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STIMULATION OF OXYGEN CONSUMPTION WITH FLUID ABSORPTION IN INSECT RECTA

BY D. F. HOULIHAN AND D. SELL

Department of Zoology, University of Aberdeen, Aberdeen, Scotland, U.K.

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SUMMARY

The oxygen consumption of excised abdomens of cockroaches and locusts has been measured before and after the injection of fluids into the ligated recta. Fluid injection caused a transient stimulation of oxygen consumption of up to 30% of the resting rate. The extra amount of oxygen consumed is positively correlated with the osmolality of the fluid injected and the amount of fluid absorbed. Parallel experiments were carried out on the time course of fluid uptake; these experiments revealed a correlation first between a rapid increase in fluid absorption and stimulation of oxygen consumption, and secondly between the final resting rate of oxygen consumption and a slower absorption of fluid. Locusts take up fluid at double the rate of cockroaches and have double the stimulation in oxygen consumption following fluid injection. In locusts the increases in oxygen consumption can also be correlated with the net movement of Na^+, K^+ and Cl^- from the rectum. The stimulation of oxygen consumption during fluid uptake is discussed in relation to the local osmosis model for fluid uptake.

INTRODUCTION

A number of studies have shown that the movement of fluid across the rectum of insects *in vitro* is dependent upon energy (Vietinghoff, 1965; Wall, 1967; Irvine & Phillips, 1971; Tolman & Steele, 1980). The general conclusion from these studies and theoretical calculations (Maddrell, 1971; Edney, 1977) seems to be that the normal oxygen consumption of an arthropod can supply sufficient energy for the recorded rates of water uptake (Phillips, 1964; Kanungo, 1965; Edney, 1966; Arlian, 1975). Houlihan (1977) reported that when the thysanuran *Petrobius* took up fluid with its eversible abdominal vesicles there was over a 200% increase in oxygen consumption. The increased consumption could be correlated positively with the amount of fluid uptake and the osmolality of the fluid in contact with the sacs.

The present paper describes a semi-isolated preparation of the cockroach and locust which allows a determination of oxygen consumption through the tracheal system before, during, and after the injection of a known amount of fluid into the rectum. The amount of fluid absorbed from the rectum was determined and the relationships between the stimulation of oxygen consumption and fluid absorption

investigated. Parallel experiments were conducted on the time course of fluid absorption from the rectum and comparisons drawn between this and oxygen consumption.

Water absorption from the insect rectum is usually linked with active ion transport and there are numerous examples of epithelia displaying a direct relationship between the rate of active transport of ions and the rate of oxygen consumption (Whittam, 1964; Silva *et al.* 1980). We have investigated these relationships in our system first by relating the changes in ionic composition of the fluid injected into the rectum to the stimulation of oxygen consumption and secondly by injecting a sucrose solution, from which the insect can take up fluid but not ions. We have also used the Na^+–K^+ activated ATPase inhibitor, ouabain, to investigate the role of this enzyme in rectal oxygen consumption. Since the ouabain molecule is too large to penetrate the rectal cuticle (Phillips & Dockrill, 1968), the haemocoel was perfused to allow the inhibitor to gain access to the transporting cells.

The complex tissue culture medium of Berridge (1966) was substituted for the saline of Mordue (1969) as the luminal fluid to determine the effects of added energy substrates and intermediary metabolites on oxygen consumption.

MATERIALS AND METHODS

Adult male and female American cockroaches, *Periplaneta americana* L., and adult male and female desert locusts, *Schistocerca gregaria* Forskål, were used. The animals were kept at 28 °C at 45–50% relative humidity on 12 h light : 12 h dark and 16 h light : 8 h dark photoperiods for cockroaches and locusts respectively. The animals were fed on dried bran and allowed access to water. The locusts also received fresh cabbage. Cockroaches were used 1–4 weeks after the final moult and locusts 2–3 weeks after the final moult. The live weight of the animals used was: cockroaches 0·64–1·43 g (mean 1·09 g), locusts 1·31 g to 3·14 g (mean 2·17 g). Batches of cockroaches and locusts were dehydrated in dry air at room temperature and a record kept of weight losses. Cockroaches were anaesthetized with CO_2 before being handled.

Fluid uptake and oxygen consumption

Dehydrated animals were anaesthetized with ether and an operation was performed through a small incision in the abdominal cuticle to isolate the rectum from the hindgut by ligation (Phillips, 1964). The animals were given 2–3 h to recover and then the rectum of each animal was washed out with distilled water. A length of polythene tubing (Portex: O.D. 1.0 mm), containing 10 mg of test fluid in the case of cockroaches and 30 mg for locusts, was sealed into the rectum with Loctite super glue. The anterior meniscus of the fluid in the tubes was level with the anus. After allowing 45 min for the glue to set the abdomen was cut from the thorax and the cut end sealed with petroleum jelly.

A constant pressure respirometer modified from Davies (1966) with 20% KOH acting as a CO_2 absorber was used to measure the oxygen consumption of individual abdomens (Fig. 1). The polythene tube sealed into the rectum fitted tightly into a hole in the rubber stopper of the respiration chamber. The hole was completely sealed with petroleum jelly. The ventral surface of the abdomen rested on the floor of the

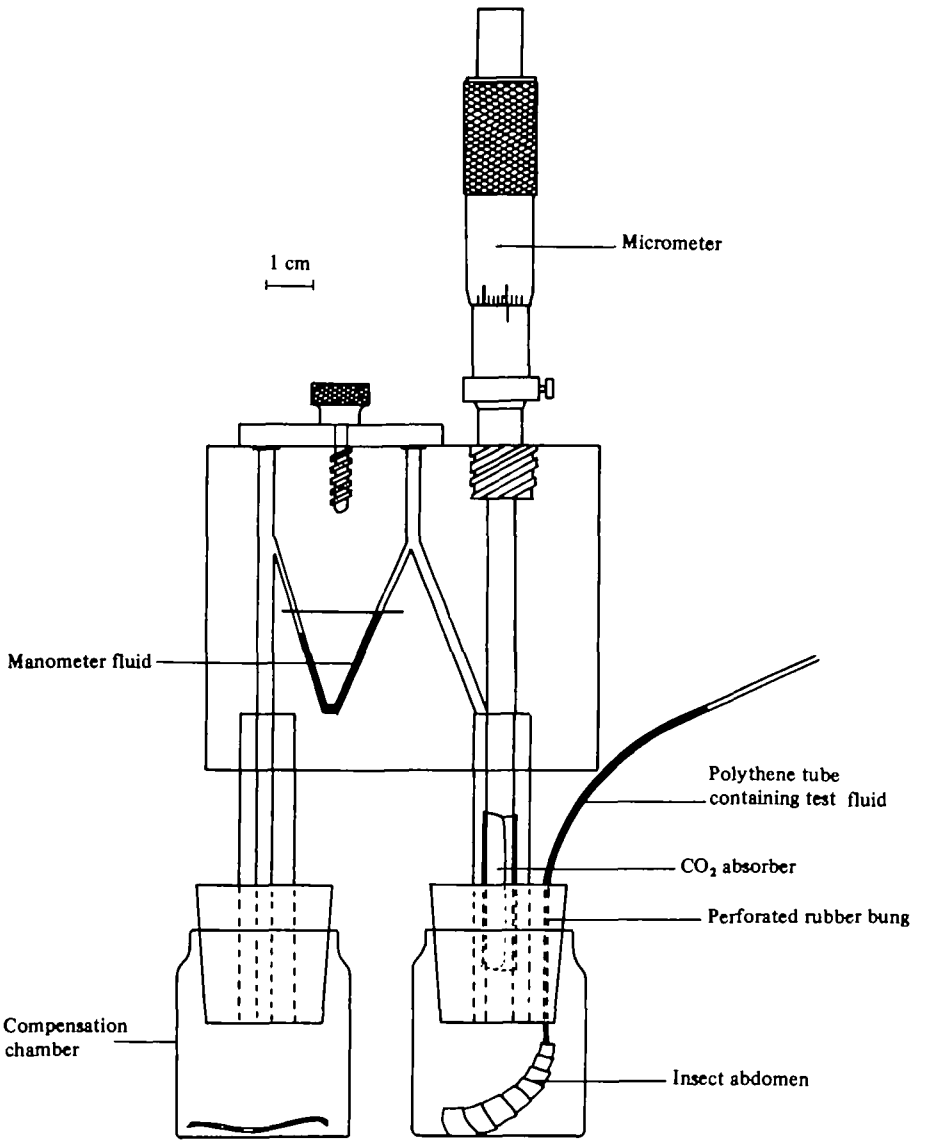

Fig. 1. Diagram of the respirometer used to determine the oxygen consumption of individual abdomens. In a constant temperature tank and with a CO_2 absorber in the arm of the respirometer, oxygen consumption resulted in the fluid levels moving and this was compensated for with the micrometer. After a period of steady oxygen consumption the fluid shown in the tube sealed into the anus was injected into the rectum. Measurements were then made for a further hour before the amount of fluid remaining in the rectum was determined.

respiration chamber. The assembled respirometer was placed in a water bath at 25 °C (± 0·2 °C), sealed 20–30 min later, and the measurements began a further 30 min later.

