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EXCRETION IN THE HOUSE CRICKET: STIMULATION OF RECTAL REABSORPTION BY HOMOGENATES OF THE CORPUS CARDIACUM

JEFFREY H. SPRING AND SULAYMA A. ALBARWANI*

Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504, USA

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Summary

1. We describe an *in vitro* perfused preparation of *Acheta domesticus* rectum which allows direct comparison of Malpighian tubule secretion and rectal absorption under identical conditions. Rectal absorption is stimulated four- to sixfold by corpora cardiaca (CC) homogenates and the stimulated rate is sufficiently rapid to account for all the fluid secreted by the tubules.

2. The time course for increased fluid absorption is similar to that required to stimulate electrogenic chloride transport in locusts and grasshoppers. Chloride is rapidly absorbed by the rectum under all conditions, along with lesser amounts of Na^+ and K^+. Unlike the situation in locusts, K^+ uptake is unaffected by CC homogenates and the stimulated absorbate is NaCl-rich, similar in composition to the NaCl-rich tubule fluid produced under stimulated conditions. The absorbate is always slightly hypo-osmotic to the perfusate, reaching a maximum differential of approximately 15 mosmol l^{-1} following CC stimulation.

3. The antidiuretic factor that reduces tubule secretion does not promote fluid reabsorption by the rectum.

Introduction

The corpora cardiaca (CC) of insects contain a plethora of factors known to alter fluid secretion by the Malpighian tubules and absorption by the hindgut (Phillips, 1983; Bradley, 1985; Spring, 1990). Bioassays of diuretic or antidiuretic activity vary widely, however, and in the majority of cases there are no direct comparisons between the output of primary urine by the Malpighian tubules and the reabsorptive capacities of the hindgut (Phillips, 1983; Spring, 1990).

Until recently, the action of regulatory factors in non-blood-feeding insects was assumed to be only partially bidirectional. Diuretic factor increased tubule secretion and inhibited rectal reabsorption whereas antidiuretics stimulated rectal absorption, leaving tubule secretion to decline because of the absence of stimulatory factors (reviewed by

*Present address: College of Medicine, Sultan Qaboos University, PO Box 32485 ALKHOD, Muscat, Sultanate of Oman.

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Gee, 1977; Phillips, 1983). In *Acheta domesticus*, this model had to be modified somewhat following the discovery of an antidiuretic factor that acted specifically to inhibit tubule secretion (Spring *et al.* 1988).

Still largely unanswered was the question of how tubule secretion and rectal reabsorption were integrated. Increasingly, there has been evidence that, at least in xeric insects, diuretic factors may promote increased hemolymph filtration without any overall water loss (Proux *et al.* 1984; Nicolson and Hanrahan, 1986; Spring, 1990) and indeed Nicolson (1991) has recently proposed that the term clearance factor might be more appropriate than diuretic factor in these insects.

The present study was designed to compare the functions and regulation of the rectum in the house cricket, *Acheta domesticus*, under *in vitro* conditions directly comparable to those employed to examine Malpighian tubule function (Spring and Hazelton, 1987; Clark and Spring, 1992). Given that the rectum was almost certain to be affected by factors in the CC, this study was designed to address the following questions. (1) What are the rates and the time course for stimulation of rectal fluid absorption? (2) Which ions are absorbed to support fluid uptake? (3) Is rectal absorption sufficiently rapid to recover secretion by the tubules? (4) Is the stimulatory (i.e. antidiuretic) factor the same as the factor that inhibits tubule secretion in *Acheta domesticus*?

Materials and methods

Experimental animals

Immature *Acheta domesticus* L. were obtained from Flucker's Cricket Farm, Inc. (Baton Rouge, LA) and reared to adulthood in the laboratory. They were maintained at 30°C, on a 14h:10 h L:D photocycle and provided with Purina cricket chow and fresh water *ad libitum*. Immature females were isolated from the population midway through their last nymphal instar and placed in separate 4l containers (20–30 crickets per container). All experiments were performed using virgin adult females, 15–30 days past their final molt.

Solutions

The same physiological saline was used both as a bathing medium and to perfuse the rectum in order to eliminate any ionic or osmotic gradients which might drive a passive water flux. The saline used in all experiments was identical to that employed by Spring and Hazelton (1987). Its composition (in mmol l^{-1}) was as follows: NaCl, 100; K_2SO_4, 10; $MgSO_4.7H_2O$, 10; $NaHCO_3$, 10; $CaCl_2.2H_2O$, 3.5; glycine, 10; glucose, 10; imidazole, 15; proline, 10; glutamine, 2; histidine, 2; leucine, 2; lysine, 4; tyrosine, 2; valine, 4; pH7.2–7.3. Bubbling the saline with water-saturated carbogen (95% O_2:5 % CO_2) reduced the pH to 7.0, where it stabilized for the remainder of the experiment. The osmotic concentration was adjusted to 300mosmol l^{-1} with deionized water.

It was subsequently discovered that the declining basal secretion rate observed by Spring and Hazelton (1987) was a function of their bicarbonate-buffered saline and that tubules maintained in a Hepes-buffered saline secreted at a constant rate for at least 3 h (Spring and Clark, 1990; Clark and Spring, 1992). Accordingly, a new set of Malpighian tubule experiments was carried out using Hepes-buffered saline. Its composition (in

mmol l^{-1}) was identical to the standard saline with the following exceptions: Hepes, 25; $NaHCO_3$, 0; imidazole, 0; pH7.2, final osmotic concentration 300–310mosmol l^{-1}.

Determination of secretion rates

For experiments involving secretion rates, crickets were dissected and secretion rates were determined as described by Spring and Hazelton (1987). The tubule preparations were allowed 45min after the first urine drop had been removed to stabilize and to establish the control secretion rate, after which experimental solutions were added. Secreted droplets were sampled at 15min intervals. Bath osmotic concentration was maintained relatively constant by adding deionized water to the bath at each measurement interval to compensate for evaporative losses (Spring and Hazelton, 1987). Often as much as 100 μl was needed to maintain the bath volume in control preparations; therefore, samples of experimental solutions in deionized water of up to 100 μl in volume were added directly to the bath, in lieu of the compensatory volume of deionized water.

