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REGULATION OF TARSAL TASTE THRESHOLD IN THE BLOWFLY, *PHORMIA REGINA*

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SUMMARY

The nutritional condition of adult blowflies (*Phormia regina* Meigen) affects their readiness to respond with proboscis extension when their tarsi contact food stimuli. Thresholds are high in sated flies (100–1000 mmol l^{-1} sucrose) and low in starved flies (1–10 mmol l^{-1} sucrose). Two feeding regimes employing aqueous sucrose were used to reveal factors regulating tarsal taste threshold in this insect: long-term feeding (*ad libitum*) and single meals administered to starved flies.

A positive logarithmic relationship was found between crop weight and tarsal taste threshold, expressed as mean acceptance threshold, in flies fed aqueous sucrose *ad libitum* for 4 days. Threshold changes after a single meal were positively correlated to both concentration and volume of the sugar solution fed. Thresholds observed in flies fed a single meal were not as high as those in *ad libitum*-fed flies having the same crop volume.

Nerve-transection experiments demonstrated that the median abdominal nerve plays no direct role in threshold regulation in either single-meal-fed or *ad libitum*-fed flies. Transection of the recurrent nerve (RN), however, significantly attenuated the post-feeding rise in tarsal threshold in starved flies fed a single meal and markedly reduced threshold in sated flies fed *ad libitum*. Thresholds for RN-transected flies subjected to either feeding regime were still significantly higher than thresholds for starved flies. Haemocoel-injected D-glucose did not significantly elevate threshold in starved flies.

These observations establish that the RN plays an important role in the regulation of tarsal taste threshold in blowflies. The effect of the RN on threshold depends largely on the prior feeding activity of the flies. It appears, however, that other factors, in addition to the recurrent nerve, affect taste threshold after feeding.

INTRODUCTION

Adult blowflies detect food by means of contact chemoreceptors on their tarsi (Wolbarsht & Dethier, 1958; McCutchan, 1969; Shiraishi & Tanabe, 1974). When these receptors contact a sufficiently sweet substance, the fly responds by extending its proboscis. This brings a second group of taste hairs, located on the aboral surface of the labellum (Wilczek, 1967), into contact with the potential food. These receptors mediate the spreading of the labellar lobes (Pollack, 1977), thus exposing a

Key words: *Phormia regina*, feeding behaviour, blowfly, food intake, recurrent nerve.

third set of chemoreceptors, the interpseudotracheal papillae. Stimulation of these papillae initiates drinking (Falk, 1975). During the drink, liquid food passes through the foregut into the crop, a thin-walled, collapsible diverticulum (Knight, 1962). Within minutes of ingestion, crop contraction and reverse peristalsis force slugs of fluid back from the crop, up the crop duct, and into the foregut, which pumps the slug of fluid food into the midgut through the proventricular valve (Thomson, 1975*b*). If the valve remains closed, the slug re-enters the crop. Blood osmolarity controls the rate of crop emptying; as osmolarity increases, the crop empties more slowly (Gelperin, 1966*a*).

Hungry blowflies respond to food more readily than sated ones. A hungry fly will respond with proboscis extension when its tarsi contact millimolar concentrations of sucrose, while a sated fly requires much higher concentrations. Similarly, a hungry fly will more readily spread its labellar lobes (Pollack, 1977) and will drink more of a given sugar solution than a sated fly (Evans & Barton Browne, 1960; Gelperin, 1966*b*). Thus, blowflies are able to adjust tarsal responsiveness and consumption by monitoring satiety cues.

Proboscis extension in the blowfly is easily observed and can be used as a measure of readiness to feed. It is the product of a complex interaction of internal and external sensory receptors, which monitor different aspects of activity in the alimentary canal and the potential for food in the environment. Information from these receptors converges in the central nervous system (CNS), where integration occurs and the appropriate behaviour is initiated. With the exception of results reported by Omand (1971), excitatory input from the labellar or tarsal taste hairs has been shown to be independent of the state of food deprivation (Kawatabi & Shiraishi, 1977; Rachman, 1979; Hall, 1980).

Two sets of internal receptors are known to provide negative feedback to feeding behaviour. One of these, associated with the recurrent nerve (RN), monitors peristalsis in the foregut. The other, located within branches of the median abdominal nerve (MAN), monitors crop volume and crop contractions. The RN, which courses along the dorsal surface of the foregut, connects the stomatogastric nervous system to the brain. Transecting the RN causes hyperphagia, the ingestion of more than twice the normal quantity of food (Dethier & Bodenstein, 1958; Dethier & Gelperin, 1967). In addition to affecting meal size, the RN may be involved in threshold regulation, as inferred from behavioural experiments in which crop duct ligations were unable to prevent the post-meal rise in threshold (Dethier & Bodenstein, 1958).

Electrophysiological and anatomical studies have revealed stretch receptor neurones in a branch of the RN in the anterior foregut (Gelperin, 1967). These receptors are excited when slugs of food pass into the anterior foregut. In addition to causing increased meal size, RN transection increases the duration of the first drink and the frequency of later small drinks (Gelperin, 1971*b*).

Isolating the brain from the thoracico-abdominal ganglion by cutting the ventral nerve cord also results in hyperphagia (Nuñez, 1964; Dethier & Gelperin, 1967). Nerve cord stretch receptors located in branches of the MAN monitor crop

expansion and send input to the thoracico-abdominal ganglion *via* the MAN (Gelperin, 1971*a*). Transecting the MAN has effects on drinking behaviour similar to RN transection (Gelperin, 1972).

