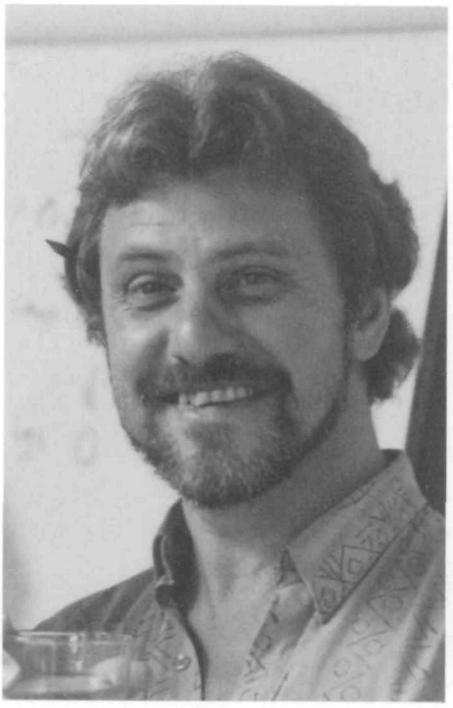

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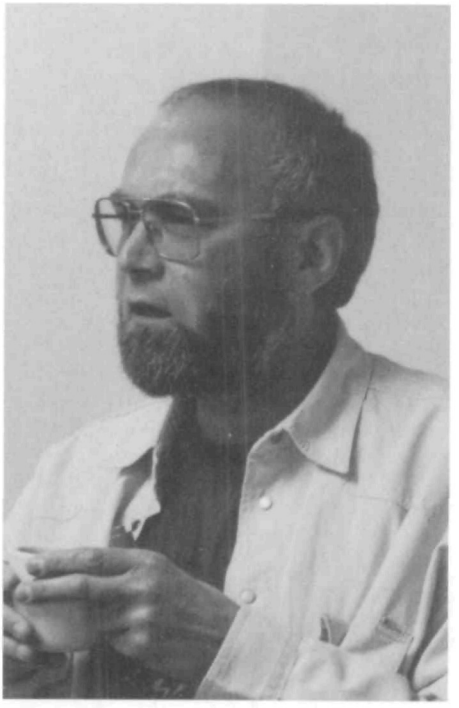

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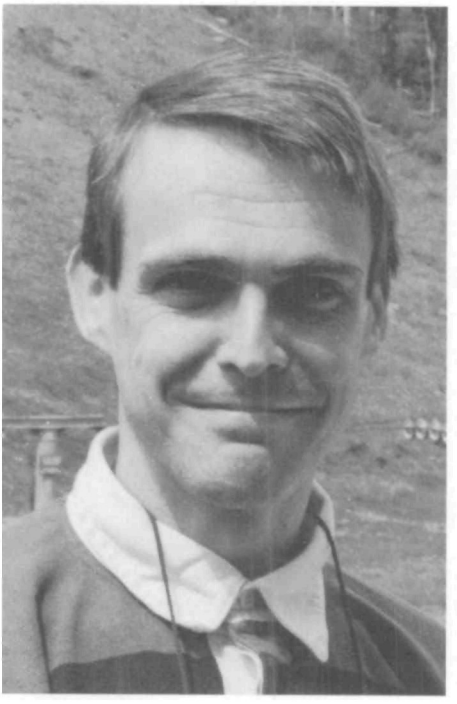

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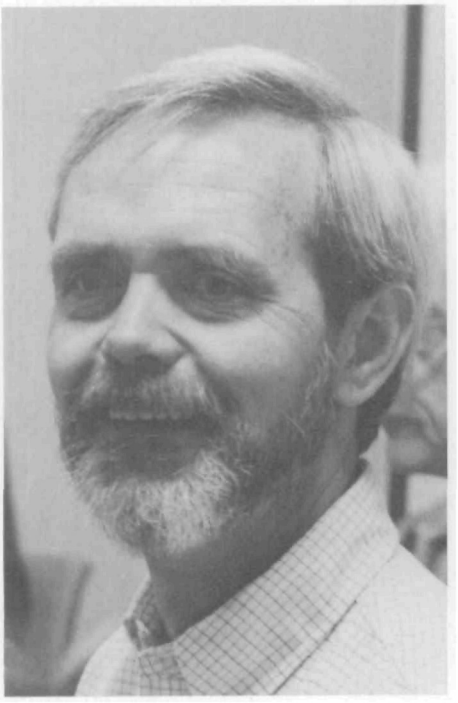

