J. Exp. Biol. (1974), 61, 749–760
With 5 figures
Printed in Great Britain

MAGNESIUM REGULATION IN MOSQUITO LARVAE (*AEDES CAMPESTRIS*) LIVING IN WATERS OF HIGH $MgSO_4$ CONTENT

BY J. KICENIUK AND J. E. PHILLIPS

Department of Zoology, University of British Columbia, Vancouver 8, B.C., Canada

(*Received* 16 *July* 1974)

SUMMARY

1. Although larvae of *A. campestris* can live in natural waters in which the magnesium content may vary seasonally from extremes of 1 to more than 100 mM, the haemolymph levels of magnesium vary only from 1·5 to 4 mM.

2. Larvae survive in water containing 90 mM-Mg and drink their own body weight every few hours, the ingested fluid and the magnesium ions being largely absorbed into the haemolymph via the midgut.

3. The urinary concentration of Mg^{2+} ions was always substantially higher than that of the external media and up to 23 times that of the haemolymph.

4. It is suggested that magnesium is not excreted across the general body surface, the Malpighian tubules and hindgut being largely responsible for removing magnesium from the haemolymph.

5. These physiological observations are discussed in relation to the environmental conditions under which larvae are naturally found.

INTRODUCTION

The Fraser River Plateau region of British Columbia possesses numerous shallow lkali ponds which become extremely concentrated in the summer due to evaporation. n some of these the concentration of $MgSO_3$ may exceed 2000 mM by late summer and he pH may rise to over 10 (Blinn, 1971; Topping, 1969). Surprisingly, larvae of the mosquito *Aedes campestris* are able to thrive in these ponds in early summer (Scudder, 1969) as well as in others containing concentrated $NaHCO_3$ but very low levels of $MgSO_4$ (Phillips & Meredith, 1969*a*, *b*; Meredith & Phillips, 1973). This information together with the findings of Phillips (unpublished) that these animals have a high drinking rate (17–100 % of body weight per day) in sodium bicarbonate waters suggests that in magnesium-rich waters magnesium may be ingested in extraordinary amounts. Several mechanisms might account for the survival of this species in such high-magnesium waters. The larvae might possess well-developed excretory mechanisms which permit them to maintain their magnesium content at low levels. Alternatively they might exhibit unusual tolerance to high haemolymph levels of this cation. A third possibility is that entry of magnesium into the haemolymph is prevented or minimized ither by reduced drinking rates or by impermeability of the gut to ingested magnesium.

Since the extent and mechanisms of magnesium regulation in insects generally have not been studied (reviewed by Maddrell, 1971), this species seemed admirably suited for investigation of this problem.

MATERIALS AND METHODS

Materials

Aedes campestris larvae were collected in late April and early May from Ctenocladus pond (located 12 miles west of Kamloops, B.C.; Blinn, 1971) and maintained in the laboratory in normal pond water at $10 \pm 0{\cdot}5$ °C. Natural particulate material in the sediment and water provided food for the larvae except when larvae were transferred to artificial media, in which case tropical fish food was used. The density of animals in storage containers was approximately 20/l, which is less than that in the pond (about 40/l). Only fourth-instar larvae were used in experiments.

Ctenocladus water on 7 May 1970 had a conductivity of 28 mmho (22 °C) with a magnesium content of 95 mM and a sodium content of 350 mM. The water temperature at the time of collection ranged from 10 °C at night to 19 °C at midday. The pH of the water was 8·8. On the date of collection in 1971 (4 May) the conductivity was 25 mmho (at 22 °C), the water temperature was 18 °C at noon, and the pH was 8·9. The magnesium concentration was 85 mM and the sodium concentration was 330 mM. The relative content of other ions in this water can be estimated from the measurements by Blinn (1971).

Mortality rate

Larvae were transferred gradually from normal Ctenocladus pond water to the test media by increasing the proportion of test solution on each of 4 days in the following proportions: 1/4, 1/2, 3/4, and full-strength test medium. Three groups, each consisting of 25 larvae, were placed in each media. Dead individuals were counted each day and removed.

Determination of magnesium content

After washing in running tap water for 3 min and drying on Kleenex, animals were individually transferred to squares of 'Parafilm', broken open with needle-pointed forceps and haemolymph was taken up in a 1 μl Drummond disposable pipette. Samples were diluted in 1 ml of 3 % EDTA solution contained in polythene vials and magnesium concentration was determined with a Techtron model AA 120 atomic absorption spectrophotometer according to the method of Willis (1960).

