

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

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(Received 4 November 1974)

SUMMARY

1. The ^{28}Mg -measured net flux of magnesium from lumen-side to haemolymph-side of the isolated and short-circuited midgut was $1.97 \pm 0.28 \mu\text{-equiv cm}^{-2} \text{ h}^{-1}$ in 8 mM-Mg^{2+} .

2. The magnesium-influx shows a delay before the tracer steady-state is attained, indicating the existence of a magnesium-transport pool equivalent to $6.7 \mu\text{-equiv/g}$ wet weight of midgut tissue.

3. Magnesium depresses the short-circuit current produced by the midgut but not the potassium transport, the depression being equal to the rate of magnesium transport.

4. Magnesium transport yields a linear Lineweaver-Burk plot with an apparent K_m of 34 mM-Mg^{2+} and an apparent V_{max} of $14.9 \mu\text{-equiv cm}^{-1} \text{ h}^{-1}$.

5. Magnesium is actively transported across the midgut and contributes to the regulation of the haemolymph magnesium concentration *in vivo*.

INTRODUCTION

Hyalophora cecropia (L.) being a phytophagous insect is characterized by high concentrations of both potassium and magnesium and by a low concentration of sodium in its haemolymph (Quatralé, 1966; Jungreis, Jatlow & Wyatt, 1973). In mature larvae the high haemolymph potassium concentration (23 mM) is nevertheless lower than that in the midgut contents (230 mM), whereas the haemolymph magnesium concentration (35 mM) is higher than that in the midgut contents (9 mM). The lower potassium concentration in haemolymph is maintained against an electrical potential difference, the lumen being some 100 mV positive with respect to the haemolymph *in vivo* (Harvey, Wood, Quatralé & Jungreis, 1975). Potassium is actively transported from haemolymph side to lumen side of the isolated midgut, rendering the lumen side positive (Harvey & Nedergaard, 1964) and presumably accounting for the maintenance of the low haemolymph potassium concentration against both electrical and chemical gradients *in vivo* (Harvey *et al.* 1975).

Quatralé (1966) and Jungreis *et al.* (1973) suggested that magnesium must be actively transported from lumen to haemolymph to account for the low magnesium

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concentration in the midgut compared to both haemolymph and foliage. However, the electrical potential difference across the midgut *in vivo* can account for the high magnesium concentration in haemolymph and for the low concentration in midgut contents compared to foliage (Harvey *et al.* 1975). On the other hand, active magnesium transport toward the haemolymph-side of the isolated midgut would account for the reversal of the potential difference described by Harvey & Zerahn (1969) when all of the potassium bathing the isolated midgut was replaced by magnesium. Finally, Wood & Harvey (1976) find many cases in which the influx of potassium exceeds the short-circuit current by as much as 20% whereas the efflux of potassium commonly measured is only 1–5% of the current (e.g. fig. 6 of Harvey & Wood, 1972). However, it was not until Jungreis asked if the midgut were sufficiently permeable to magnesium to allow this ion to move passively from lumen to haemolymph, where it complexes with phosphates released from sugars at the end of larval life (Jungreis, 1973, 1974; Jungreis, Daily & Hereth, 1975), that magnesium fluxes across the isolated midgut were finally measured. Direct evidence for active magnesium transport from lumen side to haemolymph side of the isolated midgut was immediately obtained under short-circuit conditions and is reported here.

METHODS

Midguts were isolated from fifth-instar larvae of *H. cecropia* fed either on leaves of *Prunus serotina* (wild black cherry) or *Salix babylonica* (weeping willow) or on synthetic diet (Riddiford, 1968) and mounted as a flat sheet in a Perspex chamber (Wood, 1975). The bathing solutions contained 32 mM-KCl, 5 mM-CaCl₂, 5 mM-Trisma base, 0.5 mM-HCl (pH = 8.3), 166 mM sucrose and specified amounts of MgCl₂. The midguts were short-circuited continuously using a three-bridge system and an automatic feedback device by the methods described previously (Wood, 1972, 1975) while the short-circuit current (I_{sc}) was monitored with an ammeter (Triplett) and recorded on a Servoscribe recorder. ²⁸Mg was obtained from the Brookhaven National Laboratory, Upton, N.Y., and ⁴⁸K was obtained from New England Nuclear, Boston, Mass., and these were added to the appropriate side of the chamber in 5 μ Ci amounts. Samples, 2 ml in volume, were removed from the cold side and standards, 0.1 ml in volume, were removed from the hot side. Radioactivity in the samples was counted by the Cerenkov effect in a liquid scintillation counter (Packard, Model 3380).