Although considerable data have been amassed establishing the roles of the MAN and foregut stretch receptors as inhibitory inputs regulating meal size, there are few data bearing on the roles of these inputs in controlling tarsal taste threshold. According to Gelperin (1966*b*), meal size is inversely proportional to threshold. However, there is little direct evidence for such a relationship. For example, although the MAN plays a role in the regulation of meal size (Gelperin, 1971*a*), there is no experimental evidence that it participates in the control of tarsal responsiveness. Our own pharmacological studies support the possibility that readiness to feed and cessation of feeding are two separate behaviours, subject to separate controls. Reserpine and *d*-amphetamine, when injected into the haemocoel, increased tarsal taste threshold while at the same time increasing the size of a 1 mol l^{-1} sucrose meal (Long, Edgecomb & Murdock, 1986; Murdock *et al.* 1985). Injection of fenfluramine, however, increased tarsal taste threshold but not meal size, indicating that the two behaviours can be separated pharmacologically. In the present paper we describe experiments to identify factors regulating tarsal taste threshold to sucrose in blowflies. Our approach was to measure mean acceptance threshold (MAT) and crop weight under various experimental conditions.

MATERIALS AND METHODS

Flies

Adult *Phormia regina* Meigen were kept in hardware cloth cages covered with tube gauze at 60% relative humidity and on a 16:8 L:D cycle with unlimited access to water. The temperature was maintained at 25 ± 1°C during the light phase of the photoperiod and 21 ± 1°C during the dark phase. Male and female flies were used indiscriminately. In the single-meal experiments, flies were starved from emergence and tested on the third day. In the experiments requiring fed flies, we began with 3-day-old starved flies, which were then provided with aqueous sucrose *ad libitum* for 4–5 days. The sucrose solutions offered ranged in concentration from 62·5 to 1000 mmol l^{-1}. To prepare flies for all experiments, individuals were briefly immobilized on ice and applicator sticks were attached perpendicular to the dorsum of the thorax with warm beeswax/resin mixture (3/2). Mounted flies were held before testing for at least 1 h in glass-covered aquaria kept moist with damp paper towels to allow the effects of cold and handling to dissipate. The fly saline used was a modification of that of Chen & Friedman (1975). It contained (in mmol l^{-1}) Na^+, 122; Cl^-, 127; K^+, 5·6; Ca^{2+}, 2·4; Mg^{2+}, 1·0; and phosphate, 5·0 (pH 6·8).

Determination of threshold

To obtain a measure of the sensitivity of the proboscis extension response to tarsal stimulation, an up-and-down bioassay requiring 40 flies was used (Sudlow, 1985). Using the statistical method of Dixon & Mood (1948), 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. Proboscis extension was considered positive if the proboscis achieved a position greater than 3 (Dethier, Solomon & Turner, 1965). Typically, over 90% of the flies per group exhibited either no response or full proboscis extension. The test solutions consisted of two-fold serial dilutions of sucrose ranging from 2000 to 0·12 mmol l^{-1}. All flies were tested on water prior to testing on aqueous sucrose. Flies responding to the water were excluded from the MAT determination (Thomson, 1977). However, in time-course experiments water responders were allowed to drink to satiety, but were not included in the MAT determination until the next time point. Following the water pre-test, the tips of the legs of each fly were dipped into one of the sucrose solutions and the response of the fly's proboscis was determined. The starting concentration for the actual assay was determined in a preliminary experiment. Individual flies were tested beginning at the lowest concentration of sucrose and proceeding up the gradient until a concentration was reached at which proboscis extension occurred. The assay began one dilution step lower. The concentration of sucrose on which subsequent flies were tested was determined by the previous fly's response. After a positive response by a fly on a given concentration, the next fly was tested on the next lower concentration; after a negative response, on the next higher concentration. The MAT obtained from each group of 40 flies was calculated according to Dixon & Mood (1948) and treated as a single measurement in determining the average MAT for a given treatment and time interval. Threshold values reported in tables and figures are logarithmic values, and all statistical comparisons were made using logarithmic values. In the text, however, the antilogs of these mean values (concentration instead of $\log_{10}$ concentration) are also reported.

Flies were tested for taste threshold at various times after experimental treatment. Data were evaluated using an analysis of variance (ANOVA). For nerve transection experiments, thresholds from RN-transected flies were paired with thresholds from sham-operated flies and the difference tested for significance ($P < 0·05$) using Student's t-test. Significance of differences among means ($P < 0·05$) for all other data were determined by the least significant difference (LSD) test (Statistical Analysis System, SAS Institute, Cary, NC).

Crop weight vs *threshold*

Flies were fed *ad libitum* for 4–5 days on 62·5, 125, 250, 500 or 1000 mmol l^{-1} sucrose. After feeding, the flies were transferred to clean cages, with fresh water available *ad libitum*. Crop weights to the nearest 0·1 mg (AE 100, Mettler) were determined by dissecting and weighing the crops at specified times after the flies had been removed from food. An MAT for each group was measured at the same time. The average crop weight was calculated from 15 crops and the mean values, like the MATs, were treated as a single measurement.

Threshold changes following a single meal

Sticks with flies attached were mounted onto Plasticine stands, with the flies in an upright position over glass microscope slides covered with paraffin wax. Drops of sucrose solution were placed on the slide under each fly using an adjustable 20 μl Pipetman (Gilson). Flies were lowered to their respective drops at 12-s intervals, thus initiating feeding. Tarsal threshold was determined in the same order as feeding. Any fly failing to consume a complete meal was removed from the experiment. Threshold was determined before and 0·17, 0·5, 1, 2 and 5 h after the start of feeding, time 0 being the initiation of feeding. For nerve-transection experiments, flies were operated on 1–3 h before feeding.

