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REGULATION OF LABELLAR AND TARSAL TASTE THRESHOLDS IN THE BLACK BLOWFLY, *PHORMIA REGINA*

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SUMMARY

Regulation of taste thresholds in the blowfly, *Phormia regina* Meigen, was investigated by manipulating the nutritional status of the insect and determining the effect on labellar and tarsal taste thresholds. Two feeding paradigms were employed: single meals administered to hungry flies, and *ad libitum* feeding of aqueous sucrose solutions for 4–5 days.

Flies which had fed on aqueous sucrose for several days exhibited high labellar and tarsal thresholds, with tarsal thresholds always higher than labellar thresholds. Food deprivation caused labellar and tarsal thresholds to decline. There was a positive logarithmic relationship between crop mass and labellar or tarsal thresholds. Consumption of single meals of aqueous sucrose caused increases in labellar thresholds similar to those for tarsal thresholds.

Labellar thresholds in flies fed *ad libitum* were not affected by transection of the recurrent nerve (RN) or the median abdominal nerve (MAN). Tarsal thresholds, in contrast, were markedly attenuated by RN transection but not by MAN transection. Transection of the RN in hungry flies prior to feeding them a single meal did not prevent the normal post-prandial rise in labellar thresholds. In earlier experiments, RN transection has been shown to attenuate the post-prandial rise in tarsal thresholds.

It appears that labellar and tarsal thresholds in the blowfly are similarly affected by consumption of aqueous sucrose. However, inhibitory feedback from the RN, which is partially responsible for elevation of tarsal thresholds after a meal, does not affect labellar thresholds. Additional, still unidentified, factor(s) may regulate both labellar and tarsal thresholds.

INTRODUCTION

Adult black blowflies *Phormia regina* first detect food by means of contact chemoreceptors in the tips of their tarsal hairs (Wolbarsht & Dethier, 1958; McCutchan, 1969; Shiraishi & Tanabe, 1974). When these hairs contact an appropriate substrate, such as aqueous sucrose, a hungry fly will evert its proboscis. Hungry flies respond to millimolar concentrations of sucrose, while replete flies

Key words: *Phormia regina*, blowfly, feeding behaviour, recurrent nerve, labellar taste threshold, tarsal taste threshold.

respond only to much higher concentrations. Proboscis extension thus serves as a measure of the readiness of flies to feed.

Extension of the proboscis brings a second group of chemosensory hairs, located on the aboral surface of the labellum, into contact with the substrate (Arab, 1957; Wilczek, 1967). These chemoreceptors mediate the spreading of the labellar lobes (Pollack, 1977). If the substrate is sufficiently stimulating, the fly spreads its labellar lobes, bringing a third set of chemoreceptors on the oral surface of the labellum into contact with the substrate. These chemoreceptors, the interpseudotracheal papillae, regulate the initiation of drinking (Falk, 1975). Once drinking starts, food is pumped into the crop, a thin-walled collapsible diverticulum of the foregut (Knight, 1962). Subsequent peristaltic contractions of the crop and crop duct force slugs of fluid out of the crop and into the foregut. Depending on the rate of absorption of the meal from the midgut, a slug of food in the crop duct will either pass through the proventricular valve into the midgut or be pumped back into the crop by reverse peristalsis (Thomson, 1975).

Flies will also evert their proboscises if their labellar chemosensory hairs are stimulated with aqueous sucrose. Little information is available about how labellar taste thresholds change after consumption of a meal of sucrose. Getting & Steinhardt (1972) presented behavioural evidence that labellar thresholds do not change after consumption of a single meal of sucrose, and Minnich (1931) reported that labellar thresholds in *Calliphora* do not drop with food deprivation. In other studies, however, labellar thresholds have been shown to rise after *ad libitum* consumption (Shiraishi & Yano, 1984; Kawabata & Shiraishi, 1977; Bowdan & Dethier, 1986) and fall during food deprivation (Bowdan & Dethier, 1986). In the present study, we have examined the effects on labellar thresholds in two different feeding paradigms, namely, long-term feeding with 62·5 or 250 mmol l^{-1} sucrose, and single-meal feedings using 3·7 or 15 μl of 250 mmol l^{-1} sucrose.

