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KINETICS OF EXTRACELLULAR SOLUTE MOVEMENT IN THE ISOLATED MIDGUT OF TOBACCO HORNWORM (*MANDUCA SEXTA*)

BY ALAN KOCH AND DAVID F. MOFFETT

Department of Zoology, Washington State University, Pullman, WA 99164-4220, USA

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SUMMARY

Measurements were made of the uptake of ^{14}C-labelled sucrose in the short-circuited isolated midgut of fifth instar larvae of the tobacco hornworm. The long-term volume of distribution after bilateral exposure indicates that the extracellular space is about $0{\cdot}26\,ml\,g^{-1}$ wet tissue, much less than has been previously suggested. The results of unilateral exposure yield a value of about $0{\cdot}04\,ml\,g^{-1}$ for luminal goblet cell cavities and $0{\cdot}22\,ml\,g^{-1}$ for interstitial space. The kinetics of uptake suggest that the interstitial space is best modelled as a distributed volume rather than as a single compartment. This mathematical result fits the physical picture of an interstitial fluid in long, narrow channels which communicate freely with the haemolymph but are nearly impermeable to sucrose at the luminal end; that is, they are sealed by septate junctions. The electrical conductance of the epithelium is high, but the transepithelial permeability to sucrose is very much lower than to K^+. Thus, the tissue is a physically tight epithelium.

INTRODUCTION

The midguts of larval lepidopterans possess a system for the active extrusion of K^+ from haemolymph to gut lumen. This system is characterized by extremely high rates of transport, a dependence of the transport on extracellular concentration of K^+, and a probable active step from the apical end of the cell into a semi-sequestered extracellular post-transport pool in the goblet cell cavity (Moffett & Koch, 1983, 1985; Zerahn, 1982; Moffett, 1979; Dow, Gupta, Hall & Harvey, 1984; Anderson & Harvey, 1966; Harvey, 1980). These characteristics make it likely that there is a reciprocal relationship between the extracellular ion composition and transport rates. The significance of this relationship depends on the ease with which the bulk external solution, either haemolymph or luminal fluid, exchanges with the extracellular fluid of the tissue. Thus an understanding of the properties of transfer between the extracellular compartments and the bulk solution is important to an understanding of the control of ion transport. Although steady-state volumes of distribution have been reported (Zerahn, 1975; Giordana & Sacchi, 1977, 1978*a*,*b*; Abramcheck,

Key words: tobacco hornworm midgut, [^{14}C]sucrose extracellular space, mathematical model.

Blankemeyer & Harvey, 1980; Sacchi, Cattaneo, Carpentieri & Giordana, 1981), a kinetic analysis has not previously been made.

Accordingly, we determined the kinetics of uptake of the extracellular marker sucrose into the posterior midgut of the larval tobacco hornworm, *Manduca sexta*.

MATERIALS AND METHODS

The morphologically distinct posterior midgut of fifth instar larvae of the tobacco hornworm, *M. sexta*, was excised from cold-anaesthetized animals. The peritrophic membrane and gut contents were discarded. Except where stated, the Malpighian tubules were carefully stripped from the gut. The gut was then mounted in a modified, reduced Ussing chamber.

The bathing solution was normally our '32K divalent-free solution', consisting of (in $mmol\,l^{-1}$): KCl, 32; Tris hydrochloride, 5; and sucrose, 166. For the uptake experiments, the solution contained [^{14}C]sucrose in an amount that allowed us to count the tissue samples to 1% accuracy in a reasonable time. The tissue was maintained under short-circuit conditions and compensation was made for solution resistance as described previously (Moffett & Koch, 1985). At the appropriate time, the solution on one or both sides was replaced with the solution containing isotope.

Uptake experiments

After a predetermined time of exposure to [^{14}C]sucrose, the chamber was drained and opened, the tissue was dismounted and blotted on filter paper, and a circular core of about 0·7 cm in diameter was cut out. The core was divided into two pieces which were then weighed to within 0·1 mg. This operation was completed within 60 s of the time the chamber had been drained. One piece was dried at 95 °C to constant mass and then reweighed. The second piece was dissolved in Baker Tissue Solulyte. Research Products Inc. 3A70B was added as a spectrum-shifting agent and, after dark adaptation, the sample was counted by liquid scintillation. Samples (20 μl) of the bathing solution were treated identically and also counted. Samples were routinely counted to 10 000 total counts. For all samples except those at 3 and 6 min, direct determination of counting efficiency was computed from the additional count rate subsequent to the addition of a known amount of isotope. Samples from the bathing solution and from tissue showed the same efficiency; that is, no quenching from tissue components was observed.