Nerve transection

In order to transect a specific nerve, each stick bearing a fly (either 3-day-old starved or 4- to 5-day *ad libitum*-fed) was held parallel to the top of a Plasticine block and pressed into it so that the insect stick juncture was positioned on the edge of the block with the head of the fly pointing upwards. After rotating the block 90° so that the ventral surface of the fly faced up, a Plasticine strip was placed loosely over the anterior region of the thorax and head to immobilize the pro- and mesothoracic legs and to provide a pinning surface for opening the head capsule. For RN transection, the posterior aspect of the head was affixed to the thorax by a droplet of wax and the block rotated back so that the head again faced upwards. The frontal ganglion was exposed by making a V-shaped incision along the frontal suture (Dethier & Gelperin, 1967) and pinning the cuticular flap to the Plasticine strip using a minuten pin bent to form a micro-staple. After applying a droplet of saline, the nerve branches connecting the labrofrontal nerves to the frontal ganglion were cut. The RN and frontal ganglion were then lifted away from the foregut. Except for the omission of the actual nerve transection, sham operations were performed in an identical manner. For MAN transection the metathoracic legs were also held down with small Plasticine strips. A micro-staple was placed over the tip of the abdomen to immobilize it. Transection of the MAN was performed under the first abdominal sternite as described by Gelperin (1971*a*). Sham operations were performed in the same way, omitting only the actual nerve transection. Following each of the first few experiments, 20% of the nerve-sectioned animals were autopsied using leucomethylene blue (Stay & Gelperin, 1966) to confirm that nerve transections had been performed successfully. Greater than 95% success was observed in those experiments. Subsequently, regular autopsies were discontinued. Inclusive of preliminary experiments, more than 600 RN- and 400 MAN-transections were performed.

Injection procedures

Injections were carried out 1 h after mounting, as described earlier (Long & Murdock, 1983). D-Glucose was dissolved in water and 1 μl of 100 or 500 $\mu g\,\mu l^{-1}$

(0·56 or 2·8 mol l^{-1} glucose, respectively) was injected into the thorax. Mean acceptance thresholds were determined 10 min and 60 min after injection.

RESULTS

Crop weight and MAT after long-term feeding

Crops dissected from flies starved after 4 days of *ad libitum* feeding initially ranged in weight from 6 to 15 mg. The initial crop weights and time to empty were greater for flies fed higher sucrose concentrations. During food deprivation, crop weights declined rapidly in the first 24 h and then more gradually (Fig. 1). Crop weights were greater for flies fed higher sucrose concentrations at all times observed. The crop weights for flies fed 62·5–250 mmol l^{-1} sucrose eventually reached a weight similar to that of a 3-day-old fly starved from emergence (0·1 and 0·2 mg). Crop weights from flies fed 500 and 1000 mmol l^{-1} sucrose, however, never quite fell back to that low level before death occurred.

Thresholds in flies starved after *ad libitum* feeding followed a pattern similar to crop weight, but the decline in MAT was more gradual (Fig. 2). After feeding sucrose solutions ranging from 62·5 to 500 mmol l^{-1} *ad libitum*, the MAT was higher at any given time for flies fed on the higher concentration. However, the MATs for flies fed 1000 mmol l^{-1} sucrose deviated from this pattern. For times up to 96 h, these flies had MATs that were consistently lower than those observed for flies fed 500 mmol l^{-1} sucrose. In general, the time required for MAT to return to the level of

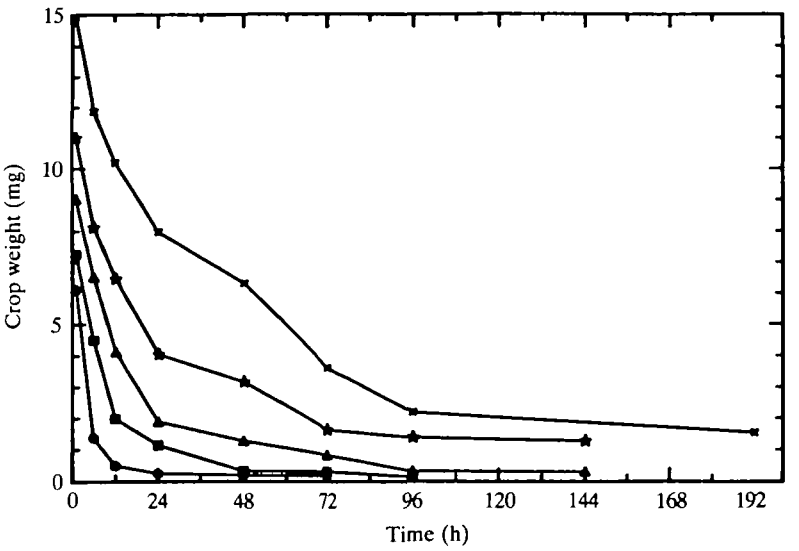

Fig. 1. Crop weights of flies starved after *ad libitum* feeding for 4 days on sucrose solutions ranging from 62·5 to 1000 mmol l^{-1}. Each point represents the mean of three determinations of average crop weights from 15 individuals. Representative S.E. of the mean: 0·8 mg. Symbols indicate the concentration fed: ● 62·5; ■ 125; ▲ 250; ★ 500; × 1000 mmol l^{-1}.

a starved fly ($0{\cdot}10$–$0{\cdot}80\,\log_{10}$ mmol l^{-1} sucrose; $1{\cdot}3$–$6{\cdot}0$ mmol l^{-1} sucrose) was shorter when the sucrose concentration fed was lower.