To measure total body content of magnesium, weighed larvae were first dried overnight in an oven at 60 °C in scintillation vials made of borosilicate glass and wet ashed according to the method of Pirie (1932). The residue was dissolved in 3 % EDTA solution for determination of magnesium as previously described.

Drinking rate

The method was similar to that of Smith (1969). Groups of 50 animals were placed in 1 ml of Ctenocladus pond water to which 0·0625 mCi of polyvinyl pyrrolidone (mol wt 30 000) labelled with ^{125}I (PVP ^{125}I; New England Nuclear Corp.) had been added. At intervals thereafter groups of ten animals were removed, rinsed, weighed and macerated individually in a drop of water on a planchet. The latter were then

dried under a heat-lamp and counted with a Nuclear Chicago, model 1042, automatic planchet counter. Drinking rate was calculated from the initial linear rate of increase in whole body ^{125}I activity.

Magnesium absorption in the gut

The method took advantage of the fact that PVP ^{125}I is not absorbed from the gut (see Results). Groups of larvae were placed into normal Ctenocladus water containing PVP ^{125}I for 30 h or 6 days. At the end of this time the whole midgut was dissected out, blot dried on filter paper, weighed and placed in 4 ml of 3 % EDTA in capped polythene vials. After a few days at room temperature to permit breakdown of the wall and release of PVP ^{125}I, the midguts were further broken up with forceps. Samples of this fluid were used for determinations of magnesium content and ^{125}I activity. The Mg/PVP ^{125}I ratios for the gut and its content were compared to those of the external medium. If no absorption of magnesium occurred in the gut, Mg/PVP ^{125}I ratios for the gut content and external medium should be the same. This method probably tended to underestimate absorption because no correction was made for magnesium in the gut wall itself.

Urine sampling

To determine whether magnesium levels were regulated via the excretory system, urine samples were taken in the following manner: Several hundred animals were acclimated for 1 week in media ranging in magnesium concentration from 1 to 85 mM. Larvae were removed as required, dabbed dry on tissue paper and placed under paraffin oil in a siliconized petri dish. Attempts were made to collect urine directly as it was released but it was found that the urine clung tenaciously to the faecal pellets. Centrifugation was therefore required to separate particles of faecal material from the urine. To do this a 30 μl Drummond pipette with the tip broken off at a 45° angle was first partially filled with paraffin oil, then faecal pellets were moved under oil into the pipette using a glass rod. When enough faecal material was collected, the pipette was sealed with beeswax and centrifuged for 20 min in a 'Clay-Adams' hematocrit centrifuge. The pipette was then cut off above the urine/oil interface and clear urine was taken up in tapered micropipettes (0·25 μl) with the aid of a micromanipulator. With adequate care, urine samples thus taken contained no oil or faecal matter. The separated faecal material was wet ashed and the magnesium content was determined as previous described.

RESULTS

Extent of magnesium regulation

Fourth-instar larvae were transferred gradually to various media in which the magnesium concentration was varied from 7 to 150 mM either by dilution of Ctenocladus pond water or by the addition of $MgSO_4$. While a high mortality rate was observed both in natural Ctenocladus pond water and in these various experimental media, none the less a substantial number of individuals were able to pupate and emerge as adults. The average mortality rate remained unchanged at about 10 % per day and was not significantly different for various media except when the magnesium concentration was raised from 100 to 150 mM (data available in Kiceniuk, 1971). In more dilute pond water (11·5 μM-Mg) larvae survived only until pupation. In