Tissues were kept in short-circuit conditions for 1 h. The isotope solution was added at various times before the end of the hour so that, although the time of exposure to ^{14}C was varied, the actual incubation times of all the tissues were identical. Both bilateral and unilateral experiments were performed. Isotope was on both sides of the tissue for bilateral experiments. For the unilateral experiments, it was placed either on the haemolymph side only (H-experiments) or on the lumen side only (L-experiments). In the unilateral experiments, 100-μl samples were collected every 12 min from the opposite chamber and counted to determine the transepithelial flux of isotope. Each of these samples was replaced with isotope-free

solution to maintain the balance of hydrostatic pressure between the two chambers. A few experiments were performed under open-circuit conditions.

Hydrolysis measurements

The amount of reducing sugar present in the bathing solutions was tested using the quantitative hexokinase reaction (Sigma glucose diagnostics procedure no. 16-U.V.). Initial bathing solutions were used as blanks so that the measurement was that of any increase in reducing sugar content during incubation.

RESULTS

Electrical activity

All preparations were monitored for electrical activity. Short-circuit current was determined at the end of 1 h of incubation, that is just before the tissue was taken out of the chamber. The average short-circuit current from 92 experiments was $870 \pm 22\,\mu A\,cm^{-2}$ (±S.E.M.). Neither time of exposure nor side of exposure to isotope had any significant effect on the current.

Invertase activity

The extent to which sucrose was hydrolysed by invertase in the gut was investigated in three preparations exposed under short-circuit conditions to the normal concentration of sucrose ($166\,mmol\,l^{-1}$) in the absence of isotope. The tissues were rinsed once to mimic the addition of isotope and then left for 48 min (equal to the longest exposure to isotope). Samples were then taken from both bathing solutions and analysed for reducing sugar content. Both dextrose and fructose react so the results were calibrated against a set of solutions containing equal concentrations of the two monosaccharides. At the end of 48 min of incubation, 1·27 % of the sucrose had been hydrolysed (Table 1). Even though there was a variation of a factor of two between different tissues, the variation was less than 10 % within each tissue.

Total water content

Every tissue was divided into two pieces and one piece was used for the determination of water content. The value obtained did not vary either with time or

Table 1. *Hydrolysis of sucrose*

Experiment	mmol of sucrose hydrolysed in 48 min: haemolymph	lumen	Average
1	3·03	2·81	2·92
2	1·44	1·50	1·47
3	2·01	1·87	1·94

Average = 2·11 = 1·27 % of 166

with side of exposure to radioactive sucrose. The data from 92 experiments were therefore pooled. Total water was estimated to be $0{\cdot}7665 \pm 0{\cdot}0026\,ml\,g^{-1}$ wet tissue (±S.E.M.).

Bilateral uptake

To estimate total tissue sucrose space, preparations were exposed to the isotope on both sides. Six experiments were performed at each of the five different times (3, 6, 12, 36 and 48 min), although it should be emphasized that all tissues had been in the short-circuit condition for 1 h. The volume of distribution of radioactive sucrose rapidly rose towards an equilibrium value of near $0{\cdot}25\,ml\,g^{-1}$ wet tissue (circles, Fig. 1). Half-time for this uptake was 4 min.

Unilateral experiments

At least six experiments were performed at each of the same times with label added only to the haemolymph (H-experiments) and six experiments at each of these times with label added only to the luminal side (L-experiments). Samples of fluid from the opposite chamber were taken every 12 min so that each of the longer experiments led to 1, 3 or 4 values for [label] in the trans solution. The ratio of trans-to-cis concentrations never exceeded 0·001.

In the L-experiments, volumes of distribution rose quite rapidly to a volume of $0{\cdot}06\,ml\,g^{-1}$ tissue (triangles, Fig. 1). The half-time for this rise was less than 2 min. The volume then showed a second component which rose much more slowly. At 48 min, the volume of distribution had reached only $0{\cdot}11\,ml\,g^{-1}$. The H-experiments

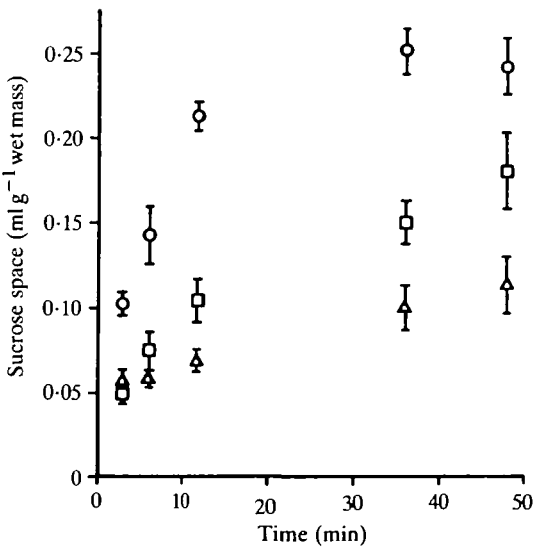

Fig. 1. Volumes of [^{14}C]sucrose distribution at times between 3 and 50 min after exposure to label. Bilateral exposures are represented by circles, basal exposures by squares and apical exposures by triangles. Values are means from at least six experiments. Vertical bars give 1 standard error.

showed a slower early uptake than did the luminal ones (squares, Fig. 1). This slower early uptake changed into a second slow component with about the same slope as that seen in the L-experiments. At 48 min, the sucrose space was 0·18 ml g^{-1} in the H-experiments.

