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SHORT COMMUNICATION

TRANSEPITHELIAL POTENTIAL AND ALKALIZATION IN AN *IN SITU* PREPARATION OF TOBACCO HORNWORM (*MANDUCA SEXTA*) MIDGUT

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The midgut of larval lepidopteran insects rapidly raises the pH of the entering food from approximately neutral values to as high as pH 12 in some species (Waterhouse, 1949; Dow, 1984). These values are among the highest achieved by biological systems and are all the more remarkable in view of the high rate of food consumption relative to body mass in lepidopteran larvae.

The isolated midgut of lepidopteran larvae secretes K^+ at a high rate by an electrogenic mechanism that generates a transepithelial electric potential (TEP) which may initially exceed 100 mV in isolated preparations (Harvey *et al.* 1968). It has been suggested (Dow, 1984, 1992; Dow and Harvey, 1988) that alkalization is a secondary process driven by the TEP, although Dow (1992) recognized that an almost unrealistically high TEP would be required *in vivo* to account for measured H^+ activity gradients of 3–5 orders of magnitude in freshly dissected animals. Values for the TEP of the midgut *in vivo* of 'around 150 mV' (Dow and Harvey, 1988) or 'between 100 and 150 mV' have been described as 'accepted' (Dow, 1992). However, to our knowledge, no experiments supporting these values have been published.

Alkalization has been studied using isolated tissues, but the pH gradients and rates of alkalization achieved by those preparations have been less impressive than those measured or estimated *in vivo*. In the work of Dow and O'Donnell (1990), the maximum pH gradient of an isolated, double-perfused preparation was about 2 pH units, a value not inconsistent with Nernstian equilibrium with the transepithelial potentials typical of such preparations. It should be emphasized that these measurements were from small volumes of solution in the microenvironment immediately adjacent to the epithelium. During hypoxic transitions, the pH gradients and transepithelial potential were lost within minutes of imposition of N_2-hypoxia and recovered on approximately the same time scale as the recovery of the TEP, suggesting that H^+ equilibrated readily across the gut epithelium under hypoxic conditions.

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Chamberlin (1990) used a pH-stat technique to measure alkalization rates of preparations mounted on flat sheets and found that luminal alkalization continued under short-circuit conditions, providing evidence for a thermodynamically active process. However, a more rigorous test of active alkalization, requiring pH-stat measurements on both sides of the gut under short-circuit conditions, was not performed. Chamberlin's experiments differ from those of Dow and O'Donnell (1990), cited above, in that an enhanced saline containing both amino acids and carboxylic acids was used. Although Chamberlin's experiments suggest active alkalization, the rates measured under open-circuit and short-circuit conditions were, on average, no higher than 8.9 and 7.3 μequiv $cm^{-2}\,h^{-1}$, respectively, whereas Dow (1984) calculated that a minimum total current of 52 μequiv h^{-1} is required to account for alkalization in a fifth-instar larva weighing 5 g. On the basis of our experience with isolated midgut preparations, the nominal surface area of the anterior and middle midgut of a 5 g larva is, at most, 2 cm^2, which would give a total current of only about 18 μequiv h^{-1}, assuming Chamberlin's (1990) short-circuit current values. Furthermore, in contrast to the results of Dow and O'Donnell (1990), the presence or absence of a TEP had almost no effect on the rate of alkalization, suggesting that electrodiffusion had almost no role in alkalization.

In the present studies, the transmembrane H^+ gradient and the TEP were measured simultaneously to determine whether $[H^+]$ approaches electrochemical equilibrium across the midgut *in situ*. The present studies assessed whether the magnitude of the TEP achieved *in vivo* was consistent with passive alkalization. If this is not so, an active process must be invoked.

Tobacco hornworms (*Manduca sexta*) were raised as in previous studies (Moffett *et al*. 1982). Larvae weighing 7–9 g, and in day 4 of the fifth instar, were used. Although larvae of this size have probably undergone the first endocrine preparations for moulting (Gilbert *et al*. 1981), in our laboratory such larvae were still actively feeding and did not show pre-moult signs. In our experience, isolated midguts from such larvae reliably had initial TEP values of 90–110 mV.

Larvae were anaesthetized by chilling to the point of immobilization (approximately 10 min), and preparative procedures were carried out with the larva embedded in crushed ice. The cuticle of the last segment was punctured paradorsally with a 20 gauge needle. A voltage bridge of polyethylene tubing (PE 20) filled with 3 mol l^{-1} KCl agar was inserted and passed anteriorly in the haemocoel, to the level of the middle midgut. The bridge was secured by a ligature to the 'horn' of the last segment. For insertion of a luminal voltage bridge, the anterior end of the animal was amputated behind the first segment and the bridge was ligated into the midgut lumen with its tip at the level of the middle midgut. A second ligature around the body of the animal, and posterior to the first, further secured the luminal bridge, precluded leakage of haemolymph and prevented an electrical short circuit between the midgut lumen and the haemocoel. The locations of the bridges were verified by dissection at the end of the experiment. The bridges were connected to a high-impedance voltmeter (Chemtrix type 60A) *via* baths containing 3 mol l^{-1} KCl. TEP was recorded at 5 or 10 min intervals, beginning as soon as the animal had returned to room temperature and continuing for 15–60 min.