Micrometer adjustments were made every 5 min. After five readings were taken and a steady rate of oxygen consumption obtained, the test fluid was introduced into the rectum by pressure from a microsyringe on the free end of the polythene tube outside the respirometer. The free end of the tube was sealed after all the fluid had been

introduced into the rectum. The micrometer was then reset to allow for the slight increase in volume resulting from the introduction of the fluid into the rectum. Measurements were then made every 5 min for 1 h. The respirometer could detect 0·7 μl changes, which is less than the lowest volume change resulting from two consecutive oxygen consumptions reported in this paper. The oxygen content of the respirometer was calculated never to have fallen below 94% of the air value with the longest duration experiments.

At the end of the experiment the fluid remaining in the rectum was aspirated into the polythene tube and weighed. The abdomen was opened and the rectum examined for signs of leakage or fluid retention. Control experiments were carried out with no animals in the respirometer. Over 3 h there were no changes in the volumes in such respirometers. Control experiments are reported in the results in which oil or air was injected into the rectum instead of fluid. All rates of oxygen consumption were converted to S.T.P.

If there was enough fluid remaining in the rectum 1 h after the injection of 800 m-osmol kg^{-1} saline, the composition in terms of Na^+, K^+ and Cl^- was determined with a Corning 435 flame photometer and Radiometer CMT 10 chloride meter. The net movement of ions was calculated from the difference in composition between the original fluid injected and the final composition.

Time course of fluid uptake

Intact animals with a ligature separating the rectum from the hindgut were prepared as described previously. A polythene tube containing a known weight of fluid was sealed into the rectum and 2–3 h later the insect was restrained with adhesive tape and the fluid injected into the rectum. After varying time periods the fluid was recovered and the weight of fluid absorbed determined. The animals were at 23–26 °C during the course of the experiments.

Fluids used were distilled water, 400 m-osmol kg^{-1} insect saline, pH 6·46 (Mordue, 1969) and 800 m-osmol kg^{-1} sucrose-adjusted Mordue saline. Sucrose was used to adjust the osmotic pressure of the saline because disaccharides are restricted in their ability to cross the rectal cuticle in locusts (Phillips & Dockrill, 1968), although this has not been tested in cockroaches (Bracke & Markovetz, 1980). Five mg% of Amaranth was added to each solution as an indicator of rectal integrity. All weighings were made to 0·1 mg. In order to show that all of the fluid could be removed from the locust rectum, injection and immediate withdrawal of 30 mg 800 m-osmol kg^{-1} saline resulted in $99{\cdot}2 \pm 0{\cdot}1\%$ ($n = 5$) recovery of the fluid. Also injection of saline containing C^{14}-sucrose resulted in $98{\cdot}2 \pm 0{\cdot}3\%$ ($n = 5$) recovery of the label after 30 min.

Where appropriate, results were subjected to regression analysis using a linear model. Groups of results or correlated results were compared using Student's *t*-test or analysis of covariance (Snedecor & Cochran, 1972). The 5% level of significance is used throughout.

Ouabain perfusion experiments

The abdomens were ligated, excised, and entubed, and small incisions were made in the epiproct and the subgenital plate to allow the perfusate to drain through the

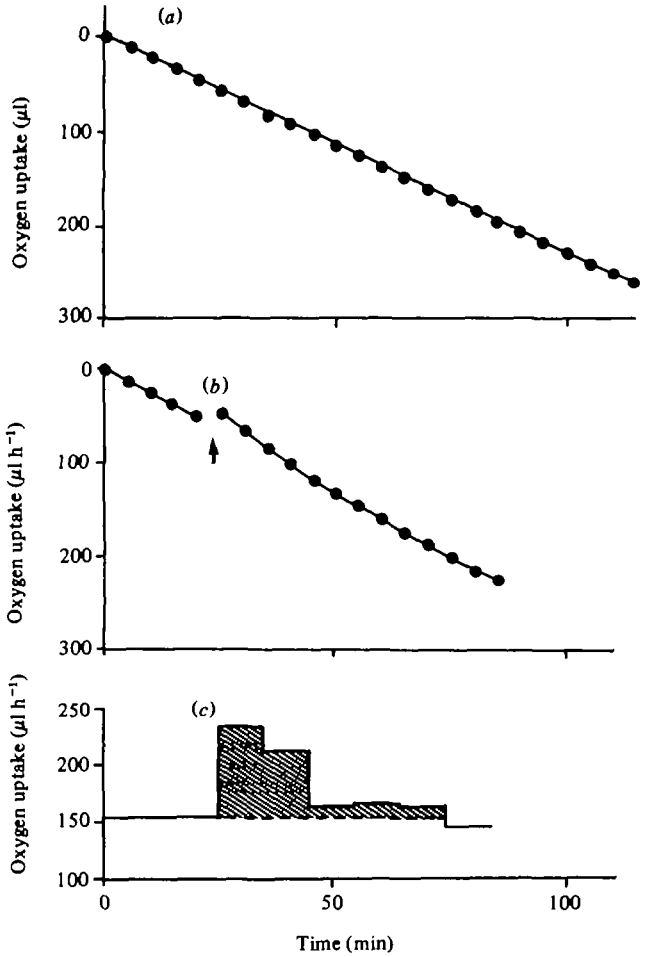

Fig. 2. Oxygen consumption of an excised cockroach abdomen. (*a*) Continuous record of oxygen consumption. The line is drawn from a regression analysis of the points. (*b*) Continuous record of oxygen consumption before and after 10 mg of an 800 m-osmol kg^{-1} saline was injected into the rectum at the point indicated by the arrow. (*c*) Diagram of the initial resting rate of oxygen consumption, and oxygen consumption rates over 10 min intervals after the injection of saline. The extra amount of oxygen consumed (shaded area) is calculated by subtracting the initial resting rate from the stimulated rates and converting from the rates to the actual extra amount of oxygen consumed.

abdomen. A needle was inserted posteriorly through the lateral aspect of tergum III and taped in position. The needle was connected via a polypropylene cannula to a reservoir containing the perfusate. The abdomen was suspended by means of the tube so that the posterior aspect faced downwards. The perfusate, Mordue insect saline containing 10^{-3} or 10^{-2} M ouabain (Irvine & Phillips, 1971), was allowed to run through the abdomen at a rate of approximately 50 ml abdomen^{-1}h^{-1} for 45–60 min before the needle was removed, the abdomen blotted lightly and the cut surfaces were sealed with petroleum jelly. Respiration was measured before and after the introduction of 800 m-osmol kg^{-1} sucrose-adjusted Mordue saline into the rectum and rectal fluid uptake was determined as above.

RESULTS

Oxygen consumption and fluid uptake in excised abdomens of cockroaches

A record of oxygen consumption from an individual uninjected abdomen is shown in Fig. 2(*a*). The line passing through the points was obtained by linear regression analysis and is significant ($P < 0.001$). The rate of oxygen consumption of the preparation calculated from the analysis is 132.4 ± 3.3 μl h^{-1} ($\pm 95\%$ confidence limits). The mean 95% confidence limit from 6 such experiments is 3·0 μl h^{-1}, which is 1·5% of the mean resting rate.

The resulting rate of oxygen consumption of the uninjected abdomen continued unchanged for up to 3 h. All the data from uninjected abdomens have been plotted on $\log^{10}$ axes in terms of live weight and oxygen consumption per animal and subjected to linear regression analysis. The value of the slope is rather low (0·5) but it is not significantly different from the more usual values of 0·72 or 1 (Keister & Buck, 1974).