The rate at which label crossed the tissue was so low that the trans concentrations were often less than twice background. Hence, these samples were counted to a lower accuracy than were any of the others reported here. Although there was no question that there was isotope crossing the tissue, the accuracy of flux estimates so derived was low. Isotope characteristically crossed from lumen to haemolymph at a faster rate than in the opposite direction. For each type of experiment, the values of trans-to-cis bathing solution concentration ratios were pooled for a 12-min average (all 12-, 36- and 48-min experiments), for a 24-min average (all 36- and 48-min experiments), for a 36-min average (all 36- and 48-minute experiments) and for a 48-min average (all 48-min experiments). A regression of these average values of concentration ratios against time was forced through zero. The slope of this line is a direct measure of the permeability of the tissue to label. The values for these permeabilities were $1{\cdot}9\times10^{-4}$ ml min^{-1} cm^{-2} for lumen-to-haemolymph flux and $8{\cdot}2\times10^{-5}$ ml min^{-1} cm^{-2} for haemolymph-to-lumen flux.

Other uptake experiments

Abramcheck *et al.* (1981) have estimated that the extracellular space of this tissue is about 0·40 ml g^{-1} tissue (0·40 ml g^{-1} divided by their value of 0·84 ml H_2O g^{-1} gives their value of 0·48 ml ECF ml^{-1} total water). This value is about 65 % higher than ours. The conditions under which they conducted their experiments were different from ours in three ways: their tissues were incubated under open-circuit conditions; they used a bathing solution that contained 1 mmol l^{-1} Ca^{2+} and 1 mmol l^{-1} Mg^{2+}; and they used middle midgut (personal communication). We conducted a small number of experiments to see which differences were crucial. Three groups of four experiments each were conducted. All were 48-min bilateral exposures. Group I was run under short-circuit conditions with the Malpighian tubules removed, but in the presence of 1 mmol l^{-1} of each of the divalent cations. Group II was conducted under open-circuit conditions with the Malpighian tubules removed, in the presence of the divalent cations. Group III was open-circuited, with the Malpighian tubules still present.

As shown in Table 2, inclusion of divalent ions (group I) had no effect either on the total water content or on the sucrose space. Open-circuit conditions (group II) probably led to a reduction of total water content; the difference between groups I and II is significant at about the 0·07 level of probability. However, these open-circuit conditions may have led to an increase in sucrose space. This increase, from 0·24 to 0·28 ml g^{-1}, was about one-half of the total increase that we saw in sucrose space. Finally, the presence of Malpighian tubules, although not affecting tissue water, did further increase the measured sucrose space.

Table 2. *Sucrose spaces in several conditions*

Group	Conditions	Total tissue water (ml g^{-1} tissue)		Sucrose space (ml g^{-1} wet tissue)		N
This paper	SC, NT, ND	0·781*	0·013	0·241	0·017	6
Group I	SC, NT, D	0·776	0·006	0·246	0·007	4
Group II	OC, NT, D	0·743	0·012	0·282	0·026	4
Group III	OC, T, D	0·747	0·009	0·337	0·025	4
Abramcheck†	OC, T, D	0·84	0·019	0·403	0·018	—

SC, short-circuit; OC, open-circuit; NT, no tubules; T, tubules present; ND, no divalent cations: D, divalent cations present.

* This is the value only from the group of six experiments with bilateral exposure of 48 min. The value of 0·767 quoted above was the average of all 92 experiments.

† From Abramcheck, Blankemeyer & Harvey (1980).

DISCUSSION

Invertase activity

The observed hydrolysis of sucrose corroborates other studies (Matsumura & Oka, cited in Ito & Tanaka, 1959; Ito & Tanaka, 1959; Hanozet, Giordana & Sacchi, 1980). The level of enzyme activity can be roughly calculated from our results. Our preparations typically weighed about 20 mg, of which 23·4 % was solid. Thus we had about 4·7 mg of protein. At 48 min, 1·27 % of 166 μmol ml^{-1} × 11 (the volume of the two chambers) had been hydrolysed at 22 °C. This is a rate of 0·1 μmol min^{-1} mg protein at a temperature of 22 °C, and is comparable with the sucrose activity of 0·242 μmol min^{-1} mg^{-1} protein found in crude homogenates of midgut from another lepidopteran larva, *Philosamia cynthia* (Hanozet *et al.* 1980).