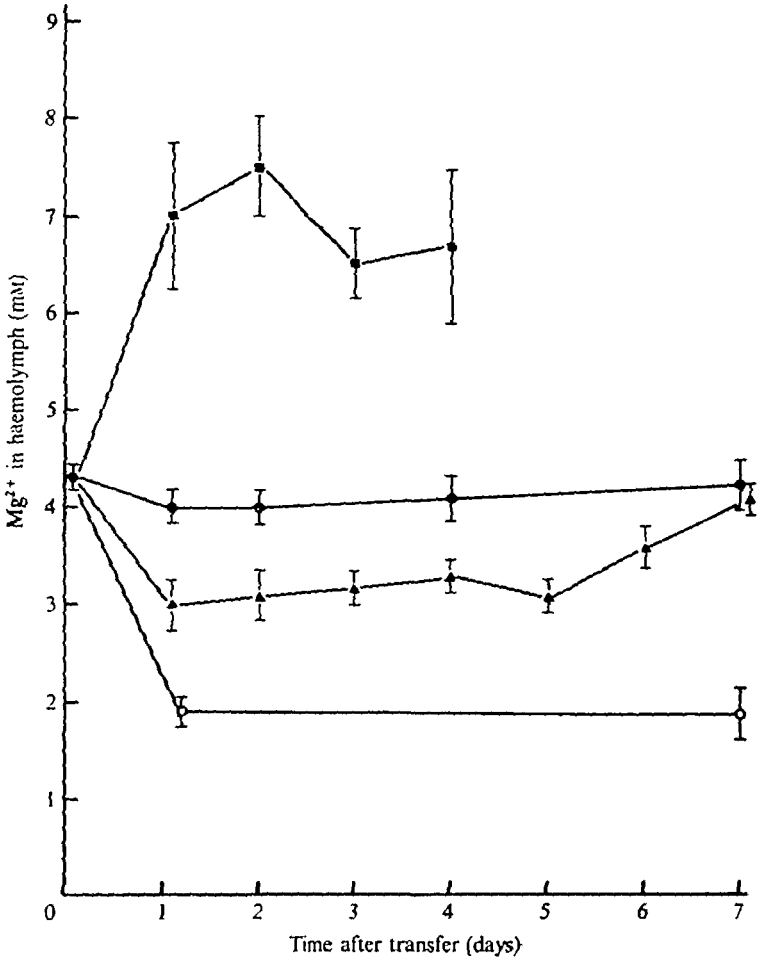

Fig. 1. The concentration of *magnesium* in the haemolymph with time after transferring larvae *from* Ctenocladus pond water to media having *different* levels of this cation. These media were prepared by diluting pond water with distilled water or by adding $MgSO_4$ to yield magnesium concentrations of 4 (○), 50 (▲), 150 (■) and 95 (●) (Ctenocladus water) mM. Vertical lines indicate ±S.E. of the mean for ten larvae.

summary, some individuals in the population are able to survive both in hypotonic and in hypertonic media, as previously observed for this species in $NaHCO_3$ waters (Phillips & Meredith, 1969*a*, *b*).

The concentrations of magnesium in haemolymph of surviving larvae at various times after transfer directly to different media are shown in Fig. 1. The haemolymph levels of magnesium responded quickly to changes in external levels during the first day but stabilized thereafter. When steady-state levels of magnesium in the haemolymph at 4 days after transfer are plotted against external concentrations (Fig. 2), a remarkable degree of regulation is apparent. Over a 10 000-fold range of external magnesium concentrations (0·01–100 mM) the haemolymph levels of this cation only increased twofold, from 2 to 4 mM.

The possibility that these low haemolymph levels of magnesium were the result of

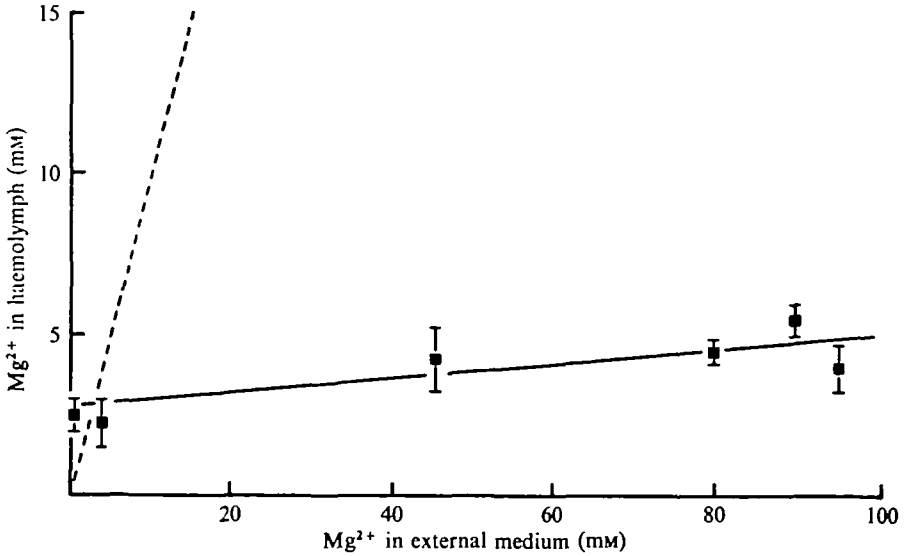

Fig. 2. The steady-state (4 day) concentrations of magnesium in haemolymph of larvae as a function of external concentration of this cation. External magnesium concentration was varied either by diluting Ctenocladus pond water or by adding $MgSO_4$ to the latter. Vertical lines indicate ±S.E. of the mean for ten larvae. The broken line indicates isotonicity.