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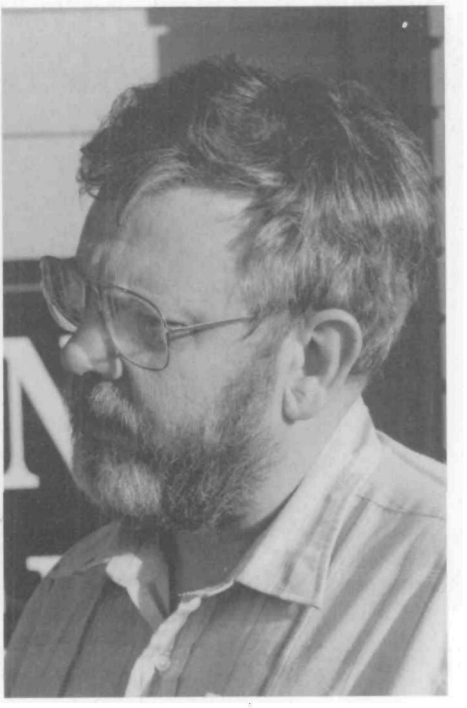

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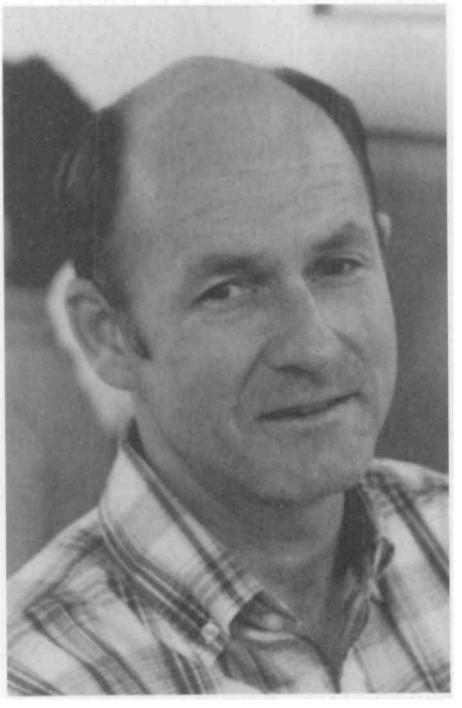

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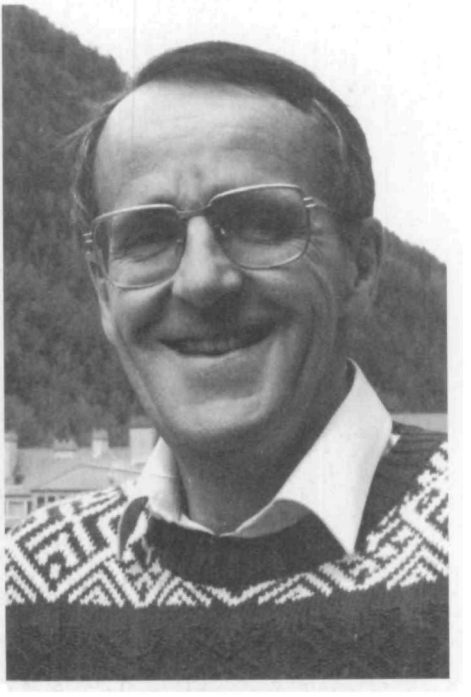

S. Maddrell

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INSECT ION HOMEOSTASIS

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Summary

The constant composition of body fluids in insects is maintained by the cooperative interaction of gastrointestinal and urinary tissues. Water follows ionic movements, which are driven by the basolateral Na^+/K^+-ATPase and/or the apical 'K^+(or Na^+) pump'. The latter now is thought to be the functional expression of a parallel arrangement of a proton-motive V-ATPase and a K^+(or Na^+)/nH^+ antiport. This review focuses on the pathways for the movement of monovalent inorganic ions through epithelia involved in ion homeostasis. A graphical summary compares the principal findings with respect to cation secretion in lepidopteran caterpillar midgut goblet cells (K^+) and in brush-border cells of Malpighian tubules (K^+, Na^+).

Gastrointestinal epithelia and insect ion homeostasis

In vertebrates, the interaction of gastrointestinal and urinary epithelia, sometimes assisted by epidermal tissues such as skin or gills, is responsible for the major portion of homeostasis. Regulation of body fluids across the cuticle-covered epidermis of insects is rare, if not impossible. In insects, the major role in maintaining ionic and water balance seems to be played by the concerted action of the gut and the tissues connected to it, such as the Malpighian tubules. The following two articles, by Moffett and Koch (1992) and by Maddrell and O'Donnell (1992), will examine the specific roles of the larval lepidopteran midgut and of the Malpighian tubules, respectively, in insect ion homeostasis.

The nature as well as the extent of homeostatic measures will depend upon the individual and optimal composition of the haemolymph, which in turn may vary with and be adapted to, the ion content of the insect's principal diet (Dow, 1986). Thus, solid-plant feeders, such as the tobacco hornworm, i.e. the caterpillar of the moth *Manduca sexta*, have to cope with a high K^+ and Mg^{2+} content in their blood (Dow, 1986; Dow *et al.* 1984). Large, electrogenic net potassium fluxes from haemolymph to gut lumen have been demonstrated (Harvey and Nedergaard, 1964; Chamberlin, 1990*a*), hence the midgut epithelium seems to contribute to K^+ excretion. Whereas the principal function of the gut is nutrient absorption, the Malpighian tubules are primarily responsible for the excretion of waste and excess ions and water. Caterpillars are no exception to this general rule (Dow and Harvey, 1988). At another extreme, blood-sucking insects such as *Rhodnius prolixus* have a high-Na^+ haemolymph; they must quickly get rid of excess

Key words: homeostasis, salt excretion, membrane pathways, K^+ channel, K^+/H^+ antiport, H^+-ATPase.

sodium, chloride and water, processes that are under hormonal control (Dow, 1986; O'Donnell and Maddrell, 1984; see also Maddrell and O'Donnell, 1992).

