J. exp. Biol. (1976), 65, 347–360
With 7 figures
Printed in Great Britain

ACTIVE TRANSPORT OF CALCIUM ACROSS THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

BY JOHN L. WOOD AND WILLIAM R. HARVEY

Department of Biology, Temple University, Philadelphia, Pennsylvania, 19122

(*Received 23 February 1976*)

SUMMARY

1. The net flux of ^{45}Ca from lumen to blood side across the isolated and short-circuited Cecropia midgut was $1{\cdot}9 \pm 0{\cdot}2$ μequiv. cm^{-2} h^{-1} in 8 mM Ca and the flux ratio was as high as 56 to 1.

2. The calcium influx was depressed by anoxia; 73 % after 30 min.

3. The kinetics of Ca transport were anomalous; the apparent K_m varied with Ca concentration from $< 0{\cdot}2$ to $> 5{\cdot}6$ mM Ca and the apparent V_{max} varied from $< 1{\cdot}3$ to $> 3{\cdot}3$ μequiv. cm^{-2} h^{-1}.

4. The calcium influx showed a delay before the tracer steady state was attained, indicating the existence in the transport route of a calcium pool equivalent to $5{\cdot}7$ μequiv/g. wet weight of midgut tissue.

5. High calcium (16 mM) depressed the short-circuit current and potassium transport from blood to lumen side across the midgut.

6. Calcium depressed magnesium transport, from lumen to blood side across the midgut, and magnesium depressed the calcium transport.

7. Ca transport by the midgut does not regulate the Ca level in the haemolymph *in vivo*; it merely aids the diffusion of calcium down its electrochemical gradient. However, Ca transport may assist the uptake of the nutrients from the midgut contents.

INTRODUCTION

The isolated Cecropia midgut produces a large short-circuit current, I_{sc} (of the order of 20 μequiv. cm^{-2} h^{-1}), which is mainly carried by potassium moving from blood to lumen side (Harvey & Nedergaard, 1964; see review by Harvey and Zerahn, 1972). It has, however, been difficult to determine whether only potassium is actively transported because the electrical properties of the midgut make it one of the most difficult of tissues to short-circuit accurately (Harvey, Haskell & Zerahn, 1967; Wood, 1972, 1977). As refinements in short-circuiting techniques were made, the ratio between the net flux of potassium and the short-circuit current increased from 83 % (Harvey & Nedergaard, 1964) to virtually 100 % (Wood, 1972; Harvey & Zehran, 1972) suggesting that potassium was the only ion that is transported by the midgut. However, when account was taken of the long isotopic mixing time for potassium transport and the correspondingly long equilibration time to attain both isotopic and tissue steady state (Wood, 1972; Harvey & Wood, 1972, 1973; Wood & Harvey, 1975), the ratio between the net fluxes of potassium in the steady state and the short-circuit

current increased to 120%, indicating that some additional ion was actively transported by the midgut. Although sodium and the other alkali metal ions are actively transported (when the concentrations of calcium and potassium are sufficiently low) they are not required for potassium transport by the isolated midgut and can be omitted during *in vitro* measurements (see Harvey & Zerahn, 1972).

The distribution of ions *in vivo* suggests that other ions might be actively transported *in vitro* and could account for the discrepancy between net K flux and I_{sc}. Thus the active K transport – demonstrated *in vitro* acts *in vivo* to maintain potassium at a low level in the haemolymph (23 mM/l) so as to oppose the large electrochemical gradient driving K into the haemolymph, the midgut contents having a K level of 284 mM/l and being 100 mV positive with respect to the haemolymph (Harvey, Wood, Quatrale & Jungreis, 1975). Although sodium is present *in vivo* (< 2 mM/l) its active transport would be inhibited by the high potassium and calcium levels in haemolymph. The other alkali metal ions are virtually absent *in vivo*. Bicarbonate transport toward the lumen may be responsible for the K flux/I_{sc} discrepancy because bicarbonate is some 10 times more concentrated in midgut contents than in blood (Turbeck & Foder, 1970). Alternatively, hydrogen transport toward the blood may be involved because hydrogen ions are more than 1000 times more concentrated in blood than in contents (Nedergaard & Harvey, 1968).

Recently the unidirectional fluxes of magnesium were studied, using ^{28}Mg, and an active transport of this cation from lumen to blood side was demonstrated (Wood, Jungreis & Harvey, 1975). This Mg transport accounts for approximately one third of the discrepancy between the net flux of K and the short-circuit current. Ironically, the *in vivo* distribution of Mg provided no reason to suspect this transport process. Although there is a chemical gradient for Mg across the midgut *in vivo* (70 m-equiv./l in the haemolymph versus 18 m-equiv./l in the midgut contents) it is more than offset by the electrical gradient of 100 mV (Harvey *et al.* 1975). The existence of this 'unnecessary' Mg pump emphasizes the weakness of attempts to deduce active transport systems from *in vivo* distributions of ions, a weakness which was further emphasized by our analysis of calcium distributions *in vivo*.

Like that for potassium, the electrochemical gradient for calcium *in vivo* is toward the blood (the [Ca] of contents being 38 m-equiv./l and of haemolymph 14 m-equiv./l). The midgut contents are 100 mV positive with respect to the blood. Active Ca transport toward the lumen would oppose this electrochemical gradient and could account for the *in vivo* distribution, but would not account for the discrepancy in the K flux/I_{sc}, nor would it explain why the concentration of calcium in the contents is less than in leaves (319 m-equiv./l) or hindgut contents (124 m-equiv./l). However, active calcium transport by the midgut toward the blood could augment passive transport down this gradient and account for the *in vitro* K flux/I_{sc} discrepancy. Active calcium transport by some other tissue toward the hindgut lumen (see Discussion) could then account for the *in vivo* calcium distribution.

Unidirectional fluxes of ^{45}Ca across the isolated, short-circuited, midgut have provided direct evidence for active calcium transport from lumen to blood side. This transport accounts for most, if not all, of the discrepancy between net K flux and short-circuit current. Some kinetic properties of this transepithelial calcium transport and its relationship to potassium and magnesium transport are described in this paper.

