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Uphill Transport of Water by Electroosmosis

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Summary. Lepismatidae are able to gain water from subsaturated atmosphere above a relative humidity of 45%, surmounting a water potential difference of at least 1.1×10^8 Pa (1,100 bar). This extraordinary task is performed by the monolayered epithelium of the posterior rectum. The particle coat of the folded apical membrane of this epithelium suggests the presence of the electrogenic, lumen-directed cation transport, which is commonly found in insects. Assuming this kind of transport and considering the anatomy of the organ, a working hypothesis for this hyposmotic water transport has been developed: The electrogenic cation transport maintains the circulation of the transported ion species across the apical membrane; the voltage-driven inward current transfers water by electroosmosis against its chemical potential from the extracellular space into the cytoplasm. Voltage and current measurements and synchronous measurements of water flow across the epithelium of the posterior rectum of *Lepisma saccharina* strongly corroborate this hypothesis. The transepithelial voltage is up to 200 mV (lumen positive); the short-circuit current averages 200 μA per cm^2 of the epithelium. Both depend acutely on oxidative metabolism as does spontaneous water uptake. Exogenous transepithelial current (I) induces, independently of anoxia, a proportional change in volume flow (J_v). The induced flow has the direction of the cation flow. Its mean coupling ratio (J_v/I) is 1.5×10^{-9} m^3/A · sec corresponding to 7 to 8 H_2O per positive unit charge. Critical evaluation of experimental data reveals that water uptake by electroosmosis may quantitatively account for *in vivo* performance without requiring any unusual assumption.

Key Words water transport · electroosmosis · insect rectum · potassium transport · membrane channels

Introduction

Numerous terrestrial arthropods have developed active mechanisms for conservation of body water besides their passive protection against water loss by an integument of low water permeability. They are able to excrete dry feces or to absorb water vapor from subsaturated atmosphere by hyposmotic water transfer against large osmotic gradients. Reviews on this latter ability have been published by Edney (1977), Noble-Nesbitt (1977), Machin (1979), Machin et al. (1982) and Rudolph and Knülle (1978, 1982).

A species-specific "critical equilibrium humidity" (CEH) or "critical equilibrium activity" (CEA) denotes the value of water activity in the air at which gain by absorption compensates for water loss by transpiration. In this respect the Lepismatidae *Thermobia domestica* (CEA = 0.45; Beament et al., 1964) and *Ctenolepisma terebrans* (CEA = 0.475; Edney, 1971) are most outstanding paradigms. Machin (1979) calculated the "pump threshold" (a term which accounts for water loss at the CEA) for *Thermobia* to correspond to 40% relative humidity. This means that the transport mechanism has to be able to transfer water from the air into the hemolymph against a pressure difference of 1.25×10^8 Pa (= 1,250 bar).

Noble-Nesbitt demonstrated (1969, 1970, 1975) that in *Thermobia* this enormous task is performed by a special organ, the "posterior rectum" or "anal sac." Its structure has been described by Noirot and Noirot-Timothée (1971).

Standing osmotic gradients have been proposed by Diamond (1981) to produce the *hypo*smotic water transport of insect recta like those of Lepismatidae. Such gradients are known to induce *iso*motic water transport in vertebrate epithelia (Diamond & Bossert, 1967; and others), and are the preferred concept for water transport against moderate activity differences (e.g., Wall, 1977; Gupta et al., 1980). The structural organization of the posterior rectum of Lepismatidae, however, does not supply the features required for a hyposmotic water transport across this huge pressure difference by a standing gradient but displays other elaborated components (*see* "Morphological Basis" and "Theoretical Basis"). Noble-Nesbitt (1977) and Phillips (1977) discussed the problem in detail and pointed to the need of alternative mechanisms.

The anal sac of Lepismatidae is an especially

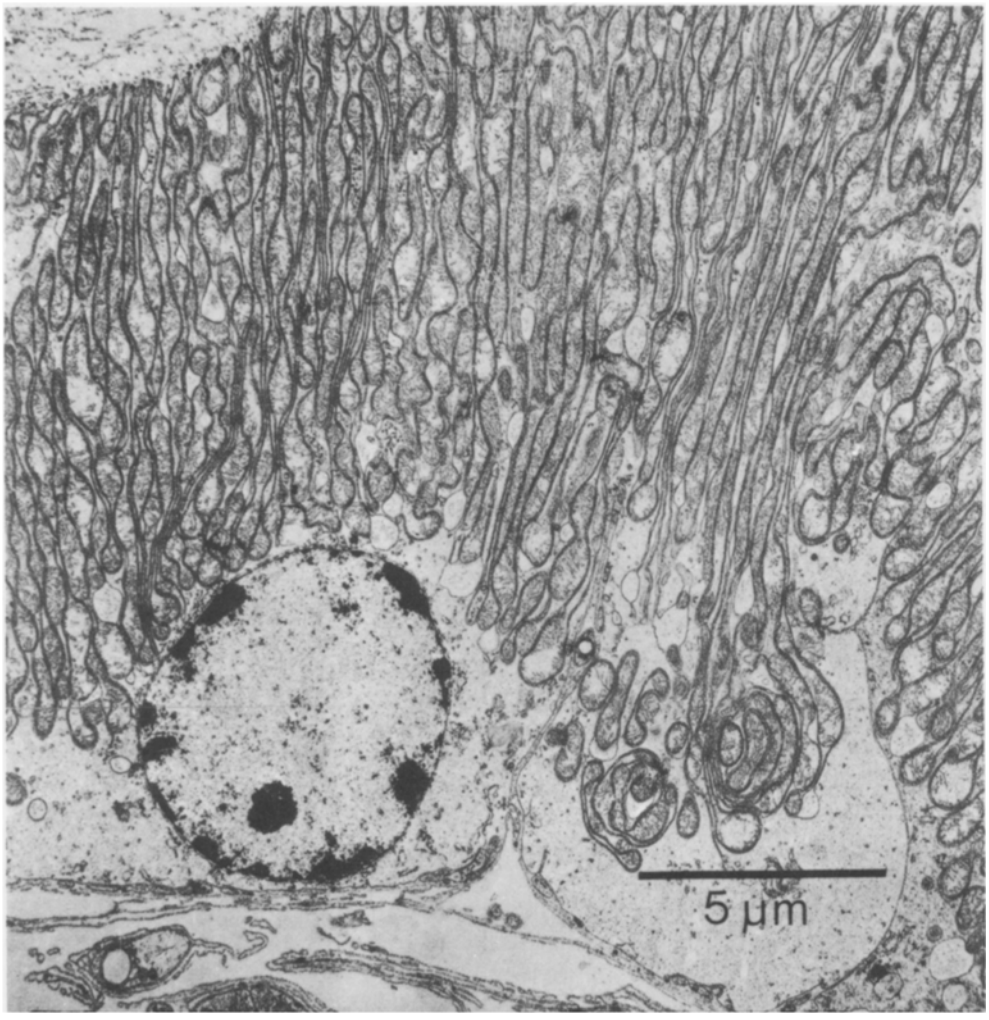

Fig. 1. Cross section through the epithelium of the anal sac. Subcuticular space at the top, hemolymph space at the bottom. (Electron micrograph by G. Neuhaus)

promising object for the analysis of mechanisms underlying uphill water transport since its unique structural simplicity (*see* "Morphological Basis") may correspond to a functional specialization for water uptake only, contrasting to the structurally complex, multifunctional recta of most other insects. The mechanism operating against an extreme gradient might also account for numerous cases where the pressure difference surmounted is less spectacular.