For both tissues, midgut and tubules, the respective driving forces which govern the excretory performance have one common principle, namely, the active transport of cations such as K^+ or Na^+ into the luminal space, achieved by the 'cation pump'. The structural correlate of this transporter was identified with the 'portasome' (Harvey *et al.* 1983; see Harvey, 1992). Now, it has become evident that K^+ (or Na^+) secretion is powered by the V-type H^+-ATPase (see below and Fig. 3). This H^+ pump is localized in the apical membrane of the midgut goblet cells and in the brush-border membrane of the Malpighian tubules (Schweikl *et al.* 1989; Wieczorek *et al.* 1989, 1991; Klein *et al.* 1991; see also Wieczorek, 1992; Klein, 1992). In all likelihood, this protein is the actual portasome (see W. R. Harvey, 1992). A K^+ pump has also been found in salivary glands (see Gupta *et al.* 1978; Harvey *et al.* 1983). Most interestingly, a non-gastrointestinal epithelial structure contains the proton pump (Klein and Zimmermann, 1991) involved in K^+ transport (Wieczorek and Gnatzy, 1985), namely, the apical membrane of the enveloping cells found next to the sensory cells in different types of sensilla. Here, the pump provides a K^+ current which recycles K^+ *via* the sensory cells, where it contributes to the receptor current (Thurm and Küppers, 1980). The localization of the V-ATPase to the apical membranes has been convincingly shown using an immunological approach (Klein and Zimmermann, 1991).

Homeostasis can only be achieved if different body compartments can communicate with each other. Ions that are secreted by the diverse gastrointestinal tissues will also be subject to a certain degree of recycling. One absorptive route in the midgut is electrogenic K^+(or Na^+)/amino acid cotransport *via* columnar cells (Giordana *et al.* 1985). The cation in question will then probably be recycled *via* gap junctions (Dow and Peacock, 1989) and the goblet cell to the gut lumen. A small paracellular backflux of potassium (Harvey and Nedergaard, 1964) down the strong electrochemical gradient will also contribute to K^+ recycling. Furthermore, the hindgut recycles ions: in the locust rectum, a standard tissue for hindgut research, the gut contents are concentrated through active chloride absorption, followed by passive movements of K^+ and water (Phillips *et al.* 1986). In the hindgut (Phillips *et al.* 1986; see Harvey *et al.* 1983), in addition, a powerful active H^+ secretion was observed which also appears to be due to a V-ATPase (see Klein, 1992).

In this review, I will sketch (see Fig. 3) the routes of K^+, Na^+, Cl^- and H^+/OH^-, which follow the electrochemical gradients that are ultimately set up by the activity of the K^+ pump whose intrinsic power source is, in many cases, the apical proton-motive V-ATPase (see Wieczorek, 1992; Klein, 1992).

The basal membrane

General aspects – is K^+ entry active or passive?

Although the ouabain-inhibitable Na^+/K^+-ATPase is almost universal, it is not involved in transport in sensilla or in the midgut of phytophagous caterpillars. Ouabain does not influence the K^+-dependent ATPase in auxiliary cells of sensilla (Wieczorek, 1982), although it does inhibit absorption in the hindgut (Phillips *et al.* 1986) and

secretion in Malpighian tubules (O'Donnell and Maddrell, 1984; Maddrell and Overton, 1988). The caterpillar midgut definitely lacks this enzyme (Jungreis and Vaughan, 1977). Thus, the apical K^+ pump will, at least in the latter tissue, depend on an alternative basolateral access pathway for K^+.

A candidate for this pathway is another typical building block of basal membranes, the spectrum of K^+ channels. As in other membranes, K^+ channels appear to exist in various versions in the midgut of the tobacco hornworm, as was recently revealed by patch-clamp analysis (Moffett and Lewis, 1990). Insects with a high-K^+ haemolymph would not need the Na^+/K^+-ATPase to maintain the indispensable high cell K^+ concentration if K^+ were able to enter the cells passively. Such passive K^+ entry (see Fig. 3) is apparently possible in the caterpillar midgut (Zeiske *et al.* 1986), where the apically located K^+ pump provides the electrical driving force to overcome the unfavourable basal chemical gradient for K^+ (Dow *et al.* 1984; Moffett and Koch, 1988*a*).