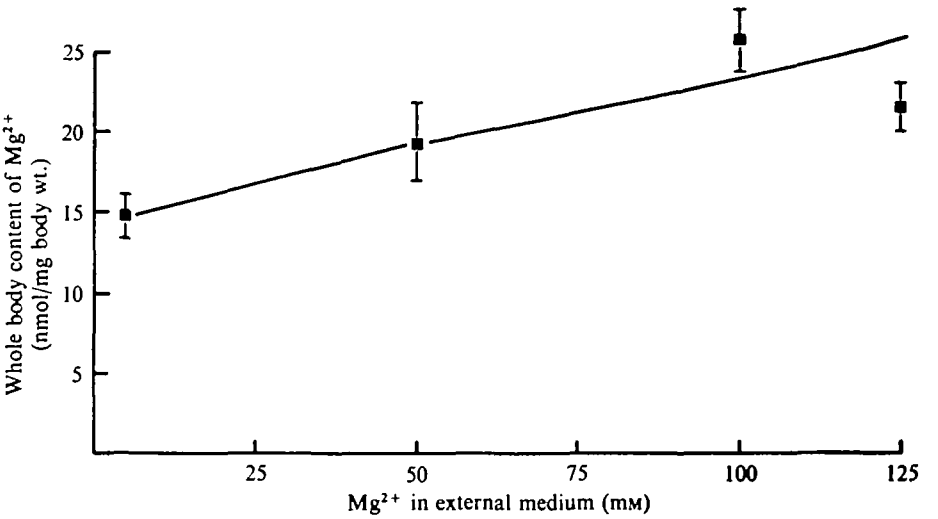

Fig. 3. The whole body content of magnesium per unit wet weight of larvae (average total weight of 4·6 mg) as a function of external concentration of this cation. Larvae were adapted for 1 week in media prepared either by diluting Ctenocladus pond water with distilled water or by adding $MgSO_4$ to the latter. Vertical lines indicate ±S.E. of the mean for ten animals.

storage of this cation (e.g. as precipitates) in particular compartments or tissues of the body was considered. The whole body contents of magnesium in larvae acclimated for a week to various external media are shown in Fig. 3. The body content of magnesium (25 m mol/kg body weight) is low compared to that in normal Ctenocladus pond water (90 mM). For a 40-fold change in external magnesium concentration, the total body content of this cation changed less than twofold; this parallels the regulatory abilities

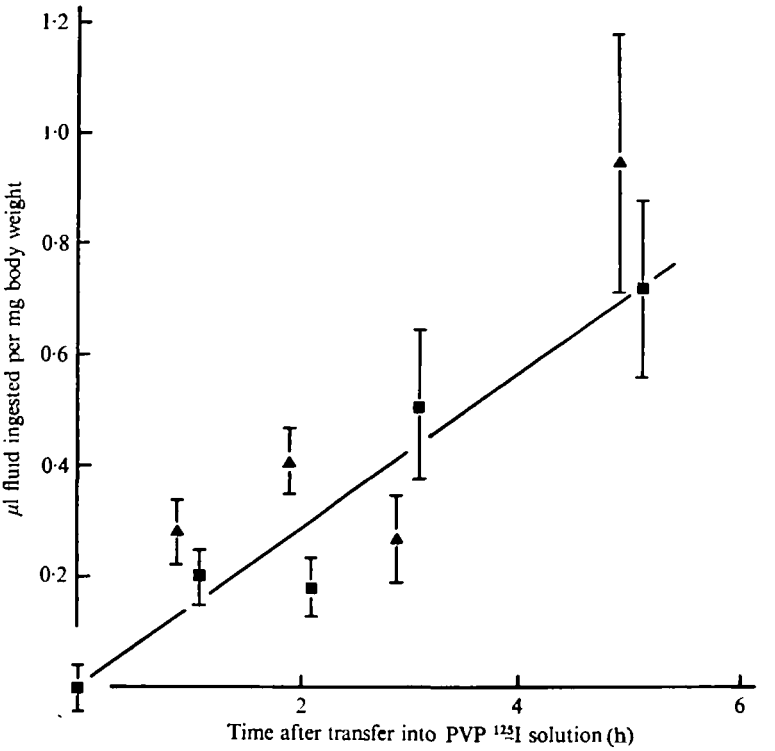

Fig. 4. The amount of Ctenocladus pond water ingested per unit wet weight of larvae with time at 10 °C (■) or 22 °C (▲). Drinking was estimated from the increase in body content of ^{125}I-activity following addition of a large inert polymer (PVP ^{125}I) to the external media. Vertical lines indicate ±S.E. of the mean for ten animals.

indicated by studies of haemolymph levels. In summary, *Aedes campestris* larvae are able to maintain their body content of magnesium at very low and constant levels in the face of drastic variations in external levels of this cation. The question now arises as to whether this is because they do not drink the water in which they live.