MATERIALS AND METHODS

Larvae of *Hyalophora cecropia* (L.) were reared on a synthetic diet (Riddiford, 1968) or on wild black cherry leaves. Midguts were isolated from fifth instar larvae using the procedure of Nedergaard & Harvey (1968), except that the larvae were chilled under ice at 0 °C for 1 h and not under frozen carbon dioxide. The isolated midgut was mounted as a flat sheet in a chamber (Wood, 1977) similar in design to that of Ussing & Zerahn (1951), except that the tissue was tied over a flange with loops of cotton thread by the method developed by Wood *et al.* (1969; see also Forte *et al.*, 1965), which accommodates the delicate nature of the midgut tissue and presumably avoids 'edge damage' (Helman & Miller, 1973). Both sides of the midgut were bathed in a solution containing 32 mM-KCl, 5 mM Tris base and 1·5 mM-HCl (pH = 8·3), 166 mM sucrose and specified amounts of $CaCl_2$. The midguts were continuously short-circuited by a three-bridge system using an automatic voltage clamp as previously described (Wood, 1977), while the short-circuit current was monitored with a Triplett ammeter and recorded on a Servoscribe recorder. The use of the three-bridge system compensates for the voltage drop in the bathing solution, but leads to 'over short-circuiting' of the tissue (by an amount proportional to the thickness of the tissue) the error being less than 10% with the midgut (Wood, 1977). Moreover, since the lumen side is negative with respect to the blood side by this small amount, 'over short-circuiting' the tissue opposes the net flux of calcium from lumen to blood side and the net fluxes of calcium (the amount of active Ca transport) are underestimated. ^{45}Ca and ^{42}K, obtained from New England Nuclear, and ^{28}Mg, obtained from Brookhaven National Laboratory, were added to the appropriate side of the chamber (12 ml in volume) in 5 μCi amounts. Samples, 1 ml in volume, were removed from the cold side at 10 min intervals. Standards, 0·1 ml in volume, were removed from the hot side and made up to 1 ml. ^{45}Ca was counted after adding 12 ml of Packard Insta gel in a Packard scintillation counter (Model 3380). ^{42}K and ^{28}Mg, when used, were counted in the liquid scintillation counter before adding cocktail by the Cerenkov effect and ^{45}Ca in the same samples was subsequently counted by adding cocktail after the ^{42}K or ^{28}Mg had decayed 15 half lives.

RESULTS

The initial unidirectional calcium flux showed a long delay before a tracer steady state was attained, whereas the subsequent unidirectional flux showed a long washout before a tracer steady state was attained (Fig. 1). The unidirectional fluxes were, therefore, calculated as the mean steady-state value during the second hour of each determination. Moreover, the order in which fluxes were measured was alternated because it was found that simply rinsing the midgut with fresh bathing solution had an unpredictable effect on the fluxes. In one experiment (1 April 1975) for example, the radiocalcium flux from blood to lumen side jumped from 0·03 to 0·91 μequiv. $cm^{-2}\,h^{-1}$ after a rinse at 120 min. The results of the typical experiment and 19 others are summarized in Table 1. The ^{45}Ca flux from lumen to blood side was always greater (by as much as 160 times) than the flux in the opposite direction in each of the 20 paired determinations and hereafter will be called the influx. Moreover, the Ca

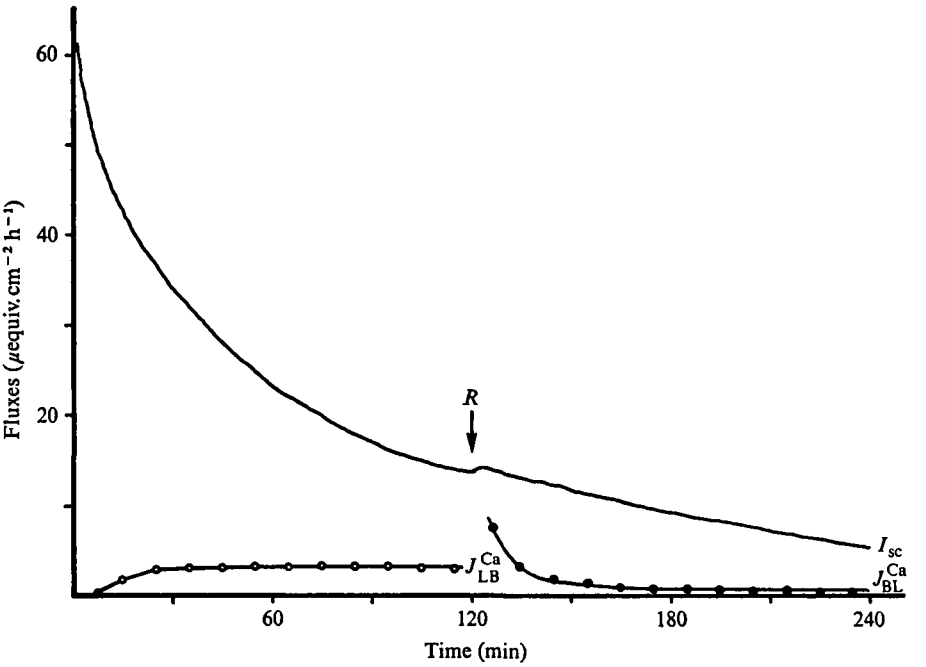

Fig. 1. A typical experiment (20 February 1975) in which the two unidirectional fluxes of ^{45}Ca in 8 mM Ca were determined sequentially and plotted on the same scale as the short-circuit current (I_{sc}) smooth curve to allow direct comparison. ^{45}Ca was added to the lumen side at 5 min and samples were taken from the blood side at 10 min intervals for 120 min. The time course of the ^{45}Ca-measured-Ca flux from lumen to blood side J^{Ca}_{LB} is shown by the open circles. At 120 min the lumen side then blood side were rinsed with fresh non-radioactive bathing solution for 1 min each (*R*). ^{45}Ca was added to the blood side at 125 min and samples were taken from the lumen side at 10 min intervals for the next 120 min. The time course of the ^{45}Ca-measured-Ca flux from blood to lumen side (J^{Ca}_{BL}) is shown by the closed circles. The initial unidirectional flux shows a long delay of about 45 min before a tracer steady state is attained, whereas the subsequent unidirectional flux shows a long washout of about 60 min before a tracer steady state is attained. The mean value of J^{Ca}_{LB} from 60 to 120 min is 3·1 μequiv. cm^{-2} h^{-1} and the mean value of J^{Ca}_{BL} from 180 to 240 min is 0·5 μequiv. cm^{-2} h^{-1}; therefore, the net flux of Ca from lumen to blood side for this experiment is 2·6 μequiv. cm^{-2} h^{-1}. (The short-circuit current produced by the midgut is directed so that either a cation is actively transported toward the lumen side or an anion toward the blood side. Since calcium and magnesium are actively transported toward the blood side this transport subtracts from the measured short-circuit current. Such fluxes of calcium and magnesium from lumen to blood have been plotted in the first quadrant in Figs 1, 2, 5, 6 and 7 for convenience rather than in the fourth quadrant.)

influx is inhibited by anoxia (Fig. 2) but this inhibition requires much longer to appear than that of the short-circuit current (K transport). This oxygen-dependent net movement of calcium under short-circuit conditions is direct evidence that calcium is actively transported from lumen to blood side across the isolated midgut.