Since both crop weight and MAT declined during starvation after feeding, MAT was plotted against crop weight to determine the nature of their relationship. A curvilinear relationship was apparent (Fig. 3). At low crop weights the MAT rose sharply with increasing crop weight. At higher crop weights MAT rose more slowly as crop weight increased. A logarithmic relationship was found to provide the best fit of the data for each sucrose concentration fed (see legend of Fig. 3). Using the logarithm of crop weight, the lines obtained from flies fed $62{\cdot}5$, 125 or 250 mmol l^{-1} sucrose lay very close together with slopes in the $1{\cdot}2$–$1{\cdot}5$ range and y-intercepts in the $1{\cdot}6$–$1{\cdot}8\,\log_{10}$ mmol l^{-1} sucrose range. For flies fed 500 and 1000 mmol l^{-1} sucrose the slopes increased to $1{\cdot}8$ and $1{\cdot}7$, respectively. The y-intercept was slightly lower for flies fed 500 mmol l^{-1} sucrose ($1{\cdot}2\,\log_{10}$ mmol l^{-1} sucrose). The y-intercept for flies fed 1000 mmol l^{-1} ($0{\cdot}8\,\log_{10}$ mmol l^{-1} sucrose) was significantly lower ($P < 0{\cdot}05$) than the y-intercepts for flies fed $62{\cdot}5$, 125 or 250 mmol l^{-1} sucrose.

Effects of single-meal feeding

Tarsal responsiveness to sucrose was depressed immediately (by $0{\cdot}17$ h) following single meals with volumes of $2{\cdot}5$ μl and higher. Threshold was maximal by 30 min post-feeding and gradually declined towards pre-feeding values (Fig. 4). When flies were fed only $1{\cdot}0$ μl of 250 mmol l^{-1} sucrose, threshold rose slightly, but there was no statistically significant difference in MAT compared with the pre-feeding value at any time. Meals of $2{\cdot}5$ μl of 250 mmol l^{-1} sucrose and higher consistently caused a

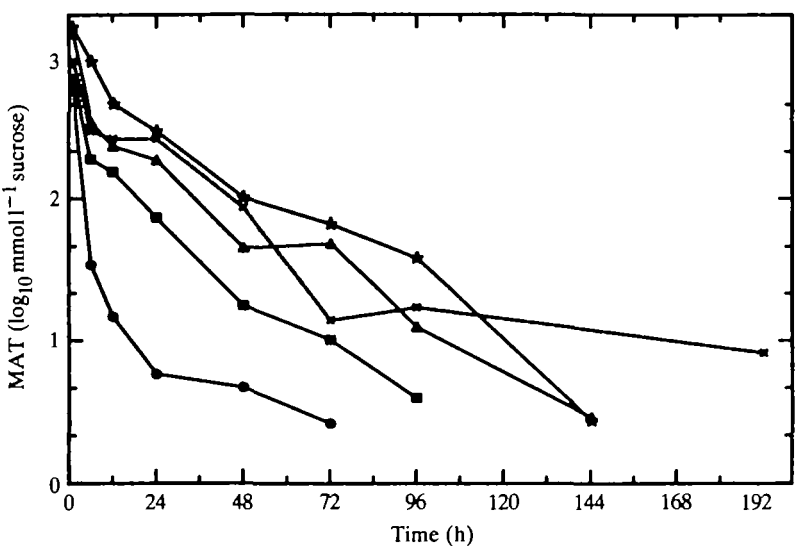

Fig. 2. Mean acceptance thresholds (MATs) from flies starved after *ad libitum* feeding for 4 days on sucrose solutions ranging from $62{\cdot}5$ to 1000 mmol l^{-1}. Each point represents the mean of three MAT determinations. Representative S.E. of the mean: $0{\cdot}2\,\log_{10}$ mmol l^{-1} sucrose. Symbols indicate the concentration fed: ● $62{\cdot}5$; ■ 125; ▲ 250; ★ 500; × 1000 mmol l^{-1}.

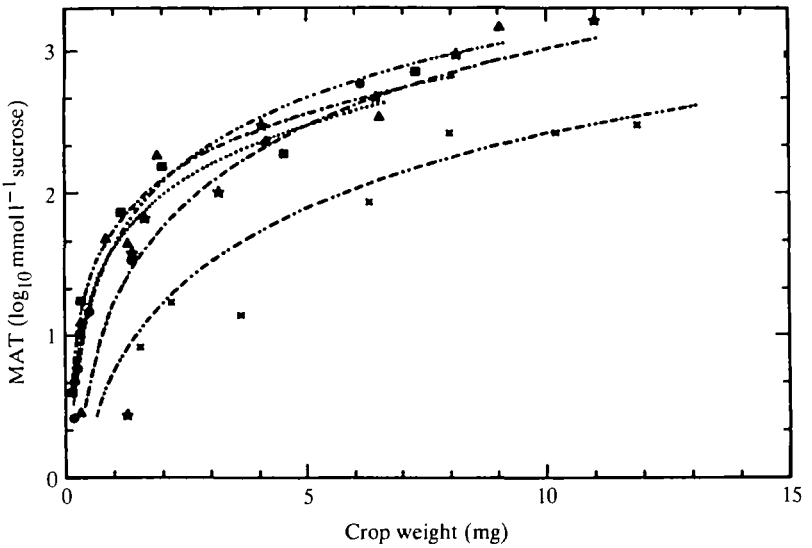

Fig. 3. Mean acceptance thresholds (MATs) *vs* mean crop weight at each time observed for flies starved after *ad libitum* feeding for 4 days on sucrose solutions ranging from 62·5 to 1000 mmol l^{-1}. Although average points are plotted, the curves were fitted and drawn using scatter data. Equations for each line: ● 62·5 mmol l^{-1} sucrose: MAT = 1·6+1·3 $\log_{10}$(crop wt); ■ 125 mmol l^{-1} sucrose: MAT = 1·8+1·2 $\log_{10}$(crop wt); ▲ 250 mmol l^{-1} sucrose: MAT = 1·6+1·5 $\log_{10}$(crop wt); ★ 500 mmol l^{-1} sucrose: MAT = 1·2+1·8 $\log_{10}$(crop wt); × 1000 mmol l^{-1} sucrose: MAT = 0·8+1·7 $\log_{10}$(crop wt). r^2 ranged from 0·55 to 0·80.

