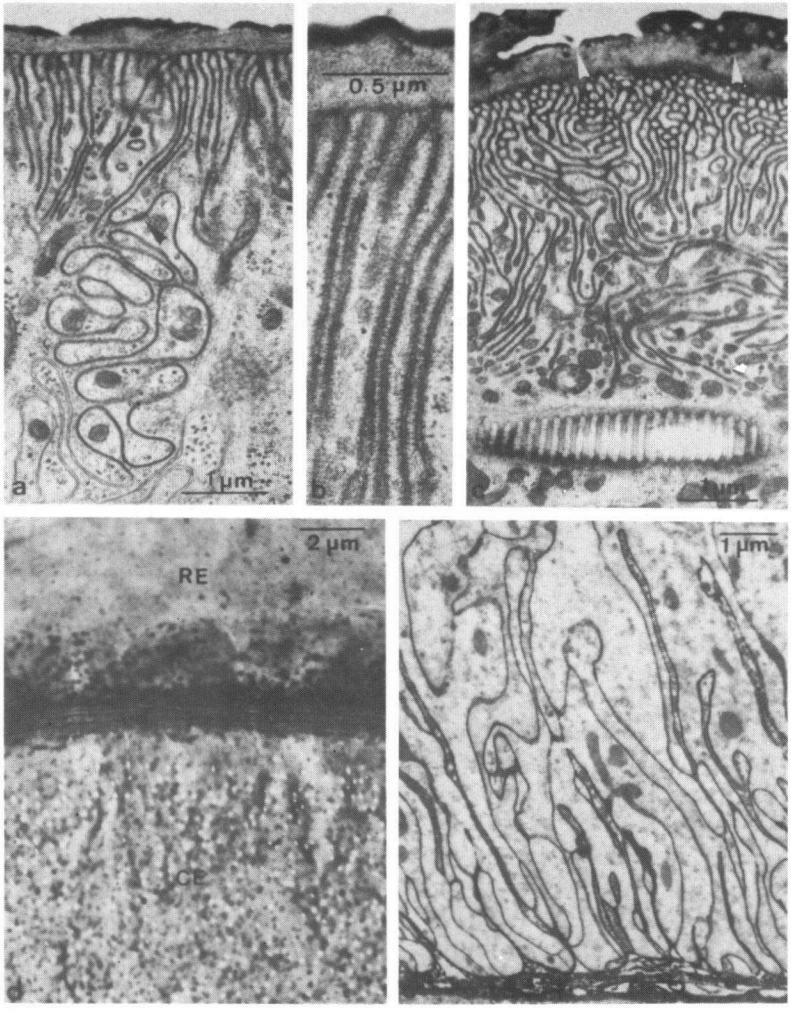

Fig. 6. Electron microscopic details of rectal chloride epithelium of *Aeshna cyanea:* (a) cross section of the apical region showing infoldings of the apical plasma membrane, zonula adhaerens (black arrowhead) and the highly folded zone of a pleated septate junction; (b) higher magnification of infolded apical plasma membrane showing membrane particles at the cytoplasmic sides; (c) oblique section of the apical region showing cuticular depressions cut in different directions (white arrowheads), the microvillous tips of the apical folds and the taenidium of a supply tracheole invaded into the apical zone of mitochondria;
(d) whole-mount preparation of the shed cuticle of a rectal gill leaflet showing the presence of cuticular depressions (white dots) over the chloride epithelium (CE) and their absence from the cuticle of the respiratory epithelium (RE); (e) section through the basal region of a chloride epithelium showing the basal labyrinth of intercellular clefts infiltrated with ruthenium red.

ION-ABSORPTIVE FUNCTION

KOCH (1934) attributed the function of ion absorption to the epithelial pads at the bases of the tracheal gills. These epithelial pads of the larval dragonfly rectum had already been described histologically in the last century (CHUN 1876; FAUSSEK 1887; SADONES 1896). More recent examinations with the electron microscope (GREVEN & RUDOLPH, 1973; WICHARD & KOMNICK, 1974c; LEADER & GREEN, 1978) revealed that these thickened areas of the gill lining epithelium can undoubtedly be classified as transporting epithelia (BERRIDGE & OSCHMAN 1972). In analogy to fine-structurally and functionally similar differentiations of other aquatic insects they were designated rectal chloride epithelia (WICHARD & KOMNICK 1974c; KOMNICK 1977a,b). Figure 7 shows diagrammatically a pair of chloride epithelia in contralateral position at the base of the gill lamella. Paired chloride epithelia are present in *Aeshna* larvae exceeding 2.5 cm body length, while the gill lamellae of smaller larvae are furnished with single chloride epithelia (KOMNICK 1977b, 1978). Single chloride epithelia are also present in the gills of *Libellula* up to the last instar.