Preliminary reports have been given (Plagemann et al., 1978; Küppers & Thurm, 1980).

Morphological Basis

Our physiological approach has been induced by the ultrastructural findings of Noirot and Noirot-Timothée (1971) on the anal sac of *Thermobia*. To clarify some physiologically relevant questions and to get quantitative morphological data on our object, a systematic morphological study was performed on the anal sac of *Lepisma* parallel to the physiological experiments (Neuhaus et al., 1978; Thurm & Neuhaus, *in preparation*). The following findings are important for understanding the physiological results.

The terminal region of the gut—the posterior rectum—is constituted by a folded, monolayered epithelium (*compare* Fig. 3). During the intermoult phase a large subcuticular space extends between the detached cuticle and the epithelium. The subcuticular space is anteriorily and posteriorily closed by tight attachment of the cuticle to specialized epithelial cells. Similar attachments of epidermal sensilla of insects proved to be tight for the migration of lanthanum ions (Keil & Thurm, 1979; Thurm & Küppers, 1980). The narrow lumen is usually filled

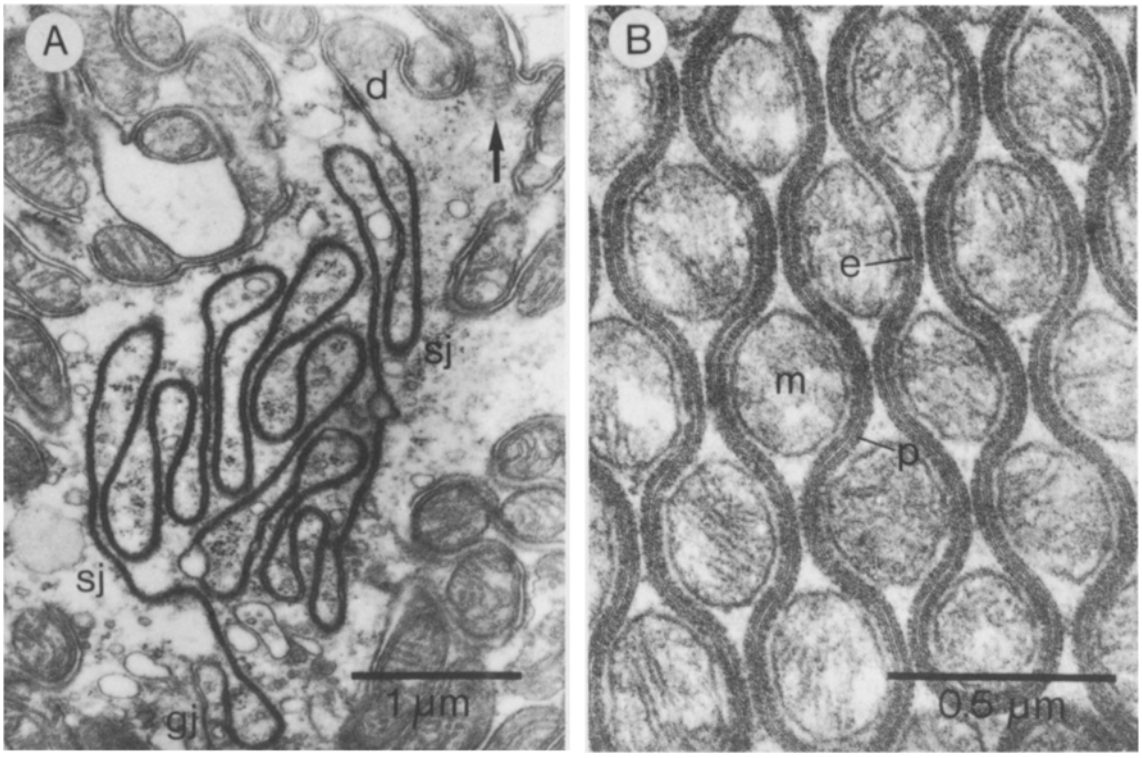

Fig. 2. (*A*) Section (perpendicular to the epithelial plane) through two adjacent cells. Arrow points towards the lumen. *d*: desmosome; *sj*: septate junction; *gj*: gap junction. (*B*) Cross section through the folds of the apical membrane (parallel to the epithelial plane). *m*: mitochondria; *p*: particle coat; *e*: extracellular space. (Electron micrographs by G. Neuhaus)

with air and is tightly closed towards the gut by a sphincter. The epithelium consists of only a single type of cells (Fig. 1). Due to regular deep infoldings, the area of the apical membrane facing the subcuticular space is enlarged by a factor of 180 compared to the smooth basal membrane. Within the resulting lamellae mitochondria are arranged at an extreme density. The intercellular clefts appear to be "carefully" sealed by very tortuous septate junctions (Fig. 2*A*). Extended lateral spaces or basal infoldings are poorly developed and never associated with mitochondria.

An electron-dense, polyanionic material (binding ruthenium-red) covers the luminal side of the epithelium and fills the narrow extracellular spaces between the lamellae. In the remaining space between epithelium and cuticle an electron-translucent fluid is enclosed (*see below*).

The apical cell membrane bears a dense coat of particles, about 12-nm thick, at its cytoplasmic side (Fig. 2*B*). This special membrane configuration has been found in various organs of insects, e.g., recta, Malpighian tubules, salivary glands, midgut (goblet-cells), and epithelial sensilla (tormogen cell) (*see* Harvey et al., 1983; for references). Many of these organs are known to transport potassium into their lumen. For lepidopteran goblet-cells (Harvey & Nedergaard, 1964; Wood et al., 1969; Blankemeyer & Harvey, 1978) and for cockroach tormogen cells (Thurm, 1974; Thurm & Küppers, 1980) it is known that it is just this type of coated membrane which bears a ouabain-insensitive cation pump; this pump transfers potassium from the cytoplasm to the extracellular space in a purely electrogenic way.