As the crop fills during feeding, two sets of stretch receptors begin to fire. The first set is associated with the foregut wall (Gelperin, 1967, 1972). The projections of these neurones run in the recurrent nerve (RN) and terminate in the anterodorsal neuropile of the suboesophageal ganglion (Edgecomb, 1986). A second set of stretch receptors is located in the first and second lateral branches of the median abdominal nerve (MAN) (Gelperin, 1971). These MAN stretch receptors fire in proportion to the amount of tension placed on the abdominal nerves by the enlarged crop. Transection of the RN (Dethier & Bodenstein, 1958; Evans & Barton Browne, 1960; Dethier & Gelperin, 1967) or of either the MAN (Gelperin, 1971) or the ventral nerve cord (Dethier & Gelperin, 1967; Nuñez, 1964) causes hyperphagia. Tarsal thresholds are markedly lowered by RN transection in *ad libitum*-fed flies but MAN transection did not affect tarsal thresholds (Edgecomb, Murdock, Smith & Stephen, 1987). There is little information available about the possible roles of information carried in the RN or MAN on labellar proboscis extension thresholds. In experiments examining the effects of nerve transection on labellar lobe spreading, Pollack (1977) found that transection of either the RN or the MAN attenuated the post-prandial decrease in responsiveness of lobe spreading. In a brief report,

Shiraishi & Yano (1984) stated that RN transection did not affect labellar thresholds, but provided no data to support their assertion. To establish if either nerve is involved in the regulation of labellar thresholds, we performed RN or MAN transections in flies that had been fed for 4–5 days and measured labellar and tarsal thresholds at various times after surgery. In a second set of experiments, we transected the RN in hungry flies and fed them a single meal of sucrose to determine if the RN affects labellar thresholds shortly after a single meal.

MATERIALS AND METHODS

Flies

Black blowflies *Phormia regina* were reared on beef liver. Adult flies were held in hardware cloth cages (12 cm × 30 cm) covered with cotton tube gauze and provided with water *ad libitum*. They were maintained at 65 % relative humidity (RH) on a 16 h:8 h light:dark cycle, at 25 ± 1 °C during photophase and 21 ± 1 °C during scotophase. Both male and female flies were used indiscriminately. In single-meal experiments, flies were starved for 3 days after eclosion. In long-term feeding studies, 3-day-old starved flies were fed either 62·5 or 250 mmol l^{-1} sucrose *ad libitum* for 4–5 days. In preparation for all experiments, flies were immobilized by cooling them for a few minutes on ice. Each fly was then fixed to an applicator stick held perpendicular to the dorsum of the thorax using a melted beeswax/rosin mixture (3/2) (Long & Murdock, 1983). Mounted flies were held for at least 1 h prior to testing in glass-covered aquaria at 80% RH to allow the effects of cooling and handling to dissipate. The fly saline contained (in mmol l^{-1}) Na^+, 122; Cl^-, 127; K^+, 5·6; Ca^{2+}, 2·4; Mg^{2+}, 1·0; phosphate buffer, 5 (pH 6·8).

Threshold determination

In the present work, the measure of feeding threshold was the mean acceptance threshold (MAT), the $\log_{10}$ of the minimum sucrose concentration to which an average fly in a given population will respond with proboscis extension upon labellar or tarsal contact. An up-and-down bioassay was employed to determine labellar and tarsal MATs (Sudlow, 1985). Because this assay has been described elsewhere for tarsal threshold determinations (Edgecomb *et al.* 1987), no further details will be given here.