The oxygen consumption from an individual before and after the injection of 10 mg of 800 m-osmol kg^{-1} saline is shown in Fig. 2(*b*). The initial resting rate over 20 min is calculated from a regression analysis of the measurements and is 158·5 μl h^{-1}. After injecting the saline and resetting the micrometer, there was an immediate increase in oxygen consumption, which then declined to close to the initial resting rate. Oxygen consumption was calculated in 10 min intervals to reveal the rate changes (Fig. 2*c*). The change in weight of the fluid in the rectum over 1 h was 2·14 mg.

Sixty-one experiments were carried out with cockroach abdomens in which there was no leakage of the injected fluid from the rectum and all the fluid was removed from the rectum at the end of the experiment. In one of the experiments there was no change in oxygen consumption after the injection of fluid into the rectum although there were changes in the weight of the recovered fluid. In the remaining experiments there was a steady initial resting rate and an immediate rise in consumption after the injection of fluid. This stimulation of consumption was judged to have ended if the rate returned to within 5% of the resting rate.

The increase in oxygen consumption following the injection of fluid into the rectum can be revealed by subtracting the resting rate from the post-injection rates over the first 10 min interval. Such increased oxygen consumption rate can be correlated with the weight of fluid absorbed from distilled water, 400, and 800 m-osmol kg^{-1} saline (Fig. 3).

The duration in the stimulation of oxygen consumption increases significantly with both the increase in the amount of fluid absorbed and the increase in the osmolality of the fluids injected (Fig. 3*b*), although there is no significant correlation with the amount of fluid absorbed from 400 m-osmol kg^{-1} saline.

We have calculated the total extra volume of oxygen consumed during the stimulated rate period by the method described in Fig. 2(*c*). The results in Fig. 4 show the correlations between the increased oxygen uptake of the stimulated period and weight change for the three fluids used with the 61 abdomens injected. Covariance analysis reveals that elevation of the 400 m-osmol kg^{-1} line is significantly higher than the distilled water line ($P < 0.05$). The slopes of the 400 and 800 m-osmol kg^{-1} regressions analyses are significantly different ($P < 0.01$) but the 800 m-osmol kg^{-1} line is not significantly higher than the 400 m-osmol kg^{-1} line.

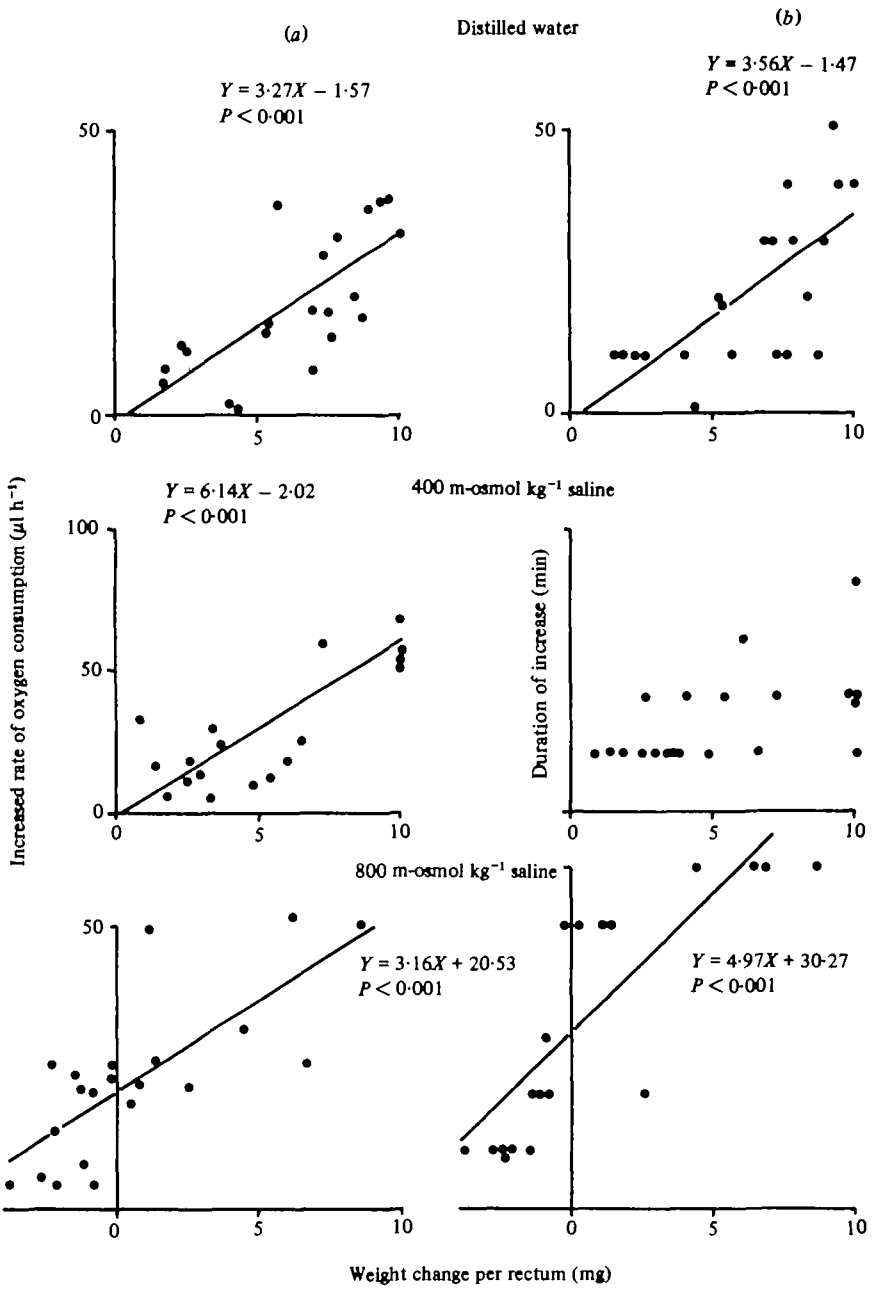

Fig. 3 (*a*) Increased rate of oxygen consumption over the first 10 min after injection of fluid into the rectum (stimulated rate minus initial resting rate) and the weight change that occurred over 1 h in the 10 mg of fluid injected into cockroach recta. (*b*) The duration of the increase in rate of oxygen consumption and the weight change that occurred in the fluid injected into the rectum. The regression analysis of the data and the level of significance of each regression is shown.

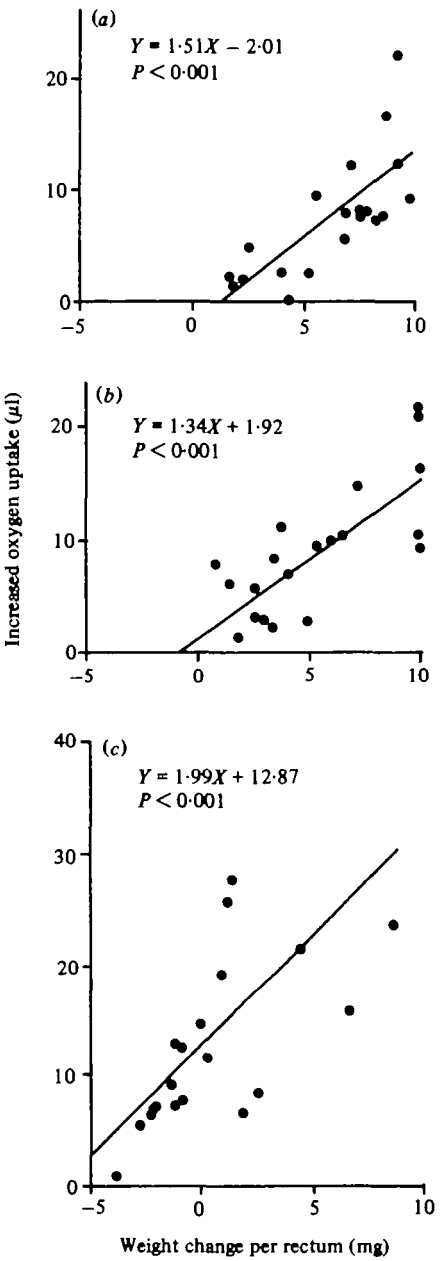

Fig. 4. Relationships between the weight change occurring over 1 h in the 10 mg of fluid injected into the cockroach rectum and the increased oxygen consumption calculated as described in Fig. 2. (*a*) Distilled water, (*b*) 400 m-osmol kg^{-1} saline, (*c*) 800 m-osmol kg^{-1} saline. The lines are drawn from linear regression analyses shown.