The kinetics of Ca transport by the midgut are anomalous, the apparent K_m increasing with the Ca concentration. The concentration velocity relationship (Fig. 3) is not a rectangular hyperbola and it shows no sign of saturation. The Lineweaver–Burk plot of the results accelerates negatively (Fig. 4) and is reminiscent of curves which are characteristic of homotropic effects between enzymes and their substrates. The apparent K_m from the lowest two Ca concentrations tested is 0·2 mM Ca and

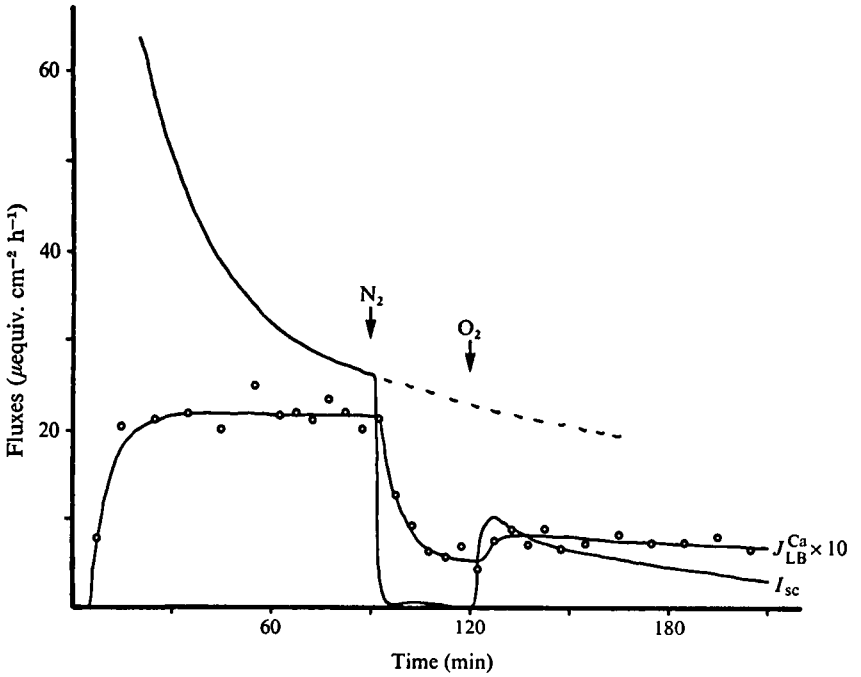

Fig. 2. The effects of anoxia on Ca transport and the short-circuit current (30 June 1975; leaf-fed). The ^{45}Ca-measured-Ca influx (open circles) in 4 mM Ca was measured for 90 min with 100% oxygen and was 2·2 μequiv. cm^{-2} h^{-1} at 90 min. At this time the stirring gas was changed within seconds to 100% pre-purified nitrogen and the Ca influx was depressed by 1·6 μequiv. cm^{-2} h^{-1} to 0·6 μequiv. cm^{-2} h^{-1} (73%) after 30 min, approaching the measured level of the efflux (see Table 1), such that J_{net}^{Ca} approached zero. At 120 min. 100% oxygen was restored and the Ca influx recovered slightly to 0·8 μequiv. cm^{-2} h^{-1} (36% of its value at 90 min). However, the simultaneous I_{sc} (smooth curve) was depressed by 26·2 μequiv. cm^{-2} h^{-1} (100%) after 30 min in 100% nitrogen and recovered to only about 44% of its projected time course in 100% oxygen (dotted line).

the apparent V_{max} is 1·3 μequiv. cm^{-2} h^{-1}, while the corresponding values from the highest two concentrations are 5·6 mM and 3·3 μequiv. cm^{-2} h^{-1} respectively. Unfortunately, the range of Ca concentrations over which the Ca kinetics can be studied is limited; concentrations below 1 mM cause the midgut to become very leaky to K, whereas those above 8 mM severely inhibit the short-circuit current and K transport (see below).

Ca transport is independent of K transport, as shown by the greatly different time courses of the Ca influx and the I_{sc} (Fig. 5). But whether Ca transport directly requires K cannot be tested, since the short-circuit current is inhibited irreversibly by K-free bathing solutions. However, both the short-circuit current and K transport are inhibited by high concentrations of Ca (Fig. 6). Since K transport is inhibited by Ca the depression in the short-circuit current upon the addition of Ca cannot be used as an indirect measure of Ca transport.

The effects of Mg on Ca transport were studied because magnesium is also actively transported from lumen to blood side of the isolated midgut (Wood *et al.*, 1975). Ca transport is depressed upon the addition of 16 mM Mg (Fig. 7). However, Mg transport is depressed more by Ca than vice versa (Table 2). As the Ca concentration is

Table 1. *Unidirectional fluxes of calcium (μequiv. cm^{-2} h^{-1}) measured with ^{45}Ca at several [Ca] (mM) across the isolated, short-circuited midgut of* Hyalophora cecropia

[Ca]	Date (1975)	Wet wt (mg)	J^{Ca}_{LB} 2nd h	J^{Ca}_{LB} 4th h	J^{Ca}_{BL} 2nd h	J^{Ca}_{BL} 4th h	$J^{Ca}_{net(LB)}$
16	10 Feb.	37	—	2·97	0·18	—	2·79
	12 Feb.	94	2·51	—	—	0·64	1·87
	13 Feb.*	42	2·28	—	—	1·86	0·41
	14 Feb.	52	—	2·80	0·13	—	2·67
Mean ± S.E.M.							2·45 ± 0·29
8	20 Feb.	47	3·08	—	—	0·52	2·56
	21 Feb.	29	—	1·70	0·03	—	1·65
	24 Feb.	30	2·62	—	—	0·73	1·89
	25 Feb.	19	—	1·66	0·03	—	1·63
Mean ± S.E.M.							1·93 ± 0·22
4	26 Feb.	58	1·90	—	—	0·29	1·61
	27 Feb.	34	—	1·46	0·02	—	1·44
	28 Feb.	46	1·76	—	—	0·54	1·22
	3 Mar.	54	—	1·45	0·04	—	1·41
Mean ± S.E.M.							1·42 ± 0·08
1	4 Mar.—1	30	—	1·19	0·01	—	1·19
	4 Mar.—2	31	—	1·04	0·04	—	1·01
	9 Mar.	58	1·30	—	—	0·15	1·16
	13 Mar.*	39	1·84	—	—	1·52	0·32
Mean ± S.E.M.							1·12 ± 0·05
0·1	5 Mar.	27	—	0·64	0·00	—	0·64
	14 Mar.	64	0·42	—	—	0·04	0·39
	16 Mar.	31	0·25	—	—	0·05	0·19
	18 Mar.	25	—	0·60	0·00	—	0·60
Mean ± S.E.M.							0·46 ± 0·10

* Values not used in calculation of mean ± S.E.M. because J^{Ca}_{BL} are atypical following rinse (see text).

increased the Ca influx increases but the Mg influx, measured both by isotope and by the depression in the short-circuit current, ΔI_{sc}, decreases. Moreover, there is poor agreement between the isotropically measured Mg influx and the depression in the short-circuit current whereas in the previous study (Wood *et al.*, 1975) the Mg influx agreed well with the depression in short-circuit current in Ca-free bathing solutions. Obviously the interaction between Ca and Mg transport is complex.