The rectal chloride epithelia are characterized by two main features in their fine structure: the rich supply of mitochondria and the profusion of plasma membrane. The first meets the energy demand of active transport, the second provides a large membrane area for the integration of transport carriers. The mitochondria preponderate in the apical cell region but are also present in the zone of basal infoldings of the plasma membrane (Figs 4c, 5). Occasionally tracheoles are found inserted into the apical zone of mitochondria (Fig. 6c). However, oxygen supply may likewise be achieved by diffusion directly from the rectal lumen. This is presumably the reason for the mitochondrial accumulation in the apical instead of the basal region close to the energy-consuming pumping sites. The basal infoldings result from basolateral interdigitation of the cells and create an extensive basal labyrinth of intercellular clefts. These clefts open into the haemocoel and apparently function as transport routes, since they are readily infiltrated with ruthenium red from the blood side across the basal lamella (Fig. 6e). On the other hand, these intercellular pathways appear to be sealed towards the rectal lumen by extensively folded septate junctions of the pleated type (Figs 5, 6a). These are associated with communicative and adhesive junctions (KUKULIES 1982). The apical plasma membrane is similarly enlarged by numerous irregular plicae that terminate with microvillous tips

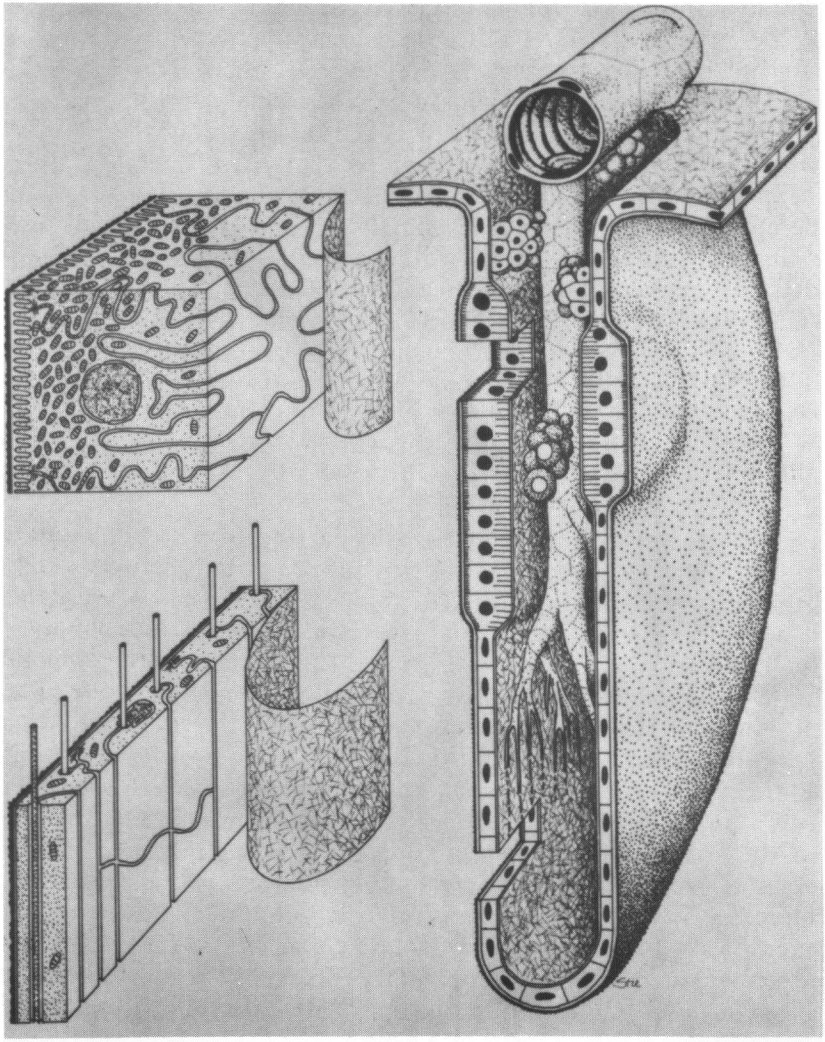

Fig. 7. Blockdiagram of a rectal gill lamella of larval *Aeshna cyanea* with enlarged portions of the (top) chloride and respiratory (bottom) epithelia (drawing by Dr. R. Stiemerling).

underneath the cuticle (Fig. 6c). The cytoplasmic face of the plicated apical plasma membrane is studded with small particles (Fig. 6b) which after the first report by GUPTA & BERRIDGE (1966) and NOIROT & NOIROT-TIMOTHÉE (1966) were noticed in various types of ion-transporting cells.