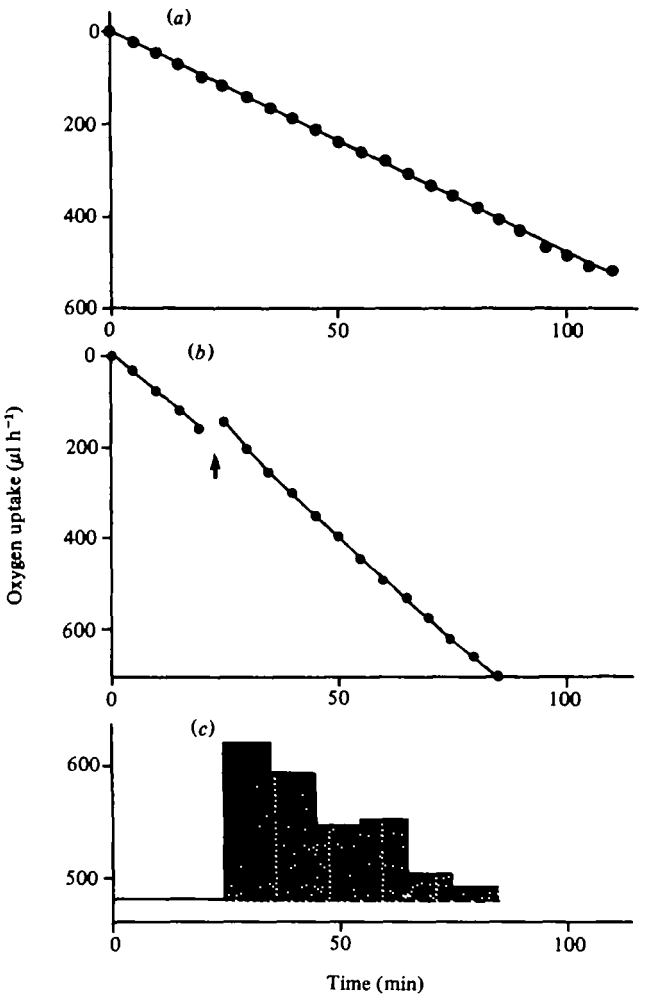

Fig. 5. Oxygen consumption of an excised locust abdomen. (*a*) Continuous record with a line drawn by regression analysis. (*b*) Continuous record of oxygen consumption before and after 30 mg of an 800 m-osmol kg^{-1} saline was injected into the rectum at the point indicated by the arrow. (*c*) Diagram of the initial resting rate of oxygen consumption and oxygen consumption rates over 10 min intervals after the injection of fluid. The extra amount of oxygen consumed (shaded area) is calculated by subtracting the initial resting rate from the stimulated rates and converting from the rates to the actual extra amount of oxygen consumed.

The results from the cockroach show a stimulation of oxygen consumption positively correlated with both the weight of fluid absorbed from the rectum and the osmolality of the fluid injected.

Oxygen consumption and fluid uptake in excised abdomens of locusts

The resting rate of oxygen consumption of locust abdomens (Fig. 5), before the injection of fluid, continued for more than 5 h with no decrease in rate and a very small deviation from a linear plot. The weight range of the abdomens used is too small to allow a plot of size and oxygen consumption. A group of six abdomens showed constant

oxygen uptake over 2h; the 95% confidence limit of the experiments is $3{\cdot}2 \pm 0{\cdot}7$ μl h^- which is 1·3% of the mean resting rate respiration.

The initial resting rate of oxygen consumption and the increased rates of consumption over 10-min intervals following injection of 30 mg of an 800 m-osmol kg^{-1} saline are shown in Fig. 5(*b*). This pattern of consumption occurred in 90 experiments. In these experiments there was no leakage of the injected fluid from the rectum and all the fluid was recovered from the rectum at the end of the experiment. All the experiments satisfied the criteria described for the cockroach data, except that the stimulation of oxygen consumption persisted throughout the hour following the injection of fluid at a rate which was at least 5% above the initial resting rate in six of the 15 distilled-water-injected abdomens and in eight of the 25 800 m-osmol kg^{-1}-injected abdomens.

The increased rate of oxygen consumption during the first 10 min interval, following injection of fluid, minus the initial resting rate, can be correlated with the weight change of fluid in the rectum in the case of distilled water (Fig. 6*a*) but not with 800 m-osmol kg^{-1} saline. The duration of the stimulation in oxygen consumption can be correlated with the weight of fluid absorbed (Fig. 6*b*).

The increase in the amount of oxygen consumed following an injection of fluid into the rectum was calculated as for the cockroach (i.e. as in Fig. 5*c*). It can be positively correlated with both the weight of fluid absorbed and the osmolality of the fluid injected (Fig. 7). When distilled water was injected into the recta, the regression analysis suggests that almost 5 mg of water can be absorbed before respiration is stimulated. However, the respiration rate always rose when distilled water was injected, so that the time course of stimulation does not show a time lag that would be expected if 5 mg could be absorbed without stimulating oxygen consumption. For 800 m-osmol kg^{-1} saline (Fig. 7), fluid uptake is always accompanied by an increase in oxygen consumption. Covariance analysis reveals that the slopes of the regressions from distilled water and 800 m-osmol kg^{-1} are not significantly different but that the elevation of the 800 m-osmol kg^{-1} line is significantly greater than that for the distilled water line ($P < 0{\cdot}05$).

Control experiments

A standard procedure of starving the animals for 18–24 h prior to the experiment was used for the controls. The gut was ligated, the rectum intubated, and the abdomen excised as described in Materials and Methods.

Ten μl of vegetable oil and 10 μl of paraffin were injected into recta from groups of five and seven excised cockroach abdomens respectively, whilst oxygen consumption was being monitored. In both cases fluid absorption occurred during 1 h after injection ($1{\cdot}1 \pm 0{\cdot}3$ mg and $4{\cdot}3 \pm 0{\cdot}9$ mg for vegetable oil and paraffin respectively). Oxygen consumption after injection continued at 99 ± 4% and 102 ± 2% of the resting rates for vegetable oil and paraffin injected controls respectively.

Ten μl of air was injected into the rectum of a group of four cockroach excised abdomens (Fig. 8). Over the first 5 min after the injection there was a decrease in oxygen consumption to 94 ± 5% of the initial resting rate. During the following 10 min oxygen consumption increased to 103 ± 1% of the resting rate. The rate then returned to the resting rate. When 30 μl of air was injected into four locust recta the oxyge

