

## THE ROLE OF WATER IN TARSAL TASTE THRESHOLDS TO SUGAR IN THE BLOWFLY *PHORMIA REGINA*

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### Summary

Thirsty blowflies whose tarsi come into contact with water will respond with proboscis extension. When flies were exposed to high or low relative humidities (RH) prior to testing, the tarsal taste thresholds to sucrose in flies that were unresponsive to water alone increased or decreased, respectively. Evidently, thirst can also be subthreshold and recognizable only as a change in threshold to aqueous sucrose.

Thresholds to sucrose and fructose, which were in the millimolar range in 3-day-old adult flies held at 55–70 % RH prior to testing, fell significantly after the flies had been held at low humidity (13–20 % RH) for several hours. There was no low-humidity-dependent drop in threshold with galactose or xylose, sugars to which flies normally have thresholds in the  $10^2$  mmol l<sup>-1</sup> range.

Flies held at low relative humidity exhibited increased tarsal thresholds to sucrose when they were injected with water or were allowed to drink water *ad libitum*. When multiple injections of water were given, thresholds rose after successive injections. Changes in threshold to sucrose occurred after injection with aqueous NaCl, the direction and magnitude being dependent on the concentration injected. The threshold declined significantly after injection of 2 µl of 75 mmol l<sup>-1</sup> NaCl, remained unchanged after injection of 150 or 300 mmol l<sup>-1</sup> NaCl, and increased after injection of 600 mmol l<sup>-1</sup> NaCl.

Thirst in blowflies can affect thresholds to food stimuli. The changes in water content of the body resulting from a water-rich meal could contribute significantly to the normal post-meal rises in tarsal thresholds.

### Introduction

The black blowfly, *Phormia regina* Meigen, can monitor internal cues relative to its state of water balance and adjust its behaviour accordingly. A sufficiently thirsty fly will extend its proboscis and imbibe water following application of water either to its tarsal or its labellar chemosensory hairs. Water-negative flies, i.e. those that do not extend their proboscis to water, can be made water-positive by desiccation or bleeding (Evans, 1961; Dethier & Evans, 1961; Barton Browne, 1968).

Key words: *Phormia regina*, feeding behaviour, blowfly, thirst, tarsal threshold.

Injection of water into the haemocoel of water-positive *P. regina* reduced the percentage of flies responding to water (Evans, 1961). Injections of NaCl at concentrations of  $175 \text{ mmol l}^{-1}$  or less reduced the duration of drinking and volume of water ingested by *Lucilia cuprina* (Barton Browne, 1968). By injecting *L. cuprina* with various salts, Barton Browne concluded that in addition to osmotic pressure, blood chloride concentration is important in regulating water ingestion.

There is electrophysiological evidence for a water receptor neurone in labellar taste hairs of *P. regina* (Evans & Mellon, 1962) and *P. terranova* (Rees, 1970). The situation is somewhat different with tarsal taste hairs. Behavioural evidence for the perception of water by tarsal hairs is clear, but there is minimal electrophysiological data. McCutchan (1969*a,b*), using the electrophysiological methods of Evans & Mellon (1962), was unable to demonstrate a water receptor in *P. regina* tarsal chemosensitive hairs. In other studies, water has been shown to elicit action potentials from more than one type of receptor neurone in *P. regina* (van der Starre, 1970) and in *C. vicina* (Den Otter & van der Starre, 1968; van der Starre, 1972).

The water receptor in labellar taste hairs of *P. regina* is sensitive to the osmolarity of the test solution (Evans & Mellon, 1962; Rees, 1970). Increasing the concentration of nonelectrolyte to which the receptor was exposed (e.g. sucrose) resulted in a decline in the response to water. The receptor was very active in the presence of nonelectrolyte in the millimolar range. It was still functional, though reduced in activity, at nonelectrolyte concentrations of  $1 \text{ osmol kg}^{-1}$  (approximately 1 molal) and higher.