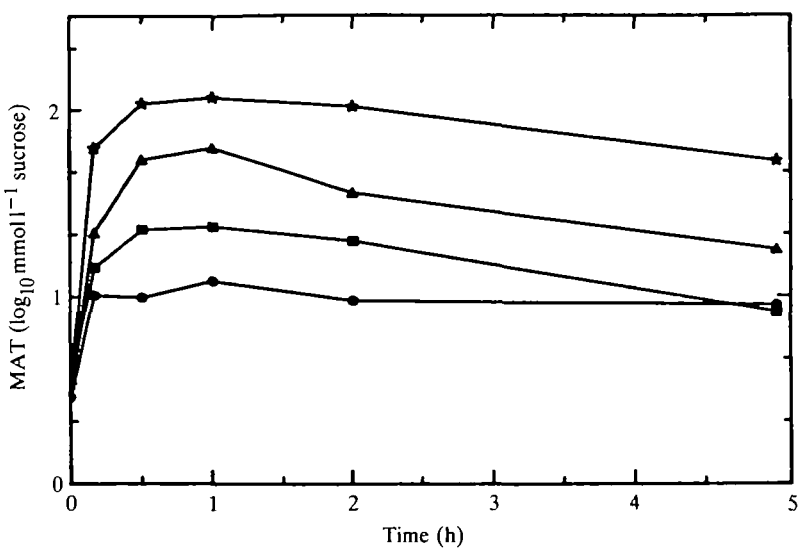

Fig. 4. Mean acceptance thresholds (MATs) for previously starved flies tested before and 0·17, 0·5, 1·0, 2·0 and 5·0 h after feeding a single meal of 250 mmol l^{-1} sucrose. The volume ranged from 1 to 15 μl. Each point is the mean of 3–6 MAT determinations. Representative S.E. of the mean: 0·13 $\log_{10}$ mmol l^{-1} sucrose. ● 1-μl meal; ■ 2·5-μl meal; ▲ 10-μl meal; ★ 15-μl meal.

significant rise in MAT, the rise being greater when greater volumes were fed. By 5 h post-feeding, flies given 2·5 μl of 250 mmol l^{-1} sucrose had MATs that were not significantly different from those of unfed flies.

Crop weights were also determined for several time points after flies had been fed single meals of 2·5, 10 or 15 μl of 250 mmol l^{-1} sucrose (Fig. 5). Crop weights roughly paralleled the MATs attained following a single meal. The larger the volume fed the higher were the crop weights at any time observed. 10 min after feeding meals of 2·5–15 μl of 250 mmol l^{-1} sucrose, 27–45 % of the meal had already passed into the midgut.

The threshold for flies fed single meals did not rise as high as the threshold for *ad libitum*-fed flies when the crop weights for the two groups were the same (Table 1). For example, at a crop weight of 9·6 ± 0·3 mg the threshold for flies fed single 15-μl meals of 250 mmol l^{-1} sucrose was 2·0 ± 0·1 (100 mmol l^{-1} sucrose) while the threshold for flies fed 250 mmol l^{-1} *ad libitum* for 4–5 days and with the same mean crop weight was 3·1 (1250 mmol l^{-1} sucrose), a difference of 1·1 $\log_{10}$ mmol l^{-1} sucrose.

Tarsal responsiveness after a single 2·5-μl meal also depended on the sucrose concentration administered. Sucrose concentrations as low as 3·96 mmol l^{-1} caused significant elevation of MAT at 0·17, 0·5 and 1 h post-feeding (Fig. 6). Flies fed 31·2 or 250 mmol l^{-1} sucrose had MATs significantly above the pre-feeding value when tested at 0·17, 0·50, 1·0 and 2·0 h post-feeding, but MATs were not different at 5 h post-feeding. The MATs for flies fed 2·5 μl of 2000 mmol l^{-1} sucrose were elevated at 0·17 h and had not yet begun to decline by 5 h post-feeding.