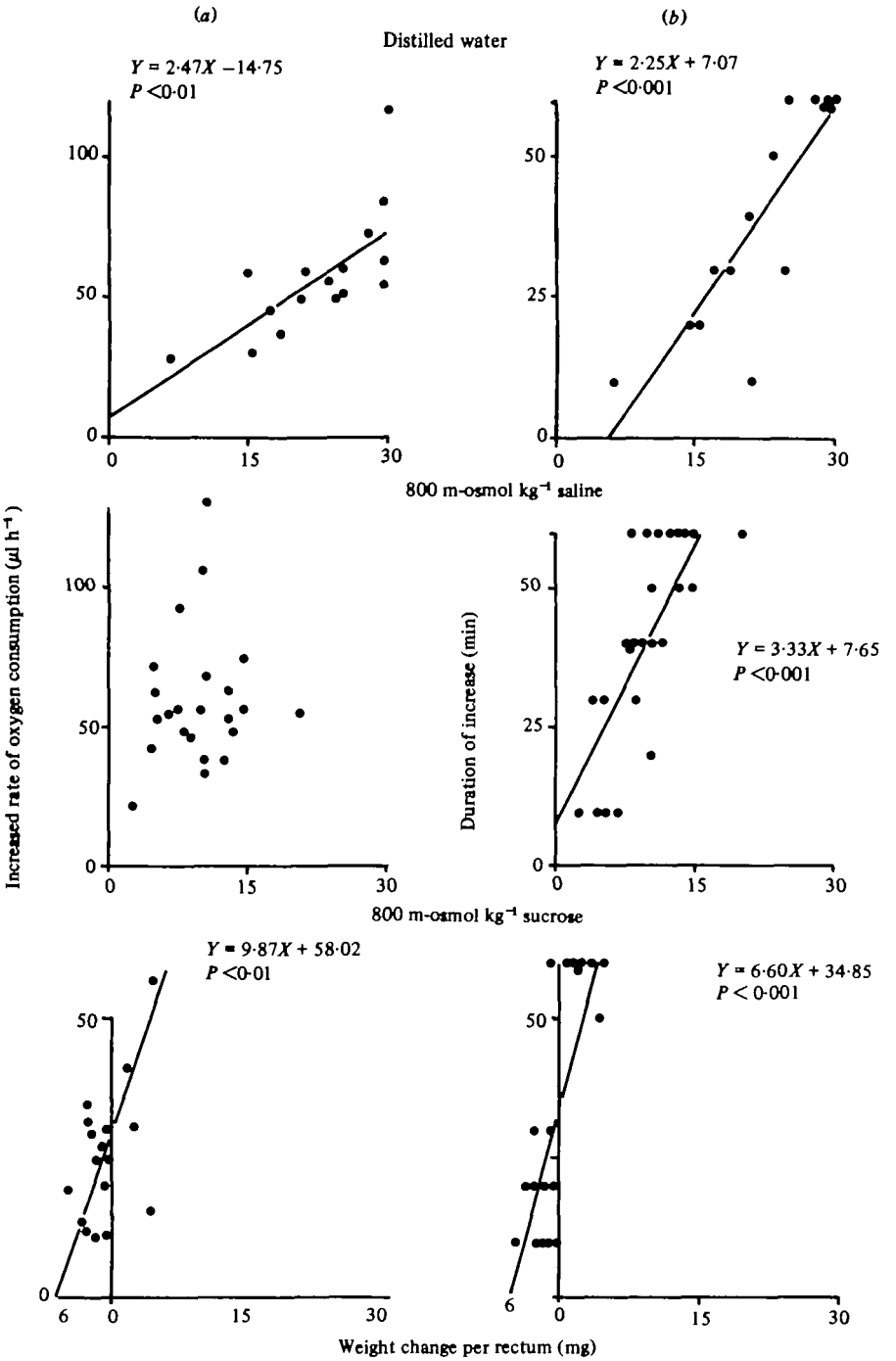

Fig. 6(*a*) The increased rate of oxygen consumption over the first 10 min after injection of fluid into the rectum (stimulated rate minus initial rate) and the weight change that occurred over 1 h in the 30 mg of fluid injected into locust recta. (*b*) The duration of the increase in rate of oxygen consumption and the weight change that occurred in the fluid injected into the rectum. The regression analysis of the data and the level of significance of each regression is shown.

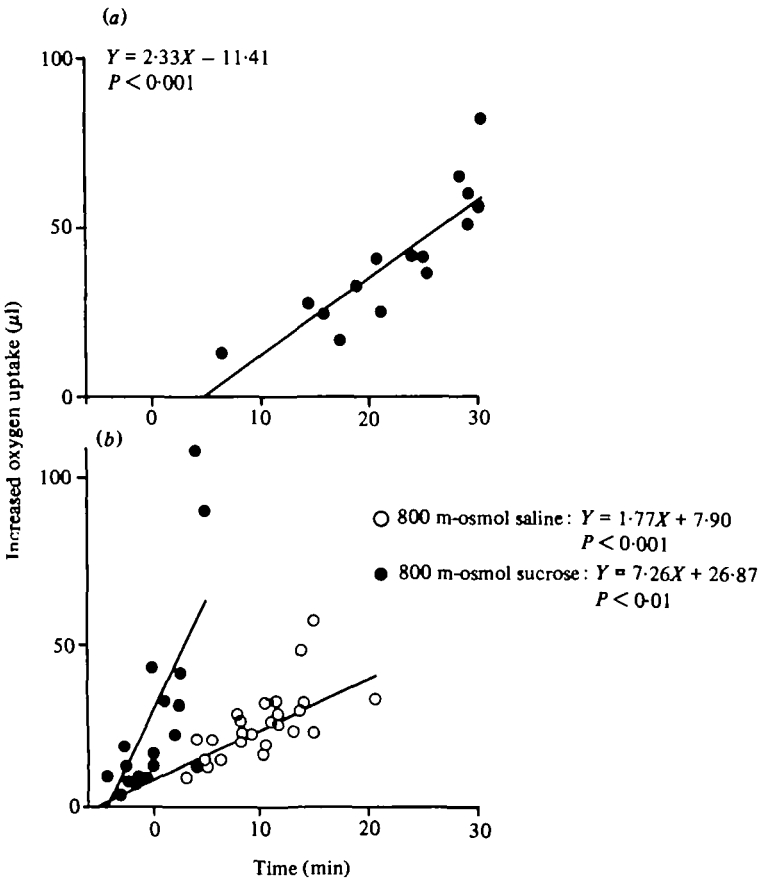

Fig. 7 (*a*) Relationships between the weight loss occurring over 1 h in the 30 mg of fluid injected into the locust rectum and the extra amount of oxygen consumed, calculated as described in Fig. 5. (*a*) Distilled water; (*b*) 800 m-osmol kg^{-1} saline and 800 m-osmol kg^{-1} sucrose. The lines are drawn from the linear regression analyses shown.

consumption rose to 105 ± 2% of the resting rate for 30 min and then returned to the resting rate (Fig. 8).

In order to investigate the influence of spiracular control and the possibility of discontinuous respiration of the abdomens (Hamilton, 1964), nine animals were prepared with their spiracles on either side of segment 8 kept open with glass filaments. Oxygen consumption was then measured as 30 mg of 800 m-osmol kg^{-1} saline was injected. The normal pattern of stimulation of oxygen consumption was recorded and there are no significant differences in the parameters measured between this group and the normal animals (Table 1).

Time course of fluid absorption

Fig. 11 shows the time course for the weight of fluid absorbed by cockroach recta after 10 mg of various fluids were introduced. Distilled water uptake rates are always significantly higher than those from the saline injections; 400 m-osmol kg^{-1} means are all significantly higher than 800 m-osmol kg^{-1} means. The distilled water results

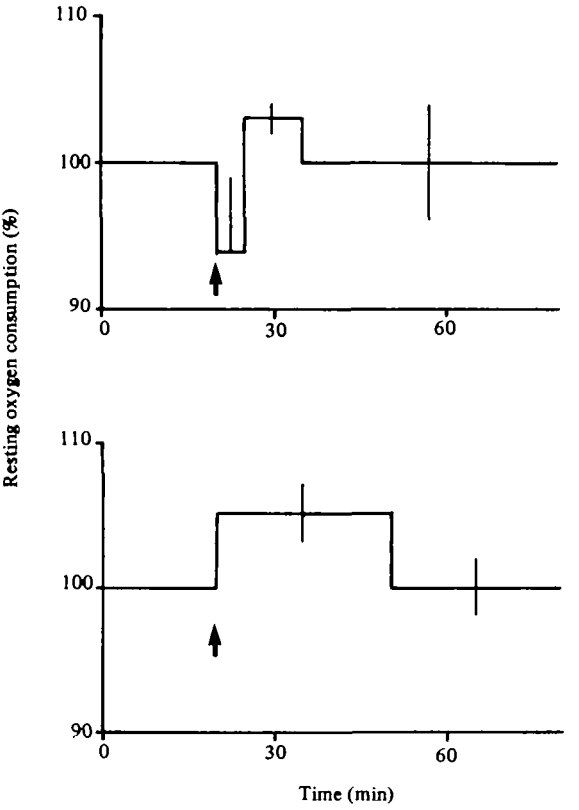

Fig. 8. Percentage change in rate of oxygen consumption of excised abdomens following the injection of air into the rectum (indicated by arrows). (*a*) Injection of 10 μl of air into cockroach abdomens. (*b*) Injection of 30 μl of air into locust abdomens. Each line (with a vertical bar) is a mean ($\pm$S.E.) for five experiments.

Table 1. *The effect of various procedures on the oxygen consumption of excised locust abdomens*

(A standard procedure of depriving the animals of food and water for 18–24 h before the experiment was adopted. Thirty mg of 800 m-osmol kg^{-1} test fluid was injected into the rectum whilst measuring oxygen consumption and the fluid was recovered 1 h later. The control group was injected with 30 mg of 800 m-osmol kg^{-1} Mordue saline. Means $\pm$S.E. are used throughout and the results of Student's *t*-tests are shown. N.S. is no significant difference with controls.)