Absorption of external magnesium

Preliminary experiments (data available in Kiceniuk, 1971) confirmed that PVP ^{125}I was not adsorbed on the body surface of larvae and did not enter the haemolymph in significant amounts. The whole gut of larvae held for 40 h in pond water containing this compound contained on average 20 times the activity per unit weight as did the external media. Virtually all the activity in the body was retained in the midgut. These experiments confirm that this compound can be used to estimate drinking rates of *A. campestris* larvae, as previously shown by Smith (1969) for *Artemia salina*. These results also indicated that 95 % of fluid ingested by these *A. campestris* larvae was absorbed in the midgut.

Fig. 4 shows the amount of fluid ingested with time after transferring larvae to Ctenocladus pond water containing PVP ^{125}I at either 10 or 22 °C. The mean rate of ingestion, which did not vary significantly over this temperature range, was 0·15 μl h^{-1} mg^{-1} or about 300 % of total body weight (4·6 mg average) per day. We considered the possibilities that drinking rate might be influenced by the presence of food

Table 1. *The concentration of magnesium in clear urine and faecal material, which were separated by centrifugation, as a function of external concentration of this cation*

(External concentrations were varied by diluting Ctenocladus pond water. Mean ± S.E. for animals which had been acclimated to experimental media for one week. The urine to external medium (U/M) and urine to haemolymph (U/M) concentration ratios of magnesium were calculated from the means.)

	Magnesium concentrations (mM)		Concentration ratios	
External medium	Urine	Faeces	U/M	U/H
1	2·8 ± 0·67	125*	2·8	0·7
5	11 ± 2·2	177 ± 12·2	2·2	2·3
17	23 ± 1·9	188 ± 4·0	1·4	5·2
35	42 ± 2·9	206 ± 34·1	1·2	9·3
85	89 ± 4·0	221 ± 6·2	1·0	23·0

* One value only.

particles (including micro-organisms) and by the external concentration of magnesium. The drinking rate was not significantly different when larvae were placed in Millipore-filtered water (0·45 μm pore diameter) or when the external concentration of magnesium was varied from 50 to 150 mM as previously described (data available from Kiceniuk, 1971).

To determine whether the large amounts of magnesium ingested were actually absorbed from the midgut into the haemolymph, larvae were placed in Ctenocladus pond water containing PVP ^{125}I and the Mg:PVP ^{125}I concentration ratios in the midgut contents and external media were determined after either 30 h or 6 days. This ratio (mean ± S.E.) was 7·6 ± 0·26 (n = 3) in the external medium and 0·061 ± 0·0086 (n = 19) in the midgut contents; that is, virtually all of the ingested magnesium was absorbed in the midgut. This means that in Ctenocladus pond water containing 85 mM-Mg, where a conservative estimate for drinking rate is 0·1 μl h^{-1} mg^{-1}, the entire body content of magnesium (Fig. 4) is turned over in about 3 h. These high rates of absorption explain why haemolymph levels of magnesium change abruptly during the first day of transfer to different media (Fig. 1).

Site of magnesium removal

The rapid absorption of magnesium by larvae which are able to maintain very low body levels of magnesium indicates the presence of efficient excretory processes for removal of this cation from the body against large concentration gradients. From our present knowledge of the mechanisms whereby salt water species of insect regulate their NaCl content (Phillips & Meredith, 1969; Meredith & Phillips, 1973; Leader, 1972), the Malpighian tubules, rectum, anal papillae, or general body surface might be possible sites of magnesium removal.

If the excretory system (Malpighian tubule–rectal complex) is completely responsible for removal of ingested magnesium, then the magnesium concentration in the excreta should equal or exceed that of the external media, allowing for some water loss across the body wall. This is indeed the case (Table 1). The urine to external medium concentration ratios varied from 1·0 at the highest to 2·8 at the lowest external