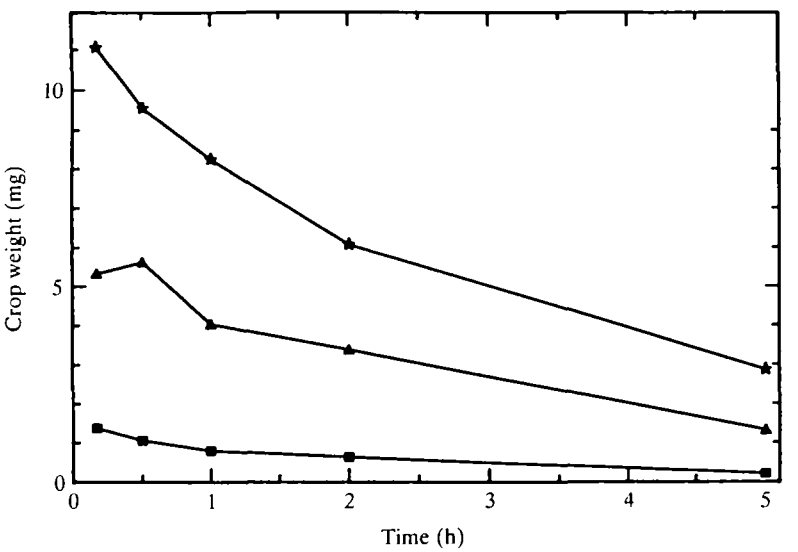

Fig. 5. Crop weights 0·17, 0·5, 1·0, 2·0 and 5·0 h after feeding a single meal of 250 mmol l^{-1} sucrose to starved flies. Each point is the average of 15 individual crops. Representative S.E. of the mean: 0·2 mg. ■ 2·5-μl meal; ▲ 10-μl meal; ★ 15-μl meal.

Effects of nerve transection on MAT

When *ad libitum*-fed flies were subjected to MAN transection, no effect on tarsal responsiveness to sucrose was evident at 2, 5 or 8 h post-operation (Table 2). By contrast, flies fed in the same way and subjected to RN transection exhibited MATs 10-fold lower than sham-operated flies (Table 2). The MATs obtained with RN-transected flies, however, did not drop to the levels observed with starved flies ($0{\cdot}10$–$0{\cdot}80\ \log_{10}$ mmol l^{-1} sucrose; $1{\cdot}3$–$6{\cdot}0$ mmol l^{-1} sucrose).

Table 1. *Tarsal thresholds in flies fed 250 mmol l^{-1} sucrose as a single 15 μl meal or* ad libitum *for 4–5 days at specific crop weights*

Crop weight* (mg ± S.E.)	MAT after a single 15-μl meal‡ ($\log_{10}$ mmol l^{-1} sucrose ± S.E.)	MAT after *ad libitum* feeding‡ ($\log_{10}$ mmol l^{-1} sucrose ± S.E.)
$11{\cdot}1 \pm 0{\cdot}2$	$1{\cdot}7 \pm 0{\cdot}02$	$3{\cdot}2$
$9{\cdot}6 \pm 0{\cdot}3$	$2{\cdot}0 \pm 0{\cdot}1$	$3{\cdot}1$
$8{\cdot}2 \pm 0{\cdot}3$	$2{\cdot}1 \pm 0{\cdot}1$	$3{\cdot}0$
$6{\cdot}1 \pm 0{\cdot}3$	$2{\cdot}0 \pm 0{\cdot}2$	$2{\cdot}8$
$2{\cdot}9 \pm 0{\cdot}3$	$1{\cdot}7 \pm 0{\cdot}1$	$2{\cdot}3$

* From Fig. 5.
† From Fig. 4.
‡ Calculated from equation from Fig. 3 for flies fed 250 mmol l^{-1} sucrose *ad libitum*: MAT = $1{\cdot}6 \pm 1{\cdot}5 \log_{10}$(crop weight).

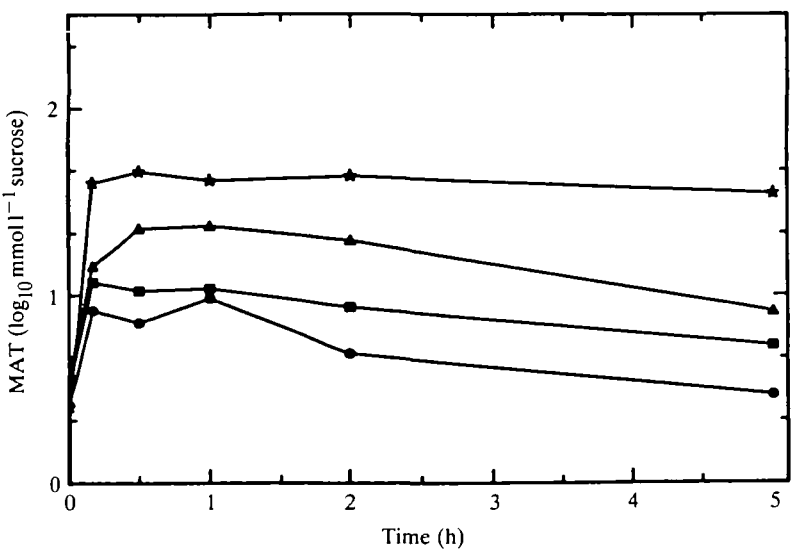

Fig. 6. Average MATs for flies tested before and 0·17, 0·5, 1·0, 2·0 and 5·0 h after consuming a single 2·5-μl meal of sucrose. The concentration fed ranged from 3·96 to 2000 mmol l^{-1} sucrose. Each point is the average of 3–5 replicate experiments, each consisting of 40 flies. Representative S.E. of the mean: $0{\cdot}13\ \log_{10}$ mmol l^{-1} sucrose. ● 3·96 mmol l^{-1} sucrose; ■ 31·2 mmol l^{-1} sucrose; ▲ 250 mmol l^{-1} sucrose; ★ 2000 mmol l^{-1} sucrose.

Experiments were also carried out to examine the effect of MAN and RN transection on threshold in starved, operated flies after consuming a single meal. When MAN-transected flies were tested for threshold prior to the meal, MATs were not different from those of sham-operated flies (Table 3). Transection of the MAN did not prevent the normal rise in threshold response to sucrose after a single 10-μl meal of 250 mmol l^{-1} sucrose. While MAN sham-operated and nerve-transected flies had MATs significantly greater than pre-feeding controls 10 min after the meal, the MATs were not significantly different from each other.