Previous studies of responses to water in blowflies have addressed responsiveness in terms of the number of flies responding to water with proboscis extension before and after a specific treatment, as well as in terms of the duration and volume of fluids imbibed by the fly. Since water receptors are highly responsive to water over the osmotic range to which hungry flies also respond to sucrose with proboscis extension ( $1\text{--}10 \text{ mmol l}^{-1}$  sucrose), it seemed possible that the tarsal water receptor input might influence tarsal thresholds to sucrose. In the present study we demonstrate that the level of hydration in hungry blowflies affects tarsal thresholds to certain sugars. This suggests that water receptor input can play an important role in determining tarsal taste threshold to food.

## Materials and methods

### *Preparation of flies*

Adult blowflies, *Phormia regina*, were kept in hardware cloth cages covered with tube gauze at 60% RH and exposed to a 16 h:8 h L:D cycle with unlimited access to water. The temperature was maintained at  $25 \pm 1^\circ\text{C}$  during the light phase of the photoperiod and  $21 \pm 1^\circ\text{C}$  during the dark phase. Only 3-day-old flies that had been allowed water but no food since eclosion were used. Adult male and female flies were used indiscriminately. To prepare flies for all experiments,

individuals were briefly immobilized on ice, and wooden applicator sticks were attached perpendicular to the dorsum of the thorax with a warm beeswax/resin mixture (3/2). To assess mass changes after changes in the ambient humidity to which the flies had been exposed, glass capillaries were used in place of moisture-sensitive wooden sticks. Mounted flies were held for at least 1 h before testing to allow the effects of cold and handling to dissipate.

#### *Determination of threshold*

To obtain a measure of the sensitivity of the proboscis extension response to tarsal stimulation, an up-and-down bioassay utilizing 40 flies was chosen (Edgecomb *et al.* 1987). The test yielded a mean acceptance threshold (MAT) – the minimum logarithmic ( $\log_{10}$ ) concentration of sucrose that will elicit proboscis extension on tarsal contact by an average fly in a given population or treatment. The test solutions consisted of twofold serial dilutions of an aqueous sugar solution, the range depending on its solubility. Flies responding to a water pretest were excluded from the MAT determination (Thomson, 1977). Following the water pretest, the tips of the legs of each water-negative fly were dipped in one of the sugar solutions and the presence or absence of proboscis extension noted. The MAT was performed and calculated according to the method of Dixon & Mood (1948) using groups of 40 flies. Each MAT value was treated as a single measurement in determining the average MAT. Threshold values reported in tables and figures are logarithmic values. In the text, however, the antilogs of these mean values (concentration instead of  $\log_{10}$  concentration) are also given when absolute threshold values are reported.

Data were evaluated using an analysis of variance (ANOVA). For experiments in which thresholds were determined before and after treatment, measurements were paired and the difference tested for significance ( $P < 0.05$ ) using a paired *t*-test. Comparisons among means were tested for significance ( $P < 0.05$ ) using Duncan's multiple-range test (Statistical Analysis System, SAS Institute, Cary, NC).

#### *Effects of relative humidity on tarsal threshold*

To determine the effects of desiccation and hydration on threshold and fly body mass, flies mounted on sticks were held in glass-covered aquaria for 3 h. Tarsal thresholds were determined before and after exposure to a high- or low-humidity environment. To form the low-humidity environment, the bottom of the aquarium was covered with silica gel, producing a measured relative humidity of 13–20% (digital hygrometer/thermometer, Fisher Scientific). To form the high-humidity environment, the bottom of the aquarium was covered with moist paper towels, producing a measured relative humidity of >90%. During the experiment the relative humidity of the room varied from 47 to 85%, but usually ranged from 55 to 70%.

*Injection procedures*

Injections were carried out 1 h after mounting, as described earlier (Long & Murdock, 1983). Distilled water, or 75, 150, 300 or 600 mmol l<sup>-1</sup> NaCl, or 1.2 mol l<sup>-1</sup> L-fucose was injected into the thorax of 3-day-old, unfed flies in volumes of 2 µl. For sham injections the needle only penetrated the cuticle. No apparent blood loss was observed during sham injections. Flies showing fluid leak after injection were discarded. Thresholds were determined 20 min after injection. When multiple injections of water were administered, flies were tested for tarsal threshold 30 min after the initial injection, then immediately reinjected and tested 30 min later. Three sequential injections of water were given.

