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SHORT COMMUNICATION

HIGH pH IN THE ECTOPERITROPHIC SPACE OF THE LARVAL LEPIDOPTERAN MIDGUT

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The midgut lumen of lepidopteran larvae is highly alkaline (Waterhouse, 1949; Berenbaum, 1980), a condition maintained in the presence of a large and opposing transapical H^+ gradient from the midgut epithelial cells. Lumen pH values of approximately 11 may be common (Dow, 1984), whereas the cell cytoplasm itself is maintained at approximately neutral pH (Dow and O'Donnell, 1990; Chao *et al.* 1991), giving rise to cell/lumen H^+ concentration ratios of 10^4 or more. Dow (1984) reported a mean pH of 12.0 in the midgut of a sphingid larva, *Acherontia atropos*, and Schultz and Lechowicz (1986) recorded a maximum reading of pH12.4 in the European gypsy moth, *Lymantria dispar*. These values are among the highest recorded in any biological system.

The high alkalinity is mediated by active K^+ secretion into the midgut lumen (Harvey and Nedergaard, 1964; Dow, 1986, 1992), a process driven by an ATP-dependent, electrogenic H^+ pump coupled with an electrophoretic K^+/nH^+ antiport in the invaginated apical membrane of goblet cells (Wieczorek *et al.* 1991). This system generates a transapical potential difference throughout the midgut (lumen positive to the cell interior) that drives protons passively across the epithelium in the basal direction. At equilibrium, observed lumen pH values agree with those predicted from the Nernst equation (Dow, 1986, 1992). Secretion of HCO_3^- across the apical membrane, and its subsequent conversion to CO_3^{2-}, appears to provide the main anion both for balancing K^+ and for titrating the luminal contents to the high pH (Dow, 1984, 1992). The various components of this model, including the primary role of the V-ATPase (H^+) pump and the way in which it produces an alkaline fluid 'downstream', were reviewed in detail by Dow (1992), Harvey (1992), Moffett and Koch (1992) and Wieczorek (1992).

That the midgut epithelium is responsible for the high luminal pH implies that the pH in the ectoperitrophic space (Richards and Richards, 1977) and in the lumen should be

Key words: *Bombyx mori*, *Choristoneura fumiferana*, *Lymantria dispar*, *Manduca sexta*, *Spodoptera eridania*, ectoperitrophic space, midgut lumen, alkaline pH, *Bacillus thuringiensis.*

similar, for two reasons. (1) The electrical gradient that drives H^+ into the cells must be established first between the interior of the cells and the ectoperitrophic space; consequently, it is from the latter that H^+ must be mobilized initially; (2) any base secreted by the epithelium into the lumen must traverse this space. The first compartment to become alkaline, therefore, should be the ectoperitrophic space, followed by the midgut lumen proper, as a result of passive equilibration across the porous peritrophic membrane. Moreover, the extent to which the lumen itself is alkalized would depend upon the extent to which the ectoperitrophic space is alkalized.

Nevertheless, based on measurements in dissected midguts, Yunovitz *et al.* (1987) reported that the midgut lumen of the lepidopteran larva *Spodoptera littoralis* (Noctuidae) was typically quite alkaline, but the pH at the apical surface of the epithelium was nearly neutral. A similar pattern had been reported earlier in dissected midguts of the lepidopteran *Erinnyis ello* (Sphingidae) (Santos *et al.* 1983). If this condition were to exist *in vivo*, however, it would call into question the above model for lepidopteran midgut H^+ regulation and the origin of the high lumen pH. This apparent contradiction prompted us to measure the pH *in situ* in the midgut lumen and ectoperitrophic space of a number of species of leaf-feeding Lepidoptera as well as in dissected midguts, to determine whether differences could be explained on the basis of experimental procedure. Haemolymph pH values were also measured.