The cuticle of the chloride epithelia differs from the cuticle of the respiratory epithelia by the occurrence of circular depressions (Figs 6c, d). These depressions are mainly due to the local absence of epicuticular layers and appear to constitute preferred diffusion pathways across the cuticle (KUKULIES 1982).

Recent work of MOENS (1973, 1975a, b, c) and HERZOG (1979, 1982) has elucidated that sodium and chloride ions account for the major part of the haemolymph osmolarity in larval *Aeshna cyanea*, and play an essential role in the osmoregulation of these animals. An important link in the overall process of osmoregulation in freshwater insects is the active uptake of salt from the dilute external medium (STOBBERT & SHAW 1974; KOMNICK 1977b). The use of radioactive sodium and chloride in the ligation experiments (SCHMITZ & KOMNICK 1975, 1976) has revealed that these ions are absorbed against a steep concentration gradient from hypo-osmotic solutions into the haemolymph through the rectum (Figs 8a, b).

When *Aeshna* larvae are transferred from fresh water into dilute sea water that is hyperosmotic with respect to the nominal value of the haemolymph in freshwater larvae (330 mosmol) the osmolarity and sodium chloride concentrations of the haemolymph rise rapidly

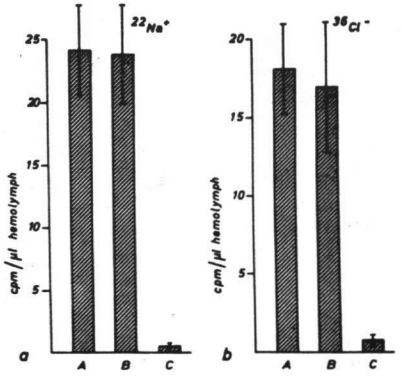

Fig. 8. Uptake of (a) sodium and (b) chloride into the haemolymph during 6 hours in external hypo-osmotic solution of 1mM radioactive NaCl. A, untreated larvae; B, larvae prevented from drinking; C, larvae prevented from rectal ventilation. (Drawn after SCHMITZ & KOMNICK, 1976).

above the values of the external medium, re-establishing a constant osmoregulatory situation at a slightly hyperosmotic level (MOENS 1975a; HERZOG 1979).

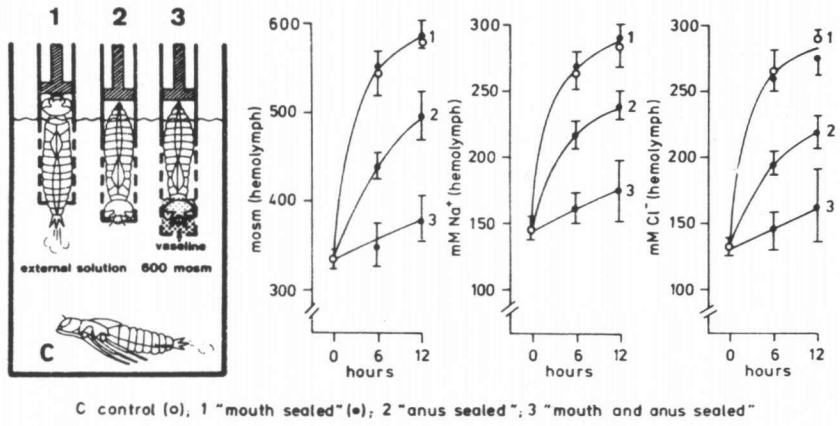

Fig. 9. Parallel rises of total osmolarity, sodium and chloride concentrations of the haemolymph after transfer from 5 mosmol to 600 mosmol external salinity (sea salt). The larvae were prevented from drinking, rectal ventilation or both by placing them into perforated plastic syringes as indicated in the left hand diagram. (Redrawn after HERZOG, 1979).

The rapid rise of the haemolymph concentrations in such transfer experiments also largely depends on the rectum. Although transfer into hyperosmotic sea salt solution induces a high drinking activity (HERZOG 1982), sealing of the mouth does not significantly change the increase in the haemolymph concentrations, whereas sealing of the anus greatly reduces the rise. There is only little elevation of the haemolymph concentrations when the larvae are prevented from both drinking and rectal ventilation (Fig. 9). It is tempting to assume that in the case of anal blockage some active ion absorption may still take place in the rectum or even in the ileum (MOENS 1980) from drinking water. However, as shown by HERZOG (1982) with the aid of substitution experiments, the initial quick rise of the haemolymph concentrations after transfer is mainly due to osmotic water extraction and passive ion influx. The oral and anal seals used largely reduce the internal and external body surfaces still in contact with the medium and therefore also reduce the surface area available for passive exchange. The results presented in Figures 8 and 9 clearly show that the rectum is not only the site of sodium-chloride absorption from the hypo-osmotic freshwater environment but also offers a large surface area for passive water and ion fluxes.

