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SHORT COMMUNICATION

LUMINAL ALKALINIZATION BY THE ISOLATED MIDGUT OF THE TOBACCO HORNWORM (*MANDUCA SEXTA*)

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The midgut contents of lepidopteran larvae are often several pH units more basic than the haemolymph (Berenbaum, 1980). One species, *Manduca sexta*, maintains a luminal pH of 11.3 (Dow, 1984) while the haemolymph has a pH of 6.7–6.8 (Dow, 1984; Dow *et al.* 1984). The midgut of *M. sexta* is divided into three morphologically distinct regions (Cioffi, 1979), and Dow (1984) has shown that the luminal pH is greatest in the anterior and middle sections of the midgut. The posterior midgut lumen, while still more basic than the haemolymph, has a pH lower than the middle and anterior sections. Whereas these results suggest that alkaline secretion may differ along the length of the midgut, no direct measurements of alkaline secretion have been made. In this study the rate of luminal alkalinization in the three midgut sections was measured *in vitro*.

Tobacco hornworms were grown from eggs or larvae purchased from Carolina Biological Supply Company (Burlington, North Carolina). The larvae were maintained at 28°C on a 16 h:8 h light:dark cycle and fed an artificial diet (Carolina Biological Supply Co.). Fifth-stage larvae weighing 3–5.3 g were used in all experiments.

Larvae were decapitated and a dorsal incision made to expose the midgut. The anterior, middle or posterior midgut was then cleaned of adhering tracheae and Malpighian tubules, opened, removed from the animal and mounted as a flat sheet in an Ussing type chamber. Each half of the chamber had a volume of 2.5 ml and was a modification of a design described previously (Hanrahan *et al.* 1984). The midgut was secured to a notched collar with cotton thread and the opening (0.196 cm^2) was backed with fine nylon screen to support the tissue. The tissue was bathed bilaterally with saline which was constantly bubbled with 100 % oxygen to maintain high oxygen tension and to circulate the saline. After reaching a steady transepithelial potential difference (PD) or short-circuit current (I_{sc}; usually 60–90 min), the midgut was bilaterally perfused with saline at a rate of 3 ml min^{-1} using a Gilson Minipuls peristaltic pump to ensure that any residual alkali was

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washed from the luminal surface. After 15 min the perfusion on the luminal side was stopped and the base secretion measured for 30 min using a pH stat technique (Radiometer PHM84 pH meter, ABU 80 autoburette and a TTT80 titrator). Vigorous oxygenation of both sides of the epithelium was maintained throughout these procedures. Luminal alkalinization was determined by the rate of titrant (0.01 or 0.1 mol l^{-1} HCl) added to maintain the initial pH. Since the anterior portion had the greatest rate of alkalinization (see below), transport was further explored in this section. Alkali secretion was measured for 90 min in K^+-free saline and under short-circuit conditions. Experiments were conducted at room temperature (22–25 °C).

The PD was measured with calomel electrodes connected to KCl agar bridges which were inserted into the saline on either side of the midgut. Current was passed *via* silver/silver chloride electrodes connected to agar bridges. PD and I_{sc}, with compensation for saline resistance, were measured with a custom-made voltage clamp (Duke University Physiology Department Technical Shop) and monitored on Soltec recorders.

A saline containing haemolymph levels of ions, amino acids and carboxylic acids was used, since a saline of similar composition has been shown to maintain midgut function *in vitro* (Chamberlin, 1989). Control saline had the following composition (in mmol l^{-1}): Hepes, 2; $MgCl_2$, 5.0; $CaCl_2$, 1.0; KOH, 5.8; NaOH, 9.0; sodium methylsulphate, 3.0; potassium citrate, 7.7; sodium succinate, 2.8; malic acid, 5.6; glucose, 2.0; trehalose, 27.0; glutamine, 9.4; serine, 8.9; proline, 7.4; glycine, 12.8; threonine, 4.6; alanine, 3.6; polyethylene glycol (M_r 400), 140. K^+-free saline consisted of the following (in mmol l^{-1}): Hepes, 2; $MgCl_2$, 5.0; $CaCl_2$, 1.0; NaOH, 9.0; sodium methylsulphate, 3.0; citric acid, 7.7; sodium succinate, 2.8; malic acid, 5.6; glucose, 2.0; trehalose, 27.0; glutamine, 9.4; serine, 8.9; proline, 7.4; glycine, 12.8; threonine, 4.6; alanine, 3.6; *N*-methyl-D-glucamine, 23.1; polyethylene glycol (M_r 400), 140. Salines were vigorously bubbled with 100% oxygen for 2 h prior to adjusting the pH to 6.7.