When labellar thresholds were to be determined, the stick to which each fly was fixed was clamped so that the fly was positioned upright, with its tarsi in contact with the bottom of a clean, inverted beaker. A water droplet was touched to several (at least four) of the largest labellar hairs by means of a small micro-inoculation loop (o.d. = 1 mm). As in previous studies (Edgecomb *et al.* 1987), a proboscis extension was defined as an excursion of the proboscis reaching at least position 3 as described by Dethier, Solomon & Turner (1965). Approximately 80 % of the proboscis extensions observed after labellar stimulation surpassed position 3. As with the tarsal MAT determinations, if a fly responded to the water pre-test with proboscis extension, it was allowed to drink but was not used again in that threshold

determination. After the water pre-test, the micro-inoculation loop was washed in distilled water and dried. The loop was then dipped into the appropriate sucrose solution and touched to the labellar hairs and the fly observed for proboscis extension.

Unless specified otherwise, the MATs presented in the text are averages for at least three replicate experiments using different generations of flies. Analyses of variance with Duncan's multiple range tests were performed on the MATs and crop masses (Statistical Analysis System, Cary, NC). Regressions were fitted by the least-squares method (Statistical Analysis System, Cary, NC). Regression equations and regression coefficients were compared by analysis of covariance and analysis of variance (ANOVA) techniques, respectively (Steel & Torrie, 1980).

Single-meal feeding

Two groups of flies were mounted for each feeding experiment, one for tarsal and the other for labellar threshold determinations. Sticks with attached flies were pressed into Plasticene stands and the flies were positioned upright over paraffin-coated microscope slides. Droplets of 250 mmol l^{-1} sucrose were placed on the microscope slide under each fly using a Pipetman (P20, Rainin, Woburn, MA) calibrated to deliver 3·7 or 15 μl of distilled water. At 15-s intervals (for tarsal thresholds) or 20-s intervals (for labellar thresholds) flies were lowered to make contact with the sucrose droplet, thus initiating feeding. Each fly was allowed 2 min to complete its meal. Any fly that failed to consume all of its sucrose droplet was discarded. Flies were tested for proboscis extension in the same order as they were fed. Labellar and tarsal thresholds were determined 0·17, 0·5, 1, 2, 3, 4 and 6 h after completion of feeding.

Long-term feeding

For the long-term feeding studies, caged 3-day-old starved flies were allowed access to one of two sucrose solutions (62·5 or 250 mmol l^{-1}) *ad libitum* for 4–5 days. The sucrose solutions were replaced every 48 h. At the end of the feeding period, the flies were transferred to a clean cage and given fresh water. Groups of flies were withdrawn from the cage and mounted for testing after various periods of food deprivation. Labellar and tarsal MATs and crop masses were measured at specified times after food removal. Crops were dissected and weighed to the nearest 0·1 mg.

Nerve transection

RN and MAN transections and sham operations were performed as described earlier (Edgecomb *et al.* 1987).

RESULTS

Long-term feeding

When flies had been fed 250 mmol l^{-1} sucrose *ad libitum* for several days and then removed from food, labellar and tarsal MATs were highest shortly (1 h) after food