Further evidence that the rectal chloride epithelia represent the sites of ion uptake is provided by chloride histochemistry and chloride autoradiography. The epithelial area stains specifically with the histochemical silver chloride reaction (Fig. 2a), the chloride precipitates exclusively on the apical surface of the epithelium (Fig. 10a). When the larvae are exposed to hypo-osmotic radioactive chloride solution prior to the histochemical reaction, the presence of label in the histochemical chloride precipitates can be demonstrated autoradiographically (Fig. 10b). This suggests that chloride is adsorbed by the chloride epithelia from the external solution. The site of the histochemical chloride precipitation coincides with a PAS-staining layer which is absent from the respiratory epithelium (Figs 10c, d). This unidentified material with the PAS-staining glyco-component might be responsible for the initial step in the absorption process.

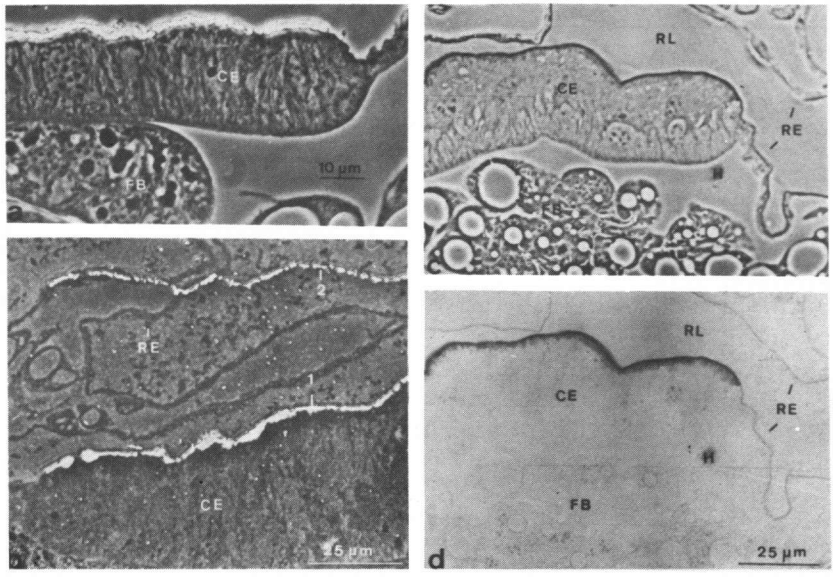

Fig. 10. Rectal chloride epithelia of *Aeshna cyanea*:
(a) histological demonstration of chloride specifically at the luminal surface of chloride epithelium by the silver chloride precipitation method;
(b) autoradiographic demonstration of $^{36}Cl^-$ adsorption by fixation with the histochemical chloride reagent after 1 day in hypo-osmotic 1 mM $Na^{36}Cl$ solution, 1 histochemical silver chloride precipitates and 2 corresponding autoradiographic silver grains separated by artifactual shift during photographic processing (from SCHMITZ & KOMNICK 1976);
(c-d) histochemical demonstration of PAS-positive amylase-resistent glyco-components in the chloride-adsorbing surface layer of chloride epithelium, (c) phase contrast, (d) bright field clearly showing heavy PAS-staining. CE chloride epithelium; FB fat-body cells; H haemocoel; RE respiratory epithelium; RL rectal lumen.

LEADER & GREEN (1978) concluded from their electrophysiological measurements on the rectum of *Uropetala* that both sodium and chloride ions are actively transported into the haemolymph. The two kinds of transported ions act as activators of two different ATPases present in the larval rectum of *Aeshna* (KOMNICK et al. 1980). In addition to the Na^+/K^+ -activated ATPase there is an anion-dependent ATPase, which is stimulated by chloride and bicarbonate ions. The two ATPases are clearly distinguishable by the effect of the sodium-transport inhibiting drug ouabain which completely inhibits the