Accuracy of our results

Sucrose hydrolysis

Since some of the sucrose is hydrolysed, there is some uncertainty in measurements of both volume of distribution and of transepithelial fluxes. We shall defer evaluation of the flux measurements and discuss only the volume measurements here.

The question of accuracy of the space measurements is not really a question of whether there is hydrolysis, but rather a question of whether sucrose and its hydrolysis products behave differently in the tissue. An erroneously low estimate of space would be computed if, for example, all the dextrose and fructose in the bathing solution remained there, but those compounds were metabolized within the tissue with the ^{14}C being lost as CO_2. Our estimates of sucrose hydrolysis rates suggest that this is not a serious problem. At 48 min, 1·3 % of the sucrose had been hydrolysed. Thus, hydrolysis could lead to an absolute error of 0·013 × 0·24 = 0·003 ml g^{-1} tissue. This error, for a 48-min exposure, is the largest error that we would expect to see; for earlier times, we would expect proportionately less error. The standard error of our

measurements was $0{\cdot}017\,ml\,g^{-1}$. Thus the error from hydrolysis would have been lost in the sample-to-sample variation.

No difference has been found between inulin and sucrose space in *M. sexta* (Abramcheck *et al.* 1980) and only a small and probably non-significant difference was found in *Bombyx mori* (Giordana & Sacchi, 1977). The results of our hydrolysis experiments suggest that the hydrolysis rate is too low to exceed normal animal variability.

Tissue water content

The water content reported by Abramcheck *et al.* (1980) was attributed to Wolfersberger *via* a personal communication and was on the basis of four determinations. Their value was $0{\cdot}84\,ml\,H_2O\,g^{-1}$ tissue. Our value of $0{\cdot}767\,ml\,g^{-1}$ tissue was on the basis of 92 determinations from the main series. Neither the addition of Ca^{2+} and Mg^{2+} nor the use of open-circuit conditions increased the water content in our hands. Indeed, there was the suggestion that tissues have a lower water content in open-circuited than in short-circuited experiments. A similar suggestion is provided by X-ray microprobe analysis of midgut in hypertonic solutions (Dow *et al.* 1984). An average of X-ray data from all portions of both columnar and goblet cells, without any weighting for relative volume, gives values of $0{\cdot}741\,ml\,H_2O\,g^{-1}$ tissue for short-circuited preparations and $0{\cdot}717\,ml\,g^{-1}$ for open-circuited ones. The slightly lower water content of these results can be explained by the use of hypertonic solutions.

It seems unlikely that differences in blotting technique can account for the experimental differences between these reports. Almost 0·5 g of adherent water would have to be added to 1 g of well-blotted tissue to elevate the water content from 0·77 to $0{\cdot}84\,ml\,g^{-1}$ wet tissue. We assume that the very high water content observed by Wolfersberger is a characteristic of the middle midgut.

Extracellular space

Our estimates of extracellular space are only about 60 % of Abramcheck's. About one-half of the difference can be attributed to the different experimental conditions; the rest may stem from the difference in the portion of the midgut that was used.

In comparison with our results, both the high water content and the high extracellular space reported by Abramcheck *et al.* (1980) can be accounted for fairly well if there is simply an additional volume of extracellular fluid present in the middle midgut. This additional volume would increase both extracellular volume and total water content. As we discussed above, we do not believe that the additional volume would be adherent to the outside of the tissue, since the additional fluid would make up one-third of the tissue sample. However, the middle midgut may be the site of absorption of organic material and water absorption may occur *pari passu* with this process. Such passive water transport would be expected to entail a swelling in the lateral interstitial spaces. In addition, goblet cell volume is greater in the middle

portion than in the posterior portion of the midgut (Cioffi, 1979). It seems possible that these functional differences might account for the high fluid volumes observed by Abramcheck *et al.* (1980).

Tight epithelium with low electrical resistance

Resistance across the epithelium was relatively low, being $50{\cdot}2\,\Omega cm^2$. This value is only 2·8 times the resistance of an equivalent sheet of bathing solution (Robinson & Stokes, 1959). For comparison, Moffett (1980) obtained a value of $55\,\Omega cm^2$ in the presence of Ca^{2+} and Mg^{2+}, a bathing solution in which I_{sc} is lower than in the solution used here (Moffett & Koch, 1983, 1985) and resistance is correspondingly higher. Tissue resistance can be converted into a value for permeability in units of $ml\,min^{-1}$ by using the diffusion coefficient of KCl. The permeability of this tissue in our chamber was $3{\cdot}32\times10^{-3}\,ml\,min^{-1}\,cm^{-2}$. We have a completely independent estimate of permeability which can be derived from the backflux of $^{42}K^+$ during isotope flux experiments (Moffett & Koch, 1985). This value is computed to be $1{\cdot}92\times10^{-3}\,ml\,min^{-1}\,cm^{-2}$. Agreement between these two estimates is satisfactory and we can say that permeability to ions of this preparation is about $2{\cdot}5\times10^{-3}\,ml\,min^{-1}\,cm^{-2}$.