Perfused rectal preparations

The head, legs, wings and cerci were removed from the cricket using dissecting scissors. The body was then pinned dorsal-side-up in a wax-filled Petri dish. The cricket was opened dorsally with a midline incision, and the cuticle was pinned away from the body. The egg masses were removed and the preparation was flooded with warm saline (30°C). The saline was constantly oxygenated with water-saturated carbogen (95% O_2:5% CO_2) throughout the period of dissection. Sufficient tracheae were removed to free the hindgut and permit it to be pinned straight. A small cut was made in the anterior hindgut, a polyethylene tube filled with saline was inserted into the rectum through the anus and the saline was flushed through the hindgut to remove all fecal material. A polyethylene cannula (PE-10, Intramedic) was inserted through the cut until it was close to the rectal pads, where it was secured with silk thread. A second cannula (PE-50, Intramedic) was inserted into the rectum, through the anus, and ligated. Both cannulae were then trimmed to a length of 4–7mm, following which the rectum and its associated cannulae were transferred to an assay chamber.

The incubation chamber (Fig. 1) was identical to that used by Spring and Hazelton (1987) for Malpighian tubule assays, with the single exception that the saline bath was larger (2.5ml total volume) to accommodate the rectum. The preparation was maintained under identical conditions to that of the Malpighian tubules; i.e. the saline was continuously oxygenated, its osmotic concentration was maintained close to 300mosmol l^{-1} throughout the experiment, and the chamber was water-jacketed to maintain the temperature between 28 and 30°C. The inlet cannula to the rectum was attached to a gas-tight syringe (Hamilton, 0.1ml) mounted to a motorized microdrive (Sage Instruments) for continuous perfusion. The perfusion apparatus was based on that of Dow (1981). The outlet cannula was fitted into the notch in the acrylic wall separating the saline and oil baths and a seal was formed using silicone grease. The rectum was perfused on the lumen side with saline infused at a rate of 11–12 μl h^{-1} unless otherwise specified. The impermeant dye Amaranth (FD&C red dye no. 2) was added to the infused saline to detect any damage or leaks in the rectal wall. In all experiments, mounted recta

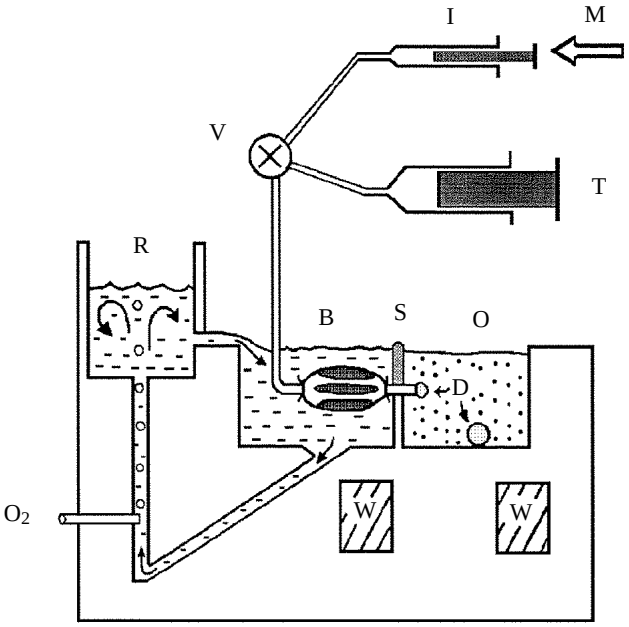

Fig. 1. Schematic diagram of the apparatus used to maintain *Acheta domesticus* recta *in vitro*. R, saline reservoir; B, saline bath; O_2, inlet for gas-lift pump. Saline circulation is in the direction of the arrows. S, silicone grease; D, perfusate droplets, forming at the end of the cannula and resting on the floor of the oil bath (O). W, water jacket for heating the preparation. I, 0.1ml injection syringe; M, motor drive; T, 3.0ml rinse syringe; V, three-way valve. For a complete explanation, see text.

were flushed completely using the rinse syringe to remove all gut fluid that might be present. The preparations were also allowed to run for 60min before beginning the experiment to allow the recta to stabilize, since rectal activity is known to fluctuate during the first hour after dissection as a result of its removal from natural hormonal and neural influences (Phillips *et al.* 1982). In control preparations, the recta were allowed to run for 3.3h following initial stabilization. Experimental preparations were allowed to run for 90min to establish baseline values, then the test solution was added to the hemocoel side (the saline bath) for 1.3h. After the test period, the chamber was rinsed with three changes of fresh saline to remove the stimulants from the bath and the rectum was incubated for an additional 90min. In all experiments, the perfusate droplets were collected under oil at 20min intervals and their volume was determined as described by Maddrell (1980). Droplets were kept under oil in separate depressions for further analysis. In one set of experiments, the effect of perfusion rate on fluid absorption was determined by decreasing the rate from 11–12 $\mu l h^{-1}$ to 6.8 $\mu l h^{-1}$.

Preparation of corpora cardiaca homogenates

The corpora cardiaca (CC) are located behind the brain, connected to it by three pairs of nerves (Raabe, 1982). They were removed using the method of Spring and Hazelton (1987). To expose the CC, a freshly severed head was placed posterior-side down on a

wax dissecting surface. A single-edged razor blade was then used to cut off the upper portion of the head capsule, with a horizontal incision being made just below the two lateral ocelli. The lower portion was placed in the normal vertical position and secured with insect pins. The bluish-white CC were then readily visible near the pars intercerebralis and could be dissected free using fine forceps and iris scissors. The CC were immediately homogenized in distilled water (4 pairs per 100 μl) in a Potter–Elvehjem ground-glass homogenizer and stored on ice until used. Homogenates of four pairs of freshly dissected CC were used for each perfused rectum experiment, whereas Malpighian tubules were stimulated with a single pair of CC.

Reversed-phase HPLC

Twenty pairs of CC were homogenized in 200 μl of distilled water and the antidiuretic fraction was isolated using reversed-phase HPLC following the method of Spring *et al.* (1988). Briefly, the aqueous solvent was 0.11% trifluoroacetic acid (solvent A), and solvent B was 0.1% trifluoroacetic acid in 60% acetonitrile. Solvents were applied as a linear gradient (25% to 80% B in 45min; 1.0mlmin^{-1} flow rate), and the eluant was monitored at 210nm. The peak known to inhibit Malpighian tubule secretion in *Acheta domesticus* (Spring *et al.* 1988) was hand-collected and dried under a stream of filtered compressed air. Dry fractions were resuspended in 500 μl of distilled water and four CC equivalents were used for each experiment.