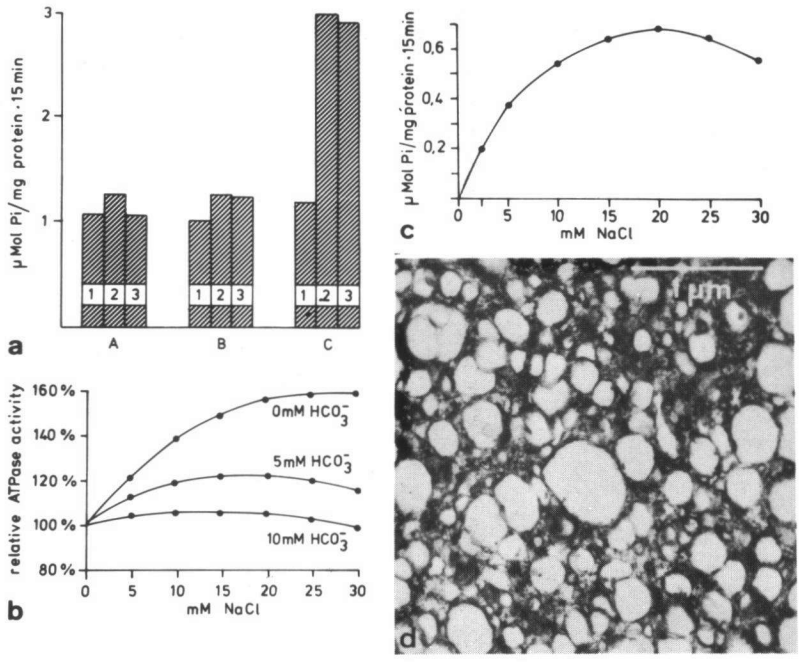

Fig. 11. (a) Biochemical demonstration of cation- and anion-activated ATPases in homogenates of the larval rectum of *Aeshna cyanea*. Columns A: Assay for Na/K-ATPase, (1) basic activity in the absence of K^+; (2) total activity in the presence of 5 mM K^+; (3) residual activity in the presence of 5 mM K^+ plus 0.1 mM ouabain. Columns B: Assay for Cl^--depending anion-ATPase, (1) basic activity in the absence of Cl^-; (2) total activity in the presence of 20 mM Cl^-; (3) activity in the presence of 20 mM Cl^- plus 0.1 mM ouabain. Columns C: Assay for HCO_3^- -depending anion-ATPase, (1) basic activity without HCO_3^-; (2) total activity in the presence of 30 mM HCO_3^-; (3) activity in the presence of 30 mM HCO_3^- plus 0.1 mM ouabain. (b) Chloride activation in the absence and presence of hydrogencarbonate activation of the anion-ATPase in rectum homogenates. (c) Chloride activation of anion-ATPase obtained in the presence of 5 mM bicarbonate, with microsomal fraction showing about 4-fold enzyme enrichment. (d) Electron micrograph of a section through the pelleted microsomal fraction used for the measurements of Fig. 11c. (From KOMNICK et al. 1980, partly redrawn).

Na^+/K^+ -ATPase without affecting the Cl^- and HCO_3^--dependent activities (Fig. 11a). On the other hand, the latter are completely inhibited by the active-chloride-transport inhibiting drug furosemide which at the same concentration has little effect on the Na^+/K^+ -ATPase (GASSNER & KOMNICK 1981a). Unlike the Na^+/K^+ -ATPase which requires the simultaneous presence of both activators sodium and potassium, chloride activation of the anion-ATPase is highest in the absence of the other activator bicarbonate and decreases with increasing bicarbonate concentrations (Fig. 11b; for further details see also BORNANCIN *et al.* 1980; GASSNER & KOMNICK 1981b). The chloride concentration for maximal ATPase activation in the presence of 5 mM bicarbonate is 20 mM (Fig. 11c). Although the Cl^- activated ATPase was found enriched in the microsomal fraction (Fig. 11d), the insufficient purity of this fraction does not allow us to determine whether or not this enzyme is located in the plasma membrane. Therefore, it remains to be clarified whether this chloride-activated ATPase is involved in active chloride transport by the rectum.

The localization of the Na^+/K^+ -ATPase, which is an emzyme of the plasma membrane and practically identical with the sodium pump (e.g. HILDEN & HOKIN 1976; SKRIVER et al. 1980a, b), was elucidated by the autoradiographic demonstration of ^{3}H-ouabain-binding sites (KOMNICK & ACHENBACH 1979). As visible in the autoradiograph (Fig. 12a) only the basal and midregion of the chloride epithelium is heavily labelled with silver grains, whereas the apical region, the fat-body cells, and the haemocoel are loosely scattered with few (background) grains. The heavily labelled region is identical with the region of extensive cellular interlocking (Fig. 12b). This result indicates that the sodium pump resides in the baso-lateral plasma membranes of the rectal chloride epithelia, as also holds true for other sodium-absorbing epithelia (DIBONA & MILLS 1979).