Treatment	Oxygen uptake of abdomen (μl O_2 h^{-1})	Increased oxygen uptake (μl O_2)	Weight of fluid absorbed (mg)	*n*
Control	229·3 $\pm$ 13·7	25·7 $\pm$ 5·4	9·2 $\pm$ 1·0	8
Spiracles open	188·7 $\pm$ 18·5 (N.S.)	20·1 $\pm$ 3·8 (N.S.)	6·5 $\pm$ 1·3 (N.S.)	11
Perfused with 10^{-3} M ouabain	171·0 $\pm$ 15·3 ($P < 0.02$)	12·6 $\pm$ 3·5 (N.S.)	6·4 $\pm$ 1·5 (N.S.)	9
Perfused with 10^{-2} M ouabain	74·2 $\pm$ 20·2 ($P < 0.01$)	3·9 $\pm$ 1·7 ($P < 0.001$)	1·3 $\pm$ 0·8 ($P < 0.001$)	8*
Berridge saline 800 m-osmol kg^{-1}	237·4 $\pm$ 37·7 (N.S.)	21·0 $\pm$ 3·3 (N.S.)	6·2 $\pm$ 1·0 (N.S.)	13

* Four abdomens showed complete inhibition of oxygen consumption.

are similar to those obtained by Wall (1967) from *in vitro* experiments. Fluid uptal results for the 400 m-osmol kg^{-1} saline are higher than Wall's *in vitro* results. However, Wall used Pringle saline. Fig. 12 shows the results from similar experiments with locusts. The distilled water means are not significantly different from those reported by Phillips (1964). Water was removed by rectal absorption until only a paste of amaranth was left between the rectal pads. Unlike the cockroach, fluid uptake occurs from 800 m-osmol kg^{-1} saline for the first hour and the rates of uptake are significantly less than those for distilled water.

Ouabain perfusion experiments

When abdomens were perfused with 10^{-3} M ouabain in saline, there was a significant reduction in tissue respiration and in the fluid-induced increase in respiration (Table 1). These findings should be interpreted cautiously since animals of different weights were used for different experiments. When 10^{-2} M ouabain was used as the perfusate, resting oxygen consumption, increased oxygen consumption, and fluid uptake were reduced significantly when compared with the controls. Total inhibition of respiration was observed to have occurred in four out of the eight preparations used. Where increases in respiration did occur with fluid injection, both the increase and the duration of the increase were markedly reduced. Fluid uptake was reduced to 13% of the control value but only fell below zero in one case.

Rectal fluid of differing composition

The introduction of the complex saline of Berridge (1966) into the rectum failed to alter significantly any of the parameters shown in Table 1 when compared with the control.

The introduction of an ion-free 800 m-osmol kg^{-1} sucrose solution into the rectum caused a marked increase in oxygen uptake rate. Regression analysis of the extra amount of oxygen consumed and fluid weight change is significantly higher in slope and elevation than for the 800 m-osmol kg^{-1} saline results (Fig. 7). The weight of fluid absorbed from sucrose solution ($0{\cdot}1 \pm 0{\cdot}6$ mg h^{-1}) is significantly less ($P < 0{\cdot}001$) than the control value shown in Table 1. The result for sucrose is not significantly different from the figure reported by Phillips (1964) for 800 m-osmol kg^{-1} xylose solution.

Oxygen consumption and net ion uptake

Significant correlations were found between the volume of fluid absorbed and the net rate of efflux of Na^+, K^+ and Cl^- from the luminal fluid 1 h after 30 mg of 800 m-osmol kg^{-1} saline was introduced into the rectum (Fig. 9). The net efflux of ions seems to indicate isoionic uptake. This is shown by the mean rates of Na^+, K^+ and Cl^- efflux which were $1{\cdot}52 \pm 0{\cdot}09$, $0{\cdot}13 \pm 0{\cdot}01$ and $1{\cdot}65 \pm 0{\cdot}13$ μmol h^{-1} $rectum^{-1}$ respectively. If there was isoionic efflux from the lumen then the mean efflux rates of Na^+, K^+ and Cl^- would be $1{\cdot}80$, $0{\cdot}065$, $1{\cdot}86$ μmol h^{-1} $rectum^{-1}$ respectively. The net efflux can also be correlated with the extra amount of oxygen consumed during fluid uptake (Fig. 10).

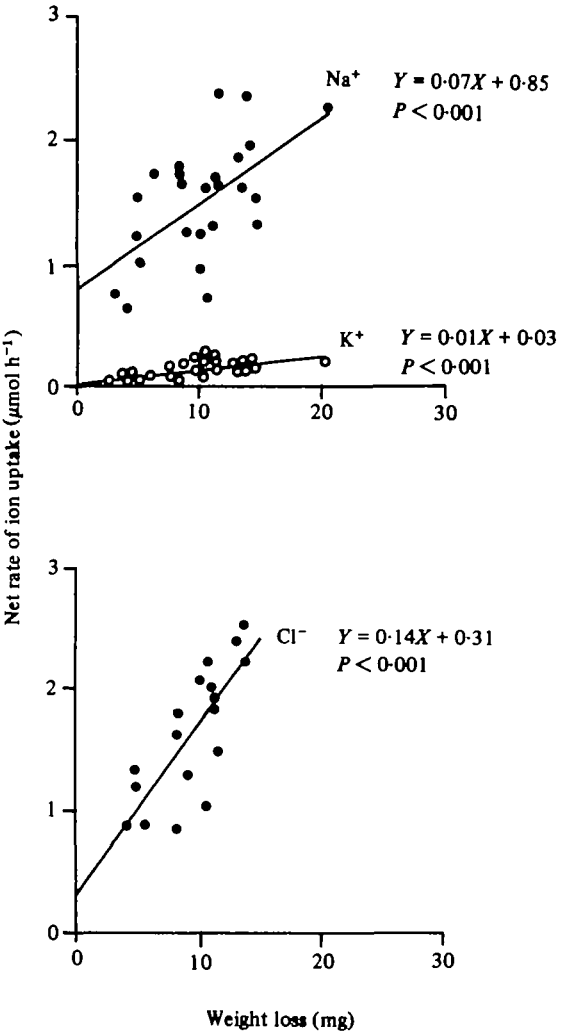

Fig. 9. Relationships between net rates of ion efflux from locust recta and the weight change occurring. Determinations were made 60 min after injecting 30 mg of 800 m-osmol kg^{-1} saline into the recta.

DISCUSSION

The principal finding to emerge from the present study is the marked stimulatory effect the injection of fluid into the rectum has on abdominal oxygen consumption in both cockroaches and locusts. The injection of air also caused a slight net increase in abdominal oxygen consumption in locusts, which may be due to the stretching of the rectal and/or abdominal muscles. This increase has been subtracted from the fluid-stimulated increase in oxygen consumption in order to calculate the amount of oxygen consumed for one mg of fluid absorbed (Table 2). This we have designated the aerobic cost of fluid uptake in Table 2. The cockroach results do not need correcting as the injection of air caused no net change in oxygen consumption.

The following discussion is concerned with whether these fluid-stimulated increases