*Crop mass after feeding*

Flies mounted on sticks were individually fed 5 µl of water or 250 mmol l<sup>-1</sup> sucrose. To ensure that the fly would drink the full volume, its tarsi were brought into contact with filter paper saturated with 2 mol l<sup>-1</sup> sucrose while guiding its extended proboscis into a capillary tube filled with water or 250 mmol l<sup>-1</sup> sucrose. The capillary tube was filled just prior to feeding the fly. Crop masses were determined to the nearest 0.1 mg (AE 100 Mettler) by dissecting and weighing crops 10, 30, 60 and 120 min after the onset of feeding. An empty crop weighs approximately 0.1 mg.

**Results***Effects of relative humidity on tarsal threshold*

Tarsal thresholds to sucrose in flies unresponsive to water (water-negative) were affected by the relative humidity to which the flies were exposed. Lowering the relative humidity to 13–20 % resulted in a significant lowering of tarsal sucrose thresholds (Table 1). Increasing the relative humidity to >90 % during the 3 h pretest period resulted in a significant increase in tarsal threshold to sucrose. Holding the flies for 3 h at room relative humidity (55–70 %) had no significant effect on the threshold of water-negative flies to sucrose.

To determine whether the chemical nature of the test sugar affected the

Table 1. *Changes in tarsal threshold to sucrose in hungry 3-day-old adult blowflies after exposure to different humidity environments for 3 h*

Humidity	<i>N</i>	MAT ± s.e. (log mmol <sup>-1</sup> sucrose)
High (>90 %)	9	0.22 ± 0.06*
Low (13–20 %)	13	-0.31 ± 0.07*
Room (47–85 %)	3	0.05 ± 0.10

Each *N* value represents a group of 40 flies.

Threshold values represent the mean difference between post- and pretreatment thresholds (MATs). Negative values indicate a decrease in threshold (increase in responsiveness).

\* *P* < 0.05.

magnitude of the threshold change in flies subjected to low humidity, thresholds to fructose, galactose and xylose were also determined and compared with those for sucrose. Two of these sugars, sucrose and fructose, have thresholds in 3-day-old starved flies which are quite low [0.72 log units ( $5.2 \text{ mmol l}^{-1}$ ) for sucrose, and 0.90 log units ( $8.0 \text{ mmol l}^{-1}$ ) for fructose in our laboratory]. The thresholds for the other two sugars chosen, galactose and xylose, are much higher [2.55 log units ( $350 \text{ mmol l}^{-1}$ ) for galactose and 2.81 log units ( $640 \text{ mmol l}^{-1}$ ) for xylose]. With both sucrose and fructose, the tarsal threshold was significantly lowered after the flies had been exposed to low humidity for 3 h (Fig. 1). However, no significant change in threshold was observed when the flies were subjected to the same conditions and tested on galactose or xylose, the two sugars with high thresholds.

#### *Effects of injection of water*

Flies previously exposed to high- or low-humidity environments were allowed to drink water for 10 min or were injected with  $2 \mu\text{l}$  of distilled water. Water intake or injection evoked no significant changes in tarsal threshold in flies previously subjected to high humidity (Table 2). However, in flies which had previously been subjected to low humidity, tarsal thresholds rose significantly when they drank water or were injected with water, by 0.54 or 0.58 log units, respectively. Injection of  $2 \mu\text{l}$  of water seemed an appropriate quantity since flies lost an average of 2.3 mg