Theoretical Basis

Since the epithelium which has to provide the metabolic energy for the uphill transfer of water does not immediately face the air, the first step in the process of water vapor uptake by Lepismatidae should be the condensation of water vapor by some hygroscopic material within the subcuticular space mediating water flow from the air to the epithelium.

Some preliminary results on the nature of the subcuticular material of *Thermobia* have been obtained in cooperation with M. Volbers and are partly reported in Küppers and Thurm (1980): it turns out that the hygroscopic property of this material is very similar to that of glycerol, though the main component is not identical with it. This mate-

rial does not need to be exuded as a hyperosmotic fluid since water is actively resorbed from it.

For the active step, water cannot be assumed to be driven by osmosis through the apical membrane of the epithelial cells since metabolism at a cytoplasmic water activity below 0.5, as required in this case, would be far beyond any paradigm.

A paracellular route of osmotic water flow is very unlikely for essentially two reasons: (i) the elaborated septate junctions between the epithelial cells suggest a tight sealing of the intercellular clefts (*cf.* Keil & Thurm, 1979); (ii) the lateral cell membranes are smooth, the intercellular clefts short, and no mitochondria, which might energize the maintenance of high osmotic concentrations within the lateral spaces or the recycling of solutes, are present.

The morphology of the anal sac reveals, however, that mechanisms appropriate to energize the water transport are located at the apical membrane. From these structures and the previous considerations it is to be expected that across the apical membrane a transport mechanism is working which raises the water potential immediately across this membrane.

Since solutes can neither leave nor enter the subcuticular space via the cuticle which faces the air, and since the subcuticular space is anteriorily and posteriorily closed, any solute transport at the epithelium, under steady-state conditions, when all solutes reached their equilibrium distribution, cannot induce a net flux but only a circulation of the transported substance (*cf.* Küppers & Thurm, 1981).

Therefore a lumen-directed electrogenic potassium transport which is suggested by the membrane structure (*see above*) would induce the circulation of potassium ions. This current circuit would be confined preferentially to the apical membrane to the degree to which the posterior and anterior sealings of the subcuticular space and the septate junctions between the epithelial cells are of high resistance.

The uphill water transport observed might be linked by electroosmosis to the inferred downhill inward current.[1] As the charge carrier cycles in the current circuit, the result would be the net flow of pure water.

According to the usual equations of irreversible thermodynamics volume flow J_v and current I are given as

$$J_v = L_p \cdot P + L_{pe} \cdot E \tag{1}$$

and

$$I = L_{pe} \cdot P + g \cdot E \tag{2}$$

where L_p is the hydraulic permeability, L_{pe} is the electroosmotic coefficient, g is the conductivity, E is the driving voltage, and P is the osmotic pressure difference calculated as $P = (RT/\bar{V}_w) \cdot \ln (a_{w1}/a_{w2})$ with $\bar{V}_w$ = molar volume of water and a_{w1}, a_{w2} = the water activities on both sides. From Eqs. (1) and (2) follows

$$P_{I=0} = -E \cdot (g/L_{pe}) \text{ and } (I/J_v)_{P=0} = g/L_{pe}$$

so that

$$P_{I=0} = -E \cdot (I/J_v)_{P=0}. \tag{3}$$

If an electroosmotic mechanism operates at optimum efficiency then $P_{I=0} = P_{J_v=0}$, so that the maximum pressure is determined by the driving voltage multiplied by the charge concentration of the fluid transported at zero pressure. (For more general and detailed theoretical treatment of electroosmosis *see* e.g. Hill, 1975; Rosenberg & Finkelstein, 1978.)

Materials and Methods

To obtain reference data of *in vivo* performance of *Lepisma saccharina* we measured its resorption of water from the vapor phase. (For water vapor condensation in the anal sac *see* Küppers and Thurm, 1980.) In the main we focussed on the transport process of the epithelium which follows water condensation. In most of these experiments we supplied the water to be transported in the fluid phase, i.e., in the same phase which provides the water for the epithelium *in vivo*. In searching for a source of electrical energy possibly driving electroosmosis, we measured transepithelial voltage (TEV), short-circuit current (SCC), and their dependence on oxidative metabolism. To look for current-water coupling channels we checked the effect of an exogenous transepithelial current on water flow.

Lepisma saccharina weighing about 10 mg on average were kept for use in a dark box for some days at relative humidity of >60%, supplied with dry fish food and sugar.

The absorption of water vapor from the atmosphere was measured similarly as done by Noble-Nesbitt (1970) for *Thermobia:* after a period of desiccation over $CaCl_2$ (32% RH) starving animals were exposed to higher humidity over defined $CaCl_2$ solutions while their weight was controlled at regular intervals by means of a balance with a resolution of 0.1 mg.

As we have not been successful in maintaining ion and water transport *in vitro* (already the first steps of dissection abolished ion transport activity), a setup for *in situ* experiments was

[1] Recently, J.E. Phillips brought to our knowledge that in 1970 he discussed this principle for the insect rectum but refused it since he did not get evidence for it in locusts (Phillips, 1970).

designed which allowed the measurements and observation of the following parameters and relations: a) transepithelial voltage (TEV) and short-circuit current (SCC) at the posterior rectum, b) dependence of voltage and current on metabolic activity, c) electrical resistance of the organ, d) water uptake by the posterior rectum and its dependence on metabolism, e) relation between water flow and transepithelial current supplied by an exogenous source. A scheme of the arrangement is shown in Fig. 3.

Prior to the experiments the animals were dehydrated for some days (as in the weighing experiments) to ensure standardized conditions of water uptake. The animals, ventral side up, were fixed under a dissection-microscope in a small Perspex® chamber with tooth cement polymerizing quickly at room temperature (Scutan® from Espe-Chemie, Seefeld Obb., FRG). The oxidative metabolism could be reversibly blocked exchanging the air within the chamber for CO_2 or N_2.

Transepithelial voltage, short-circuit current, and resistance were measured via a pair of double-barrelled electrolyte-filled micropipettes and Ag/AgCl electrodes which also allowed the application of exogenous current. The pipettes inserted into the posterior rectum were fire-polished at a tip diameter of 50 μm. Stable electrical contact was achieved by the injection of a small amount of electrolyte (0.5 mol/liter KCl, if not otherwise stated) through the electrode tip into the lumen. (A use of electrolyte-free solutions would not be *a priori* more "physiological" for the investigation of mechanisms of water vapor uptake, since those solutions in contrast to water vapor would wash out possibly required ions from the system.) The similarly constructed pair of reference electrodes filled with the same electrolyte as the rectal electrodes was placed in the haemolymph space by penetrating the body wall of the abdomen.