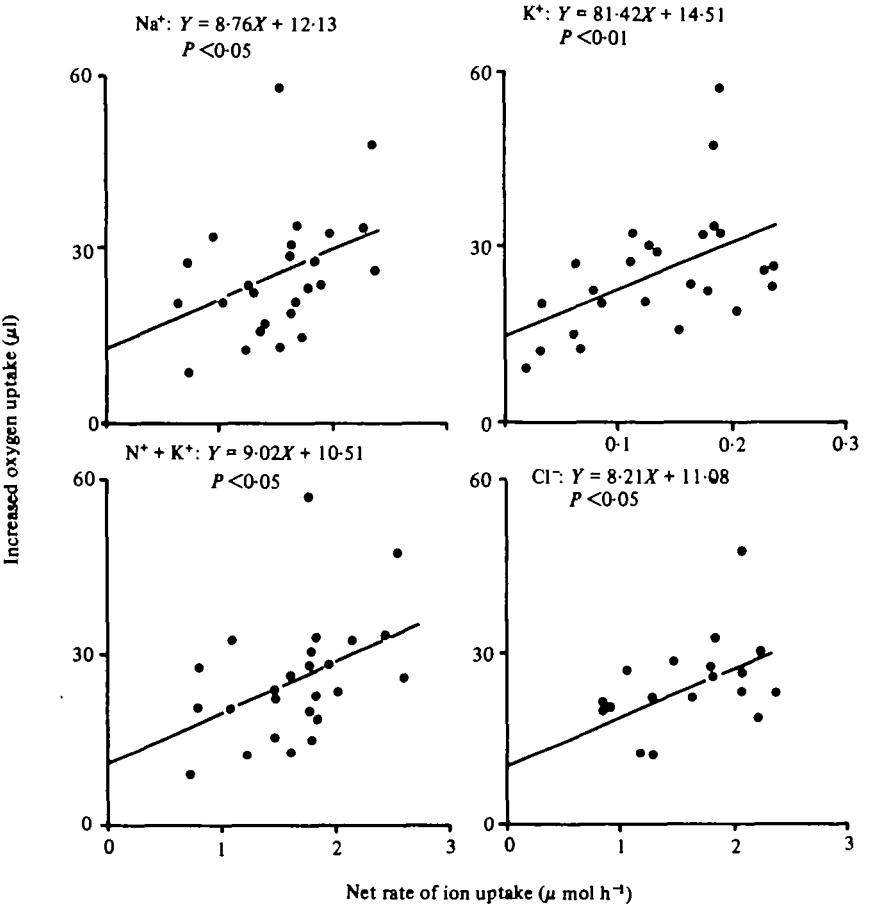

Fig. 10. Relationships between the net rates of ion efflux determined by analysis of the fluid recovered 60 min after injecting 30 mg of 800 m-osmol kg^{-1} into the locust rectum and the increased oxygen consumption during fluid uptake. The lines are drawn from the linear regression analyses shown.

in oxygen consumption are linked to the uptake of fluid from the rectum or to other phenomena. First it must be stated that when distilled water is injected into the rectum water may move into the rectal cells by osmosis and the oxygen consumption of the cells will increase with the effort of maintaining cell volume (MacKnight & Leaf, 1977), or may decrease due to membrane damage or cell lysis (MacKnight & Leaf, 1977; Hill & Hill, 1978). The possibility of these processes occurring together with the movement of ions into the rectal lumen makes the interpretation of the distilled water results difficult.

When 400 or 800 m-osmol kg^{-1} salines are injected into the recta the osmotic pressure of the lumen fluid is within the limits of that found in nature in both cockroaches and locusts (e.g. from 400 to 1900 m-osmol kg^{-1}, Phillips, 1977). It is therefore unlikely that the stimulation of oxygen consumption with these salines is due to the maintenance of cell volume or cell damage described above.

There are two lines of evidence which suggest that the fluid-stimulated increases in oxygen consumption can be ascribed to an aerobic cost of fluid uptake. First,

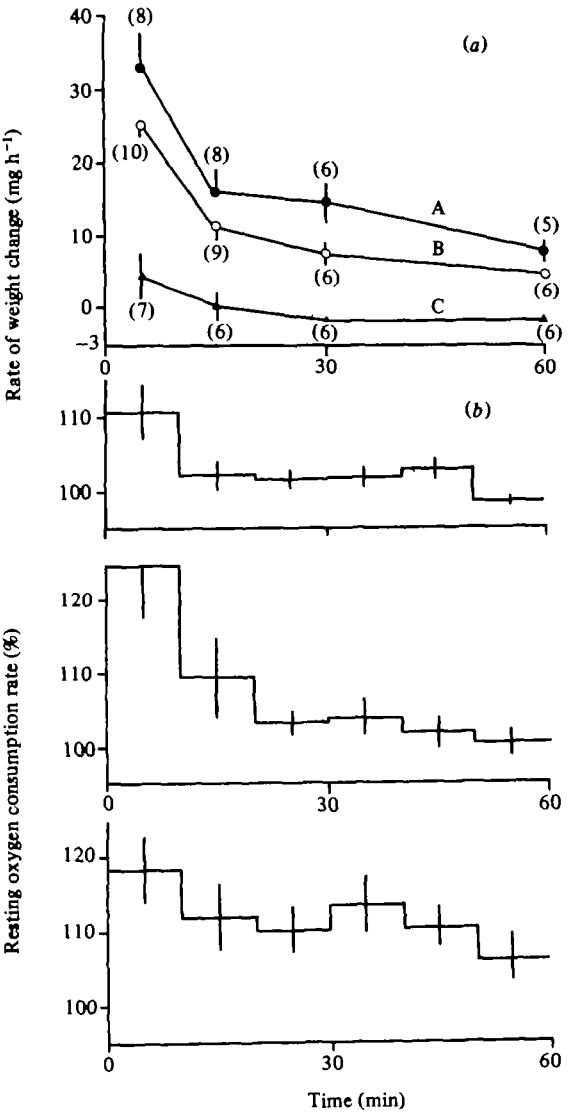

Fig. 11. Rectal fluid uptake and the associated changes in cockroach abdominal oxygen consumption. (*a*) Rate of weight change occurring during 1 h in the 10 mg of fluid injected into the rectum. Means ± S.E. (no. of observations) are given. (*b*) The change in abdominal respiration during 1 h pooled from Fig. 3 expressed as a percentage of the resting abdominal respiration rate after the injection of 10 mg of A, distilled water; B, 400 m-osmol kg^{-1} saline; C, 800 m-osmol kg^{-1} saline into the rectum. Means ± S.E. are given.

comparison between the species reveals that locusts take up fluid at double the rate of cockroaches and have double the stimulation in oxygen consumption following fluid injection (comparison of Figs. 11 and 12). Secondly, in both species the uptake of fluid falls into a rapid initial phase and a secondary phase of slower fluid uptake and these correlate with the time courses of the fluid-stimulated oxygen consumption (Figs. 11 and 12). The rapid initial decline in fluid uptake from insect recta over the first hour after fluid is introduced has been noted in previous studies on rectal absorption both

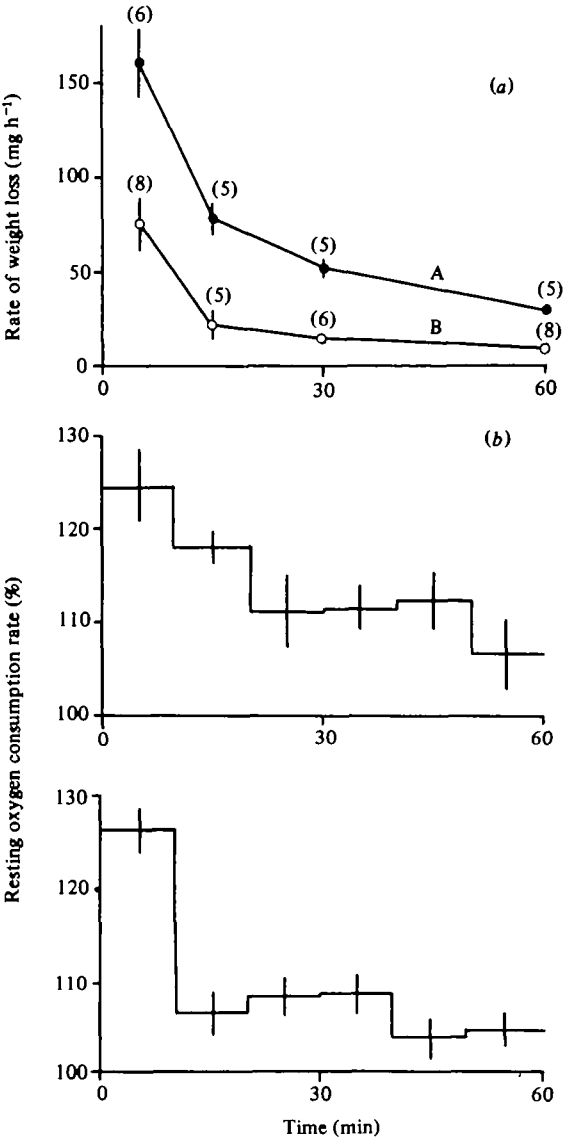

Fig. 12. Rectal fluid uptake and the associated changes in locust abdominal oxygen consumption. (*a*) Rate of weight loss occurring during 1 h in the 30 mg of fluid injected into the rectum. Means ±S.E. (no. of observations) are given. (*b*) The change in abdominal respiration during 1 h pooled from Fig. 6 expressed as a percentage of the resting abdominal respiration rate after the injection of 30 mg of A, distilled water; B, 800 m-osmol kg^{-1} saline into the rectum. Means ±S.E. are given.

in vitro and *in vivo* (Phillips, 1964; Wall, 1967; Mordue, 1969; Vietinghoff *et al.* 1969; Irvine & Phillips, 1971; Balshin & Phillips, 1971; Goh & Phillips, 1978). Goh & Phillips (1978) describe the initial phase as being a transient state of increased fluid uptake which is followed by a reduction in the rate of absorption to a steady state value. For both cockroaches and locusts it is arguable if a steady state can be obtained in the present experiments when distilled water is introduced into the rectum, since th

rectal contents are almost totally absorbed in 60 min after injection and have come into equilibrium with the osmotic pressure of the blood.