Volume and rate determinations

In experiments where volume and rate of fluid absorption were to be determined, a known quantity of ^{14}C-labelled inulin (specific activity 100 MBq g^{-1}) was added to the perfusing saline. Samples of 45 μl were taken from a stock isotope solution and added to 10ml of perfusing saline to bring the final specific activity to approximately 100ctsmin^{-1} $\mu\mathrm{l}^{-1}$. Each collected perfusate droplet (volume 2.5–4.5 μl) was transferred to a scintillation vial containing 0.5ml of distilled water. 10 ml of fluor (Scintiverse I, Fisher Scientific) was added to the vial and the radioactivity was measured using a Beckman LS 6000 scintillation counter. Triplicate 1 μl samples of the infusate were counted in the same manner to determine accurately the initial specific activity. Absorption was calculated from the increase in specific activity of the perfusate relative to the infusate. Since the precise volume of each perfusate droplet was known, absorption could be calculated both as a percentage and as the absolute volume absorbed.

Osmotic concentration determinations

During all experiments, the osmotic concentration of the bathing saline was measured at 20min intervals using a Wescor 5100B vapor pressure osmometer. The osmotic concentration was found to rise by 5–10mosmoll^{-1} between measurements due to evaporation from the bath. Distilled water was therefore added in order to maintain the bath osmotic concentration between 300 and 310mosmoll^{-1}. Owing to the small size of the perfusate droplets (2–4 μl), their osmotic concentration was measured by freezing point depression with a Clifton nanoliter osmometer (Clifton Technical Physics) using a 10–20nl sample from each droplet. These measurements were taken immediately

following the collection of the droplet to avoid errors caused by evaporation (Spring and Hazelton, 1987).

Elemental concentration determinations

Elemental concentrations of sodium, potassium, chlorine, magnesium, sulphur and calcium were measured by energy-dispersive X-ray microanalysis (EDS) using the method of Spring and Hazelton (1987). Nickel finder grids were cleaned, filmed with 2 % formvar (dissolved in chloroform), rendered conductive by coating them with carbon and then glued to aluminum sample stubs. Capillary tubes (Fisher Scientific) were cleaned with acetic acid and deionized water. The dry tubes were drawn into micropipettes with a vertical microelectrode puller (Stoelting) and coated with Sigmacoat (Sigma) to prevent wetting of the external surface. A micropipette was secured to a micromanipulator and connected to an oil-filled syringe (Hamilton, 0.1ml) by polyethylene tubing (PE 90, Intramedic), which was also filled with paraffin oil. The volume of the perfusate remaining after the removal of a sample for osmotic concentration determination was measured and the droplet was mixed under oil with a known volume of 100mmol l^{-1} cobalt acetate to give a final cobalt concentration of 25mmol l^{-1}. Picoliter samples of the mixed droplets were transferred to the nickel grid. Full grids were dried *in vacuo* and carbon-coated to prevent rehydration. Droplets were examined with a Hitachi S-450 scanning electron microscope and the elemental concentrations were determined using a Kevex 7700 series analyzer. EDS spectra were measured using an accelerating voltage of 20kV, a specimen current of 2×10^{-10} A and a collection time of 100s. At least three samples from each droplet were analyzed and the mean value was used for further calculations. The ratios of the elements to the internal cobalt standard were measured and the millimolar concentrations were determined from standard curves for each element and the calculated cobalt concentration in the original mixed droplet.

Results

Control absorption rate

The perfused rectum was allowed to stabilize for 1h in the saline bath under conditions identical to those used in experiments. After the 1h stabilization period, control absorption rates were followed for a 3.3h period. The rectum exhibited a constant rate of absorption throughout the experiment. At a perfusion rate of 12 $\mu l h^{-1}$, the absorption rate was approximately 1.3 $\mu l h^{-1}$ (10% of the infused volume). This constant absorption rate provided a stable baseline for further experiments (Fig. 2).

Effects of corpus cardiacum homogenates on fluid absorption

The CC homogenate gradually increased fluid absorption by the rectum to a maximum of 6.6 $\mu l h^{-1}$ after 80min. When the homogenate was rinsed from the bath with fresh saline, the absorption rate fell immediately to 2.7 $\mu l h^{-1}$, or about 40% of the maximum rate. Subsequently, the rate decreased gradually to a level that did not differ significantly ($P>0.05$; Student's *t*-test) from the control value (Fig. 2).

To determine the maximum rate of absorption and whether the rate would decline

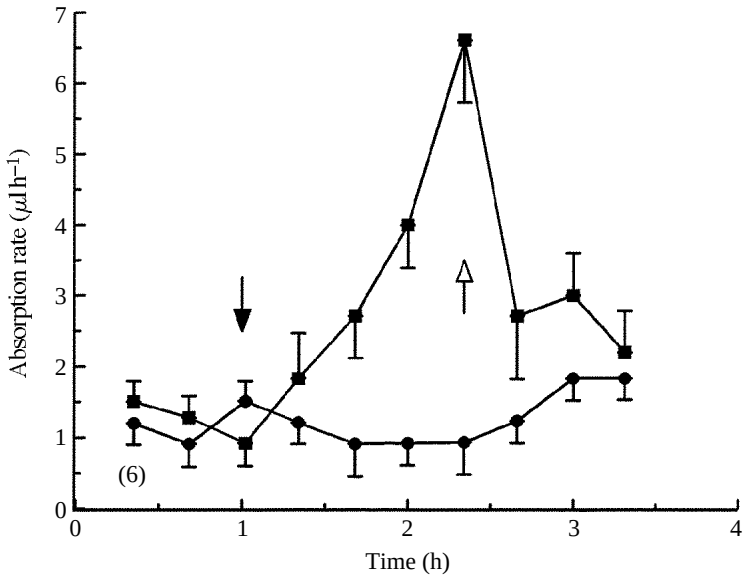

Fig. 2. Fluid absorption by the non-everted rectum perfused at $12\,\mu l h^{-1}$. Filled circles indicate control absorption rates. Filled squares show the effects of the homogenate of four pairs of corpora cardiaca (CC) added at the filled arrow and removed with three rinses of fresh saline at the open arrow. Vertical bars indicate S.E.M. The number of replicates is shown in parentheses.

spontaneously, preparations were allowed to run for 3h post-stimulation without rinsing (Fig. 3). As before, the change in absorption rate was sigmoidal, with the largest increase occurring between 20 and 40min post-stimulation, so that absorption had reached 90% of its maximum rate within 1h. Thereafter, the absorption rate increased gradually, peaking after 100min. There was no immediate decline in absorption rate and it remained elevated for a further 60min, beginning to drop very slowly during the third hour. Note that the basal and peak absorption rates are considerably lower than those observed in Fig. 2 owing to the lowered perfusion rate.