In these experiments, we measured two different values of transepithelial isotope flux. The haemolymph-to-lumen flux was $8\times10^{-5}\,ml\,min^{-1}\,cm^{-2}$. Isotope flux was about twice that value in the opposite direction. Hydrolysis of sucrose does occur and the similarity of concentrations of the products of hydrolysis that we found on the two sides of each tissue suggests that the hydrolysis products cross the gut easily. Intestinal saccharase is on the luminal border (Hanozet *et al.* 1980) and we would therefore expect that some of the lumen-to-haemolymph flux would be of hydrolysis product. Because any material that crossed the gut would be greatly diluted, very little flux in the reverse direction would be expected to be of hydrolysis product. We suggest that the haemolymph-to-lumen isotope flux that we measured is a measure of the true transintestinal flux of sucrose, but that the flux in the opposite direction is made up of two components: flux of sucrose and flux of hydrolysis products. The permeability to sucrose computed from the haemolymph-to-lumen flux we found was only one-thirtieth of the ionic permeability computed above. If any of the haemolymph-to-lumen flux of ^{14}C that we measured in these experiments was hydrolysis product, then the sucrose permeability is an even smaller fraction of the ionic permeability than computed above.

Two conclusions can be drawn from comparison of these different estimates of permeability. The first is that the midgut is truly a tight epithelium to non-electrolytes, but one with a low electrical resistance. This fits a pattern that has been found in a number of other epithelial tissues (Augustus, Bijman & van Os, 1978; Wood & Moreton, 1978; Hanrahan & Phillips, 1984). The second conclusion comes from the agreement between the total electrical resistance and the permeability of the tissue to K^+. The agreement suggests that the tissue is nearly impermeable to

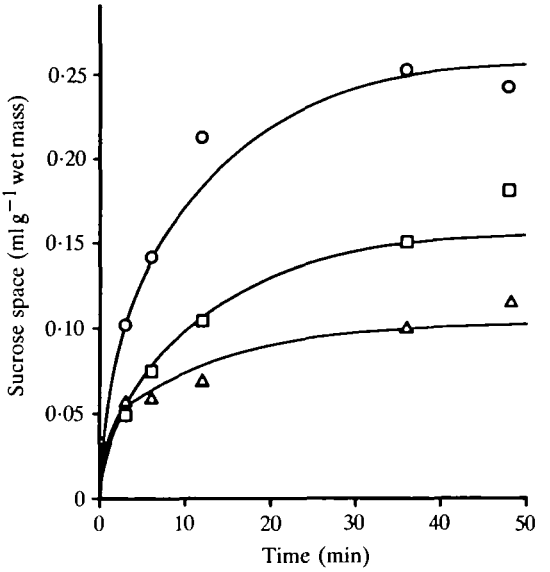

Fig. 2. Fits of the model to the data. For comparison, data points from Fig. 1 are shown (circles, bilateral exposure; squares, basal exposure; triangles, apical exposure).

chloride. This conclusion is compatible with other experiments from this laboratory (unpublished observations).

Models of the extracellular fluid

The data presented here allow us to construct a tentative model of the extracellular fluid in this tissue. This model yields the fits to the experimental data shown in Fig. 2. To show how the model agrees with the experimental findings, the data points from Fig. 1 are superimposed on the curves generated from the model. The main features of this model are as follows:

(1) Extracellular fluid is composed of two moieties, goblet fluid and interstitial fluid.

(2) The goblet compartment communicates with the luminal bathing solution and this exchange is quite rapid.

(3) The interstitial fluid communicates freely with the basal bathing solution.

(4) Transepithelial movement of sucrose is quite slow. It is limited by septate junctions near the apical border of the intestinal cells.

Because of the slow transepithelial movement, the unilateral experiments measure different things. At early times, L-experiments show only the contribution of the goblet cavity. Later, as some material does cross the septate junctions, a second contribution from the interstitial space is apparent. Conversely, H-experiments only measure the uptake in the interstitial fluid. Since isotope activity in the luminal

bathing solution was always low, the goblet cavity would never be expected to contribute to the space measured in the H-experiments.

At late times, both L- and H-experiments show contributions from material in the interstitial space. However, in neither case is the space measured for the interstitial fluid an accurate measure of the interstitial fluid volume. So long as there is material passing across the epithelium, the steady-state activity is lower in the interstitial fluid than in the unilaterally labelled bathing solution. Just how different the steady-state activity of the interstitial fluid is from the bathing solution is determined by a single parameter which is the ratio of the tight septate junction permeability to the effective permeability through the interstitial space. This parameter, which we shall call 'M', is more precisely defined and discussed below.