Phillips (1980) attributes the rapid phase of fluid uptake to the build-up of osmotic gradients within the rectal wall prior to transport when the rectum is in equilibrium with faecal material of high osmotic pressure. When fluid is introduced into the rectum there is an initial increased rate of uptake until the gradients equilibrate. This hypothesis implies that the active processes generating the gradients will occur before the fluid is introduced into the rectum and does not predict an immediate increase in oxygen consumption. Our results, however, suggest that, although gradients may well be present before fluid is introduced, there is an immediate start in the aerobic energy demanding steps as soon as fluid is introduced into the rectum. Fluid transport linked with stimulation of oxygen consumption continues until both decline to the secondary slower phase, presumably due to the loss of solutes or metabolites. We will now discuss what the energy-demanding steps causing the stimulation of oxygen consumption might be.

One current model for the absorption of fluid from the rectum is that ions and/or organic solutes are pumped into narrow intercellular spaces generating hyperosmotic conditions. Fluid moves from the lumen across the intercellular junctions and into the intercellular spaces by osmosis and passes via intercellular spaces and channels into the subepithelial spaces and hence to the blood (Oschman & Wall, 1969; reviewed by Wall, 1971 and Phillips, 1980). As the fluid moves through the channels there may be recovery and recycling of ions within the epithelium so that the absorbate may be hyposmotic to the luminal fluid (Phillips, 1964; Wall, 1967; Phillips, 1969). Support for this local osmosis model comes from micropuncture studies in *Periplaneta* rectum (Wall, Oschman & Schmidt-Nielsen, 1970) and microprobe analysis of *Calliphora* rectum (Gupta *et al.* 1980).

Assuming that the above model for fluid transport is in operation in the rectum and is immediately turned on when fluid is introduced into the rectum, we can identify at least 3 energy-demanding steps: 1, ion uptake by the apical membrane; 2, active transport of ions into the intercellular channels; 3, active transport of ions out of the intercellular channels for the purposes of ion recycling. We have calculated the theoretical costs of generating the necessary hyperosmotic gradients for fluid uptake, considering first only the active transport of ions into the intercellular channels. The mechanism by which ions are pumped into the intercellular spaces could be a Na^+–K^+ activated ATPase in the tissue. This enzyme has been identified in rectal tissue of both *Periplaneta* (Tolman & Steele, 1976) and *Schistocerca* (Peacock, 1977), and studies that relate the transport of Na^+ and K^+ to oxygen consumption have been carried out (Whittam & Willis, 1963; Harris, Balaban & Mandel, 1980; Shuttleworth & Thompson, 1980; Silva *et al.* 1980).

We have assumed that the osmotic pressure in the intercellular spaces is 100 m-osmol kg^{-1} higher than that of the lumen (Gupta *et al.* 1980) and that the gradient is established by the secretion of NaCl to form a solution equal in amount to the amount of fluid absorbed. We have also assumed that the sodium to oxygen ratio is 18 mol Na^+ : 1 mol O_2 (Shuttleworth & Thompson, 1980) although other ratios have been found (Whittam & Willis, 1963; Silva *et al.* 1980).

Table 2. *Aerobic cost of fluid uptake in terms of μl of oxygen for 1 mg of fluid transported*

(The observed cost is calculated from the increases in oxygen uptake for 10 mg weight loss obtained from the relevant regression equations divided by ten. The theoretical costs are calculated as described in the text and are also in μl O_2 mg^{-1} fluid transported.)

	Periplaneta americana		*Schistocerca gregaria*		*Petrobius brevistylis* (Houlihan, 1977)	
Fluid tested	Observed	Theoretical	Observed	Theoretical	Observed	Theoretical
Distilled water	1·31	0·07	1·19	0·07	0·15	0·13
215 m-osmol kg^{-1} NaCl	—	—	—	—	0·30	0·31
330 m-osmol kg^{-1} NaCl	—	—	—	—	0·95	0·40
400 m-osmol kg^{-1} Mordue saline	1·53	0·3	—	0·07	—	—
800 m-osmol kg^{-1} Mordue saline	3·27	0·6	1·81	0·6	—	—
800 m-osmol kg^{-1} sucrose solution	—	—	9·39	0·6	—	—

The results from such calculations are shown in Table 2 and reveal that the theoretical costs are very much less than the observed values. We are probably underestimating the osmotic pressure generated in the intercellular channels and have no way of estimating the amount of ion recycling and apical ion uptake.

We have also included in Table 2 the observed costs of fluid uptake across the vesicular transporting epithelium of *Petrobius brevistylis* Carp. (Houlihan, 1977). Not only are the measured costs of fluid transport for *Petrobius* similar to those reported for insect recta, but also *Petrobius* shows the increase in oxygen consumed with fluid transport correlated to the concentration of the substrate solution and the amount of uptake as found in the present experiments.

The theoretical costs for *Petrobius* were calculated on the assumption that the local hyperosmotic gradients generated within the epithelium are equal to the measurements made of the osmotic pressure of the absorbates produced by the transporting cells of *Petrobius* (Houlihan & Sell, 1981). Just as in the case of the rectum, without knowledge of the actual concentrations generated in the intercellular or intracellular channels we are probably underestimating the theoretical costs of transport for this reason alone.

In *Petrobius* there are close correspondences between the theoretical costs of fluid uptake and the measured costs, the range being 42–103%. In *Petrobius* the uptake is rapid and the very short (5–10 μm) intracellular channels probably allow for little ion recycling.

The results with ouabain show reduced fluid uptake and reduced fluid-stimulated oxygen consumption with 10^{-2} M ouabain and a slight effect on oxygen consumption at 10^{-3} M. These results might be taken to reinforce the linkage between oxygen consumption, the Na^+–K^+ ATPase of the intercellular channels, the generation of hyperosmotic conditions, and the fluid absorption from the rectum. However, ouabain, even at 10^{-2} M, did not abolish fluid uptake against an osmotic gradient. A similar result has been obtained by Irvine & Phillips (1971) for an *in vitro* preparation. Also there are problems with interpreting the effects of ouabain since it acts on other ion-transporting sites as well as on the Na^+–K^+ ATPase involved in fluid transport (Shuttleworth & Thompson, 1980).

The results of injecting a sucrose solution into the rectum is a greatly increased cost

transport (Fig. 7, Table 2), whereas the calculated cost of transport is the same as with 800 m-osmol kg^{-1} saline. Presumably, with sucrose there is no active transport across the apical membrane but only the generation of hyperosmotic conditions and ion recycling. As there is a considerable difference between the theoretical and the observed costs, based solely on the generation of the intercellular gradients, this result implies and reinforces the earlier speculation that ion recovery from the intercellular channels is a major contributor to the aerobic costs of fluid uptake.

The net efflux of ions from the rectum can be correlated with the uptake of fluid. As this uptake appears to be isoionic to the luminal contents, it may be the result of a solvent drag effect (Berridge & Oschman, 1972). Therefore, the correlation between ion movement and extra amount of oxygen consumed is possibly not an indication of the activity of the apical active processes known to be present for K^+ and Cl^- (Irvine & Phillips, 1971).

When 800 m-osmol kg^{-1} saline is injected into the rectum, the mean percentage increase in oxygen consumption for the whole animal is 9% and 6% of the oxygen uptake for resting cockroaches and locusts respectively, based on resting rates given in Keister & Buck (1974). These values are higher than the estimate of 0·24% of Phillips (1964).

Throughout this discussion we have concentrated on the aerobic costs of fluid transport. Although anaerobic metabolism has been implicated in fluid transport *in vitro* (Irvine & Phillips, 1971), we have no evidence for this *in vivo*. Preliminary experiments with Dr G. Gäde have shown a reduction in arginine phosphate concentration during fluid uptake but the levels of this energy source could only provide enough ATP equivalent to 6–7% of extra oxygen consumed by locust recta.

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