The main elements of the electronic measuring device were: a high impedance amplifier which provided an AC-offset current for resistance control, a voltage-clamp circuit, and a potentiometer recorder.

To observe water uptake, a glass capillary about 100 mm in length and 100 to 150 μm in diameter, with a clean wetable inner surface, was connected to the voltage-recording barrel of the intrarectal electrode pair. Attention had to be paid to the sealing of the open ends of both barrels and especially to the sealing between the anus of the animal and the electrode tip. Silicon-rubber gave the best results. (The tracheal system remained open as pathway for O_2 supply to the tissue.) The capillary was connected with a vial of larger diameter by a thin polyethylene-tube to avoid capillary attraction and to adjust the hydrostatic pressure to zero. The movement of an air bubble within the capillary indicated flow of solution. The hydraulic resistance of this arrangement was low enough to be neglected. (In a capillary of 160 μm internal diameter, for instance, a pressure of 1 Pa caused a flow of 80 nl/hr.) In order to record volume flow a pointer was repeatedly adjusted to one meniscus of the bubble by means of a micro-screw coupled to a potentiometer. The voltage differences could be read as nanoliters from a chart-recorder after appropriate calibration.

The resolution of this method was < 0.1 nl. In any single experiment, however, the calculation of absolute volume flow may include an error of up to $\pm$ 10% resulting from the determination of the diameter of the capillary. In addition, temperature changes of $\pm$ 1 K would have caused an erroneous reading of $\pm$ 2 nl. Since no special temperature-regulating device had been installed, an additional error of flow around $\pm$ 2 nl/hr cannot be excluded.

Due to surface charges, the electrode tip may contribute electroosmotic artifacts depending on the glass quality and the tip diameter. Control experiments ascertained that such artifacts were not detectable in the current range of 0 $\pm$ 40 μA.

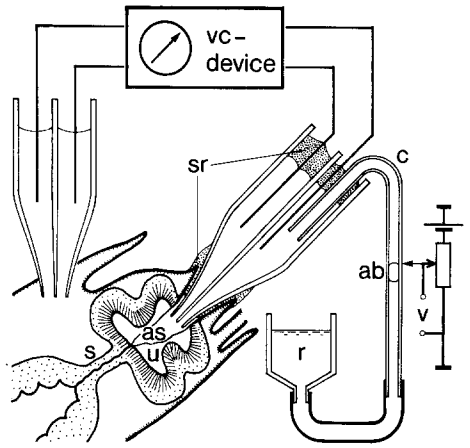

Fig. 3. Experimental setup for electroosmotic measurements. *vc-device*: voltage-clamp device; *sr*: silicon rubber; *as*: lumen of the anal sac; *u*: subcuticular space; *s*: sphincter; *c*: calibrated cannula; *ab*: air bubble; *r*: reservoir; *v*: voltage related to volume flow. Further explanation in the text. Outer diameter of the anal sac: 300 to 400 μm. Setup not in scale

Results

A. Weighing Experiments

Individually weighed animals were dehydrated without access to food for 3 days at 32% RH. As shown in Fig. 4 the loss of weight by starvation and transpiration during this period is about 23% of the initial weight. If these animals are returned to air with about 84% RH they gain 95% of their initial weight within 2 days. If the anus is sealed with Scutan or Silicon-rubber after dehydration, water uptake is prevented and weight loss by starvation and (reduced) transpiration further continues. From the difference between both curves and the average weight of these animals ($\approx$ 9 mg) the mean rate of water uptake at 84% RH is calculated to be 50 to 60 nl/hr ($\approx 1.5 \times 10^{-14}$ m^3/sec) during the first day.

B. Voltage, Short-Circuit Current, and Resistance Across the Wall of the Posterior Rectum

When the tip of a capillary electrode was brought into contact with the luminal surface of the posterior rectum by injection of some electrolyte, a voltage in the range of 100 to 200 mV was usually measured, the lumen side being positive with respect to

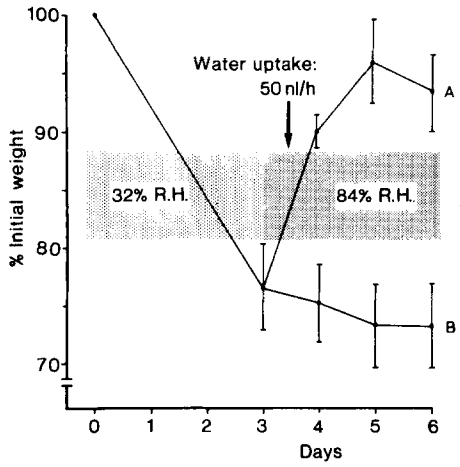

Fig. 4. Weight loss and gain of *Lepisma* over dry $CaCl_2$ and saturated KCl, respectively. *A:* anus open; *B:* anus sealed. Bars indicate standard errors

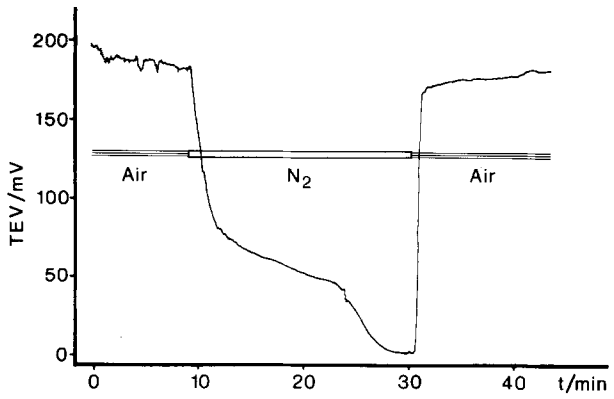

Fig. 5. Changes of transepithelial voltage when the air within the experimental chamber is replaced by N_2 and vice versa (open anus)

hemolymph. Further advance of the electrode by a few hundred microns generally caused a stepwise and reversible drop of the voltage to values below zero. From these observations we conclude that the voltage primarily measured is generated across the wall of the posterior rectum. Since nearly the total voltage depends on oxidative metabolism (*see below*), we see its origin in the epithelial component of this wall. The voltage drop produced by further advancing the electrode-tip and its reversibility suggest that the sphincter which separates anal sac and anterior rectum was forced to open. This would induce low resistance contact to the anterior rectum and higher resistance towards the posterior rectum. Vice versa we conclude that the sphincter usually remained closed during the experiments when this mechanical effect was avoided (*see below*). Occasionally occurring transient voltage drops seem to reflect spontaneous openings of the sphincter.