The synopsis of the available results justifies the following picture of the ion transport across the rectal chloride epithelia (Fig. 13). The operation of the sodium pump in the baso-lateral plasma membranes actively transports sodium out of the cells into the labyrinthine intercellular spaces against the high sodium concentration of the haemolymph and against the electrical charge which is positive on the haemolymph side (LEADER & GREEN 1978). The basal labyrinth directly communicates with the haemocoel via the basal lamina as seen from the infiltration with ruthenium red (Fig. 6e). The active sodium export from the cells reduces the cellular sodium concentration so that further sodium ions may passively enter the cells from

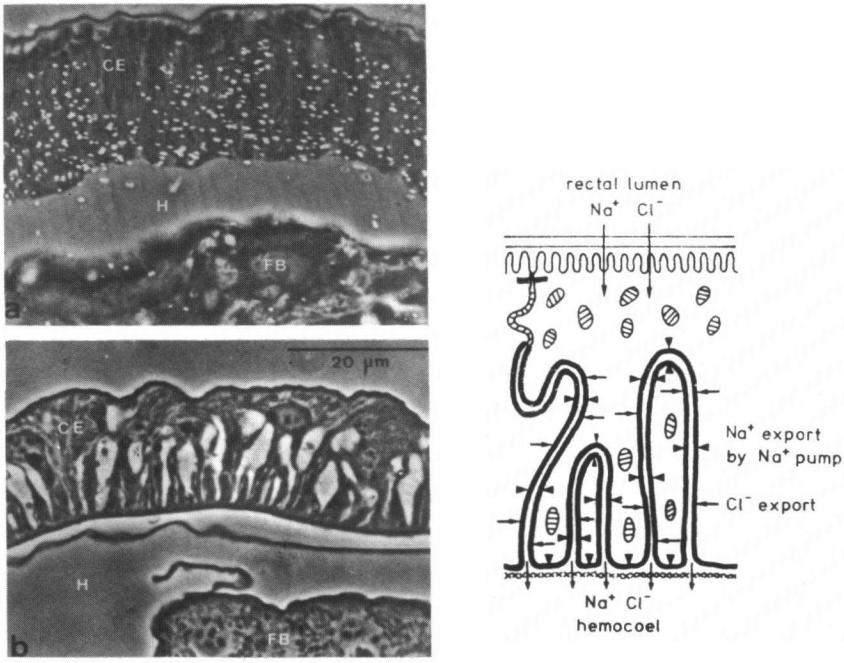

Fig. 12. (a) Autoradiographic demonstration of ouabain-binding sites (white dots) i.e. sodium pump sites (from KOMNICK & ACHENBACH 1979). (b) Cross section of rectal chloride epithelium after hyperosmotic fixation, showing dilations of basal labyrinth as a consequence of cellular shrinkage. The extension of the basal labyrinth, i.e. of the baso-lateral plasma membranes, coincides with the distribution of autoradiographic label in Fig. 12 (a).

Fig. 13. Diagram of transport routes and localization of Na^+ pump sites (thick lines) in the rectal chloride epithelia.

the rectal lumen. Passive sodium influx through the cuticle and apical cell membrane is presumably also favoured by electrical attraction of the internal negative charges which seem to be characteristic of animal cells. However, it remains to be clarified whether the potential of the apical plasma membrane is negative and large enough to account also for passive sodium influx from extremely low external concentrations. The transepithelial route of chloride ions is probably very closely associated with the route of sodium ions for reason of electroneutrality. The present knowledge of transepithelial chloride transport in the rectum of larval dragonflies ends with the electrophysiological evidence for a chloride pump (LEADER & GREEN 1978) and biochemical evidence for a chloride-activated anion-ATPase (KOMNICK *et al.* 1980; GASSNER & KOMNICK 1981a).

ACKNOWLEDGEMENTS

The author is grateful for financial support by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen and by the Deutsche Forschungsgemeinschaft, to Mrs. I. BAAS for technical assistance, to Dr. R. STIEMERLING for the drawings and to Dr. W.L. PETERS, Florida A & M University, for his support in collecting *Progomphus obscurus*.

REFERENCES

BABAK, E. & O. FOUSTKA 1907. Untersuchungen über den Auslösungsreiz der Atembewegungen bei Libellenlarven (und Arthropoden überhaupt). Arch. ges. Physiol. 119: 530-548.

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

BORNANCIN, M., G. DeRENZIS & R. NAON 1980. Cl^--HCO^- -ATPase in the gills of the rainbow trout: evidence for its microsomal localization. Am. J. Physiol. 238: 251-259.

DIBONA, D.R. & J.W. MILLS 1979. Distribution of Na^+- pump sites in transporting epithelia. Fed. Proc. 38: 134-143.

CHUN, C. 1876. Über den Bau, die Entwicklung und physiologische Bedeutung der Rectaldrüsen bei den Insekten. Abh. senckenb. naturforsch. Ges. 10: 27-56.