In hypoxic conditions (5 % O_2) and then only with very low potassium concentrations, the basal step for K^+ entry was postulated to be active (Chao *et al.* 1990; Moffett and Koch, 1988*a*). Amazingly, it was hypothesized that up to 70 % of the saturable, Ba^{2+}-blockable K^+ current might proceed, even under standard conditions, *via* this active basal pathway (see Fig. 3).

The K^+ channel

In Malpighian tubules (O'Donnell and Maddrell, 1984; Van Kerkhove *et al.* 1989) and also in midgut (Moffett and Koch, 1988*a*; Chao *et al.* 1990) the basal membranes were found to behave like almost perfect K^+ electrodes. In both cases, Ba^{2+} could block the basal conductance. This was also the case in the locust rectum, where basal barium generated a Lorentzian conduction noise in the K^+ current (Hanrahan *et al.* 1986).

The K^+ channel in *M. sexta* midgut has been intensively studied. In our experiments on *M. sexta* midgut (Zeiske *et al.* 1986) we also observed, with Ba^{2+} in the internal saline, a Lorentzian noise in the K^+ current. The kinetics of the current blockage by Ba^{2+}, which is, in standard conditions, clearly competitive with basal K^+ (Zeiske *et al.* 1986; Schirmanns and Zeiske, 1991), was fully consistent with the analysis from noise data. Transport impairment was also seen with lidocaine (Moffett and Koch, 1991) and quinidine (Alpert, 1989), both known inhibitors of volume-regulated K^+ channels (Dawson, 1987; Van Driessche and Hillyard, 1985). In contrast, tetraethylammonium (TEA^+) and Cs^+, which are also K^+ channel inhibitors (Van Driessche and Zeiske, 1985), are ineffective (K. Schirmanns and W. Zeiske, in preparation). Down to a basal pH of 6.8, which is about the pH measured *in vivo* (Dow, 1984), the channel remains insensitive to pH changes in the bathing solutions (Chao *et al.* 1991).

Neither microelectrode experiments nor noise analysis were able to distinguish between goblet and columnar cells, which are effectively coupled under all conditions (Dow and Peacock, 1989; Moffett and Koch, 1988*a*). However, Moffett and Lewis (1990) achieved a patch-clamp of the goblet cell basal membrane and detected four types of K^+ channels, two of which could be activated in the presence of Ba^{2+} rather than being blocked. It is not clear which channel plays the dominant part in K^+ secretion. Thus, although disputed (Chao *et al.* 1990), it is reasonable to assume that basal K^+ channels are

the major entry sites of potassium for secretion. This conclusion establishes a novel paradigm for K^+ secretory processes (see Fig. 3).

Haemolymph-side halogen ions, but not nitrate or thiocyanate, stimulate basal K^+ transfer (Zeiske *et al.* 1990, 1992*b*). Surprisingly, Zeiske *et al.* (1992*b*) found that the drugs 9-anthracene-carboxylate (9-AC), furosemide (Furo), diphenyl carboxylate (DPC) and di-isothiocyanatostilbene sulphonate (DIDS), which inhibit chloride transporters, reduced the K^+ current when applied basally in the presence of either chloride or gluconate (see Fig. 3 and Fig. 1 for DPC). The effect of chloride was studied more closely (Zeiske *et al.* 1990, 1992*b*). It was found that the saturating K^+ current (I_K) was increased in such a way that K_m and I_{max} were multiplied by the same factor (Fig. 2). The discovery of the ubiquitous bumetanide/furosemide-sensitive cation/Cl^- symport in Malpighian tubule basal membranes (O'Donnell and Maddrell, 1984) prompted us to investigate whether a volume increase, which may be expected to result from the activation of such a transporter, could trigger an increase of I_K. Indeed, basal *hypo*tonicity, which presumably leads to cell swelling, augmented I_K whereas strong *hyper*tonicity almost abolished I_K (Alpert, 1989; Zeiske *et al.* 1990). However, the kinetics of the chloride stimulation of I_K was unaffected by anisotonicity (Alpert, 1989; Zeiske *et al.* 1990). Furthermore, as sketched in Fig. 2, the rise of I_K caused by swelling (following hypotonicity), as a possible mediator of the 'Cl^- effect', is caused by an increase in only I_{max} at constant K_m. This clearly differs from the effect of halides on the I_K kinetics, where both Michaelis–Menten parameters are changed by the same factor. Noise analysis finally revealed that chloride, acting at the blood side of the K^+ channel, increases the single-channel K^+ current but concomitantly *lowers* the area density of available channels (Zeiske and Marin, 1992).