Some animals in these experiments failed to exhibit a significant voltage. Morphological investigation of such an animal revealed it to be in the moulting phase during which the complex of apical membrane folds and mitochondria is lacking (Thurm & Neuhaus, *in preparation*). For those specimen exhibiting a significant transepithelial voltage (TEV) the value measured at the beginning of an experiment was on average 155 ± 25 mV (M ± SD, $n = 31$), and maximum values were around 200 mV. The TEV is rather stable during free access of air to the lumen of the anal sac. On sealing the anus, as necessary for measurements of water uptake, this voltage usually declined to values somewhat below 100 mV, where it stayed for several hours.

If the voltage across the posterior rectum wall is clamped to zero a short-circuit current (SCC) is obtained. Since it was not possible in these experiments to match the ion concentrations across the preparation (*see below*) short-circuit condition is realized only with respect to the voltage. The initial mean of this current was 4 ± 1 μA (maximum 9 μA) measured at the completely electrolyte-filled anal sac. (The filling was microscopically controlled before sealing the anus.)

Transepithelial voltage and short-circuit current acutely depend on oxidative metabolism as revealed by substituting N_2 or CO_2 for air within the experimental chamber. A rather typical time course of the TEV during anoxia is represented by Fig. 5. The time for a complete decay of the voltage may vary from 3 to 25 min. This time appears to shorten with repeated anoxia. At re-entry of air into the chamber the voltage rises rapidly to almost its initial value. This increase is especially fast when the lumen of the posterior rectum is open to the surrounding atmosphere: 7 mV/sec in Fig. 5. The time course of the SCC resembles that of the related TEV.

The *I/V* plot shows no significant deviation from linearity within the range studied (0 ± 700 mV).

The resistance of epithelium and cuticle of completely electrolyte-filled anal sacs was on average 50 ± 5 KΩ (determined from the slope of *I/V* plot). During anoxia this resistance increased by 45 ± 10% (Fig. 6). If the resistance is tentatively ascribed to the epithelial area of the posterior rectum a specific resistance of ≦ 0.75 kΩ cm^2 is calculated for the cell layer in its metabolically active state (geometrical data from Neuhaus and Thurm, *in preparation*, taking into account the sizes of the specimen studied).

C. Observation of Water Uptake

The volume flow induced by the posterior rectum can be measured only as far as the sphincter remains closed during experiments, thus preventing bulk-flow of solution into or out of the larger vol-

ume of the anterior rectum and gut. Direct evidence on this topic, in addition to that given above in *B*, was obtained by the following procedure: In two of the experiments for measuring water flow (including a period of anoxia) the electrolyte within the luminal electrodes was intensely stained by procian blue, a dye that does not pass through cell membranes. Subsequent dissection of the animals showed a high concentration of the dye within the lumen of the anal sac, whereas only traces of dye have been found in the part anterior to the sphincter, indicating that no significant exchange of fluid had occurred.

A spontaneous and continuous net volume uptake via the posterior rectum occurred under oxygen supply. It could be observed for more than twelve hours under the experimental conditions. During this time the uptake rate decayed only slowly but showed major fluctuations which may have reflected movements of the animal. The mean uptake rate found at the beginning of the experiments was 55 ± 12 nl/hr (M ± SE, 5 animals) when the fluid supplied to the anal sac contained 0.5 mol/liter KCl. The volume uptake ceases reversibly when oxidative metabolism is inhibited by CO_2 (or N_2) (Fig. 6). During this inhibition a slight outflow of a few nl/hr has often been found. The difference between the time course of the TEV and that of fluid uptake will be discussed below. The total fluid volume which the animals took up in the course of an experiment was up to 500 nl, that is 500 times the volume of the lumen of the anal sac plus its subcuticular space; it is 15 times the epithelial volume of this organ; and it corresponds to about 5% of the animal's weight.

According to these findings we consider the recorded volume flow to represent the active uptake of fluid into the hemolymph space via the epithelium of the anal sac.

The osmotic pressure difference against which water was taken up in these experiments using a supply of 0.5 mol/liter KCl solution (experimental standard condition) was at least 7×10^5 Pa (≃ 0.3 Osm/kg) since the osmotic pressure of the hemolymph of *Lepisma* is 1.4×10^6 Pa (Kästner, 1973). We must take into account, however, that the difference is much greater: If—as *in vivo*—pure water is taken up by the epithelium it must be concluded that the concentration of the electrolyte within the lumen will increase. From uptake rate, diffusion constants, geometry of the capillary tip, and volume of the lumen of the posterior rectum (< 1 nl) it follows that a 0.5-mol/liter KCl solution should have been concentrated to saturation within the first few minutes of the experiment. That means that spontaneous water flow measured by using this or similarly concentrated KCl solution occurred against a constant osmotic pressure difference of

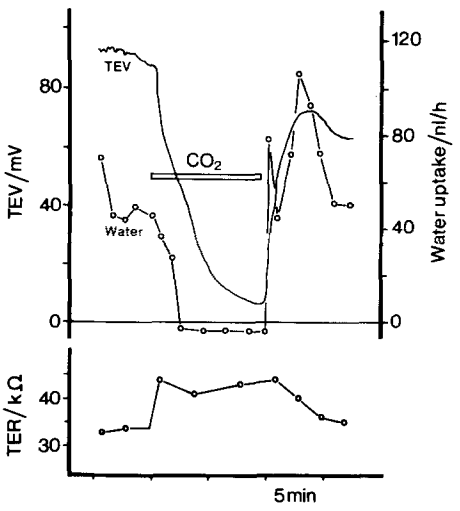

Fig. 6. Dependence of transepithelial voltage (TEV), transepithelial resistance (TER), and water uptake on oxidative metabolism (anus sealed)

2.4×10^7 Pa corresponding to that produced by 84% RH.

We also used some other electrolytes for these experiments, for example a 2 + 2 mol/liter KCl + NaCl solution (water activity of the saturated solution is 0.75). With this solution the uptake rate was 24 ± 7 nl/hr (M ± SE, 6 animals). Using 9 mol/liter Na-propionate + 2 mol/liter KCl with an osmotic pressure corresponding to 60 to 65% RH (unsaturated), water uptake ceased (−5 ± 1 nl/hr, M ± SE, 4 animals). These solutions reduced the SCC by 35 and 65%, respectively, compared to that measured with KCl. EM inspection of a Na-propionate-KCl-treated anal sac showed considerable swelling of the epithelial cells and strong reduction of the extracellular space between the lamellae (Thurm & Neuhaus, *in preparation*).

D. Current-Induced Volume Flow

Amount and direction of spontaneous fluid flow can be changed by an exogenous transepithelial current (TEC). Inward current increases and outward current decreases the spontaneous volume uptake. In the experiment of Fig. 7 spontaneous uptake at zero TEC was doubled by an inward current of about 10μA and was completely abolished by an outward current of the same size. The slope of the relation between volume flow and TEC in this experiment was 6 ± 0.6 nl/μA hr (M ± SE). The average of these coupling ratios determined from 4 specimens was 5.5 ± 1 nl/μA hr (M ± SE) (= 1.5 ± 0.3) × 10^{-9} m^3/A · sec). This ratio corresponds to 7 to 8 H_2O/monovalent cation.