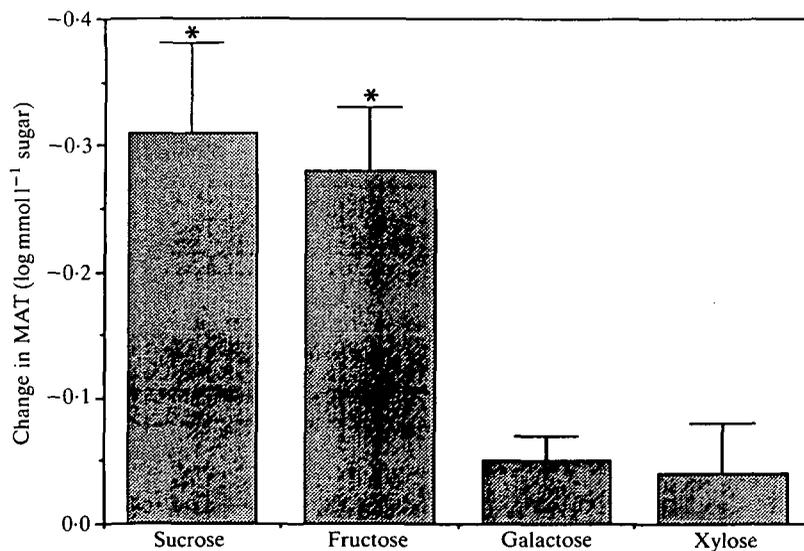


Fig. 1. Effects of subthreshold thirst on the responses of blowflies to low-threshold (sucrose and fructose) and high-threshold (galactose and xylose) sugars. MATs were determined for 3-day-old hungry flies prior to and after being held for 3 h at 13–20% RH. The difference in MAT values after treatment is plotted for each sugar, negative values indicating a decrease in threshold (i.e. an increase in responsiveness) due to low relative humidity. Error bars represent 1 s.e. An asterisk indicates a significant change ( $P < 0.05$ ) in threshold after exposure to low humidity.

Table 2. *Changes in tarsal threshold to sucrose after imbibition of water ad libitum for 10 min, or injection of 2 µl of water in flies previously exposed to different humidity environments for 3 h*

Humidity	Water <i>ad libitum</i> MAT ± s.e. (%)	Water injection MAT ± s.e.	Control MAT ± s.e.
High (>90%)	0.33 ± 0.15	0.12 ± 0.11	0.05 ± 0.07
Low (13–20%)	0.54 ± 0.05*	0.58 ± 0.09*	0.25 ± 0.10
Room (47–85%)	—	—	0.16 ± 0.05

Thresholds were determined 20 min after the injection or initiation of the drink.

At least three groups of 40 flies each were used for each humidity and treatment.

% threshold values (MAT) given represent the changes in  $\log \text{mmol l}^{-1}$  sucrose ± s.e.

Flies were induced to drink by touching the labellum with the drop of water.

Control flies were held for the same time prior to testing as the treated flies.

\*  $P < 0.05$ .

in body mass after 3 h in low humidity and consumed more than 2 µl when subsequently allowed to drink water *ad libitum* (Table 3).

When 3-day-old hungry flies held at ambient humidity were fed 5 µl of distilled water, the average crop mass during the initial 2 h following imbibition ranged from 4.0 to 2.8 mg with no significant change observed (Fig. 2). In contrast, mean crop masses in flies treated in a similar manner and fed 5 µl of 250  $\text{mmol l}^{-1}$  sucrose declined significantly during the first 2 h following imbibition (Fig. 2). By 1 h after the start of the drink, the mean crop mass (1.7 mg) had fallen significantly from the 10 and 30 min values (4.2 and 3.6 mg, respectively). At 2 h the mean crop mass (0.8 mg) was significantly lower than at all earlier times. The mean crop mass for flies fed 250  $\text{mmol l}^{-1}$  sucrose was also significantly lower than that in water-fed flies 2 h after the start of the drink (Fig. 2).

In a separate experiment to determine the effects of multiple injections of water on tarsal threshold to sucrose, flies were given three successive injections of 2 µl of

Table 3. *Changes in mean fly mass after exposure of 3-day-old hungry blowflies to high or low humidity environments for 3 h*

Humidity	Changes in mean fly mass (mg ± s.e.)	
	After 3 h exposure	After water <i>ad libitum</i>
High (>90%)	0.5 ± 0.09	3.3 ± 1.1
Low (13–20%)	-2.3 ± 0.10*	4.3 ± 0.9*

The flies were allowed to drink water *ad libitum* and were reweighed after the exposure to different humidities.

Net mass change was determined using flies mounted on glass capillary tubes.

Flies were induced to drink by touching the labellum with the drop of water.

\*  $P < 0.05$ .

$N \geq 20$ .