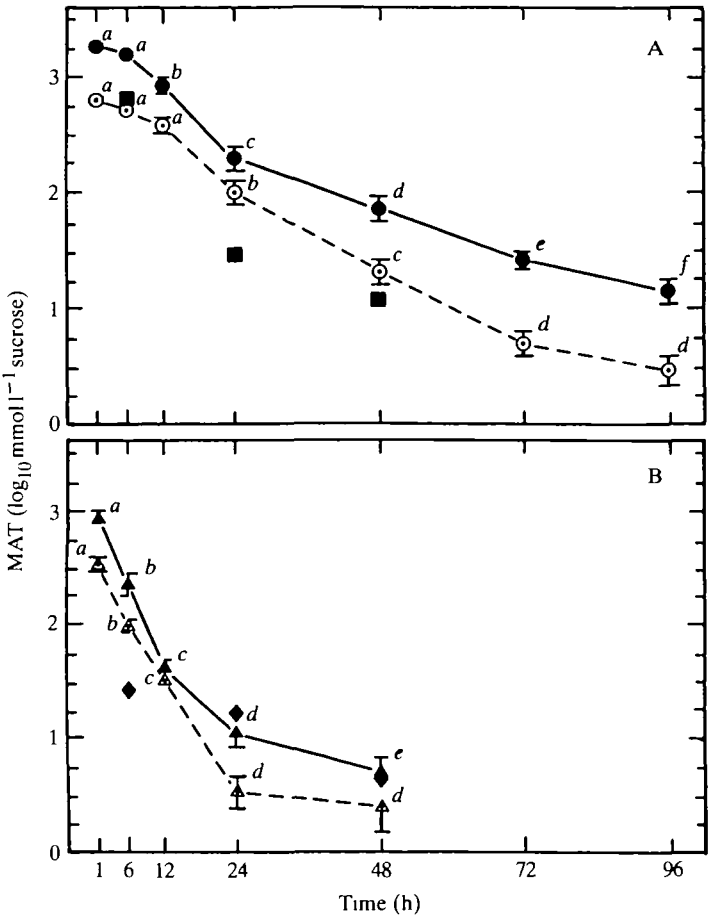

Fig. 1. Tarsal and labellar mean acceptance thresholds (MATs) in blowflies fed *ad libitum* for 4–5 days on 62·5 or 250 mmol l^{-1} sucrose. Each point represents the mean MAT ± S.E. of 5–9 determinations, each consisting of 40 flies. (A) 250 mmol l^{-1} sucrose; ●——●, tarsal MAT; ○---○, labellar MAT. (B) 62·5 mmol l^{-1} sucrose; ▲——▲, tarsal MAT; △---△, labellar MAT. Points followed by the same letters within each experimental group are not significantly different ($P > 0{\cdot}05$, ANOVA with Duncan's multiple range test). Data from Bowdan & Dethier (1986) for labellar MATs (◆) and tarsal MATs (■) at 6, 24 and 48 h of starvation are included for comparison, see Discussion.

deprivation began; MATs were 2·80 and 3·26 log units, respectively (Fig. 1A). Labellar MATs did not change significantly during the first 12 h of deprivation. Tarsal MATs were unchanged over the first 6 h of deprivation. Thereafter, labellar and tarsal thresholds fell as food deprivation continued. Tarsal MATs were significantly higher than labellar MATs ($P < 0{\cdot}05$, ANOVA) at all times except at 24 h of food deprivation.

As with flies fed 250 mmol l^{-1} sucrose *ad libitum*, labellar and tarsal MATs were also elevated in flies fed 62·5 mmol l^{-1} sucrose *ad libitum* for 4–5 days. Following removal of the 62·5 mmol l^{-1} sucrose, tarsal MATs were significantly higher than