RESULTS

The two unidirectional fluxes of magnesium measured by ²⁸Mg across the isolated and short-circuited midgut are summarized in Table 1. The ²⁸Mg flux from lumen side to haemolymph side was greater than the flux in the opposite direction in each of eight paired determinations, and hereafter will be called the influx. The influx showed a delay before a tracer steady-state was attained (Fig. 1), indicating that a sizeable pool exists in the route of magnesium transport across the midgut. A net movement of magnesium toward the haemolymph was found in one midgut isolated from a larva fed on willow and bathed in Ca²⁺-free solutions, in six midguts isolated from larvae fed on black cherry, and in one midgut isolated from a larva fed on synthetic diet.

Table 1. ^{28}Mg -measured magnesium fluxes ($\mu\text{-equiv cm}^{-2} \text{h}^{-1}$) in three time-periods at three magnesium concentrations (mM)

| Time (min) | 60-120 | | 180-240 | | 300-360 | | Net flux L → H |
|------------|--------|-------|---------|-------|---------|-------|-------------------|
| | L → H | H → L | L → H | H → L | L → H | H → L | |
| 1 | — | — | 0.45 | — | — | 0.28 | 0.17 |
| 5 | — | 0.03* | 1.24* | — | — | — | 1.21* |
| | — | 0.03 | 1.70 | — | — | — | 1.67 |
| 8 | 4.71 | — | — | — | — | — | — |
| | 3.14 | — | — | — | — | — | — |
| | 2.32† | — | 1.97† | — | — | 0.91† | 1.41† |
| | 3.82 | — | — | 0.84 | — | — | 2.98 |
| | — | 0.06 | 1.76 | — | — | — | 1.70 |
| | — | 0.13 | 2.28 | — | — | — | 2.15 |
| | — | — | — | 0.16 | 1.76 | — | 1.60 |

* Animal fed willow leaves; Ca^{2+} -free bathing solution.

† Animal fed synthetic diet.

All other animals fed wild cherry leaves.

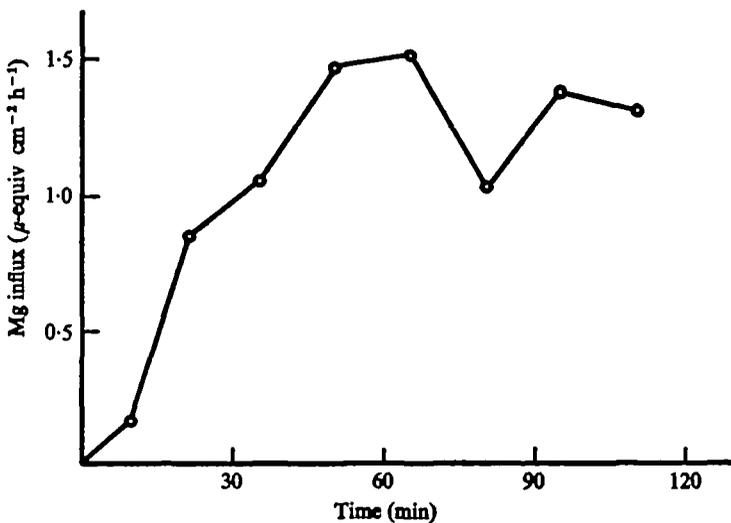


Fig. 1. The time-course of the ^{28}Mg -measured magnesium flux from lumen side to haemolymph side of the isolated midgut (influx) in 8 mM- Mg^{2+} , showing a delay in the attainment of the tracer steady state. The short-circuit current at 60 min after adding the tracer was $9.3 \mu\text{-equiv cm}^{-2} \text{h}^{-1}$.

This net movement of magnesium under short-circuit conditions is direct evidence that magnesium is actively transported from lumen side to haemolymph side of the midgut.

The short-circuit current produced by the midgut is decreased by increasing concentrations of magnesium as shown in Fig. 2 and in two similar experiments (data not shown). Since the result could be either a direct contribution of active magnesium transport or an indirect effect caused by inhibition of potassium transport, the effects of magnesium on potassium transport were determined (Fig. 3). The result obtained in this experiment was that, while the short-circuit current (I_{sc}) was depressed by