In the L-experiments, the volume of distribution rose to about 0·05 ml g^{-1} tissue within the first 3 min. There was then a much slower second component of uptake. It seems reasonable to suggest that the early rapid uptake was into goblet cell cavity and that the slower later uptake was entry across the septate junction into interstitial fluid. If this is a correct analysis, then goblet cavity volume is in the range of 0·03–0·05 ml g^{-1} tissue and transfer of sucrose into the goblet cavity is very fast; faster than we could follow accurately with 3-min resolution. (These experiments were done in chambers that kept the tissue short-circuited. The turnover time from solution reservoir to tissue is about 1 min in the smallest of these chambers. Three minutes was the shortest time we could use and describe the addition of isotope as a step function.)

Our total sucrose space was about 0·26 ml g^{-1} tissue. Approximately 0·04 ml of this is goblet cavity volume and so about 0·22 ml is interstitial space. Interstitial fluid is frequently modelled as one or several well-mixed compartments. Parameters can be chosen so that these models fit the data on total volume and rates of uptake fairly well. However, all models with only one or two compartments that fit our data for volumes of distribution predict transepithelial fluxes at least 10 times higher than those we measured. Thus a simple compartmental model of the interstitial fluid fails to explain all the data.

Histological studies of intestine show that it is made up of a layer of epithelial cells which are closely joined at the apical end by septate junctions, but which leave open intercellular clefts to communicate with the bathing solution at the basal surface (Cioffi, 1979; Hakim & Baldwin, 1984). Indeed, much of the interstitial fluid seems to be in these lateral interstitial spaces. Once material is in these long narrow spaces, limitation to movement comes from the time needed for diffusion in distance, rather than from crossing sharp boundaries.

Since no simple compartmental model adequately described the results, we chose to solve the more realistic model of diffusion through long unmixed channels which are open to the haemolymph, but closed at the luminal end with a membrane of low permeability. This model is mathematically more complex than compartmental models, but it is physically as simple or simpler. A one-compartment model of the interstitial fluid requires three physical parameters: the volume and two boundary

permeabilities. Each additional compartment entails the addition of two more physical parameters. The diffusion model is always completed with three physical parameters: the volume, the permeability at the apical boundary, and the normalized diffusion coefficient (the ratio of the diffusion coefficient to the square of the thickness of the tissue). Further, we can place some bounds on the possible values for the diffusion coefficient; it certainly cannot be higher than the diffusion coefficient of sucrose free in solution. Solutions of this diffusion problem fit the experimental data both for volumes of distribution and transintestinal flux reasonably well. In addition, the way the solution behaves adds some insight to the way solute exchange takes place in this tissue.

The diffusion model treats the midgut, when mounted in a chamber, as a sheet. There is no membrane-like surface barrier to diffusion from the basal side and the whole interstitial volume can be filled from the basal side. This is a diffusion process because almost all of the interstitial fluid is in the lateral interstitial spaces. These are long and narrow and are not well-mixed compartments. At the apical end of the lateral intercellular spaces, a septate junction comprises a membrane-like barrier to diffusion. Sucrose can cross this barrier, but only slowly. The two sides of the intestine thus have two different kinds of barriers for entry of solute from extracellular to interstitial fluid. The main factor controlling entry of solute from the luminal side is the impedance of crossing the septate junction. The main factor controlling entry from the haemolymph side is diffusion in distance through the long, unstirred intercellular channels. In addition to the interstitial fluid, there is a goblet cavity compartment. The goblet cavities open to the apical surface and act as a well-stirred compartment. Fig. 3 gives a diagram of the tissue.

The goblet cavities act as normal compartments and the solutions are the standard ones of compartmental kinetics. The ratio of isotope activities between goblet fluid and bathing solution is:

$$C_{gob}/C_{bath} = 1 - \exp(-t/\tau), \tag{1}$$

where τ is V_{gob}/λ and λ is the admittance of the goblet pore. (Functional dependence is indicated by parentheses. Multiplication is indicated by square brackets or curly braces.)

The ratio of activities between interstitial fluid and bathing solution depends on the experimental conditions. With bilateral exposure, this ratio is:

$$C_{bilat}/C_{bath} = 1 - \sum_{i=1}^{\infty} \left\{ M\left[\sin\left(\frac{\gamma_i x}{l}\right) + \sin\left(\gamma_i - \frac{\gamma_i x}{l}\right)\right] + \gamma_i \cos\left(\gamma_i - \frac{\gamma_i x}{l}\right)\right\} \frac{\gamma_i}{A_i} \exp\left(-\frac{\gamma_i^2 Dt}{l^2}\right), \tag{2}$$

where x is the distance from the basal side; l is the thickness of the gut, in cm; D is the diffusion coefficient in $cm^2 s^{-1}$; α is the junctional permeability in $cm\,s^{-1}$; M is a

dimensionless parameter = α/D^{-1}; γ_i is the i^{th} root of the function $\gamma_i\cot(\gamma_i) + M = 0$; and

$$A_i = \gamma_i^2[\gamma_i\sin(\gamma_i) - \{1 + M\}\cos(\gamma_i)]. \tag{3}$$

The average activity ratio over the whole tissue is:

$$C_{bilat}/C_{bath} = 1 - 2\sum_{i=1}^{\infty} \frac{2M[1 - \cos(\gamma_i)] + \gamma_i\sin(\gamma_i)}{A_i} \exp\left(-\frac{\gamma_i^2 Dt}{l^2}\right). \tag{4}$$