We also tried to assess the basal channel's ion selectivity. As already shown for *Hyalophora cecropia* (Zerahn, 1979), in the intact *M. sexta* midgut epithelium Rb^+ was a good substitute for K^+, whereas no transepithelial current was obtained with Na^+, Cs^+, NH_4^+ or Tl^+ (Schirmanns and Zeiske, 1991), although the latter two are good K^+

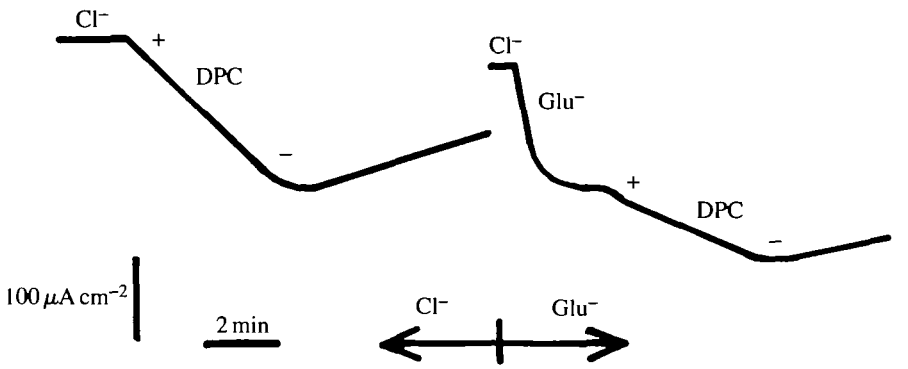

Fig. 1. Depression of secretory K^+ current (32 mmol l^{-1} K^+, standard saline) in *M. sexta* midgut by removal of Cl^- (substituted by gluconate, Glu^-), or addition of basal diphenyl carboxylate (DPC) (1 mmol l^{-1}). Arrows pointing to the left/right: ambient Cl^-/Glu^-, respectively. (From Alpert, 1989.)

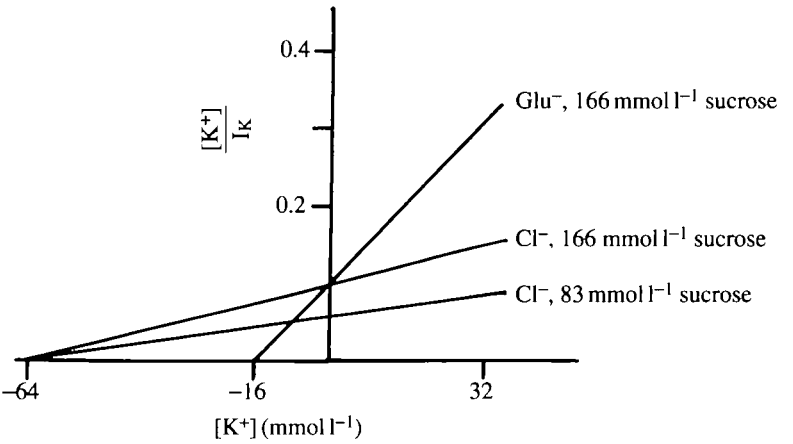

Fig. 2. A sketch of the Hanes diagram for the saturating relationship between the secretory K^+ current (I_K) and basal [K^+] in *M. sexta* midgut for three haemolymph-side conditions: isotonic chloride saline with 166 mmol l^{-1} sucrose; hypotonic (83 mmol l^{-1} sucrose) chloride saline; isotonic gluconate (Glu^-) saline. The abscissa intercepts give the negative value of the Michaelis constants, K_m; values are means from Alpert (1989), Zeiske *et al.* (1990) and Zeiske *et al.* (1992*b*). Arbitrary units on the ordinate. Note that the slope is the reciprocal of I_{max} and the ordinate intercept is K_m/I_{max}.

substitutes in other systems (Van Driessche and Zeiske, 1985). Instead, small amounts of basal and especially apical Tl^+ quickly nullified an established K^+ current, an effect already reported by Zerahn (1982) for the midgut of *H. cecropia*, where Tl^+ is a competitive blocker of the K^+ current. To determine whether thallium could pass the basal *M. sexta* K^+ channel prior to its presumed blocking of the apical K^+ pump in competition with K^+ (Zerahn, 1982), we 'functionally' eliminated (Dawson, 1987) the apical epithelial membranes by using the antibiotic amphotericin B (Schirmanns and Zeiske, 1991). This manoeuvre enabled us to estimate basal permeability ratios from bi-ionic reversal potentials. Thallium substituted perfectly for K^+ in the channel; therefore, it must inhibit the K^+ pump (competing with K^+ for the antiport?). The permeability for Rb^+ is only 0.4 times that of K^+; NH_4^+, Na^+ and Cs^+ are impermeant.

So far, the pharmacological and selectivity profiles of the midgut K^+ channel differ appreciably from those of other K^+ channels, e.g. in excitable (Hille, 1991) or epithelial (Van Driessche and Zeiske, 1985; Zeiske, 1990) membranes. Moreover, while the 'typical' K^+ channels usually exhibit multi-site, single-file permeability characteristics (Van Driessche and Zeiske, 1985; Hille, 1991; Zeiske, 1990), this is not the case for midgut in the physiological ion concentration range (Schirmanns and Zeiske, 1991). The permeability pattern and the alteration of the I_K kinetics by halides (Fig. 2) seem to be satisfactorily described (K. Schirmanns and W. Zeiske, in preparation) by a one-site, two-barrier model, as discussed in detail by Hille (1991).