Unlike MAN transection, RN transection markedly attenuated the post-meal rise in MAT (Table 3). At both 10 and 30 min following a 10-μl meal, the MAT was significantly lower in RN-transected flies than in sham-operated ones. Despite this significant lowering, MATs obtained were still significantly higher than pre-feeding MATs at all times tested. When RN-transected flies were fed 15 μl of 250 mmol l^{-1}

Table 2. *Effects of nerve transection on MAT following* ad libitum *feeding on 250 mmol l^{-1} sucrose*

	Median abdominal nerve		Recurrent nerve	
Time after operation (h)	Sham-operated	Nerve-transected	Sham-operated	Nerve-transected
2·0	2·79 ± 0·14	2·81 ± 0·30	2·78 ± 0·18	1·67 ± 0·08*
5·0	2·86 ± 0·13	2·92 ± 0·16	2·46 ± 0·25	1·65 ± 0·16*
8·0	2·72 ± 0·09	2·95 ± 0·20	2·72 ± 0·20	1·71 ± 0·19*

* Significantly different ($P < 0·05$) from sham-operated flies based on paired t-test.

Values reported are average MAT ($\log_{10}$ mmol l^{-1} sucrose) ± S.E. of the mean from at least three replicate experiments.

Table 3. *Effects of nerve transection on MAT following single 10- or 15-μl meals of 250 mmol l^{-1} sucrose*

	Median abdominal nerve		Recurrent nerve			
	10-μl meal		10-μl meal		15-μl meal	
Time after feeding (h)	Sham-operated	Nerve-transected	Sham-operated	Nerve-transected	Sham-operated	Nerve-transected
Pre-feeding	0·93 ± 0·07†	0·68 ± 0·38	0·06 ± 0·25	0·12 ± 0·21	−0·02 ± 0·21	0·08 ± 0·24
0·17	1·74 ± 0·15	1·64 ± 0·38	1·42 ± 0·16	1·06 ± 0·18*	1·65 ± 0·17	1·36 ± 0·18*
0·50	1·79 ± 0·20	1·45 ± 0·39	1·57 ± 0·13	1·06 ± 0·19*	1·98 ± 0·14	1·41 ± 0·19*
1·0	1·91 ± 0·19	1·79 ± 0·26	1·57 ± 0·28	1·38 ± 0·35	1·80 ± 0·33	1·19 ± 0·32*
2·0	1·83 ± 0·14	1·59 ± 0·28	1·52 ± 0·26	1·38 ± 0·29	1·64 ± 0·24	1·24 ± 0·28*
5·0	1·37 ± 0·19	1·51 ± 0·20	1·23 ± 0·27	0·86 ± 0·29	1·32 ± 0·34	1·02 ± 0·25

* Significantly different ($P < 0·05$) from sham-operated flies based on paired t-test.

Values reported are average MAT ($\log_{10}$ mmol l^{-1} sucrose) ± S.E. of the mean from at least three replicate experiments.

† Differences between pre-feeding values for MAN- and RN-transected flies may be due to differences in surgery. For MAN transection, a small wound was made and no saline was required for the operation. For RN transection, a large wound subject to desiccation was made and up to several microlitres of saline had to be added.

sucrose, the MATs were significantly lower than those for sham-operated flies at 0·17, 0·5, 1 and 2 h post-feeding. As with RN-transected, *ad libitum*-fed flies, MATs for RN-transected flies fed a single meal were significantly higher than MATs for unfed flies at all times tested.

Effects of injected glucose on threshold

Haemocoel injection of 100 μg fly^{-1} or 500 μg fly^{-1} of D-glucose into starved flies had no significant effect on MAT when tested 10 and 60 min post-injection. Saline-injected flies had MATs ± S.E. of the mean of 0·30 ± 0·06 and 0·05 ± 0·06 (2·0 and 1·1 mmol l^{-1} sucrose) 10 and 60 min after injection. Flies injected with 100 μg glucose had MATs of 0·28 ± 0·03 and 0·09 ± 0·09 (1·9 and 1·2 mmol l^{-1} sucrose) while flies injected with 500 μg glucose had MATs of 0·53 ± 0·20 and 0·26 ± 0·13 (3·4 and 1·8 mmol l^{-1} sucrose) 10 and 60 min after injection, respectively.

DISCUSSION

These results establish that the recurrent nerve plays an important role in the control of tarsal taste threshold in adult *Phormia regina*. However, the specific nature of its control on threshold remains to be elucidated. One clue to the linkage between tarsal threshold and the recurrent nerve is the observation that the logarithm of crop weight is positively related to tarsal threshold to sucrose after *ad libitum* feeding (see legend of Fig. 3). A similar relationship between crop volume and tarsal taste threshold in *P. regina* has been observed with D-glucose (Barton Browne & Evans, 1960).