While the spontaneous water uptake ceases during anoxia, an exogenous current restores water

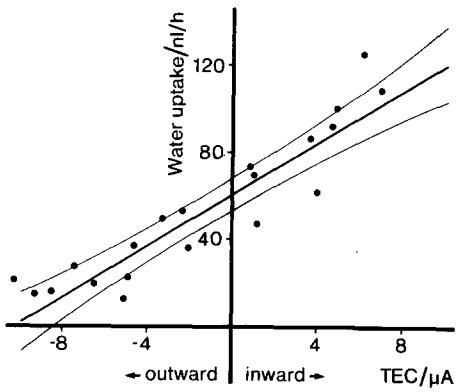

Fig. 7. Influence of exogenous current (TEC) on spontaneous volume flow. The 95%-confidence interval for the regression line is given. Data of one animal

flow. In the specimen of Fig. 8 the mean coupling ratio was increased by 20% during such periods of blocked oxidative metabolism; but it was only with $P_o \leqq 0.2$ different from that in air (6.6 ± 0.7 nl/μA hr in air and 7.9 ± 1.5 nl/μA hr in CO_2).

When the anal sac was filled with 9 mol/liter Na-propionate + 2 mol/liter KCl (which has, as mentioned, serious effects on the structure, the SCC and the spontaneous uptake), the coupling ratio was 4.6 ± 1 nl/μA hr ($n = 3$).

Discussion

Transport Phenomena

Volume Fluxes

Our weighing experiments on dehydrated individuals reveal the ability of *Lepisma saccharina* to absorb water vapor from the atmosphere as is known also from its relatives *Thermobia domestica* (Beament et al., 1964) and *Ctenolepisma terebrans* (Edney, 1971). We did not aim at an exact determination of the CEH; the rapid increase in weight at 84% RH (Fig. 4) suggests that the CEH should be appreciably below this value (for calculations following below we assume that it will correspond to that of *Thermobia,* i.e., 45% RH). The inhibition of rehydration by blocking the anus may not strictly prove the involvement of the posterior rectum (*cf.* Okasha, 1971), however, the anal route for the uptake of water from the vapor phase is well established for *Thermobia* by more sophisticated experiments of Noble-Nesbitt (1975).

We obtained direct evidence on the anal route of water uptake by our observation that local supply of fluid water to the anus yields uptake rates similar to those measured for uptake from the vapor phase at the corresponding vapor pressure. Since a water-soluble dye brought into the lumen entered neither the epithelium nor the anterior rectum during metabolism-dependent water uptake, one has to conclude that it is the last partition of the gut, the posterior rectum, which transfers water against an osmotic gradient by a metabolism-dependent water pump.

Within the posterior rectum we found exogenous transepidermal current to induce a volume flow. Such current-volume coupling may be produced either by true electroosmosis or by "pseudo-electroosmosis" caused by transport-number differences and unstirred layers (*see* Barry & Hope, 1969*a*). To consider the latter first: A current, passing e.g. a cation-selective membrane from "out" to "in," will elevate the solute concentration in the unstirred layer at the inside and decrease it on the outside. This again may cause osmotic water flow, depending on the size of the concentration change and the osmotic permeability of the membrane. An estimation of the volume flow possibly produced by "pseudo-electroosmosis"[2] reveals that it can contribute only a small portion to the current-induced flow found.

Therefore we conclude that the volume flow induced by transepithelial current is essentially caused by true electroosmosis. The identity of the directions of volume and current fluxes indicates the net volume flux to be coupled to cations.

The ratio of apparent water-to-charge coupling, measured as net J_v/I in the active state of energy metabolism is (1.5 ± 0.3) × 10^{-9} $m^3/A \cdot sec$. It re-

[2] Adopting 100 μm as an upper estimation for the thickness of the unstirred layer at the "in"-side, limited by the streaming hemolymph, we arrive (according to Barry and Diamond, 1984) at a possible elevation of the concentration within the cells by only about 15 mmol/liter. (Values used: maximal current density in the free cytoplasm 3 $mA \cdot cm^{-2}$, a reduced diffusion constant for KCl 10^{-5} $cm^2 \cdot sec^{-1}$, and difference of the transport numbers = 0.5.)

For the outside, i.e., the lumen of the anal sac together with the electrode, we do not feel able to assign any definite value to the thickness of the unstirred layer. Therefore, we may assume the worst case: solute concentration on the outside approaching zero. Exogenous inward current thus would have generated an inward osmotic gradient of max. 0.6 osmol (water to hemolymph). If this gradient should produce the current-induced change in volume flow found to be maximally ~ 100 nl/hr, this would require an osmotic permeability of 160 nl/hr · osmol. However, the dependence of endogenous water uptake on the water activity within the lumen (*see* "Results") indicates that the osmotic permeability of the organ must be below 3 nl/hr · osmol.

For endogenous water uptake any significance of transport number effects is unlikely since there is no net current flow.

sembles the movement of about 7 to 8 H_2O/positive unit charge and a charge concentration of 6.5×10^8 A · sec/m^3 within the permeating volume (ion concentration 6.5 mol/liter; for corrections and possible dependence on water activity in the lumen *see below*).

Rosenberg and Finkelstein (1978) determined for gramicidin A channels (generally considered to be suitable models for the principal properties of ion-selective channels in cell membranes) that about 6.5 H_2O pass the channel together with one cation (*see also* Schagina et al., 1978). According to Levitt (1984) the coupling ratio in gramicidin channels varies from 7 H_2O/K^+ at low K^+ activities to 5 H_2O/K^+ at 2.5 molal activity. For K^+ channels of mammalian sarcoplasmic recticulum, Miller (1982) calculated from streaming potentials that only 2 to 3 H_2O pass the channel with one K^+ ion. Barry and Hope (1969*b*) found a coupling ratio of ~ 31 H_2O/ion at algal cell membranes.

Endogenous Voltage and Current Source

The voltage generated by the epithelium of the posterior rectum reached peak values of 200 mV (lumen positive) when the lumen was exposed to air. An average short-circuit current of 4 ± 1 μA was measured under these conditions. The oxygen dependence of this voltage and current and their steep increase at the end of a period of anoxia (7 mV/sec in Fig. 5) points to an electrogenic voltage rather than to a diffusion potential depending on concentration differences. A similar behavior of the TEV has been observed for the electrogenic potassium transport at insect sensilla (Thurm & Wessel, 1979; Thurm & Küppers, 1980; Wieczorek, 1982) and at the midgut of Lepidopteran larvae (Blankemeyer & Harvey, 1978) for which electrogenic K^+-outward transport has been established. As the voltage approaches zero during prolonged anoxia, passive voltage sources (equilibrium potentials) on both sides of the epithelium obviously compensate each other.