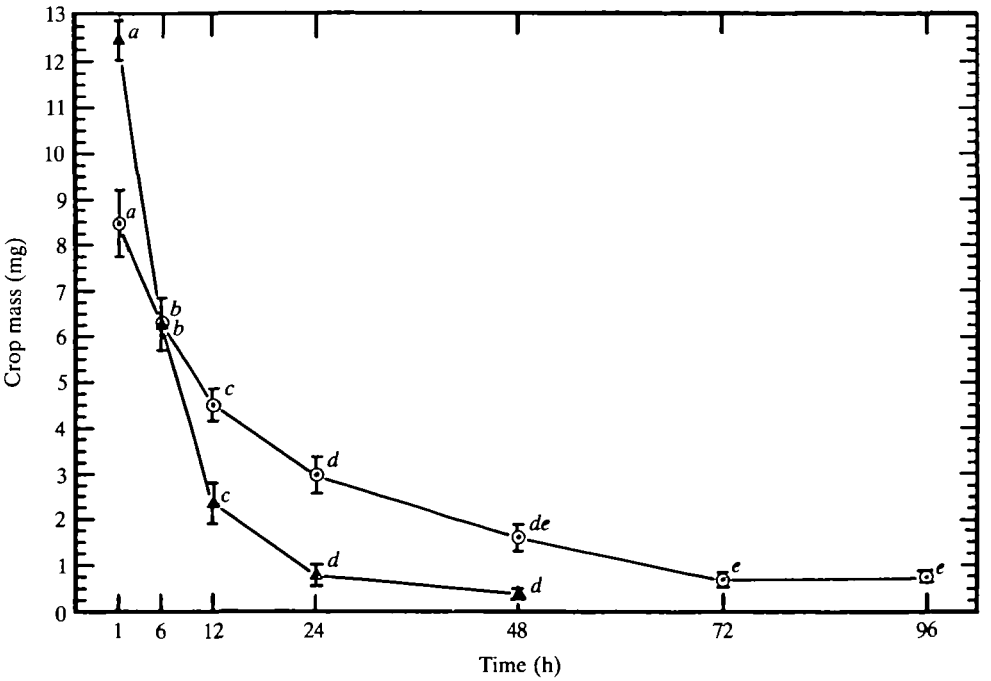

Fig. 2. Crop masses in blowflies after *ad libitum* feeding for 4–5 days on 62·5 (△——△) or 250 mmol l^{-1} (○——○) sucrose. Each point represents the mean ±S.E. of 5–9 average crop masses of 15 individuals. Points followed by the same letters within each experimental group are not significantly different ($P > 0{\cdot}05$, ANOVA with Duncan's multiple range test).

labellar MATs ($P < 0{\cdot}05$, Duncan's test; Fig. 1B), except at 12 and 48 h. Labellar and tarsal MATs had returned to near pre-feeding levels by 24 h after removal from the 62·5 mmol l^{-1} sucrose.

Using the data obtained with both concentrations of sucrose over the first 48 h of food deprivation, an ANOVA was performed based on a 2×2×5 factorial design (labellar/tarsal×concentration×time). When the regressions for labellar or tarsal thresholds *vs* time for the two concentrations of sucrose were compared, no significant difference was found among the linear and quadratic components of these four equations ($P > 0{\cdot}05$, $F_{4,40} = 2{\cdot}519$). The labellar and tarsal MATs were higher for flies fed 250 mmol l^{-1} sucrose than for flies fed 62·5 mmol l^{-1} sucrose ($P < 0{\cdot}0032$, $F_{1,40} = 9{\cdot}85$). The labellar and tarsal thresholds for each concentration were pooled and the resulting regressions of threshold *vs* time were compared. The regression coefficients of the combined labellar and tarsal MATs of flies fed 250 mmol l^{-1} sucrose were significantly different from those of flies fed 62·5 mmol l^{-1} sucrose ($P < 0{\cdot}0001$, $F_{4,40} = 9{\cdot}99$).

Crop masses in flies fed either 62·5 or 250 mmol l^{-1} sucrose *ad libitum* were highest 1 h following removal of food. Subsequently, crop masses declined logarithmically (Fig. 2). One hour after food removal crop masses were significantly higher in flies fed 62·5 mmol l^{-1} sucrose than in flies fed 250 mmol l^{-1} sucrose ($P < 0{\cdot}05$,

Duncan's test). Crops emptied more quickly in flies fed 62·5 mmol l^{-1} sucrose, however, and their masses reached pre-feeding levels by 24 h. Flies fed 250 mmol l^{-1} sucrose exhibited a slower loss of crop mass; crop masses reached pre-feeding levels after 72 h.