Species from five families were chosen, representing a broad spectrum of leaf-feeding Lepidoptera: *Bombyx mori* (Bombycidae), *Choristoneura fumiferana* (Tortricidae), *Lymantria dispar* (Lymantriidae), *Manduca sexta* (Sphingidae) and *Spodoptera eridania* (Noctuidae). Neither *Erinnys ello* nor *Spodoptera littoralis* was available, which precluded direct comparisons with the species used by Santos *et al.* (1983) and Yunovitz *et al.* (1987), but other species from the same families (*M. sexta* and *S. eridania*, respectively) were included.

Bombyx mori, *C. fumiferana* and *L. dispar* larvae were from laboratory stocks of the Forest Pest Management Institute (Sault Sainte Marie, Ontario). *Manduca sexta* was from Carolina Biological (Burlington, NC) and *S. eridania* was from Rohm & Haas (Springhouse, PA). Experimental insects were a mixture of males and females, reared under controlled temperature, relative humidity and photoperiod, and fed either artificial medium or foliage. All were fifth-instar larvae, except for *C. fumiferana*, which were sixth-instar larvae. Only insects that were determined to be actively feeding were used.

A combination semi-microelectrode with a 1.3mm (o.d.) tip (Microelectrodes Inc., Londonderry, NH), suitable for volumes down to 0.5 μl, was used for the pH measurements. Insects were placed on filter paper and transected just anterior to the first pair of abdominal prolegs, through the middle region of the midgut. The anterior portion of the larva was discarded and haemolymph exuding from the wound in the posterior portion was blotted by the filter paper. The probe was inserted 3–10mm (depending on insect size) through the cut in the posterior portion of the larva into the compartment of interest, and the pH was measured. The middle region of the midgut was chosen for these measurements as it was expected that it would provide maximum pH values and minimize variability. The order in which measurements were made in each insect was (1) ectoperitrophic space, (2) midgut lumen and (3) haemocoel; all three measurements

were completed in about 10–20s. Twenty-five larvae of each species were measured. The pH meter was adjusted periodically for drift and changes in ambient temperature.

Longitudinal pH gradients were measured in two species, *M. sexta* and *S. eridania*. Larvae were treated as described above, except that the cut was made just posterior to the third pair of thoracic legs, through the anterior region of the midgut. The gradient was measured *in situ*, in an anterior-to-posterior direction, first in the ectoperitrophic space and then in the lumen. In all insects, the epithelium was cut longitudinally following the last lumen measurement and the peritrophic membrane was examined to verify that it had not been ruptured.

Measurements made *in situ* show that the ectoperitrophic space and midgut lumen are highly and equally alkaline in every species tested (Table 1). This result is consistent with current models for the origin of the alkaline pH in the lepidopteran midgut and would appear to be the condition *in vivo*. However, the results contradict previous conclusions that the pH at the apical surface of the epithelium is nearly neutral or even less alkaline than in the midgut lumen. Moreover, as illustrated for *S. eridania* (Fig. 1), the longitudinal pH profile observed in the ectoperitrophic space is identical to the profile typically observed in the lumen of the lepidopteran midgut, with the pH rising to a peak in the middle region and declining again in the posterior region (Dow, 1984; Dow and Harvey, 1988). The pattern in *M. sexta* (not shown) was the same. There is no evidence, therefore, to support the claim that the peritrophic membrane protects the epithelium from the high pH in the lumen (Yunovitz *et al.* 1987). The possibility of contamination from lumen contents during pH measurements in the ectoperitrophic space can be discounted, since the former are gel-like in consistency and were easily avoided by the probe. Moreover, the integrity of the peritrophic membrane was confirmed visually following the pH gradient measurements in each insect.

Midgut pH was measured *ex situ* in six *S. eridania* larvae, in a manner similar to that of Santos *et al.* (1983) and Yunovitz *et al.* (1987). The midguts were removed and blotted, the epithelium was cut longitudinally, and the pH was measured on its apical surface and in the food bolus. The values were compared with those measured *in situ* in the same

Table 1. *pH measurements* in situ *in the midgut (middle region) and haemolymph of five species of Lepidoptera*

		pH		
Species	Mass (mg)	Ectoperitrophic space	Midgut lumen	Haemolymph
L. dispar	708±35	10.2±0.13	10.4±0.12	6.6±0.07
S. eridania	633±22	10.3±0.07	10.3±0.07	6.6±0.04
C. fumiferana	102±8	10.4±0.13	10.5±0.12	6.7±0.04
M. sexta	5410±350	10.8±0.22	10.8±0.25	6.6±0.05
B. mori	803±32	11.1±0.14	11.2±0.15	6.8±0.06

Masses are means ± S.E.M., N=10; pH values are mean ± 95% confidence limit, N=25; pH was measured in each insect in all three compartments.