DISCUSSION

Active calcium transport from lumen to blood side across the isolated midgut is demonstrated by the net flux of calcium under short-circuit conditions (Fig. 1, Table 1), the influx being oxygen dependent (Fig. 2). This Ca transport occurs in a bathing solution containing, in addition to Ca, only K, Tris, Cl and sucrose at pH = 8·3 with 100% oxygen as the stirring gas. The Ca transport is present in midguts isolated throughout the feeding, fifth instar but is greatly reduced in those isolated during the moult from fourth to fifth instar (three experiments) and could not be demonstrated

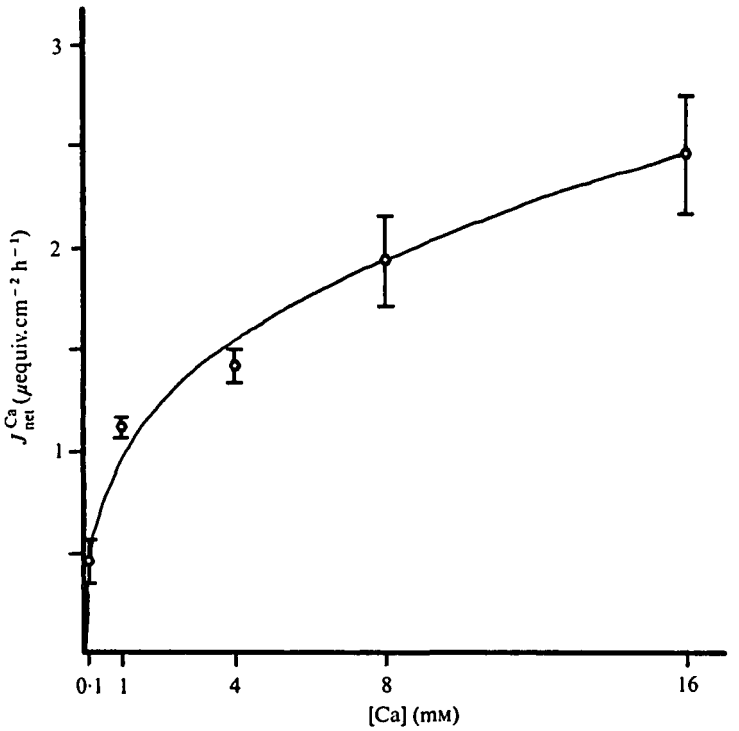

Fig. 3. Concentration–velocity relationship between the net flux of Ca from lumen to blood side (J_{net}^{Ca}) and the concentration of Ca in the bathing solutions taken from Table 1. The curve through the open hexagons was fitted by inspection and the bars are ± one S.E.M. The curve is not a rectangular hyperbola and shows no sign of saturation at 16 mM Ca.

in those isolated 36 h after gut evacuation (two experiments). The kinetics of Ca transport are anomalous (Figs. 3, 4), behaving as though a homotropic effect were occurring, although it may never be possible to ascribe a specific mechanism to net flux data.

Ca transport is independent of K transport (Fig. 5) showing little or no decay. This result implies that the decay in the short circuit current is caused by a decay in the K pump specifically and not by a generalized decay in the tissue. High Ca (16 mM) inhibits K transport (Fig. 6). High intracellular Ca may cause the epithelial cells to become uncoupled electrically (Rose & Loewenstein, 1971), thereby reducing the effective size of the K pool. High intracellular Ca could interfere directly with the K pump or its required energy production. It is more likely that high Ca limits the entry of K into the cellular K pool, thereby slowing down the K pump, since even low Ca (5 mM) inhibits Na transport by limiting the entry of Na into the cellular pool awaiting transport by the alkali metal ion pump (Harvey & Wood, 1972). In this case less energy would be used by the K pump and more energy would be available for Ca transport and could account for the anomalous kinetics of Ca transport (Figs. 3, 4).

Indeed, Ca transport has a much slower response to anoxia than K transport (Fig. 2), suggesting that the Ca pump has a much higher affinity for a shared energy substrate, such as ATP, than does the K pump. However, this simple suggestion is ruled out by the faster recovery of the K pump compared to the Ca pump when oxygen is restored (Harvey & Wood, unpublished data). This rapid loss of K transport followed

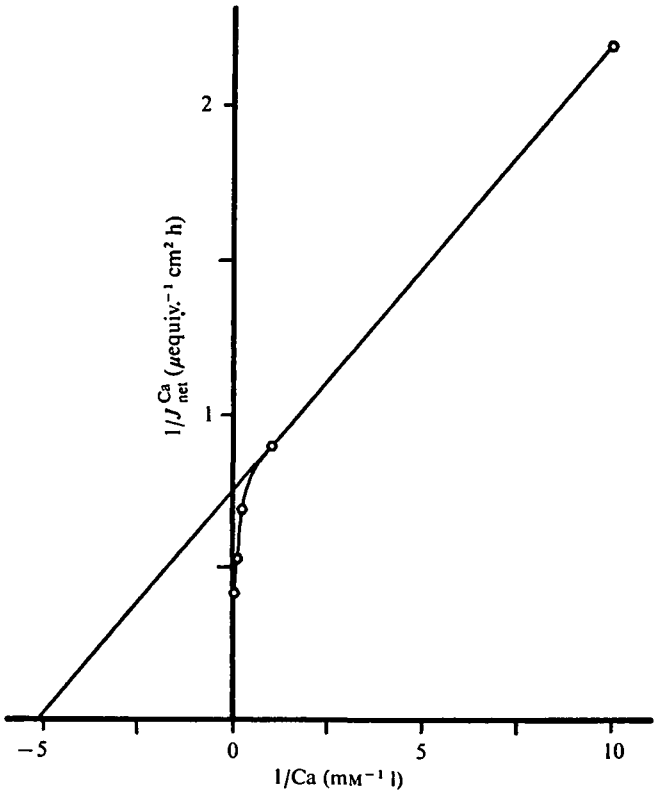

Fig. 4. Lineweaver–Burk plot of the concentration–velocity relationship between the net flux of Ca from lumen to blood side (J^{Ca}_{net}) and the concentration of Ca in the bathing solutions. The resulting curve is not a straight line and the possible explanations for this result are discussed in the text. An approximation of the limiting apparent K_M was obtained by connecting the points corresponding to the two lowest Ca concentrations tested and was found to be about 0·2 mM Ca. The limiting apparent V_{max} approaches infinity.

by a slower loss of Ca transport in nitrogen and the rapid recovery of K transport followed by a slower recovery of Ca transport in oxygen, at the very least suggests that K transport is more directly coupled to electron transport than is Ca transport. Supporting this suggestion Mandel, Moffet & Jobsis (1975) have found a strong correlation between the redox state of cytochrome b_5 (see Shappirio & Williams, 1957) and K transport.