Effects of perfusion rate on fluid absorption

Recta were normally perfused with physiological saline at the rate of $12\,\mu l h^{-1}$ in all experiments, a rate 1.5- to threefold higher than the rate of primary urine formation by the tubule mass under similar conditions (Spring and Hazelton, 1987). Accordingly, one experiment was performed to investigate whether this high perfusion rate affected the absorption ability of the rectum, i.e whether dwell time influenced the absorption rate. This question was important inasmuch as all previous studies have used sac preparations, not perfused ones (e.g. Wall, 1967; Mordue, 1969; Goh and Phillips, 1978; Proux *et al.* 1984; Fournier *et al.* 1987). The volume of an *Acheta domesticus* rectal sac preparation is 3–4 μl (J. H. Spring, unpublished observations), so the normal perfusion rate, assuming zero absorption, represents a dwell time of only 15–20min. In this

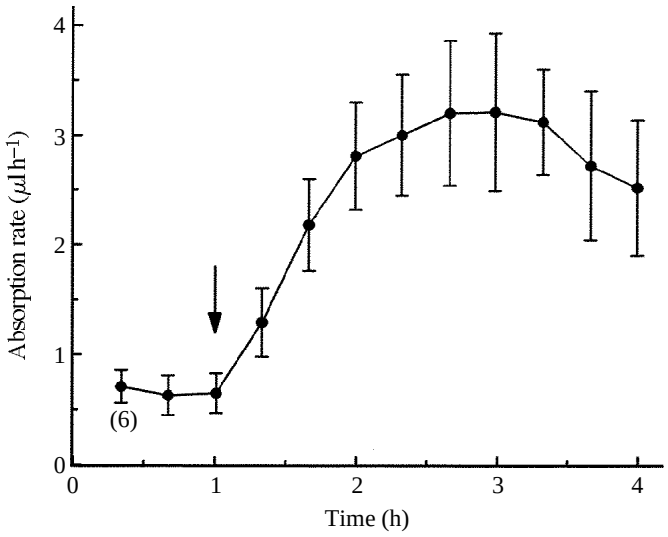

Fig. 3. Prolonged effect of CC stimulation on absorption. A homogenate of four pairs of CC was added at the arrow. Each point represents the mean ±1 S.E.M. The number of replicates is shown in parentheses. Perfusion rate was 6.8 μlh^{-1}.

experiment, the perfusion rate was decreased to 6.8 μlh^{-1}, the minimum reproducible infusion rate of which our apparatus was capable, and one which approximated maximum tubule fluid output *in vitro*. Absolute values and percentage of fluid absorbed by the recta perfused at two different rates are compared in Fig. 4. In absolute terms, recta perfused at the higher rate consistently absorbed fluid at a higher rate (Fig. 4A). However, the rectum was found to absorb a constant percentage of the infusate regardless of perfusion rate (Fig. 4B) and regardless of whether it was stimulated by CC homogenates. In both cases, the percentage absorbed was approximately 10% in control preparations and increased when the rectum was stimulated with CC homogenate to a maximum absorption rate of 47% after 80 min.

Comparison of tubule secretion and rectal absorption

The responses of the Malpighian tubules and the rectum to CC homogenates are compared in Fig. 5. When Malpighian tubules were maintained in Hepes-buffered saline, the secretion rate remained relatively constant for at least 3h (J. H. Spring, unpublished observations) in contrast to the constant decline in rate observed in bicarbonate-buffered saline (Spring and Hazelton, 1987). The response to CC homogenates was also changed in that the decline observed following peak stimulation was less sustained, and tubules continued to secrete at an elevated rate for at least 2h post-stimulation (Fig. 5). The patterns of response – rapid rise, plateau, slow decline – are the same in both tissues, although these changes occur much faster in the tubules. Rise time is 15min, compared with 60min for the rectum, and the plateau lasts 30 min rather than 80min. The rectal perfusion rate for this experiment was 6.8 μlh^{-1}, which is

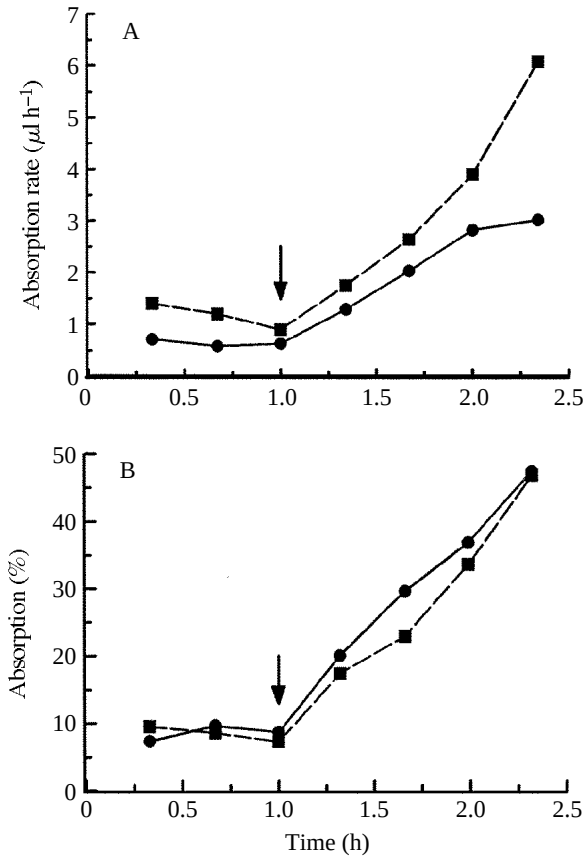

Fig. 4. Comparison of fluid absorption by the non-everted rectum perfused at two different rates. Preparations were stimulated by the addition of homogenates of four pairs of CC at the arrow. (A) Comparison of absolute absorption rates. (B) Comparison of percentage of perfusate absorbed. Filled circles and solid line indicate a perfusion rate of 6.8 μl h^{-1}. Filled squares and dashed line indicate a perfusion rate of 12 μl h^{-1}. Each point is the mean of six replicates. Data are from Figs 2 and 3.

slightly less than the maximum fluid output by the tubules (8.0 μl h^{-1}; Spring and Hazelton, 1987), so these values are likely to be a reasonable approximation of *in vivo* conditions.