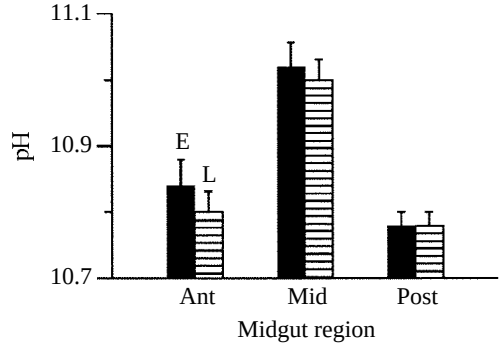

Fig. 1. Longitudinal pH gradients in the larval midgut of *S. eridania*. Midgut regions: Ant, anterior; Mid, middle; Post, posterior; E, ectoperitrophic space; L, lumen. Means + S.E.M., *N*=5.

insects in the ectoperitrophic space and midgut lumen, respectively. The results indicated that, *ex situ*, the epithelium cannot maintain the high pH on its apical surface. Measurements made immediately following dissection showed a marked drop in pH, to more than 2units below the value recorded *in situ* in the ectoperitrophic space. Mean values (±95% confidence limit) *in situ* were 10.8±0.22 in the ectoperitrophic space and 10.8±0.14 in the lumen; mean values in the dissected midgut were 8.2±0.38 at the epithelial surface and 10.4±0.21 in the food bolus. The slightly higher *in situ* values compared with the mean values recorded for *S. eridania* in Table 1 may reflect differences in larval age or time of day when the measurements were made. Such temporal influences on midgut pH have been observed in *L. dispar* (Schultz and Lechowicz, 1986).

In an additional five *S. eridania* larvae, time-course changes in the pH of dissected material were measured in air and in nitrogen. Between measurements, the tissue was kept in a Petri dish with moistened filter paper. Nitrogen exposure was carried out by continuous flushing of the interior of the Petri dish with N_2 at 14–69kPa. The pH at the apical surface of the epithelium exposed to air continued to decline steadily with time (Fig. 2). The effect was considerably enhanced during hypoxia when the tissue was exposed to N_2 (Fig. 2), confirming that establishment of the steady-state H^+ gradient across the midgut epithelium is ultimately an active, energy-requiring process dependent on oxidative metabolism. This conclusion is consistent with previous observations made in isolated midgut preparations, which show that K^+ pumping declines during hypoxia (Haskell *et al.* 1965; Dow *et al.* 1984), as do tissue ATP levels (Mandel *et al.* 1980) and the transepithelial pH gradient (Dow and O'Donnell, 1990).

One factor contributing to the rapid decline in pH at the apical surface of the dissected midgut must certainly be the disruption of the tracheal system and, consequently, a general suppression of O_2-dependent active-transport processes and homeostatic mechanisms (Harvey and Zerahn, 1972). A second factor would be the removal of external K^+, which would lead to a cessation of the steady-state K^+ cycling between basal and apical surfaces of the epithelium. The effect would be to abolish the transapical electrical potential difference (Harvey and Zerahn, 1972; Moffett and Koch, 1988; Chao