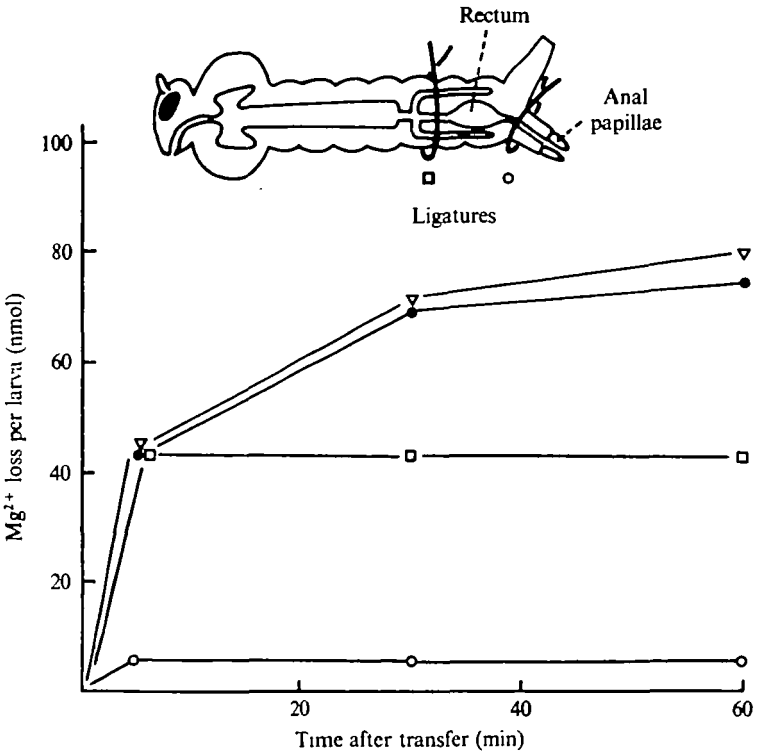

Fig. 5. The total loss of magnesium per larvae with time after transferring rinsed animals from Ctenocladus pond water to droplets of distilled water under liquid paraffin. Individual larvae were placed in 1 μl droplets (open symbols) or 20 larvae we placed in 1 ml of water (solid symbols) and the increase in external magnesium levels followed. Open symbols are mean values for ten animals. The loss from normal larvae (▽, ●) is compared with that from animals which has been ligated at the anal segment (i.e. posterior to the rectum, ○) or at the penultimate adbominal segment (i.e. anterior to rectum, □).

magnesium concentrations. The urine to haemolymph concentration ratios rose steadily as the external concentration of magnesium increased to attain a ratio of 23 when the external level of this cation was 85 mM. The magnesium content of faecal material was in all cases several times that of the urine, presumably due to precipitates or binding of magnesium to the anionic sites of macromolecules. Thus, if the rate of fluid excretion from the anus is equal to or slightly less than the rate of fluid ingestion, the Malpighian–rectal system could account completely for removal of ingested magnesium.

Unfortunately a method of accurately measuring rate of fluid excretion under steady-state conditions was not available. On handling, larvae tended immediately to void their rectal contents, leading to overestimates of fluid excretion rates. However, it was possible to distinguish between the general surface of the body wall and the excretory system as sites of magnesium removal by ligation of larvae at the appropriate site and by then following the rate of magnesium concentration increase in small (1 μl) droplets of distilled water into which larvae were placed under liquid paraffin. It should be emphasized that ligated larvae swim actively without any apparent decrease in viability for at least 24 h. All experiments were completed within 1 h of ligation.

When the ligature was posterior to the rectum and siphon (i.e. at the anal segment) there was a very small increase in magnesium concentration during the first 5 min but none thereafter (Fig. 5). We believe this apparent very small initial loss is due to external magnesium from fluid in crevasses of the body. The body wall is not a site of magnesium removal from the larvae because no loss of this cation to the external medium occurred after 5 min. The same was true when the ligature was placed just anterior to the rectum but posterior to the point where Malpighian tubules join the gut; i.e. at the anterior border of the penultimate abdominal segment (Fig. 5). However, under these conditions the initial loss during the first 5 min was greatly increased presumably due to voiding of the rectal contents. This interpretation is supported by the observation that unligated larvae lose the same amount of magnesium during the first 5 min (Fig. 5). Thereafter unligated larvae continued to lose magnesium at a greatly diminished rate (5–30 min) which may have more closely approximated the steady-state rate of excretion since the original rectal contents had already been voided. The loss rate continued to decline (30–60 min) possibly due to the low concentration of magnesium in the bathing fluid, which the larvae had by then ingested in substantial amounts. These experiments exclude the body wall and again implicate the excretory system as the site of magnesium removal.