CUVIER, G. 1798. Mémoire sur la manière dont se fait la nutrition des insectes. Mém. Soc. Hist. nat., Paris VII, 49 (cited after Tillyard, 1916).

DUFOUR, L. 1852. Etudes anatomiques et physiologiques, et observations sur les larves des libellules. Annls Sci. nat. 3e serie, 17: 65-110.

FAUSSEK, K. 1887. Beiträge zur Histologie des Darmkanals der Insekten. Z. wiss. Zool. 45: 694-712.

GASSNER, D. & H. KOMNICK 1981a. The loop diuretic furosemide as non-competitive inhibitor of Cl^-/HCO_3^- -ATPases of vertebrate kidneys and insect rectum. Comp. Biochem. Physiol. Part C (in press).

GASSNER, D. & H. KOMNICK 1981b. Nachweis und Eigenschaften einer Cl^-/HCO_3^- ATPases in der Vogelsalzdrüse. Eur. J. Cell Biol. 25: 108-119.

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

GUPTA, B.L. & M.J. BERRIDGE 1966. A coat of repeating subunits on the cytoplasmic surface of the plasma membrane in the rectal papillae of the blowfly, Calliphora erythrocephala (Meig.) studied in situ by electron microscopy. J. Cell Biol. 29: 376-382.

HAGEN, H. 1880. Beitrag zur Kenntnis des Tracheensystems der Libellen-Larven. Zool. Anz. 3: 157-161.

HERZOG, H.U. 1979. Einfluss verschiedener Aussenkonzentrationen auf die Hämolymphe und rektalen Chloridepithelien von Libellenlarven. Dipl. Arbeit Math. Nat. Fakultät Univ. Bonn.

HERZOG, H.U. 1982. Effects of various external media on the haemolymph of larval Aeshna cyanea. Adv. Odonatol. 1: 63-68

HILDEN, S. & L. HOKIN 1976. Coupled Na^+-K^+ transport in vesicles containing a purified (NaK)-ATPase and only phosphatidyl choline. Biochem.Biophys.Res.Com.69 :521-527.

HUGHES, G.M. & P.J. MILL 1966. Patterns of ventilation in dragonfly larvae. J. exp. Biol. 44: 317-333.

IMMS, A.D. 1973. A general textbook of entomology. 9th ed. Chapman & Hall, London.

KOCH, H. 1934. Essai d'interprétation de la soi-disant "réduction vitale" de sels d'argent par certains organes d'arthropodes. Annls Soc. r. Sci. med. nat. Brux. Ser. B 54: 346-361.

KOCH, H.J.-A. 1936. Recherches sur la physiologie du système trachéen clos. Mém Acad. r. Belg. Ser. 2,16: 1-98.

KOMNICK, H., 1977a. Chloridzellen und Chloridepithelien als osmoregulatorische Anpassungen bei Wasserinsekten. Verh. dt. zool. Ges.Erlangen 111-112.

KOMNICK, H. 1977b. 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. & U. ACHENBACH 1979. Comparative biochemical histochemical and autoradiographic studies of Na^+/K^+ -ATPase in the rectum of dragonfly larvae (Odonata : Aeshnidae). Eur. J. Cell Biol. 20: 92-100.

KOMNICK, H., J. BONGERS & W. FISCHER 1982. Lipid absorption in the midgut of larval Aeshna cyanea. Adv. Odonatol. 1: 93-104.

KOMNICK, H., M.H.J. SCHMITZ & H. HINSSEN 1980. Biochemischer Nachweis von HCO_3^--und Cl^--abhängigen ATPase- Aktivitäten im Rectum von anisopteren Libellenlarven und Hemmung der rectalen Chloridaufnahme durch Thiocyanat. Eur. J. Cell Biol. 20: 217-227.

KROGH, A. 1920. Studien über Tracheenrespiration. II. Über Gasdiffusion in den Tracheen. Arch. ges. Physiol. 179: 95-112.

KROGH, A. 1941. A comparative physiology of respiratory mechanisms. University Press, Pennsylvania.

KUKULIES, J. 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. Adv. Odonatol. 1: 105-115.

LEADER, J.P. & L.B. GREEN 1978. Active transport of chloride and sodium by the rectal chamber of the larvae of the dragonfly Uropetala carovei. J. Insect Physiol. 24: 685--692.

MOENS, J. 1973. Study of the water balance in larvae of Aeshna cyanea (Müller) by means of measurement of changes in total body weight, with special reference to the method. (Anisoptera : Aeshnidae). Odonatologica 2: 91-98.