Due to filling and sealing the anus for observation of water flow, voltage and short-circuit current declined to values around 90 mV and 1.6 μA, respectively, when the electrode contained 0.5 mol/liter KCl (standard conditions). From this steady-state current and from morphological data (Neuhaus et al., 1978) the density of the SCC can be calculated to be about 200 μA/cm^2 epithelial area. Transport currents across cell membranes usually do not exceed densities around 20 μA/cm^2 (*see* Thurm, 1974). These data strongly suggest that the ion transport is located at the highly enlarged area

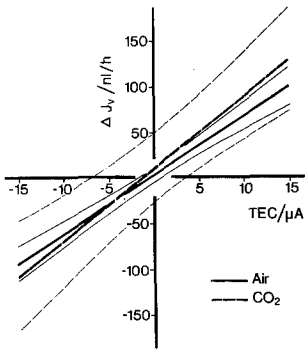

Fig. 8. Changes of volume flow ΔJ_v by an exogenous current in air and in CO_2 (spontaneous flow subtracted). The 95%-confidence intervals for the regression lines are given. All data from the same animal

of the apical membrane, as opposed to the basolateral membranes. The transepithelial SCC density, however, appears to be low compared to that found at the midgut of *Cecropia* (250 μA/cm^2 on average, Harvey and Nedergaard, 1964), where the potassium-transporting goblet cells constitute only the minor part of the epithelium, or compared to the current density at the sensory epithelium of the haltere of *Musca* (600 μA/cm^2, Thurm, 1974). In view of the elaborated apical membrane-mitochondria complex one might expect that the transport capacity of the posterior rectum should rather exceed that of these examples. The low current density found may indicate appreciable series resistances to the apical membrane, represented by the cuticle and/or the basal membrane.

During anoxia the resistance of the preparation increased by 45% on average. For *Cecropia* midgut Blankemeyer and Harvey (1978) reported a significant increase of the resistance of the transporting membrane during metabolic inhibition which they interpret as an increase of the resistance of the electrogenic transport itself. Such increase of transport resistance has been inferred also for cockroach sensilla from impedance measurements in the active and blocked state of the transport (Küppers, 1984; *see also* Thurm, 1974; Küppers & Thurm, 1979). Further below we argue that in the anal sac of *Lepisma* the resistance of active transport may be high compared to the total resistance of the apical membrane. If this holds true one has to assume that beside the transport resistance some resistance in series (basal membrane) and/or in parallel (ion channels of the apical membrane) is increased by anoxia in order to account for the amount of transepithelial resistance change.

Summarizing these aspects we infer that the luminal membrane of the posterior rectum bears an electrogenic ion pump. This inference is corrobo-

rated and specified by the particle coat of the apical membrane (portasomes: Harvey, 1980): in thoroughly studied insect organs it proved to be associated with electrogenic K^+-outward transport (*see* "Morphological Basis").

Endogenous Electroosmosis?

For understanding the water transport mechanism of the posterior rectum the powerful ion transport located within the same epithelium should be most significant. However, in this case it is impossible, as pointed out above, that the principle which may link water flux to ion transport could be chemiosmosis. But the water-to-cation-coupling property found within the rectal wall and the electrical circuit conditions given make an electroosmotic water flow across the apical membrane a cogent consequence of the experimental findings, on the premises that two specifications of these findings are valid: (i) the ion transport found is a lumen-directed electrogenic transport of cations; (ii) both, the ion transport and the water-cation-coupling channels, are located at the apical membrane of the epithelium. These premises are inferred from the experimental results and corroborated by comparative considerations but are not yet directly proven.

In the following it remains to be examined how far endogenous electroosmosis, as characterized by the data given, can account for the quantitative performance of water-vapor resorption *in vivo*.

At a membrane voltage of 200 mV and with the coupling ratio determined in our experiments (1.5×10^{-9} $m^3/A \cdot sec$ or 6.5×10^8 $A \cdot sec/m^3$, respectively) one arrives (using Eq. 3) at 1.3×10^8 Pa, which corresponds to about 40% RH, just the pump threshold calculated by Machin (1979) for *Thermobia*.

The measured coupling ratio, however, needs some interpretations: For a transepidermal current flow the circuit differs from that of an endogenous intraepidermal current flow in two respects: (i) an active cation outward transport as the endogenous current source will produce a passive pure cation inward current in the steady state, independent of the relative permeabilities (*see* "Theoretical Basis"). An exogenous current, however, should be carried by anions and cations according to the permeabilities of the epithelium so that in the experiment an additional anion-conductance may become effective. (ii) Whereas in the intraepidermal closed circuit the conductance of the active transport system lies in series to that of passive membrane channels, these conductances lie in parallel for an exogenous transepidermal current. Both anion and transport conductances will diminish J_v/I for an exogenous current compared to J_v/I of an endogenous electroosmotic flux. Therefore the calculation above may yield an overestimation of the pump threshold. A consequence of these considerations is, that even complete independence of water flow from exogenous current (in the case of equal water coupling to anions and cations) would not have disproved the hypothesis.

In order to be in accord with the pump threshold cited despite these reservations, we have to assume that either the conductivity in parallel to the cation channels is low or that the coupling ratio decreases with the water activity in the subcuticular space (*see* Levitt, 1984; the above value has been measured at relatively high water activity) or that both are true. The advantage of an inverse dependence of J_v/I on the pressure gradient is obvious since it would reduce energy dissipation at any humidity above the threshold.

From the coupling ratio measured at rather high water activity one calculates that a minimum current of about 9 μA might account for the rate of spontaneous uptake found at the corresponding relative humidity. In view of comparative data for the short-circuit current density (20 $\mu A/cm^2$), the membrane area (2 cm^2) and the elaborated structure, this current strength is in a conceivable range. Any comparison between the current in the *in vivo* circuit and in the shorted circuit (*cf.* 4 μA measured) defies, however, further critical examination, until there are separate measurements of the conductivity for the apical membrane.