Ca transport interacts with Mg transport (Fig. 7, Table 2) in a way suggesting that they compete for the same ion pump, but with Ca having a much higher affinity (K_m = 0·2 mM for Ca compared to K_m = 34 mM for Mg). However, the interaction could be due to competition for availability of a common energy substrate by separate pumps as suggested for Ca and K above. Finally, high Ca could limit the entry of Mg into a cellular pool thereby reducing Mg transport. A further complication is that the ^{28}Mg-measured-Mg influx does not account for the depression in the I_{sc} upon the addition of Mg to the bathing solution in the presence of Ca whereas in Ca-free solutions the measured net flux of Mg agreed with the I_{sc} depression from 1 to 8 mM Mg (Wood *et al.* 1975). The present findings suggest that Ca, Mg and K interact in a very complicated fashion, possibly by turning on yet another ion pump.

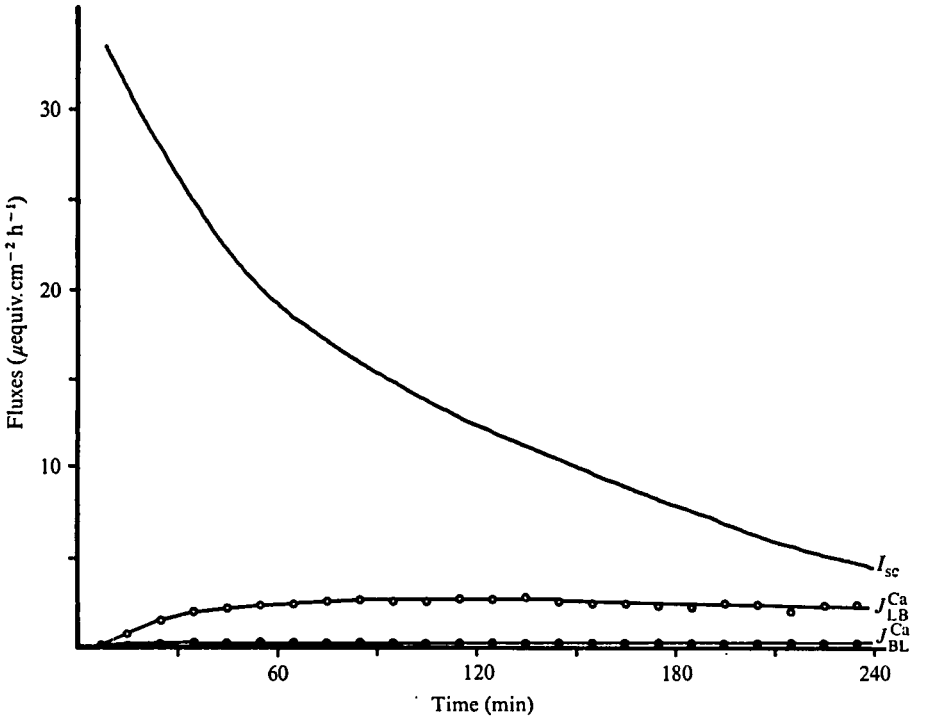

Fig. 5. The independence of Ca transport in 8 mM Ca and the short-circuit current (14 April 1975). After a tracer steady state was attained, the ^{45}Ca-measured-Ca influx (J^{Ca}_{LB}, open circles) declined by only 0·3 μequiv. cm^{-2} h^{-1} (11 %) from 90 to 240 min, whereas the simultaneous I_{sc} declined 11·0 μequiv. cm^{-2} h^{-1} (71 %) over the same time period. In a separate experiment (1 May 1975) the ^{45}Ca-measured-Ca efflux (J^{Ca}_{BL}, closed circles) was found to be constant from 90 to 240 min. Therefore, Ca transport is independent of the I_{sc} (and K transport). Moreover, the size of the Ca pool (S^{Ca}_{1}) corresponding to the delay in the attainment of the tracer influx steady state (J° = 2·8 μequiv. cm^{-2} h^{-1}, α = 3·2 h^{-1}) is 0·88 μequiv. Ca cm^{-2} (5·7 μequiv. Ca/g wet weight or about 80 % of the estimated tissue Ca).

Ca transport *in vivo* has not been demonstrated because neither the activities of Ca in the midgut contents and haemolymph nor the pH optimum of the Ca pump have been determined. However, if the midgut Ca pump operates in the same direction *in vivo* as it does *in vitro* then it cannot be regulating the Ca level in the haemolymph since Ca transport from lumen to blood would only augment the diffusion of Ca down its electrochemical gradient into the haemolymph. This accelerated movement of calcium into the haemolymph would account for the drastic reduction of the Ca level from leaves to midgut contents. Obviously some other tissue would have to regulate the Ca level in haemolymph, a possible candidate being the Malpighian tubules which empty into the hindgut where high Ca levels are found again (Quatrale, 1966). The puzzle presented by this 'unnecessary' Ca pump is added to the similar one presented by the 'unnecessary' Mg pump. Possibly both Mg and Ca transport are involved in nutrient uptake, substituting for the now classical role of Na transport in vertebrate nutrient uptake by this virtually Na-free, plant-eating insect. Before considering this prospect it seems worthwhile to compare Ca transport by the midgut with that in vertebrate intestine.

Ca transport by the vertebrate intestine has been recognized and studied for many years