Note that tubule secretion is always two- to threefold greater than rectal absorption. If the secretion and absorption rates are shifted so that both are assumed to be equal at the time of stimulation (Fig. 5, inset), two points become evident. First, stimulation of reabsorption is much slower, so that it is a full hour after stimulation before the rates match. Second, in the stimulated condition, once the initial lag is over, the increase in rectal absorption is more than sufficient to compensate for all of the increased secretion by the tubules, so that there need be no net increase in the final excreted fluid volume in the whole insect.

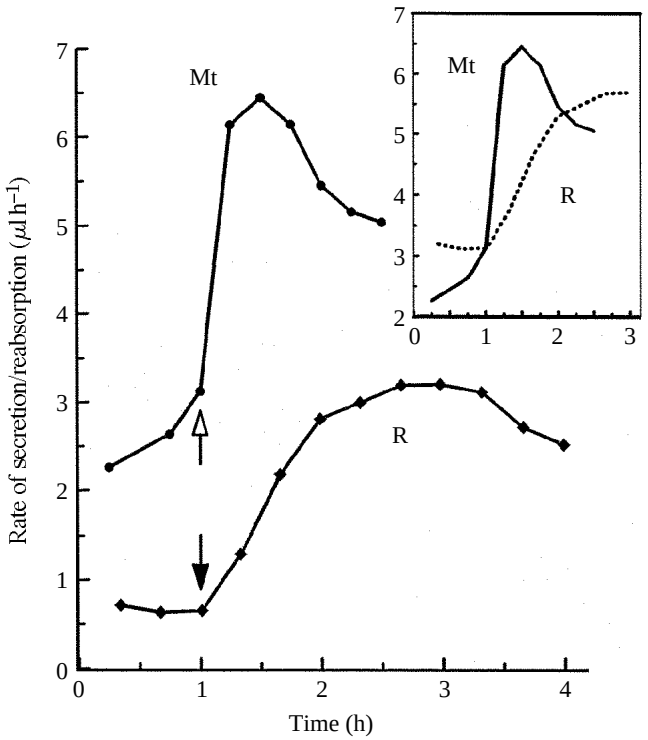

Fig. 5. Comparison between rates of Malpighian tubule secretion (filled circles, Mt) and rectal reabsorption (filled diamonds, R). Tissue stimulated with homogenate of one pair (Mt) or four pairs of CC (R) was added at the arrows. Each point is the mean of six replicates. Perfusion rate was 6.8 μl h^{-1}; absorption data from Fig. 3. Inset shows data replotted so that secretion is equal to absorption at the time of stimulation (1h).

Effect of HPLC-purified antidiuretic factor on fluid absorption

Given that CC homogenates had a strongly antidiuretic effect on the rectum, it was important to determine whether the antidiuretic factor (ADF) that stimulated fluid absorption by the rectum was the same as the one that inhibited tubule secretion. Accordingly, the fraction that inhibited secretion by Malpighian tubules (Spring *et al.* 1988) was isolated using HPLC and tested on the rectum. The test ADF fraction concentration (four CC equivalents in 2.5ml) was identical to that used in previous experiments. As shown in Fig. 6, the ADF fraction had no effect on absorption, with volume and percentage of fluid absorbed being unchanged before and after the addition of the fraction.

Changes in perfusate osmotic concentration

The osmotic concentration of the perfusate remained essentially identical to that of the saline bath throughout the control period of the experiment. Stimulation of the rectum by CC homogenates, however, caused the osmotic concentration of the perfusate to become hyperosmotic to the saline bath (Fig. 7). When the CC homogenates were replaced by

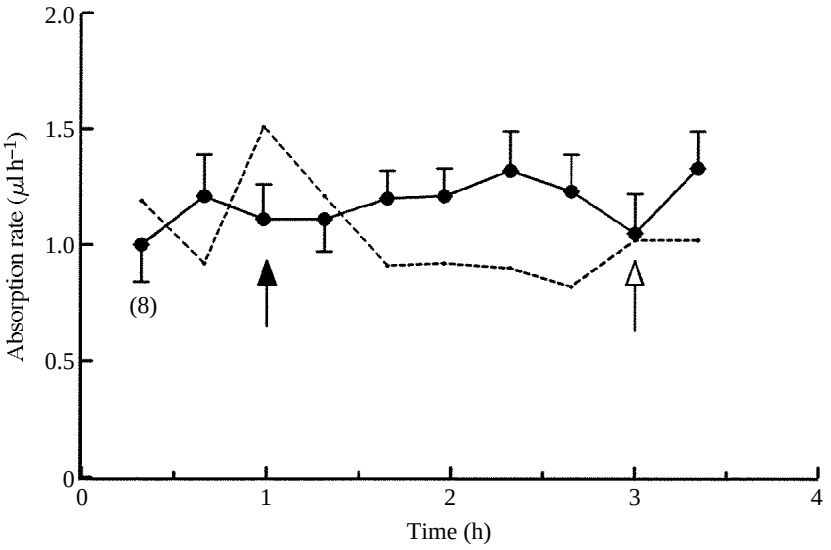

Fig. 6. Effect of HPLC-purified antidiuretic factor (ADF) fraction on fluid absorption by non-everted rectum perfused at 12 μl h^{-1}. Filled circles and solid line indicate that four CC equivalents of ADF were added at the solid arrow and removed with three rinses of fresh saline at the open arrow. Dashed line indicates control (unstimulated) values; data from Fig. 2. Each point represents the mean ± S.E.M. with the number of replicates indicated in parentheses.

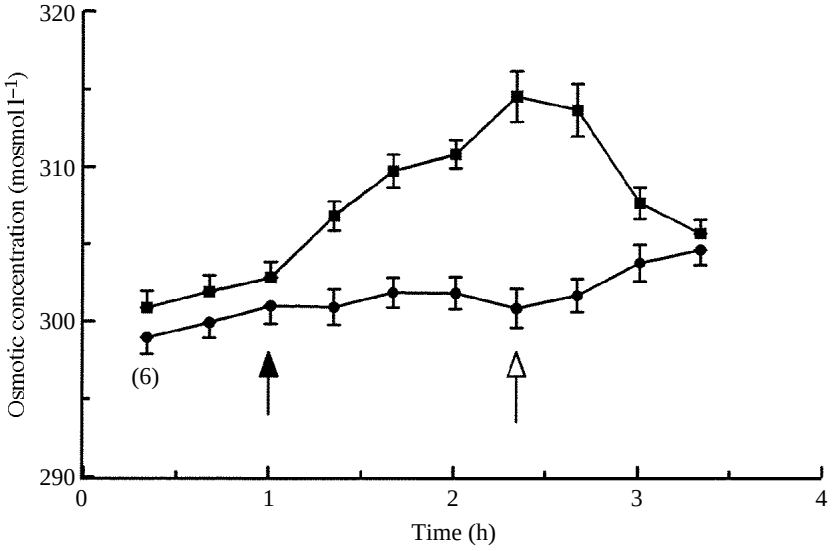

Fig. 7. Effect of CC homogenates on the osmotic concentration of the perfusate for a non-everted rectum perfused at 12 μl h^{-1}. Filled circles indicate unstimulated controls. Filled squares indicate the addition of a homogenate of four pairs of CC at the solid arrow and removed with three rinses of fresh saline at the open arrow. Each point indicates the mean ± S.E.M. The number of replicates is indicated in parentheses.