The sucrose space of the tissue is the sum of the true interstitial fluid volume (V_{in}) times the average activity ratio in interstitial fluid and the product of the goblet volume and its activity ratio:

$$\text{sucrose space} = V_{in}\left[\frac{C_{bilat}}{C_{bath}}\right] + V_{gob}[1 - \exp(-t/\tau)], \tag{5}$$

where V_{gob} is the goblet volume.

For the L-experiments, the goblet contribution is the same as in the bilateral experiments, but the solution for the interstitial fluid differs. The sucrose space for L-experiments is:

$$V_{in}\left[\frac{M/2}{1 + M}\right] - 2\sum \frac{M[1 - \cos(\gamma_i)]}{A_i} \exp\left(-\frac{\gamma_i^2 Dt}{l^2}\right) + V_{gob}[1 - \exp(-t/\tau)]. \tag{6}$$

H-experiments contain no contribution from the goblet cells. The sucrose space for H-experiments is:

$$V_{in}\left[\frac{1 + M/2}{1 + M}\right] - 2\sum \frac{M[1 - \cos(\gamma_i)] + \gamma_i\sin(\gamma_i)}{A_i} \exp\left(\frac{-\gamma_i^2 Dt}{l^2}\right). \tag{7}$$

Solutions can also be obtained for the transepithelial flux in unilateral experiments. The solutions predict the same flux in either direction. The flux comes to a steady value within a few minutes and will stay constant as long as the activity on the trans side remains zero. The permeability, in $ml\,min^{-1}\,cm^{-2}$, derived from this steady flux is:

$$\text{permeability} = V_{in}\alpha\left[\frac{1}{1 + M}\right]. \tag{8}$$

Each of the interstitial space relationships is made up of two parts. The first term, either 1·0 or some function of M, is the equilibrium value. When it is 1·0, the measured sucrose space is the same as the true interstitial space. When it differs from 1·0, it describes how the values differ. Note that this steady-state term differs between the two unilateral exposures and that the way it differs depends on M. When M is very small, the apical junctions are nearly impermeable and the steady-state space from luminal exposure is nearly zero. That is, transfer across the apical junction is so slow that the intercellular clefts empty out to the haemolymph as rapidly as material can enter from the lumen. With small values of M, the steady-

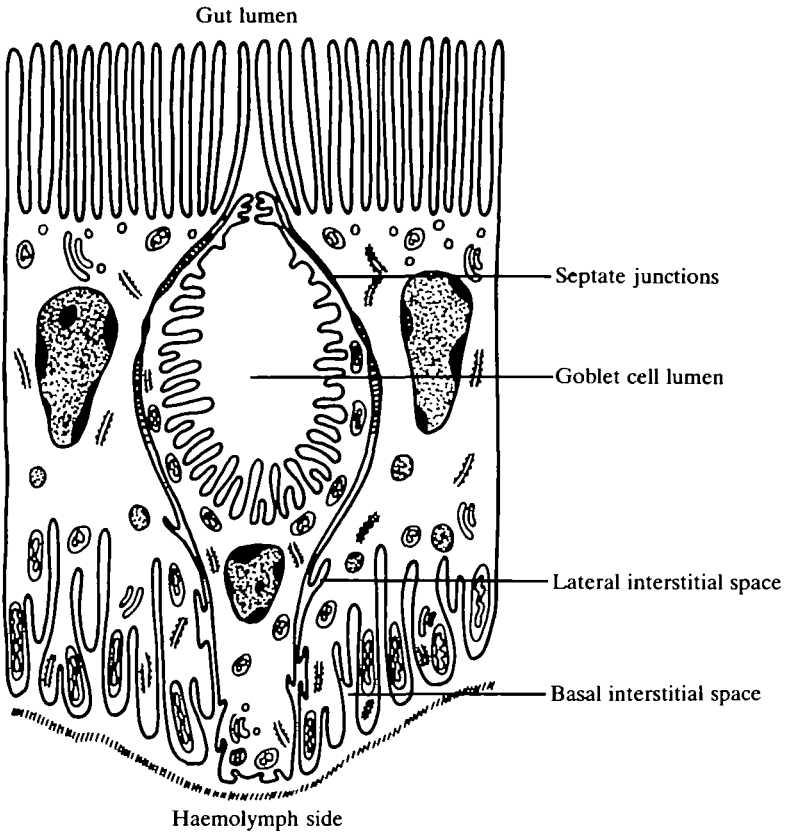

Fig. 3. Ultrastructural correlates of extracellular spaces in midgut (modified from Cioffi, 1979). The figure shows a goblet cell flanked by two columnar cells. Haemolymph extracellular spaces include contributions from basal infoldings. Luminal spaces include those between columnar cell microvilli and goblet cell lumina.