MOENS, J. 1975a. Ionic regulation of the haemolymph in the larvae of the dragonfly Aeshna cyanea (Müller) (Odonata : Anisoptera). Archs int. Physiol. Biochim. 83: 443-451.

MOENS, J. 1975b. Osmoregulation and the regulation of free amino acid concentration in the haemolymph of the larvae of Aeshna cyanea (Müller) (Anisoptera : Aeshnidae). Odonatologica 4: 169-176.

MOENS, J. 1975c. Changes of haemolymph volume in larvae of Anax imperator Leach and Aeshna cyanea (Müller) during starvation in different experimental media (Anisoptera : Aeshnidae). Odonatologica 4: 219-224.

MOENS, J. 1980. Microscopic and ultrastructural study of different epithelial elements in the ileum of larvae of anisopteran dragonflies. Odonatologica 9: 309-315.

NOIROT, M.Ch. & C. NOIROT-TIMOTHÉE 1966. Revêtement de la membrane cytoplasmique et absorption des ions dans les papilles rectales d'un Termite (Insecta: Isoptera). C.r. Acad. Sci. Paris 263: 1099-1102.

OGUMA, K. 1913. On the rectal tracheal gills of a libellulid nymph and their fate during the course of metamorphosis. Berl. ent. Z. 58: 211-225.

OUSTALET, E. 1869. Note sur la respiration chez les nymphes des libellules. Annls Sci.nat. 5e serie 11: 370-386.

POUPART, F. 1702. Letter concerning the insect called Libellula. Phil. Trans. 22: 673 (cited after Tillyard, 1916).

REAUMUR, R.A.F. de, 1742. Mémoire pour servir à l'histoire des Insectes 6: 389 (cited after Tillyard, 1916).

RICH, S.G. 1918. The gill chamber of dragonfly nymphs. J. Morph. 31: 317-349.

SADONES, J. 1896. L'appareil digestif et la respiration larvaire des Odonates. Cellule 11: 273-327.

SCHMITZ, M. & H. KOMNICK 1975. Osmoregulatorische Aufnahme von $^{22}Na^+$ und $^{36}Cl^-$ durch das Rectum von Libellenlarven. Verh. dt. zool. Ges. Karlsruhe 128.

SCHMITZ, M. & H. KOMNICK 1976. Rectale Chloridepithelien und osmoregulatorische Salzaufnahme durch den Enddarm von zygopteren und anisopteren Libellenlarven. J. Insect Physiol. 23: 875-883.

SKRIVER, E., A.B. MAUNSBACH, B.M. ANNER & P.L. JØRGENSEN 1980a. Electron microscopy of phospholipid vesicles reconstituted with purified renal Na, K-ATPase. Cell Biol. Intern. Rep. 4: 585-591.

SKRIVER, E., A.B. MAUNSBACH & P.L. JØRGENSEN 1980b. Ultrastructure of Na, K-transport vesicles reconstituted with purified renal Na, K - ATPase. J. Cell Biol. 86: 746-754.

SMITH, D.S. 1966. The organization of flight muscle fibers in the Odonata. J. Cell Biol. 28: 109-126.

STOBBART, R.H. & J. SHAW 1974. Salt and water balance; excretion. In: The physiology of insecta. 2nd (M. Rockstein ed. V 361-446. Academic Press, New York and London.

SUCKOW, F.W.L. 1828. Respiration der Insekten, insbesondere über die Darmrespiration der Aeschna grandis. Heusingers Z. organ. Physik 2: 24-49.

SWAMMERDAM, J. 1680. Bibliae naturae (cited after Dufour, 1852).

TILLYARD, R.J. 1916. A study of the rectal breathing apparatus in the larvae of anisopterid dragonflies. J. Linn. Soc. Zool. 33:127-196.

TONNER, F. 1936. Mechanik und Koordination der Atem-Schwimmbewegungen bei Libellenlarven. Z. wiss. Zool. 147: 433-454.

WICHARD, W. & H. KOMNICK 1974a. Structure and function of the respiratory epithelium in the tracheal gills of stonefly larvae. J. Insect Physiol. 20: 2397-2406.

WICHARD, W. & H. KOMNICK 1974b. Zur Feinstruktur der rektalen Tracheenkiemen von anisopteren Libellenlarven. I. Das respiratorische Epithel. Odonatologica 3: 121-127.

WICHARD, W. & H. KOMNICK 1974c. Zur Feinstruktur der rektalen Tracheenkiemen von anisopteren Libellenlarven. II. Das rektale Chloridepithel. Odonatologica 3: 129-135.

WIGGLESWORTH, V.B. 1965. The principles of insect physiology. 6th ed. Methuen, London.