DISCUSSION

This study has shown that fourth-instar larvae of *Aedes campestris* maintain their haemolymph levels of magnesium relatively independent of external conditions, as previously shown for NaCl (Phillips & Meredith, 1969*a*, *b*). The magnesium content is low in the midgut but very high in fluid within the excretory system. The total weight of the latter, however, is probably less than 5 % of body weight. Therefore, estimates of total body magnesium suggest that intracellular levels of this cation may be much higher than those in the haemolymph; nevertheless total magnesium content remains relatively independent of external concentration. This extraordinary regulatory capacity is a valuable adaptation because it allows larvae of this species to inhabit numerous saline ponds which are very productive in terms of organic matter (Blinn, 1969) and which are inhabited by only a few other animal species, none of which are obvious predators.

The severe selection pressures which have given rise to these regulatory abilities in waters of such high magnesium content are evident from field observations. The salt-water ponds on the Fraser River Plateau are relatively dilute at the time mosquito larvae hatch but the salinity increases rapidly within a matter of weeks. The rate and extent of this increase is quite variable from year to year depending on climatic conconditions. Animals used in this study were collected in particularly dry years (1970, 1971) when the total osmolality of Ctenocladus pond water was several times higher than that of haemolymph of *A. campestris* larvae. A high mortality rate was observed amongst the natural population such that the bottom of the pond was covered with dead larvae to a depth of several millimeters and surviving larvae appeared grossly shrunken, which indicated difficulties in regulating body volume. Nevertheless many individuals pupated and emerged as adults. A few weeks later when the magnesium vel had risen to 125 mM, larvae which had hatched later in the season did not survive

pupation. (Obviously the correct timing of hatching and rapid development are critical to survival.) This suggests an upper tolerance limit between 95 and 125 mM-Mg, which was confirmed by mortality rates in the laboratory. Sodium and sulphate levels are several times higher than magnesium concentrations in this pond (Blinn, 1971); therefore, it would be premature to designate high magnesium levels as the limiting factor. Regardless, there was obviously a severe selection for good hypo-regulators that could excrete magnesium (and sodium and sulphate also) at high rates. By contrast, there was heavy precipitation in the spring of 1974 and the osmolality of Ctenocladus pond water was only half that of haemolymph from *A. campestris* larvae collected quite late in the season (Phillips & Maddrell, 1975). Little natural mortality was observed and the larvae were not shrunken in appearance. Clearly the capacities within the population for hyper-regulation are called upon in some years.

These field observations are presented to justify the laboratory studies reported in this paper. While our results may hold for only a small fraction of the total population of *A. campestris*, we believe they reflect a natural and common situation for surviving individuals at the upper limit of the salinity tolerance range in high $MgSO_4$ waters. After transferring these larvae to media of different salinities, mortality was low (10–20 %) at the time new steady-state levels of haemolymph magnesium were achieved (1–2 days). We believe, therefore, that the regulatory abilities apparent in Fig. 2 reside within individuals and are not a consequence of further selection from within a variable population.

The rates of drinking reported for *A. campestris* are several times higher than those observed by Phillips (17–100 % body weight/day) for the same species in $NaHCO_3$ waters, varying in concentration from 0·01 to 1·2 osM. While this discrepancy might be due to the use of a selected group of hypo-regulators in the present study, a more likely explanation is the shrunken condition of the latter larvae, which had lost up to a third of their body water. Phillips (unpublished observation) observed that when larvae from bicarbonate waters were pushed to their upper osmotic tolerance limits so that body volume decreased more than 5–10 %, drastic increases in drinking also occurred. Presumably such larvae desperately attempt to restore volume and hence the high internal hydrostatic pressure which is essential for mobility in an animal with a hydrostatic skeleton.

Considering the composition of $MgSO_4$ and $NaHCO_3$ ponds, it is perhaps surprising that *A. campestris* larvae drink so rapidly even at moderate and low salinities when loss of water across the body wall is low or absent. The explanation might be that while there is abundant organic nutrient in the flocculent sediment on which larvae appeared to feed, only a small percentage of the ingested carbon compounds are available in forms which are readily assimilated. Therefore large amounts of sediment containing mostly water must be consumed. This material can be observed in a concentrated form as a consequence of fluid removal in the midgut. Thus to take advantage of an abundant but dilute external energy source, high rates of fluid intake and the high cost of osmoregulation cannot be avoided.