Most preparations displayed periods of vigorous motor activity that introduced motion

artefacts into the TEP measurement and threatened the integrity of the preparation. This behaviour was found to be inhibited by tactile stimulation of the body surface and was minimized by cradling the animal in an acrylic tube that loosely fitted the body.

Measurements of H^+ activity were made with pH electrodes (Transidyne General, model 801) intended for intracellular use. Extensive preliminary calibrations using acetate, Tris and Taps as well as the standard phthalate, phosphate and borate buffers showed that these electrodes were insensitive to buffer composition and to changes in K^+ concentration. For the measurements reported here, the electrodes were calibrated at three points in acetate, phosphate and borate buffer solutions (VWR Scientific, West Chester, PA) immediately before each measurement, and a calibration curve was generated by fitting to the Nernst equation.

The H^+ activities of the haemolymph and the midgut contents were measured at the end of each experiment as follows. A small incision was made in the body wall, and a pH electrode secured in a micromanipulator was positioned with its tip in the haemolymph adjacent to the midgut for measurement of haemolymph pH. The haemolymph pooled in the incision was then sponged away and the pH electrode was plunged directly through the wall of the middle midgut into the lumen, for measurement of luminal pH. The implanted haemolymph and luminal voltage bridges were used as references for these measurements.

The transepithelial potentials of the *in situ* preparation typically rose or fell slightly after mounting, but showed no systemic changes over experiments lasting up to 1 h. The mean values of final transepithelial potential, haemolymph pH and lumen pH for six experiments are given in Table 1. For each experiment, the TEP predicted by the Nernst equation, for a passive H^+ distribution on the basis of the measured pH difference, was compared with the final measured value. If H^+ were distributed passively, 58 mV of TEP would be required to account for each unit of pH difference. The mean of these comparisons (Table 1) shows measured TEP smaller than predicted TEP by a value equivalent to almost 2 pH units. This pattern was seen in every experiment; the smallest difference observed was 52 mV, corresponding to slightly less than 1 pH unit. In summary, the pH gradient across the middle midgut in the *in situ* preparation was always at least an order of magnitude larger than that expected for a purely passive distribution. The present studies treat the midgut epithelium as a ‘black box’. Irrespective of the mechanism, if the system as a whole is in steady state, persistent non-equilibrium

Table 1. *Measured (TEP) and predicted (TEP_{pred}) transepithelial potentials of the midgut of* Manduca sexta

	Haemolymph pH	Lumen pH	pH difference	TEP (mV)	TEP_{pred}–TEP (mV)
Mean	6.64	10.05	3.41	91.2	106.4
S.D.	0.33	0.46	0.32	14.82	29.06

Predicted values were calculated from the Nernst equation on the basis of passive H^+ distribution. Values given are the means and S.D. for six experiments.

distribution of H^+ is evidence for active transport of H^+, or an equivalent ion, across the midgut epithelium.

The midgut preparation described here has the advantages of spiracular respiration and complete haemolymph. The haemolymph and luminal pH values (Table 1) recorded at the end of the experiments are similar to those obtained from rapidly dissected animals (Dow, 1984). Evidence for an essentially steady state is also provided by the stability of the TEP over experimental periods that are long compared with the apparent rates of diffusion of H^+ across the epithelium (Dow and O'Donnell, 1990) and comparable to the normal gut turnover time for this animal. The stability of the TEP of this preparation is better than that of the isolated preparations immediately after mounting. Although the TEP of the isolated preparation characteristically declines steadily over time, the magnitudes of the TEP values recorded from the *in situ* preparation are not markedly different from those typical of the isolated preparation during the first hour after mounting, even those bathed in a minimal saline containing only KCl, sucrose and buffer. This result suggests that, at least in this respect, the isolated preparation corresponds reasonably closely to the physiology of the midgut tissue *in vivo*.

Despite several decades of intensive study of the transport activities of the lepidopteran midgut, there is relatively little information available to support hypotheses about mechanisms of active alkalization. The association of carbonic anhydrase with the goblet cell apical membrane in the anterior and middle midgut (Ridgway and Moffett, 1986) suggests that the goblet cell could be responsible for the anionic component of active alkalization. Azuma *et al*. (1991) have shown that a soluble alkaline phosphatase is also associated with goblet cell apical membranes and have suggested that this enzyme is identical to the HCO_3^--sensitive ATPase reported earlier (Deaton, 1984) and that it may be responsible for alkalization. However, the concentration of this alkaline phosphatase is highest in the posterior midgut, the region presumed to be least active in alkalization. These results have led Dow (1992) to propose that columnar cells are involved in the anionic component of base secretion. Evidence for Cl^-/HCO_3^- exchange across the apical membrane of posterior midgut columnar cells has been presented (Chao *et al*. 1989), but similar investigations on middle midgut and anterior midgut columnar cells have not yet been carried out. The spiracular respiration of the preparation described here is assumed to preserve a haemolymphal P_{CO_2} similar to that of intact animals, in contrast with isolated preparations, which are typically bathed in solutions equilibrated with 100 % oxygen. This factor could well be important for a mechanism that involves secretion of bicarbonate or carbonate.

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