fresh saline, the osmotic concentration of the perfusate decreased gradually to control values, so that, 60min after rinsing, control and experimental osmotic concentrations were the same. For the perfusate to become hyperosmotic, the rectum must be absorbing a hypo-osmotic fluid, i.e. proportionally more water than salts.

Changes in elemental composition of perfusate

The concentrations of sodium and potassium in the perfusate from control and stimulated preparations are shown in Fig. 8. Under both conditions, potassium concentration in the perfusate ranged from 10 to 16mmol l^{-1}, without any pattern of change between control and experimental preparations. This shows that 20–50% of the potassium entering the rectum is absorbed and that the mechanism of its absorption is not affected by CC homogenates. Sodium concentrations in perfusates, however, showed high variability. This is due, at least in part, to the EDS technique: the location of the sodium peak on the rising shoulder of the background makes it difficult to measure accurately using EDS alone (Spring and Hazelton, 1987). Despite the variability, perfusate sodium concentrations are consistently 15–30mmol l^{-1} below bath concentrations, indicating that sodium is reabsorbed by the rectum. In contrast to

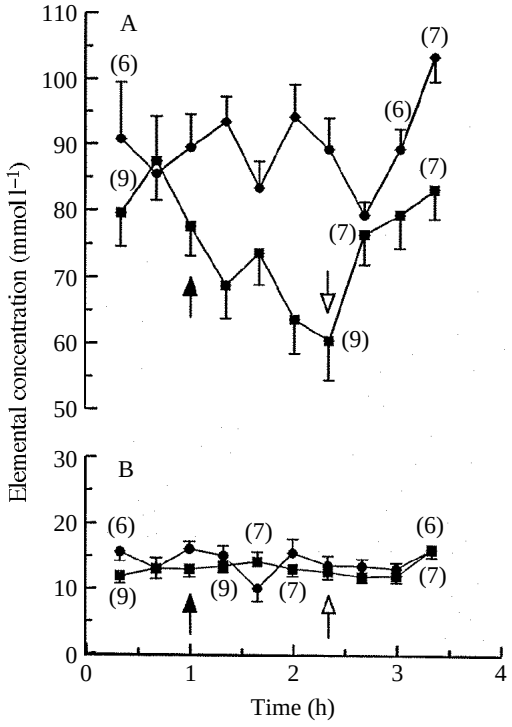

Fig. 8. Comparison of perfusate concentrations of elemental sodium (A) and potassium (B) for non-everted rectum perfused at 12 $\mu l h^{-1}$. Filled circles indicate control values. Filled squares indicate that a homogenate of four pairs of CC was added at the filled arrow and removed with three rinses of fresh saline at the open arrow. Each point represents the mean ± S.E.M. for the number of replicates indicated in parentheses.

potassium, sodium concentration was markedly decreased when the rectum was stimulated by CC homogenates, reaching its lowest concentration (62mmol l^{-1}) 80min post-stimulation. At that time the concentrations were significantly different (P<0.05, Student's t-test). Removal of CC homogenates from the saline bath caused the sodium concentration to rise to control levels, with the bulk of the increase occurring in the first 20min post-rinse interval.

Magnesium, sulphur and calcium concentrations showed no changes when the rectum was stimulated with CC homogenates (Fig. 9). The initial magnesium concentration in the perfusate from the control preparation was 7.2mmol l^{-1}; it showed a slight and gradual decrease with time to 5.2mmol l^{-1} at the end of the experiment but was unaffected by the addition of CC homogenates. Sulphur concentrations in the perfusate ranged from 11.4 to 13.6mmol l^{-1}, whereas calcium concentration was relatively constant throughout the experiment and showed no change when the rectum was stimulated.

Chlorine concentrations in the perfusate from control preparations ranged from 50 to 75mmol l^{-1} (Fig. 10). This is 45–70% of the infusate concentration (107mmol l^{-1}),

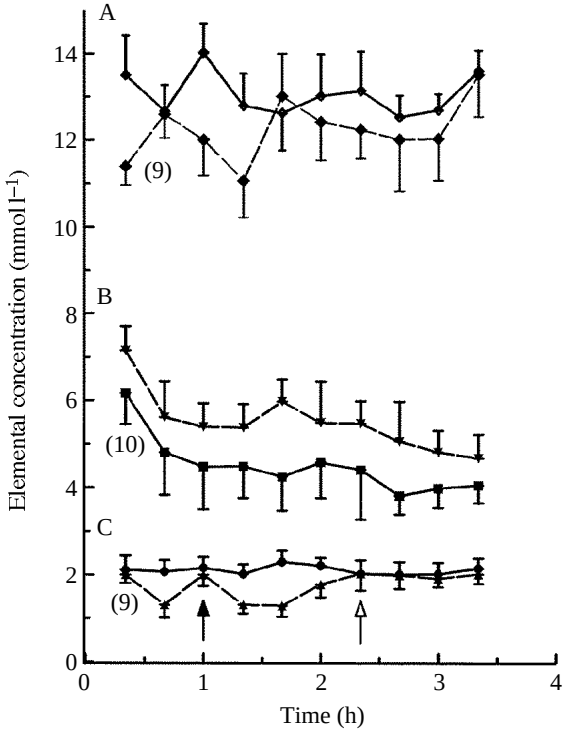

Fig. 9. Comparison of perfusate concentrations of elemental sulphur (A), magnesium (B) and calcium (C) in non-everted rectal preparations perfused at 12 μl h^{-1}. Filled triangles and diamonds and dashed lines indicate control values. Filled circles and squares and diamonds and solid lines indicate the effect of a homogenate of four pairs of CC added at the filled arrow and removed with three rinses of fresh saline at the open arrow. Each point represents mean ± S.E.M. for the number of replicates indicated in parentheses.