We have concluded that the net magnesium loss observed between 5 and 30 min of transferring larvae to magnesium-free media gives a reasonable estimate of excretion rates (Fig. 5). If this interpretation is correct, then the excretory system may be alone responsible for removal of ingested magnesium. Specifically for larvae in Ctenocladu

Water, the calculated mean rate of magnesium excretion per larvae is 54 nmol h^{-1} (Fig. 5) and the estimated rate of ingestion is 42–83 nmol h^{-1}, using observed mean drinking rates of 0·1 to 0·2 μl h^{-1} mg^{-1} for different groups of larvae (Fig. 4). Secretion of some magnesium by anal papillae cannot be rigorously excluded because the small internal pool of this cation which is present in the few segments posterior to the ligature might be quickly depleted so that no loss is observed after 5 min. Nevertheless, the excretory system is clearly the major site of magnesium loss. This is directly confirmed by the observation of Phillips & Maddrell (1975) that the full compliment of Malpighian tubules of *A. campestris* actively secrete Mg^{2+} ions by a saturable mechanism at rates comparable to those reported in the present paper for loss via the anus, at least at moderate external concentrations.

The principal anion (> 90 %) in Ctenocladus pond is sulphate, at a concentration of about 250 mM in the water used in the present experiments (estimated from Blinn, 1971). Since we have now established that nearly all the ingested fluid and magnesium are absorbed in the midgut, presumably most of the sulphate is also assimilated. This conclusion suggests that the Malpighian tubules might also possess an even more powerful sulphate transport mechanism. Subsequent studies have indicated that this is indeed the case (Maddrell & Phillips, 1975). Thus the situation in *A. campestris* and possibly other salt-water insect species is analogous to that of marine fishes whose nephrons possess separate secretory mechanisms to deal with the high levels of Mg and SO_4 ions in their natural environment (Hickman & Trump, 1969). A difference is that SO_4^{2-} ions almost completely replaces Cl^- ions in Ctenocladus and similar ponds (Blinn, 1971).

We wish to thank Miss Joan Meredith for technical assistance and Dr Simon Maddrell for reading the manuscript. This work was supported by operating grants from the National Research Council of Canada.

REFERENCES

Blinn, D. W. (1969). Autoecology of *Ctenocladus* (Chlorophyceae) in saline environments. Ph.D. thesis, University of British Columbia.

Blinn, D. W. (1971). Dynamics of major ions in some permanent and semipermanent saline systems. *Hydrobiologia* **38**, 225–38.

Hickman, C. P. & Trump, B. F. (1969). The kidney. In *Fish Physiology*, vol. 1 (ed. Hoar and Randall), pp. 91–240. New York: Academic Press.

Kiceniuk, J. W. (1971). Magnesium regulation in *Aedes campestris* larvae. M.Sc. Thesis, University of British Columbia.

Leader, J. P. (1972). Osmoregulation in the larvae of the marine caddis fly, *Philanisus plebeius* (Walk) (Trichoptera). *J. exp. Biol.* **57**, 821–38.

Maddrell, S. H. P. (1971). The mechanisms of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.

Maddrell, S. H. P. & Phillips, J. E. (1975). Active transport of sulphate ions by the Malpighian tubules of the larvae of the mosquito, *Aedes campestris*. *J. exp. Biol.* (In the Press).

Meredith, J. & Phillips, J. E. (1973). Rectal ultrastructure in salt- and freshwater mosquito larvae in relation to physiological state. *Z. Zellforsch. mikrosk. Anat.* **138**, 1–22.

Phillips, J. E. & Maddrell, S. H. P. (1975). Active transport of magnesium by the Malpighian tubules of the larvae of the mosquito, *Aedes campestris*. *J. exp. Biol.* **61**, 761–771.

Phillips, J. E. & Meredith, J. (1969*a*). Osmotic and ionic regulation in a salt water mosquito larva *Aedes campestris*. *Am. Zool.* **9**, 588.

Phillips, J. E. & Meredith, J. (1969*b*). Active sodium and chloride transport by anal papillae of a salt water mosquito larva (*Aedes campestris*). *Nature, Lond.* **222**, 168–9.

PIRIE, N. W. (1932). The metabolism of methionine and related sulphides. *Biochem. J.* **26**, 2041–5.
SCUDDER, G. G. E. (1969). The fauna of saline lakes of the Fraser Plateau in British Columbia. *Verh. Internat. Verein. Limnol.* **17**, 430–9.
SMITH, P. G. (1969). The ionic regulation of *Artemia salina* L. II. Fluxes of sodium, chloride and water. *J. exp. Biol.* **51**, 739–57.
TOPPING, M. S. (1969). Giant chromosomes, ecology and adaptation in *Chironomus tentans*. Ph.D. thesis, University of British Columbia.
WILLIS, J. B. (1960). Spectrophotometric method for determination of Ca and Mg in biological fluids. *Spectrochim. Acta* **16**, 273–8.