Other ion transport pathways

Of the Na^+-dependent transporters besides the Na^+/K^+-ATPase, only the bumetanide-

inhibitable $Na^+/K^+/2Cl^-$ symport, which represents the first step in NaCl secretion in Malpighian tubules, has been demonstrated in tubules of *Drosophila hydei* (Bertram *et al.* 1991) and *Rhodnius prolixus* (O'Donnell and Maddrell, 1984) which have a high-Na^+ blood. In *R. prolixus,* the appropriate choice of solution composition could 'switch' this transporter to a pure NaCl or a KCl mode. In view of the sensitivity of the midgut K^+ channels towards furosemide and other drugs with an affinity for Cl^- transporters, it is tempting to suppose that there is some molecular kinship.

Anion movements across the basal membranes of insect epithelia have been poorly studied, with the exception of the cation/chloride cotransporter (see above). In locust rectum (Phillips *et al.* 1986), a cyclic-AMP-sensitive, basal, passive Cl^- permeability was described, but in lepidopteran midgut and Malpighian tubules, basal conductive pathways for Cl^- have not been established or have been explicitly ruled out, as in *M. sexta* midgut goblet and columnar cells (Chao *et al.* 1989).

The apical membrane

The V-type H^+-ATPase (see also Wieczorek, 1992)

V- and F-type H^+-ATPases appear to be close relatives (see Nelson, 1992). In contrast to V-type proton pumps, in mitochondria-rich cells of amphibian epidermis (Ehrenfeld *et al.* 1985) and urinary bladder (Fanestil and Park, 1981) primary active proton pumps have been tentatively classified as F-type, mainly because of their inhibitor profiles [*N,N'*-dicyclohexylcarbodiimide (DCCD), oligomycin]. Naturally, with intact epithelial preparations, misinterpretations can arise as oligomycin is a well-known mitochondrial inhibitor. In addition, most substances used as blockers of H^+-ATPases (Wieczorek *et al.* 1986, 1989; Bertram *et al.* 1991), such as DCCD, 7-chloro-4-nitro-benz-2-oxa-1,3-diazole (NBD-Cl) or *N*-ethylmaleimide (NEM), are not very selective from the chemical point of view and might attack more or less any protein.

The V-type ATPase is sensitive to micromolar concentrations of NEM (Wieczorek *et al.* 1989; Bertram *et al.* 1991) but is insensitive to azide and vanadate (F- or E-type H^+-ATPase blockers, respectively). Cytosolic-side nitrate ions inhibit the V-ATPase in midgut goblet cell apical membrane vesicles (Wieczorek *et al.* 1986). In the epithelium *in vitro*, nitrate blocks the apical cation pump only after the *basal* membrane has been permeabilized for the anion with amphotericin B (K. Schirmanns and W. Zeiske, in preparation). Amiloride is known for its specific submicromolar inhibition of apical Na^+ channels in epithelial cells of vertebrates (Van Driessche and Zeiske, 1985) and, as most recently reported, also of invertebrates (Zeiske *et al.* 1992*a*). At submillimolar concentrations, it affects the H^+ pump in *M. sexta* midgut (Wieczorek *et al.* 1991) and it also affects the parallel K^+/H^+ antiport (see below). Today, the most specific and highly sensitive test for a participation of the V-type H^+ pump is the antibiotic bafilomycin A_1, which is structurally related to oligomycin. Bafilomycin inhibits the V-type H^+-ATPase in nano- to micromolar concentrations (Wieczorek *et al.* 1991; Bertram *et al.* 1991).

The K^+/nH^+ antiporter

The apical antiporter is sensitive to the hallucinogen harmaline (Wieczorek *et al.* 1991)

and to amiloride, which is reminiscent of its effect on the Na^+/H^+ exchanger in plasma membranes (Aronson *et al.* 1982). Amiloride blocks it at concentrations almost 10 times lower than that acting on the pump (Wieczorek *et al.* 1991). The antiport seems to operate electrogenically, exchanging at least two protons for one potassium (Chao *et al.* 1991; Wieczorek *et al.* 1991). Evidence for this postulate comes from the finding that the pH gradient from midgut goblet cavity to cytosol is insufficient to drive K^+ out of the cell against a large electrochemical gradient. Rb^+, Li^+ and Na^+ can substitute for K^+ (Wieczorek *et al.* 1991), whereas Tl^+ may be a competitive inhibitor (see above).

Are there anionic shunts?