Table 1 shows that all three midgut sections engage in *in vitro* luminal alkalinization under open-circuit conditions. The rate of alkalinization is greatest in the anterior section and can be maintained at a constant rate for at least 90 min (Fig. 1). This result is consistent with the observation that the highest pH is found in the anterior and middle regions of the midgut lumen *in vivo* (Dow, 1984). The

Table 1. *Luminal alkalinization under open-circuit conditions by the three morphologically distinct regions of* Manduca sexta *midgut*

Midgut section	Luminal alkalinization (μequiv cm^{-2} h^{-1})
Anterior	8.9±0.7
Middle	2.8±0.2
Posterior	3.5±0.4

Values are expressed as mean±S.E., N=6.

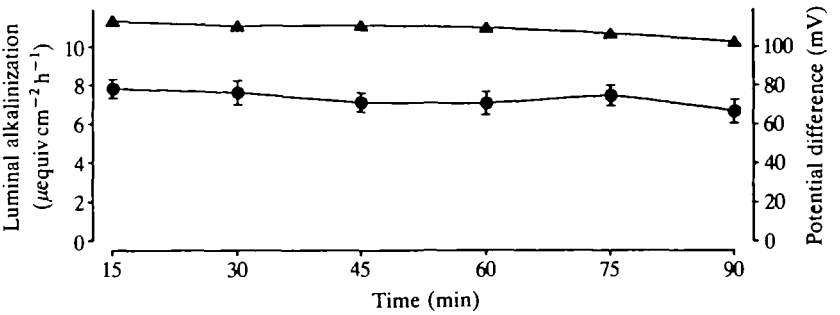

Fig. 1. Luminal alkalinization by the anterior midgut under open-circuit conditions. Values for the rate of alkalinization (●) and potential difference (▲) are means±S.E., N=5. Error bars have been omitted if they are smaller than the size of the symbol.

observation that luminal pH falls in the posterior section of the midgut *in vivo* may reflect a lower rate of base secretion (or H^+ absorption), although reflux of Malpighian tubule fluid or rectal contents cannot be discounted.

The chemical nature of the secreted alkali was not investigated in this study. Secretion of ammonia is a possibility since amino acids were present in the saline and the midgut tissue can oxidize some amino acids (Chamberlin, 1987, 1989). Carbonic anhydrase is found in the epithelial cells of the midgut (Ridgway and Moffett, 1986) and therefore HCO_3^- may be produced and secreted. Under the conditions of the present study, exogenous CO_2 is minimal and therefore HCO_3^- would be synthesized from endogenously produced CO_2. The posterior midgut section consumes oxygen at a rate of 213 μmol $O_2\,g^{-1}\,h^{-1}$ (Mandel *et al.* 1980) and this is equivalent to a rate of 23.8 μmol $O_2\,cm^{-2}\,h^{-1}$ using the conversion factor reported by Cioffi and Harvey (1981). With respiratory quotients ranging from 0.7 to 1, HCO_3^- could be produced at a rate sufficient to account for the luminal alkalinization seen in this study. The cell types responsible for this putative HCO_3^- transport may differ along the length of the midgut, since histochemical studies reveal that carbonic anhydrase is found predominantly in the goblet cells in the anterior and middle regions of the midgut, while this enzyme is localized in the columnar cells in the posterior region (Ridgway and Moffett, 1986).

It should be emphasized that HCO_3^- *per se* may not be the species which is transported by the midgut epithelium. Secretion of OH^- or absorption of H^+ could also produce luminal alkalinization. Wieczorek *et al.* (1989) have recently proposed that K^+ secretion occurs *via* an apically located K^+/H^+ exchanger which is energized by the action of a H^+ pump on the same membrane. If a Cl^-/OH^- (Cl^-/HCO_3^-) exchanger were located on the apical membrane, the net result would be KOH ($KHCO_3$) secretion. In support of this view, Chao *et al.* (1989) have recently shown a Cl^-/HCO_3^- exchange mechanism in the apical membrane of midgut cells.

Luminal alkalinization is maintained (7.3±0.7 μequiv $cm^{-2}\,h^{-1}$, N=5) under short-circuit conditions when no transepithelial pH or electrical gradient is

present, suggesting that this process is active. Under the conditions of this study, however, the passive secretion of an anion from the cell cannot be discounted (assuming an intracellular pH greater than 6.7). In contrast, if this alkalinization occurs *via* active transepithelial transport it may account for some of the disparity between the I_{sc} and ion fluxes measured under similar conditions (Chamberlin, 1990). Under open-circuit conditions, K^+ secretion far exceeds Na^+ absorption and Cl^- secretion (Chamberlin, 1990) and, under such conditions, base secretion may contribute to maintenance of electroneutrality.

Exposure to K^+-free saline for 45 min depressed the rate of open-circuit base secretion by 66.7±1.8% (N=3). As suggested by Dow and Harvey (1988) and Dow and Peacock (1989), luminal alkalization is dependent, to a great extent, on the large K^+-dependent transepithelial potential. The high rates of *in vitro* luminal alkalinization measured in this study indicate that the mechanisms of base secretion and/or acid absorption should be readily described with further experimentation.

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