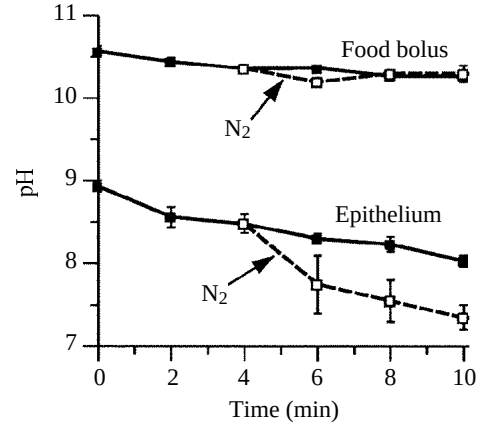

Fig. 2. pH changes in the dissected midgut of *S. eridania* larvae, initially in air and (at arrows, dashed line) in N_2 (open squares). The midgut was removed, blotted and cut longitudinally, and the food bolus, enveloped in the peritrophic membrane, was taken out. Epithelium pH was measured on the apical surface while the latter was wrapped around the electrode tip. Means ± S.E.M. of five insects from 0 to 4min. After 4min, two of the five preparations were exposed to N_2; three remained in contact with air. Points without error bars are larger than the S.E.M.

et al. 1991) and to dissipate the pH gradient that depends on it. Base secretion on the luminal side of the isolated midgut of *M. sexta* is reduced by more than two thirds when the epithelium is exposed to K^+-free saline (Chamberlin, 1990).

The precipitous drop in pH at the onset of hypoxia (Fig. 2) reflects the abrupt disruption of oxidative phosphorylation, general cessation of active transport processes, and collapse of electrical and chemical gradients. Additionally, there may also be bulk leakage of goblet cavity contents onto the apical surface of the epithelium (Moffett and Koch, 1988). The pH in the goblet cavity is very nearly neutral (Chao *et al.* 1991). During periods of hypoxia, and in other situations in which K^+ transport is inhibited, the entire contents of the cavity can be extruded (Moffett and Koch, 1988).

In the light of the results obtained in this study, it seems likely that the nearly neutral pH recorded by Yunovitz *et al.* (1987) at the apical surface of the dissected midgut of *S. littoralis* and the low-alkaline pH observed earlier by Santos *et al.* (1983) in *E. ello* are a consequence of their experimental procedures and do not represent conditions *in vivo*. The dissected food bolus (midgut lumen *in situ*) remains highly alkaline, in agreement with these authors, and, predictably, there is no effect of N_2 (Fig. 2), since the bolus is metabolically isolated from any living tissue that might alter its pH. The proposition that constant proton flow from the lumen to the apical surface of the epithelium could generate a neutral pH at the latter (Yunovitz *et al.* 1987) is untenable, since it would require that either the luminal contents or the peritrophic membrane (itself a non-cellular and non-respiring structure) are somehow actively secreting H^+ against a very steep electrochemical gradient. The absence of any pH change in the dissected food bolus in response to N_2 (Fig. 2) is evidence that such secretion does not occur. Proton flow from the lumen across the peritrophic membrane can only occur as a result of passive diffusion

into an alkaline (rather than neutral) ectoperitrophic space. In any case, direct *in vitro* measurements have now demonstrated that the isolated midgut secretes alkali on its apical side (Chamberlin, 1990) and is capable of establishing and maintaining a standing transepithelial pH gradient (Dow and O'Donnell, 1990). Finally, Dadd (1975) found equally high pH values in the midgut lumen and ectoperitrophic space of mosquito larvae *in situ*.

The high pH in both the midgut lumen and ectoperitrophic space in Lepidoptera is significant for the mode of action and specificity of *Bacillus thuringiensis* as an insect pathogen. The insecticidal activity requires solubilization and digestion of the parasporal crystal in a high-pH medium, which the lepidopteran midgut provides. Neutralizing the pH of toxin solutions has been shown to reduce their toxicity in cell assays (Johnson, 1981) and in the isolated midgut (Yunovitz *et al*. 1987), effects that are often the result of molecular aggregation (Fast, 1981). Recently it was shown that even prior to molecular aggregation, lowering the alkalinity of toxin solutions suppressed activity, probably as a result of changes in molecular charge (Gringorten *et al*. 1992). The high pH in the ectoperitrophic space makes it unlikely that such suppression occurs as toxin diffuses across this space and interacts with the midgut epithelium.

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