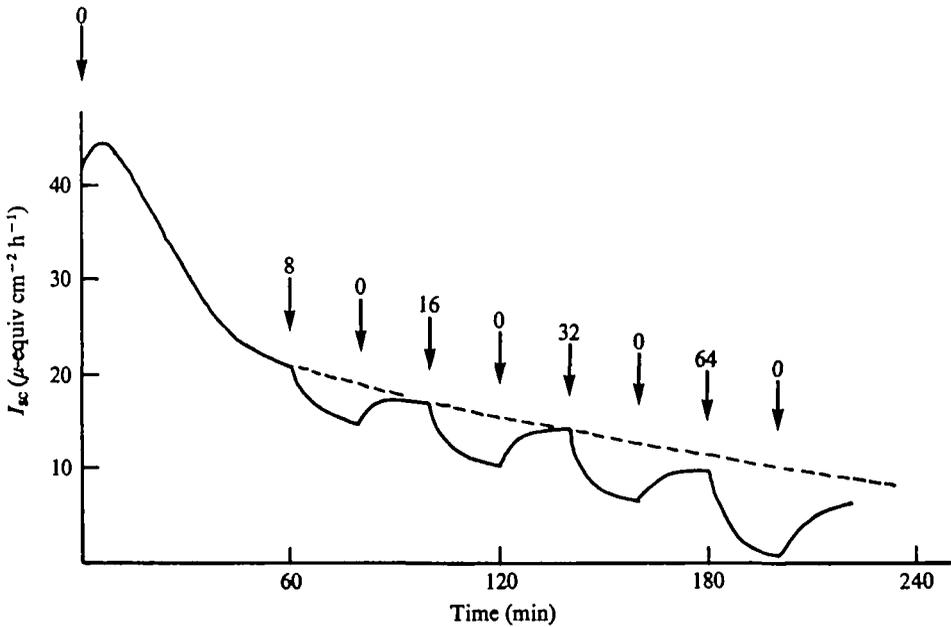


Fig. 2. The time-course of the short-circuit current in the concentrations of magnesium indicated by the numbers above the arrows (mM). The dotted line shows the projected time-course of the short-circuit current (I_{sc}) with no magnesium added. The net flux of magnesium from lumen side to haemolymph side is given by the vertical distance between the I_{sc} at time, t , and its projected value at this same time.

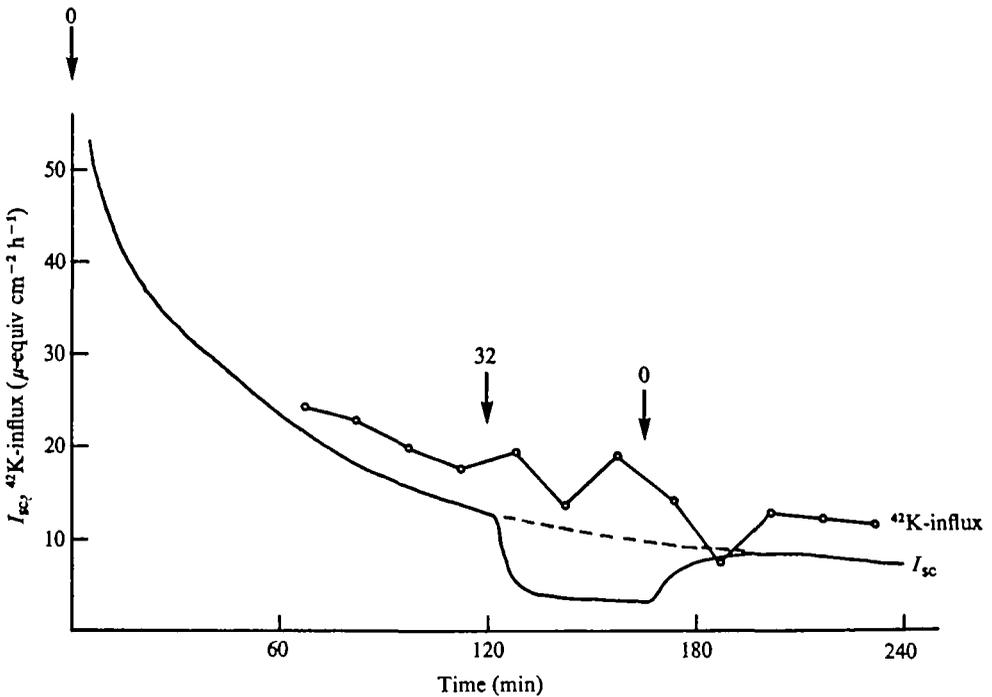


Fig. 3. The time-course of the short-circuit current (I_{sc}) and the ^{42}K -measured potassium flux from haemolymph side to lumen side (open circles) in 0 and 32 mM- Mg^{2+} (numbers above arrows). The dotted line shows the projected time-course of the I_{sc} in 0 Mg^{2+} .