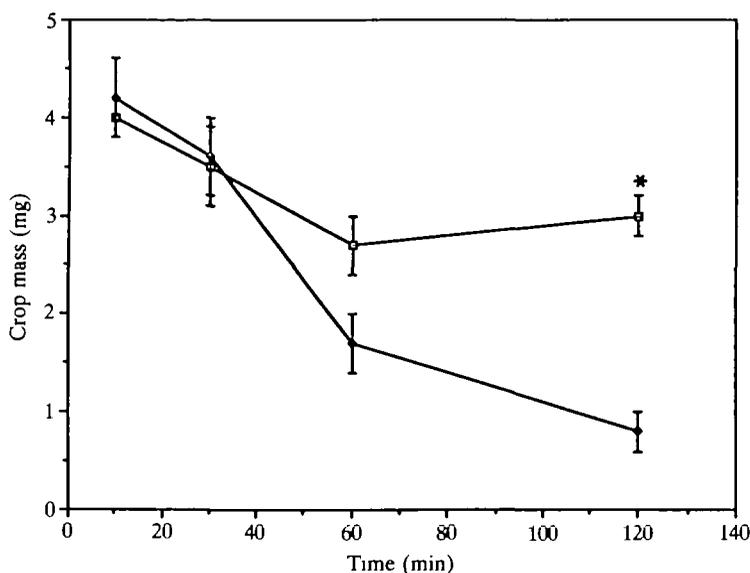


Fig. 2. Crop masses at various times after feeding  $5 \mu\text{l}$  of either water ( $\square$ ) or  $250 \text{ mmol l}^{-1}$  sucrose ( $\bullet$ ). Vertical bars at each point indicate  $1 \pm \text{s.e.}$  The asterisk indicates a significant difference ( $P < 0.05$ ) in crop masses between water-fed and sugar-fed insects at the same time after feeding.

water. Control flies held at ambient relative humidity were merely sham injected. The tarsal thresholds after water injection were significantly higher than the threshold after each corresponding sham injection (Table 4). The thresholds for sham-injected flies were significantly elevated over pre-injection values after the second and third sham injections. This increase is possibly the result of stress or injury.

#### Injection of NaCl

Injection of NaCl ( $2 \mu\text{l}/\text{fly}$ ) affected the threshold to sucrose in water-negative flies in a concentration-dependent manner (Fig. 3). Flies were injected with  $2 \mu\text{l}$  of

Table 4. Tarsal thresholds to sucrose in 3-day-old hungry blowflies after multiple water injections

Injection	MAT ( $\log_{10} \text{ mmol l}^{-1}$ sucrose)	
	Sham injection	Water injection
Uninjected	$0.99 \pm 0.07$	$0.96 \pm 0.07$
First injection	$1.13 \pm 0.08$	$1.46 \pm 0.07^*$
Second injection	$1.31 \pm 0.05$	$1.76 \pm 0.12^*$
Third injection	$1.28 \pm 0.08$	$1.99 \pm 0.20^*$

Values are MATs  $\pm$  s.e. ( $N = 3$  groups of 40 flies each).

\* Significantly elevated ( $P < 0.05$ ) over sham-injected flies.