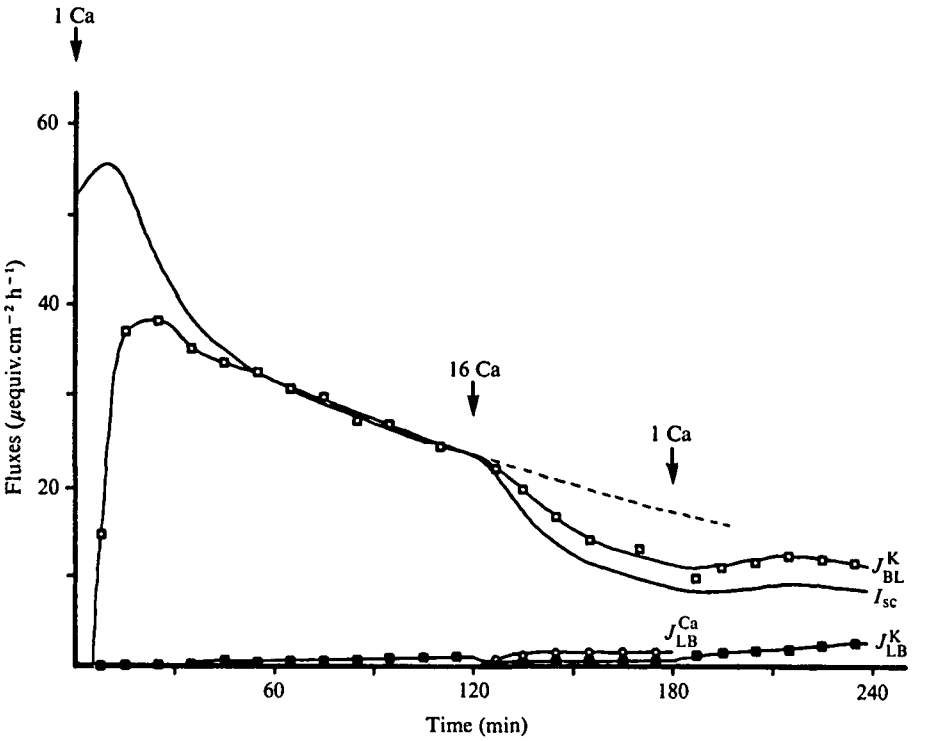

Fig. 6. The effects of Ca on K transport (6 March 1975). The midgut was initially bathed in 1 mM Ca, changed to 16 mM Ca at 120 min and returned to 1 mM Ca at 180 min. The I_{sc} (smooth curve) was depressed by 8·6 μequiv. cm^{-2} h^{-1} (51 %) after 60 min in 16 mM Ca and recovered by only about 38 % when 1 mM Ca was restored. The ^{45}Ca-measured-Ca influx (open circles) in 16 mM Ca was only 1·5 μequiv. cm^{-2} h^{-1} and cannot account for the severe depression in the I_{sc}. The simultaneous ^{42}K-measured-K influx (J^K_{BL}, open squares) in 32 mM K followed the same time course as that of the I_{sc} after the tracer steady state was attained during the first 120 min period in 1 mM Ca. In 16 mM Ca the K influx was depressed by 5·8 μequiv. cm^{-2} h^{-1} after 60 min. When 1 mM Ca was restored, the K influx remained greater than the I_{sc}. In a separate, but similar experiment (8 March 1975), the ^{42}K-measured-K efflux (J^K_{LB}, closed square) in 32 mM K was small (< 1·1 μequiv. cm^{-2} h^{-1}) during the first 120 min in 1 mM Ca. dropped to 0·4 μequiv. cm^{-2} h^{-1} when 16 mM Ca was added, and increased greatly to 2·2 μequiv. cm^{-2} h^{-1} when 1 mM Ca was returned. During the first 120 min in 1 mM Ca, the I_{sc} was carried almost entirely by the net flux of K. When 16 m*M* Ca was added, the I_{sc}, the K influx, and the K efflux were depressed, while the Ca influx was increased and the I_{sc} was then carried by the net flux of K plus the (negative) net flux of Ca. Finally, when 1 mM Ca was restored, the I_{sc}, the K influx and K efflux were increased and the I_{sc} was again carried almost entirely by the net flux of K. In summary, high Ca (16 mM) inhibits K transport, and the depression of the I_{sc} is not a measure of net Ca transport.

(see review by Harrison & Harrison, 1974). The similarities of Ca transport in vertebrate intestine and insect midgut are striking. Ca transport by the rat duodenum has a similarly low apparent K_m of 1·25 mM and an apparent V_{max} of 0·71 μequiv. cm^{-2} h^{-1} (Walling & Rothman, 1969). The Ca transport by duodenum requires Na with an optimal concentration at about 50 mM. Although Ca transport by the rat ileum has the same Na optimum, this segment does not require Na and is inhibited by high Na (Harrison & Harrison, 1974). The effects of Na on Ca transport by the midgut have not been tested but Ca transport occurs in the absence or added Na *in vitro* and only low levels of Na are found *in vivo* (Harvey *et al.* 1975). Ca transport in vertebrate

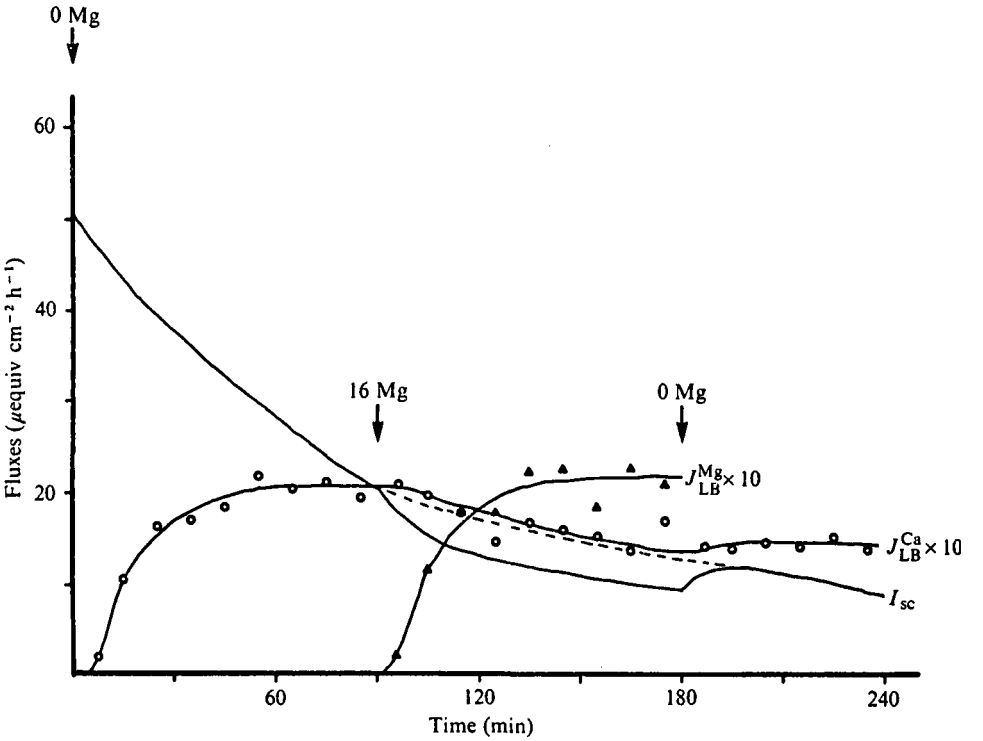

Fig. 7. The effects of Mg on Ca transport (12 March 1975(−2)). The midgut was initially bathed in a solution containing 0 Mg, then changed to 16 mM Mg, and finally returned to 0 Mg. The I_{sc} (smooth curve) was depressed by 3·4 μequiv. cm^{-2} h^{-1} after 90 min in 16 mM Mg. The ^{28}Mg-measured-Mg influx (J_{LB}^{Mg}, open triangles) after the tracer steady state was attained was 2·2 μequiv. cm^{-2} h^{-1} at 180 min. The ^{45}Ca-measured-Ca influx (J_{LB}^{Ca}, open circles) in 8 mM Ca and 0 mM Mg was 2·0 μequiv. cm^{-2} h^{-1} at 90 min. When 16 mM Mg was added, the Ca influx was depressed 0·7 μequiv. cm^{-2} h^{-1} (34%) after 90 min. When 0 mM Mg was returned the Ca-influx recovered slightly (70% of its value at 90 min), but it is very difficult to predict what the Ca influx would have been if Mg had not been added. Therefore, Mg depresses Ca transport, but not necessarily by competing for the same pump.