The apical membranes in Malpighian tubules contain Cl^- channels (Bertram *et al.* 1991; O'Donnell and Maddrell, 1984). This arrangement shunts the apical electrical gradient set up by the proton pump and mass fluxes of ions (and water) become possible. Chloride channels have been found in biochemical experiments using midgut vesicles (Wieczorek *et al.* 1989). Following Dow (1984), apical anion channels might be a pathway for bicarbonate/carbonate to leave the cell, which could explain very elegantly the generation of the extremely alkaline luminal pH that develops in the *in vivo* and *in vitro* intestine preparation (Dow, 1984, 1986; Dow and Harvey, 1988; Chamberlin, 1990*b*; W. R. Harvey, 1992). The required electrical isolation of the apical membrane is similar to that observed with enveloping cells in sensilla (Harvey *et al.* 1983; Thurm and Küppers, 1980), in that a physical enclosure of the cavities is accomplished using valve-like structures (see also Moffett and Koch, 1992) with a low permeability for Ni^{2+} (Moffett and Koch, 1988*b*) and the dye Lucifer Yellow (Dow and Peacock, 1989; Moffett and Koch, 1988*b*). Although the goblet mucus content will, perhaps, buffer the local pH (Chao *et al.* 1991) and restrict proton movement, it is difficult to understand how a high transepithelial flux of potassium (Harvey and Nedergaard, 1964; Chamberlin, 1990*a*) can be achieved if the valves are not highly permeable to K^+ (cf. Moffett and Koch, 1992). Nevertheless, with a finite paracellular permeability for H^+/OH^-, the transepithelial K^+-dependent potential difference might also generate a similar transepithelial distribution of H^+. However, any shunt or channel would have to operate so as to retain most of the more than 150 mV potential difference across the apical membrane of both goblet and columnar cells and the 120 mV transepithelial potential difference measured *in vivo*.

Regulation of the K^+ pump and K^+ secretion

A cellular response to hormonal stimulation will also involve the apical K^+ (or Na^+) pump. Hormonal stimulation has been observed in Malpighian tubules (O'Donnell and Maddrell, 1984) and salivary glands (Gupta *et al.* 1978; Berridge and Schlue, 1978). In caterpillar midgut, haemolymph factors may be stimulatory (Wolfersberger and Giangiacomo, 1983). However, simple molecules such as Cl^- (Zeiske *et al.* 1990, 1992*b*), citrate (Chamberlin, 1989; Thomas and May, 1984) or high concentrations of Mg^{2+} and Ca^{2+} (Thomas and May, 1984) in the haemolymph stimulate K^+ secretion in

caterpillar midgut at standard [K^+]. Small amounts of these divalent cations inhibit I_K at lower [K^+] (Moffett and Koch, 1983, 1985).

In salivary glands (Berridge and Schlue, 1978), cyclic AMP and Ca^{2+} are possible