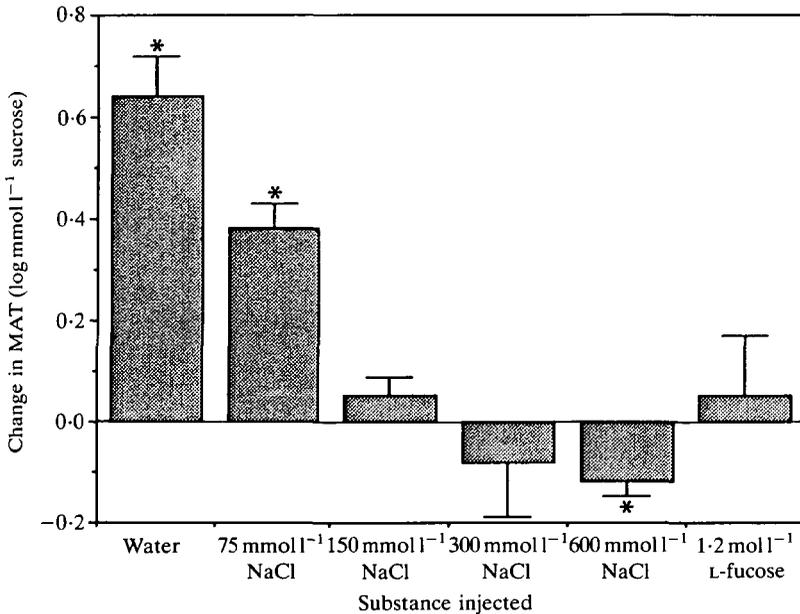


Fig. 3. The effect of injection of 2  $\mu$ l of water, aqueous NaCl at various concentrations or L-fucose, on tarsal thresholds to aqueous sucrose. Three-day-old unfed adult *Phormia regina* held at ambient relative humidity (55–70% RH) prior to testing were used. Values given represent the change in MAT (log mmol l<sup>-1</sup> sucrose) after treatment. Error bars represent 1 s.e. An asterisk indicates a significant change ( $P < 0.05$ ) in threshold 20 min after injection.

distilled water. The threshold to sucrose rose significantly (0.64 log units) 20 min after injection. When 75 mmol l<sup>-1</sup> NaCl was injected, the increase in threshold (0.38 log units) was less, but the difference was still significant. Threshold to sucrose was not affected by injection of either 150 or 300 mmol l<sup>-1</sup> NaCl. Injection of 600 mmol l<sup>-1</sup> NaCl, however, significantly lowered threshold by 0.12 log units. To determine whether the molality of the solution was the important factor, 2  $\mu$ l of 1.2 mol l<sup>-1</sup> L-fucose, a nonmetabolizable sugar, at the approximate molality of 600 mmol l<sup>-1</sup> NaCl, was injected. The slight increase in threshold observed was not statistically significant (Fig. 3).

### Discussion

Responsiveness of water-negative flies to aqueous sucrose in the tarsal taste proboscis extension test is influenced by the relative humidity to which flies have been exposed prior to testing. Flies taken from room humidity (typically 55–70% RH) and placed in high- or low-humidity environments for 3 h had thresholds to sucrose which increased or decreased, respectively (Table 1). Exposure to low relative humidities results in substantial weight loss (Table 3) and thirst, which was often subthreshold, i.e. insufficiently intense to evoke proboscis extension when

the tarsi contacted water, but capable of lowering thresholds to certain aqueous sugar solutions. Thirst, therefore, is an important criterion that requires consideration when measuring tarsal thresholds to aqueous food.

This subthreshold effect of thirst on tarsal threshold to sugar was dependent on the chemical structure of the sugar used. When low-threshold sugars, sucrose or fructose, were tested, a decrease in threshold was observed following exposure to low relative humidity (Fig. 1). In contrast, no change in tarsal threshold was observed when flies subjected to the same treatment were tested on high-threshold sugars, galactose or xylose. At high sugar concentrations the water receptor firing rate in labellar hairs is below optimum (60% relative action potential frequency for sucrose) (Rees, 1970). Since water receptor activity becomes less as the osmolarity increases, it is possible that the tarsal sugar receptor alone contributes to proboscis extension at higher osmolarities. This suggests that the mechanism involved lies in the water receptor–proboscis extension pathway. Changes in sugar receptor activity due to changes in the state of hydration are possible, but unlikely. Feeding flies increases the state of hydration as measured by lowered blood osmolarity (Bolwig, 1953), and sugar receptor activity from labellar or tarsal taste hairs has been shown to be unaffected by feeding (Kawabati & Shiraishi, 1977; Rachman, 1979; Hall, 1980; but see Omand, 1971). It thus appears that tarsal thresholds to sucrose and fructose in water-negative flies result from an integration of the input from both water and sugar receptors.

When the level of hydration of the fly is increased, either by imbibition or by injection of water, the tarsal threshold to aqueous sucrose rises. This subthreshold effect of thirst on tarsal threshold to sucrose is graded and depends on the level of hydration of the fly. Since injection of water and imbibition of water have a similar effect on tarsal thresholds to sucrose, the mechanism underlying this effect probably occurs outside the alimentary canal. This inference is supported by our observations that changes in tarsal threshold can be induced merely by placing unfed flies in high- or low-humidity environments. It appears that the blowfly somehow monitors its state of hydration and alters its utilization of the input from the water receptor according to whether it is in or out of water balance.