state sucrose space after haemolymph exposure is nearly the whole interstitial space because the clefts act nearly as blind-end tubes. Conversely, when apical permeability is very high and M is very large, the two unilateral exposures approach the same final value of one-half the true interstitial volume. This is what would be expected with very high apical permeability, where the apical end of the cleft is effectively open, as is the basal end. The second term, inside the summation sign, is just a set of weighting factors for the exponential terms that describe the transient behaviour.

These solutions are all complicated. The values of γ_i are solutions of a transcendental equation, although one that is tabulated (Carslaw & Jaeger, 1959). The terms in the solutions then are complicated functions of γ and are infinite series. It would be convenient if these solutions could be approximated with fairly simple functions, preferably functions in which the parameters reflected the physical properties of the tissue. However, the values of the physical parameters (l, α, D, V_{in} and V_{gob}) that match the data on uptake lead to approximations that require many

terms of the infinite series to predict the volume and flux results. Indeed, the inability to approximate the diffusion solution with only a few terms of the solution is equivalent to the statement that the diffusion model fits our data better than would a simple compartmental model.

We do not suggest that the solutions obtained here be routinely used to determine the physical parameters in a sheet of transporting epithelium. The significance of these solutions, rather, is that the tissue conforms to a diffusion model; that is, one in which the interstitial space is not uniform in concentration. One immediate consequence of this statement is that, for tissues with high rates of active transport, diffusion to the basolateral membrane might limit transport rate. This would appear experimentally as an extreme dependence of transport on the degree of mixing, a finding that is certainly true in this tissue. A second consequence of the diffusion model is really a restatement of one of our major reasons for choosing it in the first place. A one-, two- or three-compartment model that fits the observed data for exchange between bulk solution and interstitial fluid would have a high transepithelial sucrose flux. A match of all the data would require a large number of interstitial compartments in series; this is really the statement that we are approaching a distributed model.

The lines in Figs 1 and 2 show the fit of the diffusion model to our data. The values of the parameters used for this fit were: $V_{gob} = 0{\cdot}042\,ml\,g^{-1}$ tissue; $\lambda = 0{\cdot}021$ ml $min^{-1}\,g^{-1}$ tissue; $V_{in} = 0{\cdot}218\,ml\,g^{-1}$ tissue; $D = 1{\cdot}8\times10^{-6}\,cm^2\,s^{-1}$; $\alpha = 2{\cdot}9\times10^{-5}$ $cm\,s^{-1}$. When the parameters are chosen to fit the experimental data, the quality of the fit is more sensitive to the value of the diffusion coefficient, D, than to the other parameters. This result is consistent with the choice of a diffusion model rather than a compartmental model for the interstitial fluid. That is, if transfer across the septate junctions were the dominant limiting factor, one would expect the results to be the same, whether one used a compartmental or a diffusion model. The diffusion model would give different results only if the diffusion process itself were the limiting factor.

Only the value of D can be directly compared to an expected value. The value that fits our data is about one-third that of sucrose free in solution. This would be the result expected if the diffusion pathway through the tissue were not straight but, rather, somewhat tortuous. The longer pathway for diffusion would appear as a reduced diffusion coefficient. This phenomenon has been found in other tissues (Creese, 1954; Brookes & MacKay, 1969; Koch, Webster & Lowell, 1981). We would estimate that the actual diffusion pathway is about 1·7 times the apparent thickness of the gut.

Giordana & Sacchi (1978*a*,*b*) and Sacchi *et al.* (1981) have used unilateral sucrose spaces in lepidopteran midguts to compute intracellular concentrations. The solutions given above show that the direct use of the unilateral sucrose space leads to a systematic error, since the equilibrium activity of sucrose in interstitial fluid is not the same as the bathing fluid activity. The resulting error in computed intracellular concentration depends on the true intracellular concentration. When the true intracellular concentration is lower than the concentration in the bathing solution,

the computed cellular concentration is erroneously high. When the true cellular concentration is higher than the concentration in the bathing solution, computed cellular concentration is erroneously low. This effect is most important with materials present in low concentrations. With our values for the parameters, we compute that the equilibrium activity of the extracellular marker in the interstitial space is about 70% of bathing solution activity after basal exposure. If a cellular component had a true concentration of 0·1 of that of the bathing solution, it would be computed to have a concentration ratio of 0·2, 100% too high. However, if the true cellular concentration ratio were 5, it would be computed at 4·5, or 10% too low.

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