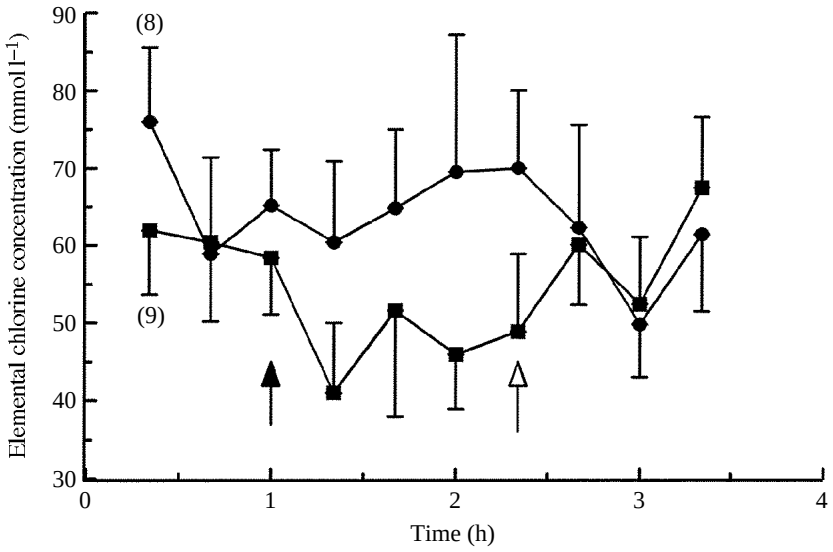

Fig. 10. Comparison of perfusate concentrations of elemental chlorine for non-everted rectum perfused at 12 μl h^{-1}. Filled circles indicated control values. Filled squares indicate that a homogenate of four pairs of CC was added at the filled arrow and removed with three rinses of fresh saline at the open arrow. Each point represents the mean ± S.E.M. for the number of replicates indicated in parentheses.

indicating that chloride is rapidly absorbed under all conditions. Addition of CC homogenates caused a decrease in perfusate chlorine concentrations from 58 to 41 mmol l^{-1}. At 80min post-stimulation, stimulated chlorine concentrations differed significantly from the controls (P<0.05, Student's t-test). Experimental chlorine concentrations increased to values identical to controls within 20min post-rinse.

Discussion

There are both advantages and disadvantages to the use of the perfused rectal preparation. With larger insects, such as *Schistocerca gregaria* (Mordue, 1969; Goh and Phillips, 1978; Proux *et al.* 1984), *Locusta migratoria* (Fournier *et al.* 1987) and *Periplaneta americana* (Wall, 1967), everted rectal sacs represent a viable and useful means of measuring fluid reabsorption. Although simple to set up and use, so that large numbers of replicates are easily obtained, sac preparations do not permit stirring or oxygenation of the hemocoel side, the tissue must be handled for each measurement, the composition of the absorbate is variable and frequently not measurable (Wall, 1967), and swelling of the tissue may induce large errors in the estimation of absorption rate (Phillips *et al.* 1982). In *Acheta domesticus*, these difficulties are exacerbated by the small size of the rectum (3–4mg, cf. 10–12mg in *Schistocerca gregaria*), which increases errors caused by fluid adsorption and weighing. The perfused preparation addressed all of these difficulties; the hemocoel (bath) side is of large volume and is constantly stirred and oxygenated; once set up, the preparation is not handled; the composition of the salines on

both sides is maintained; and tissue swelling is accounted for. On the negative side, perfused preparations require more elaborate and expensive equipment, measurement techniques are time-consuming and difficult to set up, and absorbate concentrations are inferred from perfusate concentrations, rather than measured directly.

Despite the difficulties associated with the perfused recta, the preparation described here allowed us to address several questions concerning rectal reabsorption. Most importantly for our study, we were able to examine fluid and ion reabsorption *in vitro* under identical conditions to those employed for the Malpighian tubule system in *Acheta domesticus*. From the data presented in Fig. 2, it is clear that the rectum is stable under these conditions, with absorption rates remaining constant for upwards of 3h. Since these experiments were designed specifically to enable close comparison between Malpighian tubule and rectal function and stimulation, it is noteworthy that the Malpighian tubules did *not* stabilize when maintained in an identical saline, showing a steadily decreasing baseline secretion rate (Spring and Hazelton, 1987). The difference appears to be due specifically to the use of the bicarbonate/CO_2 buffering system, since tubules are completely stable in a Hepes-buffered saline of otherwise identical composition (Kim and Spring, 1992; Clark and Spring, 1992). It is not clear whether this represents a difference in tolerance to, or requirement for, bicarbonate between the two tissues, or whether the intracellular pH of the tubules was affected in bicarbonate-buffered saline. On the basis of work with separate distal and mid-tubule segments, Kim and Spring (1992) have suggested that H^+/HCO_3^- transport may play a significant role in driving tubule fluid formation.

Time courses for responses to CC homogenates differ between these two tissues. Tubules respond to CC immediately, reaching their maximal rate of secretion within 15–30min. The rectum, in contrast, takes 3–4 times as long to reach its maximal rate of reabsorption, with the maximal increase in rate occurring 20–40min post-stimulation. The time profile for rectal stimulation is very similar to the changes in short-circuit current (I_{sc}) observed in *Schistocerca gregaria* (Spring and Phillips, 1980*a*) and *Romalea microptera* (Spring, 1986) in response to CC homogenates, suggesting that similar transport mechanisms occur in all three insects, and that they take some time to attain maximal transport capacity. Tubules bathed in Hepes-buffered saline respond to CC homogenates in a similar manner to the rectum, exhibiting a rapid rise, followed by a plateau, then a gradual decline in secretory rate. The tubules respond to stimulation much more rapidly than the rectum, however, passing through all these stages in about one-third of the time required by the latter. The CC factor(s) that stimulate rectal absorption do not appear to degrade rapidly, as shown in Fig. 3. When the CC homogenate was rinsed from the bathing saline, however, absorption by the rectum immediately slowed, dropping to 40% of the peak rate within 20min (Fig. 2). Clearly, the prolonged stimulation of rectal absorption is due to the stability of the stimulating factor(s) in the bath, rather than to a prolonged response by the tissue to a short exposure to a stimulant.