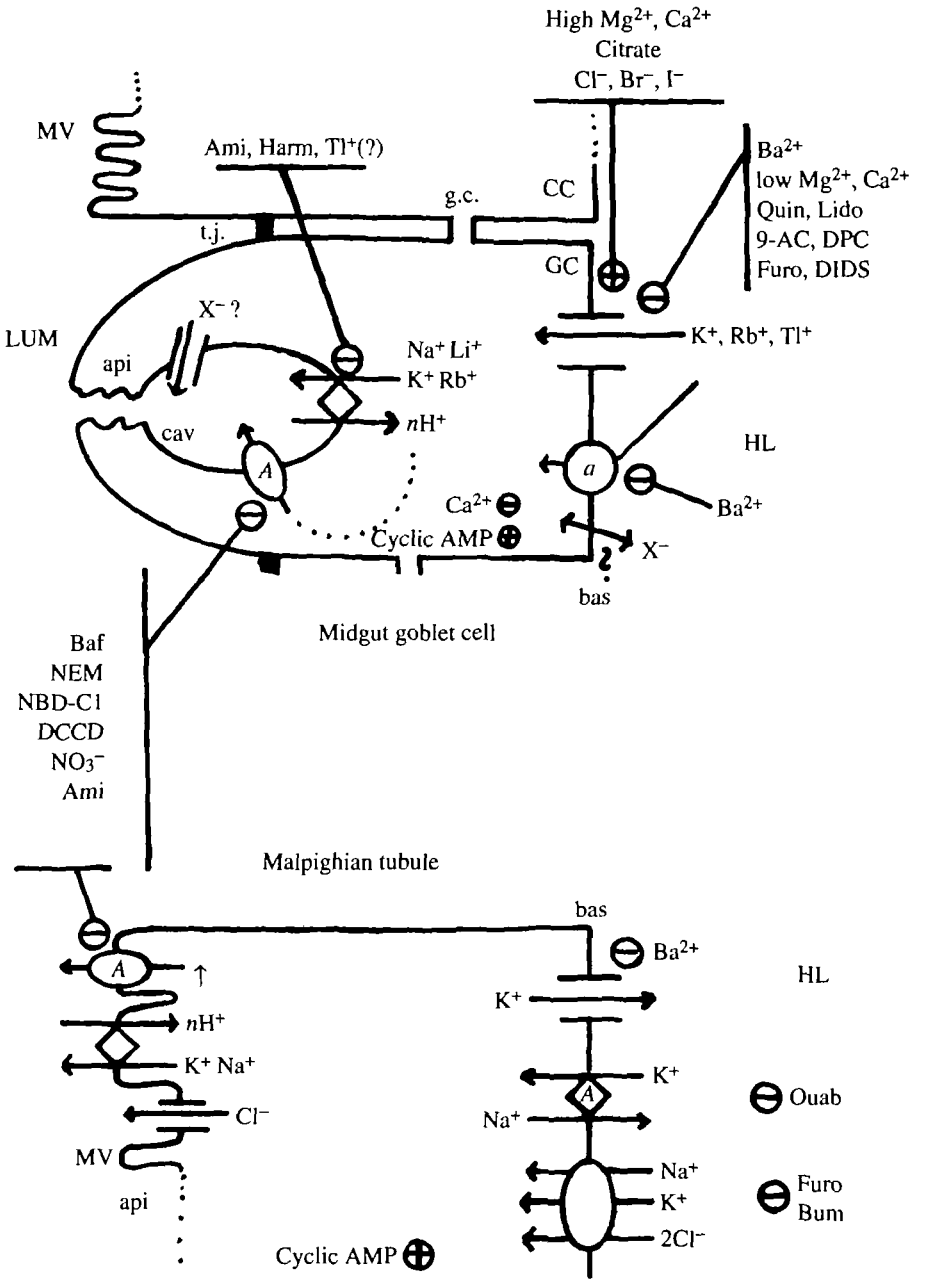

Fig. 3

intracellular messengers. The nucleotide also seems to be involved in diuresis by Malpighian tubules (O'Donnell and Maddrell, 1984; Spring and Hazelton, 1987; Van Kerkhove *et al.* 1989) and augments the K^+-carried short-circuit current in *M. sexta* midgut (Wolfersberger and Giangiacomo, 1983). Attempts to raise cell Ca^{2+} concentration with serotonin, caffeine or the calcium ionophore A23187 were without effect on the K^+ current in central midgut (Wolfersberger and Giangiacomo, 1983); however, raising intracellular calcium concentration led to a reduction of K^+ transport in the posterior midgut (Moffett *et al.* 1983). No information is available about the control of K^+ transport by intracellular pH, a major ion channel regulator in vertebrate epithelia (Harvey *et al.* 1988; see B. J. Harvey, 1992). In view of the reported allosteric influences of cytosolic protons on proton pumps or proton/cation antiport in other systems (Ehrenfeld *et al.* 1985; Aronson *et al.* 1982), a similar effect can also be expected in insect epithelia. Since H^+ pumping and antiport are electrogenic, they should also be influenced by a change in membrane potential, which would couple basal and apical membranes most effectively (Moffett and Koch, 1988*b*).

Conclusion and perspectives

This review focuses on monovalent ion movements across the midgut goblet cells of phytophagous caterpillars and the brush-border cells of Malpighian tubule. The main features are incorporated in Fig. 3. In both tissues, and also in some other insect epithelial structures, a prominent electrogenic cation secretion across the apical membrane is powered by a V-ATPase. In insects with low-K^+ haemolymph, basolateral $Na^+/K^+/Cl^-$ symport and the Na^+/K^+-ATPase contribute to the energization of secretory epithelial cation movements; however, in insects with a K^+-rich haemolymph, we find K^+ channels as the main basal access path and a Na^+/K^+-ATPase is not detectable. The K^+ channels have some unusual properties that are reminiscent of KCl symporters. With a dominant electrogenic cation secretion, the pathways of accompanying anion fluxes allow, together with special morphological features, very different modes of transport and goals to be achieved. The solution of ion homeostatic problems in insects involves a dominant use of the V-type H^+-pump. In vertebrates, the role of this H^+-ATPase in ion-translocating processes, such as in kidney or frog skin, has only recently been realized (for a review, see B. J. Harvey, 1992).

Fig. 3. Schematic representation of ion movements during $K^+(Na^+)$ secretion by *M. sexta* midgut goblet cell (GC; upper part of figure) and Malpighian tubule (lower part). Active transport is ATP-driven (*A*) or of unknown driving force (*a*). Channels are represented by parallel lines; active ion transporters, ion exchangers and symports have closed symbols. X^- denotes anions such as Cl^- or HCO_3^-. Small circles showing minus signs indicate inhibitory effects, while those showing 'plus' signs indicate stimulation. Luminal side (LUM), haemolymphal side (HL), microvillus (MV), apical membrane (api), basal membrane (bas), columnar cell (CC), tight junction (t.j.), gap junction (g.j.), goblet cavity (cav), quinidine (Quin), lidocaine (Lido), ouabain (Ouab), bumetanide (Bum), amiloride (Ami), harmaline (Harm), bafilomycin (Baf). For other abbreviations, see text. The magnitudes of chemical and electrical gradients may be found in the respective figures in the articles by W. R. Harvey (1992), Moffett and Koch (1992) and Wieczorek (1992).

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