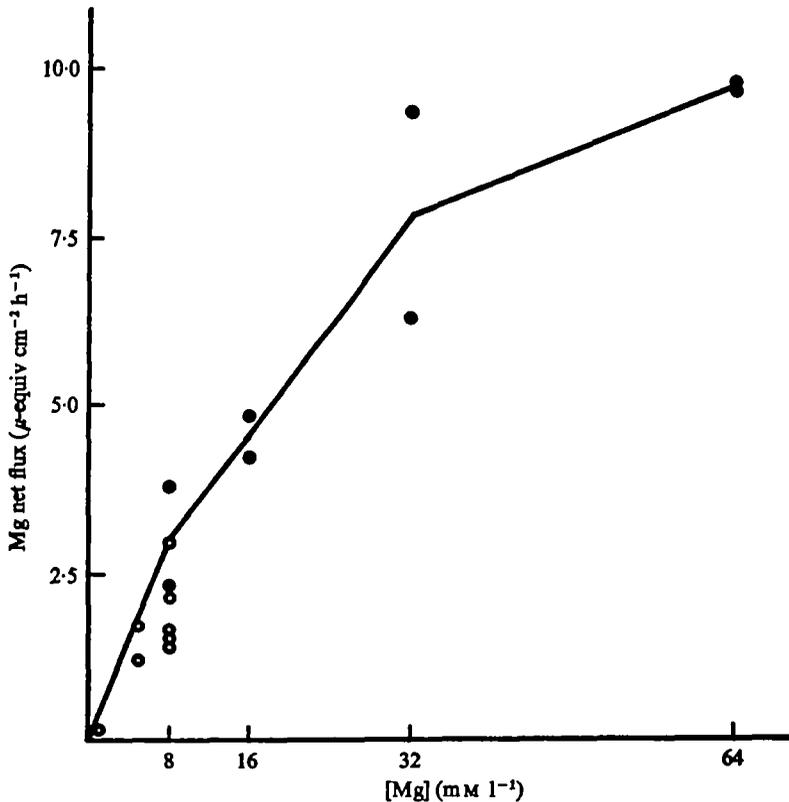


Fig. 4. The concentration-velocity curve for magnesium transport from the direct measurement of the ^{28}Mg net flux in Table 1 (open circles) and from the indirect measurement by the depression of the I_{sc} as in Fig. 2 (closed circles). The line connects the midpoints of the depression of the I_{sc} from two separate experiments.

$7.5 \mu\text{-equiv cm}^{-2} \text{ h}^{-1}$ the ^{42}K -measured potassium influx (haemolymph side to lumen side) was unaffected. A similar result was obtained in two similar experiments. Therefore the depression of the I_{sc} following the addition of magnesium is an indirect but valid measure of active magnesium transport by the midgut under these conditions.

Magnesium transport increases hyperbolically as the concentration of magnesium in the bathing solutions is increased (Fig. 4). The concentration-velocity curve was taken both from the ^{28}Mg -measured magnesium net flux directly and also indirectly from the depression in the I_{sc} resulting from the addition of magnesium to the bathing solutions. The concentration-velocity curve yields a linear Lineweaver-Burk plot (Fig. 5) with an apparent K_m of 34 mM-Mg^{2+} and an apparent V_{max} of $14.9 \mu\text{-equiv cm}^{-2} \text{ h}^{-1}$.

DISCUSSION

Magnesium is actively transported from lumen side to haemolymph side of the isolated and short-circuited midgut as determined directly from the net flux of magnesium using ^{28}Mg (Table 1) and indirectly from the depression in the short-circuit current upon addition of magnesium (Fig. 2). Magnesium transport is dependent on the concentrations of magnesium in the bathing solutions (Fig. 4) and yields a linear