Table 2. *Effects of adding* 16 *mM Mg on Ca influx,* I_{sc} *and Mg influx* (*μequiv. cm*$^{-2}$ *h*$^{-1}$) *at* 3[*Ca*] (*mM*) *across the isolated, short-circuited midgut of* Hyalophora cecropia

[Ca]	Date (1975)	Wet wt (mg)	(0 Mg) J_{LB}^{Ca}	(+16 Mg) J_{LB}^{Ca}	(+16 Mg) ΔI_{sc}	(+16 Mg) J_{LB}^{Mg}
1	7 Apr.	56	1·04	0·75	5·97	—
8	12 Mar.(−1)	49	1·57	1·19	3·73	2·16
	12 Mar.(−2)	47	2·01	1·34	3·36	2·16
16	17 Feb.	29	3·80	2·69	5·22	—
	11 Mar.	103	2·84	2·46	1·49	1·57

intestine is depressed by increasing Mg levels and vice versa (Schachter & Rosen, 1959; Lifshitz, Harrison & Harrison, 1967) as is Ca transport by the midgut (Table 2) but it is not known whether or not there is a common transport system for Ca and Mg (Harrison & Harrison, 1974). Ca transport in the chick ileum is greatly enhanced by vitamin D_3 fed to the donor animal several hours before the ileum is isolated (Adams & Norman, 1970). Diet-fed Cecropia larvae have an absolute requirement for high levels of cholesterol (2·6 mM) (Riddiford, 1968). Part of this high cholesterol requirement is elimated by uniform dispersion of cholesterol in the diet, but cholesterol, which is not sythesized by silkworms, is a precursor in ecdysone synthesis (see review, by Svoboda, Kaplanis, Robbins & Thompson, 1975) accounting for the dietary requirement. Nevertheless, one can speculate that cholesterol stimulates Ca transport in Cecropia. Supporting this speculation is the observation that larvae fed with excess cholesterol have a higher rate of Ca transport than leaf-fed larvae (Wood & Harvey, unpublished data; compare Fig. 2 with 4 mM Ca in Table 1). However, cellulose phosphate reduces Ca transport in mammalian intestine (Dent, Harper & Parfitt, 1964) suggesting that the lower Ca transport by midguts isolated from leaf-fed larvae may be due to a lower level of induction of Ca transport reflecting the reduction in the activity of Ca by cellulose phosphate in their midgut contents. In this case the inhibitory effects of high Ca on K transport (Fig. 6) may not be found *in vivo* where the Ca levels are high, but where the Ca activities may be low.

Ca transport in vertebrates is affected by amino acids and sugars but the effects of these substances on Ca transport by the midgut have not been tested. In rat intestine Ca transport is stimulated by L-lysine and L-arginine but glycine has no effect (Wasserman, Comar & Nold, 1956), whereas in the chick intestine Ca transport is inhibited by lycine (Wasserman, Comar, Schooley & Lengemann, 1957). The midgut actively transports lycine, methylamine and α-aminoisobutyric acid from lumen to blood side but transports isobutyric acid from blood to lumen side (Nedergaard, 1973). This amino acid transport across the midgut is affected by the pH and the potential difference across the tissue, but is independent of K transport and does not require added Na. The interesting hypothesis that amino acid transport is coupled to Ca and/or Mg transport across the midgut was not tested by Nedergaard, although her solutions contained 5 mM Ca and 5 mM Mg, Ca and Mg transport by the midgut being unknown then. Moreover, Ca transport in vertebrates is stimulated by sugars and hexitols when these are present in the lumen of the distal intestine (Wasserman & Comar, 1959). Possibly sugar transport could be coupled to Ca and/or Mg transport by the midgut. Finally, the role of the mitochondria-lined basal infoldings of the columnar cells (see Anderson & Harvey, 1966 for structure of the midgut) remains a question (Keynes, 1973). We postulate that the mitochondria in the folds of the columnar cells are involved in the transport of Ca and Mg and the co-transport of amino acids and sugars from lumen to blood, whereas the mitochondria in the apical projections of the goblet cells are involved in transport of K from blood to lumen.

The kinetics of Ca transport across the midgut suggest that the transepithelial transport may use a pump located in the basal plasma membrane and that the Ca transport route may be through the cells. First, the apparent limiting K_m is less than 0·2 mM, which is approximately two orders of magnitude less than that found for the alkali metal ions or magnesium, and approaches the low K_m which must characterize

pumps that regulate intracellular calcium levels (see Rasmussen, Goodman & Tenehouse 1972). Secondly, the delay in the attainment of a tracer steady state (Figs 1, 2, 5 and 7) indicates that there is a pool of calcium in the transport route across the midgut (see Wood & Harvey, 1975). From the data in Fig. 5 the Ca pool would be 5·7 μequiv./g wet weight compared to a total calcium level of 6·9 μequiv./ml tissue water in freshly isolated midguts (Harvey *et al.* 1975). A large part of this pool may be chemically bound. Nevertheless this large pool suggests that the Ca transport is through the cells and implies that large fluxes occur across the plasma membranes. The low Ca concentration in the cytosol ($< 10^{-5}$ M in cells generally, Papworth & Patrick, 1970) would then be determined mainly by the kinetics of plasma membrane Ca transport in this epithelial tissue rather than by the kinetics of Ca accumulation by organelles like mitochondria or sarcoplasmic reticulum as argued by Rasmussen *et al.* (1972) from data on muscle.

This prospect of large active Ca effluxes across the plasma membrane of the midgut cells suggests a solution to the puzzle of the 'unnecessary' Ca pump which may have important consequences for epithelial transport theory. The transepithelial Ca transport may be simply a by-product of a universally occurring active Ca extrusion from cells combined with the asymmetry of the midgut cells. Even though the cells must be in a steady state, with the sum of outward Ca fluxes at the two surfaces equal to the sum of inward Ca fluxes, it is unlikely that Ca entry and active Ca exit would be the same size at either the basal or apical surfaces and therefore a net transepithelial Ca flux would be inevitable, its direction depending on which surface happened to have the greatest active Ca efflux compared to influx. Indeed, transepithelial Ca transport may be universal since epithelial cells generally are thought to pump Ca out and are usually asymmetrical. Finally, the same logic would predict that any epithelium possessing Na–K exchange pumps would exhibit active transepithelial sodium transport.

This research was supported in part by a research grant (AI-05903) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

REFERENCES

ADAMS, T. H. & NORMAN, A. W. (1970). Studies on the mechanism of action of calciferol 1. Basic parameters of vitamin D-mediated calcium transport. *J. biol. Chem.* **245**, 4421.