As demonstrated recently, tarsal MATs are positively correlated with the logarithm of the crop mass (Edgecomb *et al.* 1987). A similar relationship was found when labellar MATs were plotted against the logarithm$_{10}$ of the crop masses. The resulting regression equations and regression lines are presented in Fig. 3. There was no significant difference between the slopes of the regression lines for labellar MATs *vs* the $\log_{10}$ crop mass and for tarsal MATs *vs* the $\log_{10}$ crop mass in flies fed 250 mmol l^{-1} sucrose ($P > 0{\cdot}05$, $F_{1,104} = 2{\cdot}49$). Similarly, there was no significant difference between the slopes of labellar and tarsal MATs *vs* the $\log_{10}$ crop mass in flies fed 62·5 mmol l^{-1} sucrose ($P > 0{\cdot}05$, $F_{1,34} = 0{\cdot}05$). The slope of the regression line for labellar MATs *vs* $\log_{10}$ crop mass in flies fed 62·5 mmol l^{-1} sucrose was significantly different from the slope of the regression-line for labellar MATs *vs* $\log_{10}$ crop mass in flies fed 250 mmol l^{-1} sucrose ($P < 0{\cdot}0005$, $F_{1,68} = 33{\cdot}7$). The slope of the regression line for tarsal MATs *vs* $\log_{10}$ crop mass from flies fed 62·5 mmol l^{-1}