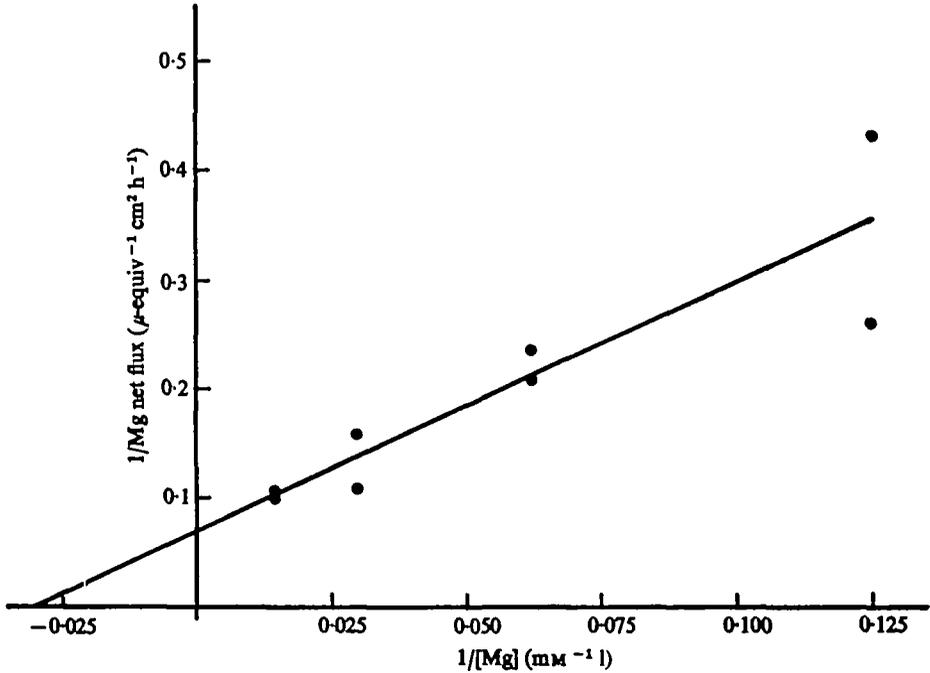


Fig. 5. The Lineweaver-Burk plot of the concentration-velocity curve from Fig. 4. The line connects the midpoints at the lowest and highest concentrations of magnesium. The apparent K_m is 34 mM-Mg²⁺ and the apparent V_{max} is 14.9 μ -equiv cm⁻² h⁻¹.

Lineweaver-Burk plot (Fig. 5) with an apparent K_m of 34 mM-Mg²⁺ and an apparent V_{max} of 14.9 μ -equiv cm⁻² h⁻¹. Therefore magnesium transport across the midgut obeys simple Michaelis-Menton kinetics similar to those found previously for potassium transport across the midgut (Harvey & Wood, unpublished data). Magnesium transport is independent of calcium (Table 1) and sodium (none was added to the bathing solutions), but its relationship to potassium transport is complicated. Although potassium transport is independent of magnesium (Fig. 3), the precise relationship between magnesium and potassium transport awaits further study.

There is a transport pool for magnesium indicated by the delay before the steady state is attained (Fig. 2), like the one found for potassium by Wood & Harvey (Wood, 1972; Harvey & Wood, 1972, 1973; Wood & Harvey, 1975), who have shown that the size of the transport pool (S_p) can be determined from tracer influx kinetics as

$$S_p = \frac{J^\infty}{\alpha},$$

where (J^∞) is the steady-state influx and (α) is the time-constant for mixing. Taking (J^∞) to be 1.5 μ -equiv cm⁻² h⁻¹ (Fig. 1), (α) to be 6.0 h⁻¹ (Fig. 1) and the wet weight to be 0.0374 g cm⁻², then the size of the transport pool for magnesium would be 6.7 μ -equiv/g wet weight in 8 mM-Mg²⁺. The total magnesium in the freshly isolated midgut is 36 μ -equiv/g wet weight in larvae fed on black cherry (Harvey *et al.* 1975) and 16 μ -equiv/g wet weight in larvae fed on synthetic diet (Jungreis, 1973). Therefore the transport pool for magnesium is equivalent to a minimum of 20% of the total magnesium in the midgut.

Active magnesium transport by the midgut plays an important role in maintaining the high level of magnesium in the haemolymph *in vivo*. The steady-state concentration of magnesium in the midgut contents is well below the apparent K_m of the isolated system but sufficiently high (9 mM compared to $K_m = 34$ mM) to yield a substantial rate of active magnesium transport toward the haemolymph *in vivo*, augmenting the passive movement of magnesium toward the haemolymph following the electrical potential gradient. Magnesium is an important ion in the haemolymph of phytophagous insects since sodium is virtually absent. It is not surprising that this important ion is regulated.

The mechanisms by which the magnesium concentration of cells and extracellular compartments is regulated are not known in any system. There is a growing literature regarding magnesium accumulation by bacterial cells (e.g. Scribner, Eisenstadt & Silver, 1974) but the question as to whether or not it is an active accumulation is unresolved. Again, magnesium is eliminated by the excretory system of vertebrates such as the ureter of trout (Beyenbach & Kirschner, 1974), but the suggestion that it involves active magnesium transport is again a deduction from *in vivo* data. Apparently the present finding of a large net flux of magnesium across the isolated midgut under short-circuit conditions is the first demonstration of active magnesium transport in an epithelial system or any other system.

This research was supported in part by a research grant (AI-09503) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, and a research grant (GB-43457) from the National Science Foundation.

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