ANDERSON, E. & HARVEY, W. R. (1966). Active transport by the Cecropia midgut. II. Fine structure of the midgut epithelium. *J. Cell Biol.* **31**, 107.

DENT, C. E., HARPER, C. M. & PARFITT, A. M. (1964). The effect of cellulose phosphate on calcium metabolism in patients with hypercalciuria. *Clin. Sci.* **27**, 417.

FORTE, J. G., ADAMS, P. H. & DAVIES, R. E. (1965). Acid secretion and phosphate metabolism in bullfrog gastric mucosa. *Biochim. biophys. Acta* **104**, 25.

HARRISON, H. E. & HARRISON, H. C., (1974). Calcium. *Biomembranes* **4**B, 793.

HARVEY, W. R., HASKELL, J. A. & ZERAHN, K. (1967). Active transport of potassium and oxygen consumption in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **46**, 235.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. Nat. Acad. Sci.* **51**, 757.

HARVEY, W. R. & WOOD, J. L. (1972). Cellular pools involved in active K-transport across the isolated Cecropia midgut. In *Role of Membranes in Secretory Processes* (ed. L. Bolis, R. D. Keynes and W. Willbrandt), p. 310. Amsterdam: North-Holland.

HARVEY, W. R. & WOOD, J. L. (1973). The route of cation transport across the silkworm midgut. In *Transport Mechanisms in Epithelia* (ed. H. H. Ussing and N. A. Thorn), p. 342. New York: Academic Press.

HARVEY, W. R., WOOD, J. L., QUATRALE, R. P. & JUNGREIS, A. M. (1975). Cation distribution across the larval and pupal midgut of the lepidopteran *Hyalophora cecropia in vivo*. *J. exp. Biol.* **63**, 321.

HARVEY. W. R. & ZERAHN. K. (1972). Active transport of potassium and other alkali metals by the isolated midgut of the silkworm. In *Current Topics in Membranes and Transport*. vol. III (ed. F. Bronner and A. Kleinzeller). p. 367. New York and London; Academic Press.

HELMAN, S. I. & MILLER, D. A. (1973). Edge damage effects on electrical measurements of the frog skin. *Am. J. Physiol.* **225**, 972.

KEYNES ,R. D. (1973). Comparative aspects of transport through epithelia. In *Transport Mechanisms in Epithelia* (ed. H. H. Ussing and N. A. Thorn) p. 505. New York: Academic Press.

LIFSHITZ, F., HARRISON, H. C. & HARRISON, H. E. (1969). Influence of parathyroid function upon the *in vitro* transport of calcium and phosphate by the rat intestine. *Endocrinology* **84**, 912.

MANDELL, M. J., MOFFETT, D. F. & JOBSIS, F. F. (1975). Redox state of respiratory chain enzymes and potassium transport in silkworm midgut. *Biochim. Biophys. Acta* (in the Press).

NEDERGAARD, S. (1973). Transport of amino acids in Cecropia midgut. In *Transport Mechanisms in Epithelia* (ed. H. H. Ussing and N. A. Thorn) p. 372. New York: Academic Press.

NEDERGAARD, S. & HARVEY, W. R. (1968). Active transport by the Cecropia midgut. IV. Specificity of the transport mechanism for potassium. *J. exp. Biol.* **48**, 13.

PAPWORTH, D. G. & PATRICK, G. (1970). The kinetics of influx of calcium and strontium into rat intestine *in vitro*. *J. Physiol.* **210**, 999.

QUATRALE, R. P. (1966). Cation concentration changes during development of the silkworm, *Hyalophora cecropia*. Ph.D. thesis. University of Massachusetts.

RASMUSSEN, H., GOODMAN, D. B. P. & TENEHOUSE, A. (1972). The role of cyclic AMP and calcium in cell activation. *Critical Reviews in Biochemistry* **1**, 95.

RIDDIFORD, L. (1968). Artificial diets for Cecropia and other Saturnid silkworms. *Science, Wash.* **160**, 1461.

ROSE, B. & LOEWENSTEIN, W. R. (1971). Junctional membrane permeability. Depression by substitution of Li for extracellular Na, and by long-term lack of Ca and Mg; restoration by cell repolarization. *J. memb. Biol.* **5**, 20.

SCHATER, D. & ROSEN, S. M. (1959). Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. *Am. J. Physiol.* **196**, 357.

SHAPPIRIO, D. G. & WILLIAMS, C. M. (1957). The cytochrome system of the Cecropia silkworm. I. Spectroscopic studies of individual tissues. *Proc. R. Soc.* B **147**, 218.

SVOBODA, J. A., KAPLANIS, J. N., ROBBINS, W. E. & THOMPSON, M. J. (1975). Recent developments in insect steroid metabolism. *A. Rev. Ent.* **20**, 205.

TURBECK, B. O. & FODER, B. 1970. Studies on a carbonic anhydrase from the midgut epithelium of larvae of lepidoptera. *Biochim. Biophys. Acta* **212**, 139.

USSING, H. H. & ZERAHN, K. (1951). Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. Scand.* **23**, 110.

WALLING, M. W. & ROTHMAN, S. S. (1969). Phosphate-independent, carrier mediated active transport of calcium by rat intestine. *Am. J. Physiol.* **217**, 1144.

WASSERMAN, R. H. & COMAR, C. L. (1959). Carbohydrates and gastrointestinal absorption of radiostrontium and radiocalcium in the rat. *Proc. Soc. exp. Biol. Med.* **101**, 314.

WASSERMAN, R. H., COMAR, C. L. & NOLD, M. M. (1956). The influence of amino acids and other organic compounds on the gastrointestinal absorption of calcium-45 and strontium-89 in the rat. *J. Nutr.* **59**, 371.

WASSERMAN, R. H., COMAR, C. L., SCHOOLEY, J. C. & LENGEMANN, F. W. (1957). Interrelated effects of L-lysine and other dietary factors on the gastrointestinal absorption of calcium-45 in the rat and chick. *J. Nutr.* **62**, 367.

WOOD, J. L. (1972). Some aspects of active potassium transport by the midgut of the silkworm, *Antheraea pernyi*. Ph.D. thesis, University of Cambridge.

WOOD, J. L. (1977). Refinements in the short-circuit technique and its application to active potassium transport across the Cecropia midgut (in preparation).

WOOD, J. L., FARRAND. P. S. & HARVEY, W. R. (1969). Active transport of potassium by the Cecropia midgut. VI. Microelectrode potential profile. *J. exp. Biol.* **50**, 169.

WOOD, J. L., JUNGREIS, A. M. & HARVEY, W. R., (1975). Active transport of magnesium across the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **63**, 313.

WOOD, J. L. & HARVEY, W. R. (1975). Active transport of potassium by the Cecropia midgut; tracer kinetic theory and transport pool size. *J. exp. Biol.* **63**, 301.