The RN carries axons of stretch receptors which monitor contractile activity in the anterior foregut, while the MAN carries axons of stretch receptors which monitor crop volume and crop muscle contractions. The fact that transection of the RN markedly lowers tarsal threshold in flies with fluid in their crops while transection of the MAN is without effect suggests that it is neither crop volume *per se*, nor crop contractions as detected by the MAN-associated stretch receptors, which is crucial for setting tarsal thresholds. It is known that after a meal, as a result of peristaltic and anti-peristaltic contractions along the crop duct, slugs of fluid move to and from the crop. Blood osmolarity in some way determines whether these slugs will enter the midgut or return to the crop. In either case, these movements excite the stretch receptors whose axons run in the RN. It is known that the frequency of crop muscle contractions increases with increasing crop volume (Thomson, 1975*a*). These crop muscle contractions, or simply the crop volume, may determine the frequency at which the crop-duct musculature undergoes reverse peristalsis. As the crop empties, its contraction rate slows, thus decreasing the frequency with which slugs of food pass along the crop duct and excite the foregut receptors. Gelperin (1972) observed an increase in action potential bursts in the RN from the foregut stretch receptors at an unspecified time in flies fed 1·0 mol l^{-1} sucrose compared with 0·1 mol l^{-1} sucrose. This may simply have been due to a greater volume of ingested sucrose remaining in the crop of the 1·0 mol l^{-1} fed fly when the measurement was made, either because

there was no control of volume during feeding or because 1·0 mol l^{-1} sucrose empties from the crop more slowly. Thus, it appears that the logarithmic relationship between tarsal threshold and crop volume in *ad libitum*-fed flies may actually result from crop-volume-dependent peristaltic activity in the crop duct.

Although the RN plays a major role in the regulation of threshold, that role varies with the prior feeding activity of the fly. The changes in threshold after RN transection were consistently and substantially higher in *ad libitum*-fed flies than in those fed single meals. When the thresholds for single-meal-fed and *ad libitum*-fed flies with the same crop volume were compared, thresholds were much higher in the *ad libitum*-fed flies, indicating that it is not just the volume contained by the crop, but also how long it is there that is important in determining tarsal threshold (Table 1). The effect of the RN on tarsal responsiveness to sucrose thus appears to be a function of age or nutritional history. Preliminary observations suggest that age does not play a major role. 10- to 15-day-old flies, previously fed and then starved until their threshold approached that of 3-day-old starved flies, exhibited rises in MAT following a single meal similar to those of 3-day-old starved flies. Nutritional history or feeding experience could affect the attained thresholds in numerous ways. Repeated activity of the foregut stretch receptors as in *ad libitum*-fed flies could alter the degree to which feeding-related interneurones in the CNS are affected by excitatory taste input. The crop and crop duct may also gradually increase their pumping rate due to some other physiological change consequent to feeding. Alternatively, the stretch receptors themselves may be altered by an earlier feeding experience.

Regardless of the mechanisms by which tarsal responsiveness to sugar is diminished by the RN after either mode of feeding, the fact remains that the RN plays less of a role in threshold regulation than might have been predicted from the literature. Inspection of Table 3 reveals that when RN-transected flies were fed either 10 or 15 μl of 250 mmol l^{-1} sucrose, much of the post-ingestion rise in threshold could not be accounted for by the RN. For example, in sham-operated flies fed 15 μl and tested 30 min later, MAT had risen 2·0 $\log_{10}$ units. Recurrent nerve-transected flies subjected to the same conditions exhibited MATs averaging 1·33 $\log_{10}$ units above the pre-feeding level, a significant increase. Dethier & Bodenstein's (1958) observation that sucrose thresholds attained a value of 0·1 mol l^{-1} when RN-transected flies were fed 1 mol l^{-1} sucrose appears to be consistent with our own observations. Although Dethier & Bodenstein evidently believed that they had observed no rise in threshold after feeding their RN-transected flies, a threshold of 0·1 mol l^{-1} is about 10 times higher than the usual threshold reported from Dethier's laboratory during that same period (Hasset, Dethier & Gans, 1950; Arab, 1957). The volume we administered as a single meal also influenced the duration of the effect of the RN on threshold. In RN-transected flies, MAT was significantly lower compared to their sham-operated counterparts for at least 2 h after a 15-μl meal, but no significant difference was observed at times later than 30 min after a 10-μl meal. This indicates that with single meals of less than 10 μl fed to starved flies, the RN plays a minor role in the regulation of tarsal threshold. Our observation that

MATs in RN-transected flies in either feeding regime converged to a similar threshold range ($1{\cdot}2$–$1{\cdot}7\log_{10}$ mmol l^{-1} sucrose, 15–50 mmol l^{-1} sucrose) is relevant. This is the same threshold range where the slope of the curve relating MAT to crop weight changes rapidly (Fig. 3). With increasing crop weight beginning at $1{\cdot}5$ mg, MAT increases gradually from 100 to 1500 mmol l^{-1} sucrose. Threshold falls rapidly, however, for crop weights less than $1{\cdot}5$ mg. At volumes of $1{\cdot}5$ μl and lower it seems unlikely that the RN is stimulated frequently, if at all. Thus, factors other than the RN probably likely play an important role in threshold regulation after small meals or at low crop volumes caused by starvation.

The nature of these other factors involved in threshold regulation is not known. No evidence has been found for an effect of blood glucose on threshold in the present study or of blood glucose or trehalose in previous experiments (Evans & Dethier, 1957; Hudson, 1958). In these glucose injection experiments, blood D-glucose levels would have been elevated well above normal values. This suggests that the non-RN-mediated, post-prandial effects on threshold are not related to absorbed sugar and are not related to activity in the MAN or the RN anterior to the brain. A decrease in locomotor activity following a meal was described by Green (1964). After many attempts to locate the cause of the observed decrease, he concluded that the corpora cardiaca released a factor into the blood after feeding. Hormones have been postulated to play roles in feeding-behaviour modulation in other insects (see Bernays & Simpson, 1982).

Our results are consistent with the following model of the regulation of feeding behaviour in the adult blowfly. Stretch receptors whose axons course in the RN and MAN are both important in the regulation of meal size. However, only the RN plays a role in the rise in threshold following a meal. In addition to the RN, other factors participate in the regulation of post-prandial tarsal taste responses. None of these other factors appears to be mediated by absorbed sugar; otherwise their nature remains to be determined.

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