What the fly monitors internally is still unclear. Our results from injecting aqueous NaCl indicate that blood osmolarity, sodium ion concentration or chloride ion concentration may be key elements mediating the effects of subthreshold thirst on tarsal thresholds to sucrose (Fig. 3). Blood osmolarity in flies has been shown to be related to the state of food and water deprivation (Bolwig, 1953). According to Bolwig, flies fed on water alone had the lowest osmotic pressure, whereas hungry and thirsty flies had the highest ones. Flies fed on sugar-water fell between these two groups. Osmolarity, however, may be less of a factor than sodium or chloride concentrations. In the present study, injection of L-fucose at the same osmolarity as  $600 \text{ mmol l}^{-1}$  NaCl did not lower threshold to sucrose (Fig. 3), which would have been expected if osmolarity were the only blood factor determining threshold.

By comparing the percentage mass loss in water-sated *L. cuprina* during water

deprivation with water responsiveness, osmotic pressure and sodium and chloride concentrations, Barton Browne & Dudzinski (1968) found that the curves relating sodium and chloride concentrations to percentage mass loss most closely resembled the curve relating water responsiveness to percentage mass loss. These relationships were investigated further by injection of various substances into thirsty or water-sated flies starved for 24 h (Barton Browne, 1968). Replacing the chloride with orthophosphate reduced the duration of the drink following injection. Also, injection of NaCl with sodium bicarbonate or glycerol resulted in no difference in the duration of water consumption. If sodium ions were a key element regulating water consumption, then the NaCl/NaHCO<sub>3</sub>-injected group should have consumed more than the NaCl/glycerol-treated flies (Barton Browne, 1968). Our results with the injection of sodium chloride or fucose on subthreshold thirst in *P. regina* are consistent with those of Barton Browne (1968).

The black blowfly thus appears to be capable of monitoring internal cues, such as osmotic pressure and chloride concentration, and altering its sensitivity to water input accordingly. It is important, however, to stress that these factors affect flies that fail to respond to water alone. How thirst regulates sensitivity to water and possibly sugar input is yet unknown. Since tarsal thresholds to sucrose in water-negative flies are affected by desiccation, hydration, consumption of water and injection of water or other substances, it seems evident that thirst and hunger cannot be easily separated.

In an earlier study of the regulation of tarsal threshold in *P. regina*, we provided experimental evidence that the recurrent nerve (RN) plays an important role in the tarsal threshold rise that follows a meal of 250 mmol l<sup>-1</sup> sucrose (Edgecomb *et al.* 1987). Nevertheless, meals of aqueous sucrose still evoked substantial rises in threshold in RN-transected flies. Neither the median abdominal nerve nor blood glucose level appeared to be responsible for this change. Based on the present studies, we propose that water itself, imbibed together with sucrose, is a major factor contributing to the threshold rise following a meal of aqueous sucrose. Several lines of evidence support this hypothesis. First, injections of water caused tarsal sucrose thresholds to rise, and the rise was proportional to the amount of water administered. The sucrose thresholds attained after 4–6 µl of water injection in normal flies (Table 4) were in the same range as sucrose thresholds attained after 10 or 15 µl meals of 250 mmol l<sup>-1</sup> sucrose fed to RN-transected flies (Edgecomb *et al.* 1987). Second, imbibition of water by flies held in low humidity conditions increased sucrose threshold (Table 2). Third, injection of 600 mmol l<sup>-1</sup> NaCl, which would have the effect of putting the fly into negative water balance, lowered the threshold to aqueous sucrose (Fig. 3). Finally, the rates of crop emptying were not the same for 250 mmol l<sup>-1</sup> sucrose and water. A hungry fly after imbibing 5 µl of water still had 3 mg in its crop 2 h after drinking. After the same time interval, a hungry fly fed 250 mmol l<sup>-1</sup> sucrose had only 0.8 mg remaining in its crop (Fig. 2). Thus, flies fed 250 mmol l<sup>-1</sup> sucrose were consuming much more water than was necessary for the maintenance of normal water balance. As a meal of sucrose is digested, monosaccharides pass into the haemolymph through the

midgut wall along with the water in the meal. This should cause a decrease in the osmotic pressure and chloride concentration of the haemolymph. Both these changes should reduce water responsiveness and hence increase tarsal threshold to sucrose in water-negative flies.

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