When recta were perfused at two different rates, although the volume absorbed was proportional to the perfusion rate, the percentage of fluid absorbed was identical over a twofold change in perfusion rate (Fig. 4). Approximately 10% of the fluid entering control recta was absorbed and a maximum of 47% was absorbed when the preparations

were stimulated with four pairs of CC. It appears that the rate at which fluid enters the rectum does not affect its absorption capability. One can speculate that the rectum may easily and rapidly establish a specific ion gradient, so that the amount of fluid absorbed is dependent on differences in concentration between the lumen and bath rather than on absolute perfusion rate. If this is the case, the gradient must be small and rapidly established since the twofold change in flow did not alter it.

Under unstimulated conditions, the rate of reabsorption by the rectum is 25–35% of the rate of tubule secretion (Fig. 5). Clearly, some other process acts to increase the rate of fluid reabsorption in order to prevent desiccation *in vivo*. The most likely explanation is that other regions of the hindgut, presumably the ileum, must also be absorbing fluid, as is seen in *Schistocerca gregaria* (Audsley *et al.* 1992). As shown in the inset of Fig. 5, however, by 1h post-stimulation, the increase in the rate of reabsorption is more than sufficient to compensate for the increase in tubule secretion. The data, then, support the suggestion that the primary function of most of the 'diuretic' or 'antidiuretic' factors may be to increase the hemolymph filtration rate rather than to alter the water balance of the insect (Spring, 1990). Perhaps Nicolson's (1991) suggestion that these factors be called 'clearance factors' is indeed more appropriate.

In the absence of any endocrine influence, which presumably occurs only *in vitro* (Spring *et al.* 1988), Hepes-buffered tubules secrete at approximately 2–3 $\mu l h^{-1}$ compared with the absorption rate of 0.6–0.7 $\mu l h^{-1}$ for the rectum. Thus, if endocrine control were removed, one might expect that crickets would constantly lose water through uncontrolled excretion. Indeed, this is precisely what Geldiay and Edwards (1976) observed in *Acheta domesticus in vivo* during the first 48h following cauterization of the median neurosecretory cells. At the time, they interpreted this diuresis to be the result of massive release of diuretic material caused by the trauma of cauterization; however, our data indicate that the water loss may simply reflect the differential in basal rates of secretion and absorption by the tubules and rectum respectively.

At this point, we do not know what the stimulating factors from the CC are. The pattern of the rate of increase in reabsorption is remarkably similar to that observed for active chloride transport in *Schistocerca gregaria* (Spring and Phillips, 1980*a*) and *Romalea microptera* (Spring, 1986), and fluid uptake is closely linked to chloride reabsorption in these insects (Goh and Phillips, 1978; Phillips *et al.* 1982; Proux *et al.* 1984). Whether the stimulant of fluid absorption in *Acheta domesticus* eventually proves to be a chloride-transport-stimulating hormone (CTSH)-like peptide (Spring and Phillips, 1980*b*; Phillips *et al.* 1980), or perhaps one of the larger neuroparsins (Fournier and Girardie, 1988), it is clearly not the same as the antidiuretic factor that inhibits fluid secretion by the Malpighian tubules (Spring *et al.* 1988).

When stimulated with CC homogenate, the rectum of *Acheta domesticus* absorbs an increasingly hypo-osmotic fluid, as indicated by the increasing osmotic concentration of the perfusate. This phenomenon, which is attributed to solute recycling in the rectal pads, has previously been documented in other species (Phillips, 1964; Wall and Oschman, 1970; Wall, 1971; Gupta *et al.* 1980). In all cases, solute recycling is characterized by a considerable degree of independence between external solute concentrations and fluid transport (Goh and Phillips, 1978). This also appears to be the

case in *Acheta domesticus*, since the rectum continues to absorb a hypo-osmotic fluid for 40–60min after the CC homogenate has been rinsed off (Fig. 6), whereas the rate of fluid transport drops dramatically within 20min post-rinse (Fig. 2). Establishment of the conditions required for solute recycling in the rectal pads (Wall, 1971; Gupta *et al.* 1980) appears to be a slow process, taking considerable time both to initiate and, once begun, to dissipate. It is noteworthy that in *Acheta domesticus*, CC-stimulated tubules produce a fluid that is hypo-osmotic to the bathing saline by 10–12 mosmol l^{-1}, a value identical to that seen in the rectal absorbate. If conditions *in vitro* accurately reflect those that occur *in vivo*, the net effect of CC stimulation would be water reabsorption at the expense of solute loss. Again, this supports the suggestion that the primary effect of the CC may be to increase the rate of hemolymph filtration, rather than to promote or inhibit water loss.

Acheta domesticus appears to be unique among phytophagous insects with respect to the ions that drive fluid transport. In *Schistocerca gregaria*, which is perhaps the best-studied plant feeder, the Malpighian tubules produce a potassium chloride-rich fluid with a sodium:potassium ratio of 1:3.5 (Hanrahan and Phillips, 1983). CC homogenates stimulate electrogenic Cl^- transport by the rectum (Spring *et al.* 1978), which is normally electroneutral, with K^+ providing the primary counterion (Hanrahan and Phillips, 1983). In *Acheta domesticus*, unstimulated tubules produce fluid with a sodium:potassium ratio of 1:3, which changes to 5:3 following CC stimulation (Spring and Hazelton, 1987). In the rectum of *Schistocerca gregaria*, the net uptake of potassium and chloride are nearly equal, with sodium conductance being roughly 10-fold lower than potassium conductance. In *Acheta domesticus*, however, we see the opposite. Potassium absorption is low, and is not altered in the presence of CC homogenates, whereas sodium absorption is considerably greater under control conditions, and the primary ion pair reabsorbed is sodium chloride, rather than potassium chloride (Figs 8, 10). While the absorption of a sodium chloride-rich fluid by the rectum admirably matches the mechanism of stimulated fluid production in *Acheta domesticus*, it remains difficult to suggest a reason for a phytophagous insect to demonstrate an ion resorption pattern normally associated with blood-feeders.

Sulphur, magnesium and calcium all appear to be conserved, i.e. the perfusate concentrations are 40–60% of the infusate concentrations. The reabsorptive mechanism does not appear to be under endocrine regulation, however, as there is no change between treated and untreated preparations.

Clearly, then, absorption by the rectum in *Acheta domesticus* is under endocrine regulation and the rectum has the capacity to reabsorb all of the output of the Malpighian tubules. The rate and ionic content of the absorbate are such that hemolymph homeostasis is maintained. While net water loss may be regulated by altering the relative transport rates of the tubules and rectum, it is highly likely that one effect of endocrine stimulation is that the hemolymph can be ‘cleared’ of unwanted solutes with no net loss of fluid. The nature of the factor(s) that regulates rectal reabsorption is at present unknown.

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