Quantitative Implications of an Electroosmotic Mechanism

The following implications of the electroosmotic mechanism outlined correspond to experimental findings:

a. The electroosmotic concept predicts an inverse correlation between the water activity in the lumen and the voltage across the apical membrane: As the luminal water activity decreases, the electrokinetic voltage across the cation channels produced by the outward-directed water gradient has to increase. This voltage has the same sign as the emf of the transport and opposes the inward current. Thus the load of the transport is reduced. It corresponds to this prediction that maximum values of the TEV (+200 mV) were recorded only when the lumen of the anal sac was exposed to rather dry ambient air (40 to 50% RH). To our knowledge a higher voltage has never been reported for an animal cell membrane. We guess that this voltage approaches the

emf of the transport being only insignificantly loaded by a current.

b. We discussed a possible dependence of the coupling ratio on the water activity. This tendency is reflected by the experiments in which the anal sac was filled with 9 mol/liter Na-propionate + 2 mol/liter KCl; in those cases the average coupling ratio was 15% lower than in our standard conditions using KCl.

c. Differences between the time courses of TEV and J_v at the onset of anoxia as shown in Fig. 6 may be expected from the fact that J_v ceases when the membrane voltage decays to the electrokinetic equilibrium potential, while equilibration of the pressure difference is attended by a small passive outflow and a corresponding slower decay of the TEV due to an induced electrokinetic voltage. Vice versa, the J_v overshoot which occurs after the end of anoxia corresponds to a decrease of the pressure difference due to the previous equilibration; its decay to the steady state may reflect approaching the new state of re-increased luminal concentration. With the methods used, however, neither time resolution nor accuracy of J_v measurements are sufficient to investigate these phenomena quantitatively.

Considerations on Efficiency

From the cited comparative data of charge concentrations in ion channels one should not precipitately infer that in biomembranes an electroosmotic mechanism might be able to absorb water from activities appreciably below 0.4, because the considerable hydraulic conductivity of lipid membranes will represent a limiting factor.

Since losses by passive water flow will be reduced by a reduction of the boundary area across which uphill flow occurs, flow density, which means density of energy conversion, should be high to keep this area small. Judging from the unique configuration of the apical membrane-mitochondria complex in the anal sac it seems that the maximal possible density of metabolic turnover and active transport might be realized in this case. Nevertheless, the area of the apical membrane is in the range of the whole outer body surface of the animal. However, for the rate of electroosmotic water flow the ion conductance actually required can be provided by a very small fraction of the apical membrane, as inferred from numerous examples of conductivities of ion-selective membranes (*cf.* usual flow rates of 10^6 to 10^8 ions/sec in an ion pore, but in the order of 10^2 ions/sec in an active transport unit).

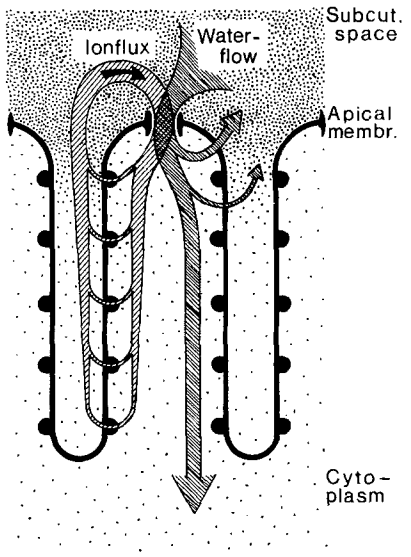

Fig. 9. Suggested pathways for ion current and water flow. High density of stippling symbolizes low water activity and vice versa

The deep apical membrane folds and the narrow extracellular spaces in between are a morphological configuration that appears to be particularly appropriate for a separation of those membrane areas devoted to active and passive transport, respectively. We suggest that the cation channels are disposed at the outermost apices of the membrane folds as illustrated by Fig. 9, while the electrogenic-transport units (portasomes) are distributed across the folded membrane as mentioned before. By this arrangement the narrow extracellular clefts might equilibrate with the osmotic pressure of the cytoplasm. Compared to the membrane area energizing the water transport this unstirred-layer effect would reduce the membrane area loaded with the osmotic gradient by two orders of magnitude. (To give a model calculation: if ion-water channels would be arranged at distances of 1 μm along the crest of each membrane fold, a ratio of about $1/(5 \times 10^4)$ would exist between the densities of passive channels and portasomes, approximately compensating the above-mentioned ratio of flow rates between passive and active transport units.) Indeed it seems that such a "remote energy supply" allows to understand the phenomenon of water vapor uptake, without the need to presume an improbably low water permeability of the bilayer proper of this cell membrane. The cell coat filling the narrow clefts (6 to 8 nm wide; *see* "Morphological Basis") may support high water activity within the clefts by reducing diffusion rates.

The considerations reveal that the principle of electroosmosis can be a proper mechanism for wa-

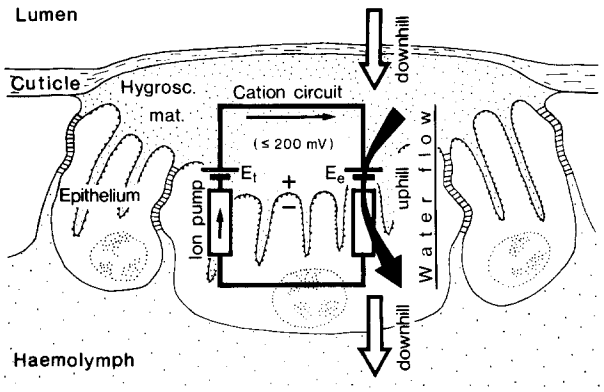

Fig. 10. Summarizing illustration of the electroosmotic concept advanced. E_t: EMF of ion transport; E_e: electrokinetic voltage

ter transport against large and very large gradients, operating on the basis of familiar functional elements.

Conclusion

Though identification and localization of the functional elements are circumstantial, both the qualitative and the quantitative aspects of our results substantiate the hypothesis and we advance the following model for the active step of water vapor uptake by *Lepisma* which is summarized in Fig. 10: The apical (luminal) membrane of the anal sac epithelium bears an electrogenic cation transport (of the type found at the *Cecropia* midgut and at sensilla of insects). This outward-directed transport generates a voltage across the apical membrane (200 mV, lumen positive). The back flow of the transported ion species driven by the membrane voltage passes through cation-selective channels. Water condensed from the atmosphere by the hygroscopic material within the subcuticular space is coupled to the inward current and thus is transferred by electroosmosis into the cytoplasm against its chemical potential difference. Consecutive flow of water from cytoplasm into the hemolymph is assumed to occur passively.

While rectal gross anatomy is very different between various groups of insects, one recognizes features at the anal sac of Lepismatidae which seem to be common to insect recta (Noirot et al., 1979): cells with a folded, particle-coated apical membrane facing a closed subcuticular space, and tortous septate junctions closing the intercellular clefts. Reasoning from these and from comparative physiological aspects (*see* Küppers & Thurm, 1980) we suggest that an electroosmotic water transport as outlined is of general significance for hyposmotic water absorption by insect recta.

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