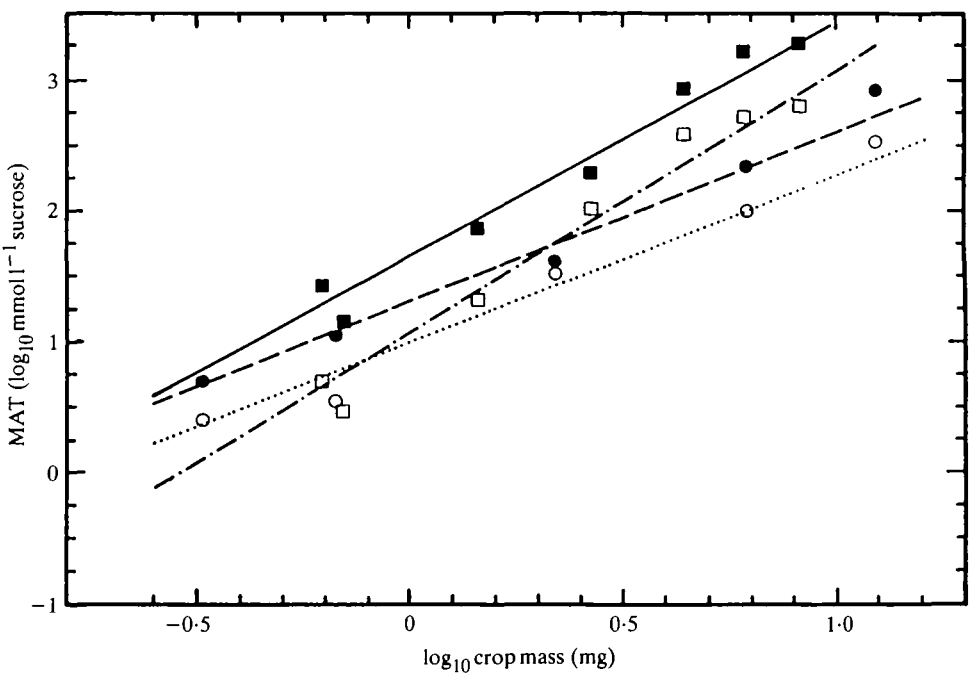

Fig. 3. Mean tarsal and labellar mean acceptance thresholds (MAT) *vs* mean log crop mass at each time observed for flies starved for various periods after *ad libitum* feeding for 4–5 days on 62·5 or 250 mmol l^{-1} sucrose. Although average points are plotted, regressions were fitted and drawn using scatter data. Equations for each line: ■——■, tarsal MAT, 250 mmol l^{-1}, MAT = 1·65+1·79 × (log crop mass), $r^2 = 0{\cdot}893$; □–·–□, labellar MAT, 250 mmol l^{-1}, MAT = 1·08+2·00 × (log crop mass), $r^2 = 0{\cdot}887$; ●– – –●, tarsal MAT, 62·5 mmol l^{-1}, MAT = 1·31+1·31 × (log crop mass), $r^2 = 0{\cdot}882$; ○·····○, labellar MAT, 62·5 mmol l^{-1}, MAT = 0·99+1·29 × (log crop mass), $r^2 = 0{\cdot}858$.

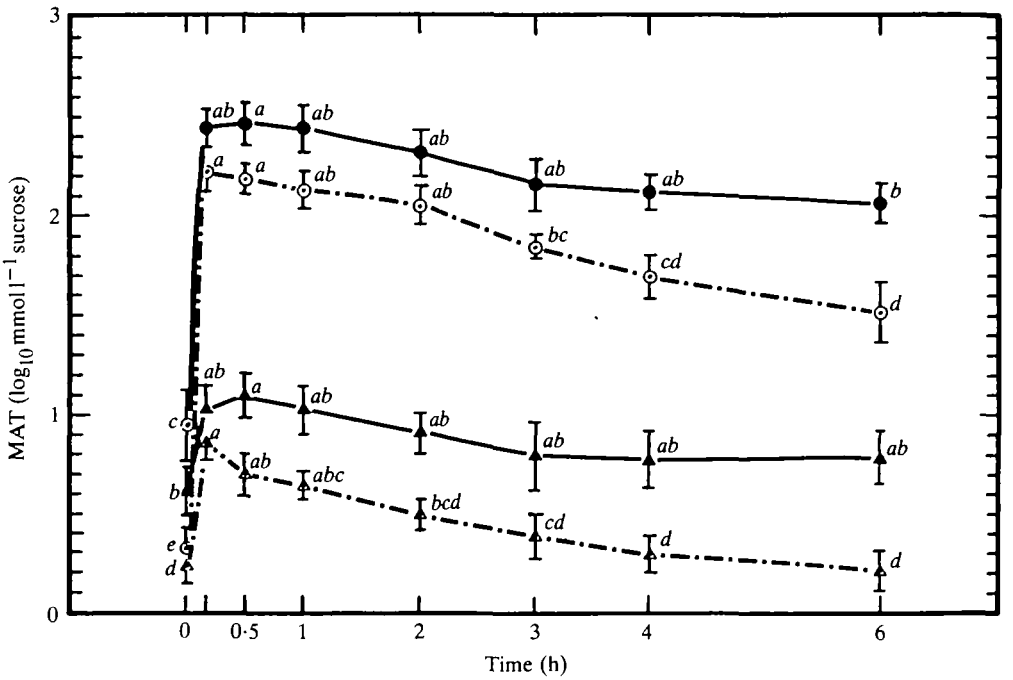

Fig. 4. Tarsal and labellar mean acceptance thresholds (MATs) for flies tested before and 0·17, 0·5, 1, 2, 3, 4 and 6 h after feeding hungry flies a single meal of either 3·7 or 15 μl of 250 mmol l^{-1} sucrose. Each point is the mean MAT ± S.E. of five determinations, each consisting of 40 flies. Symbols represent the type of MAT determination and the volume fed. ●——●, tarsal MAT, 15 μl; ○–·–○, labellar MAT, 15 μl; ▲——▲, tarsal MAT, 3·7 μl; △–·–△, labellar MAT, 3·7 μl. Points within each experimental group followed by the same letters are not significantly different ($P > 0·05$, ANOVA with Duncan's multiple range test).

sucrose was also significantly different from that for flies fed 250 mmol l^{-1} sucrose ($P < 0·0005$, $F_{1,68} = 42·2$).

Single-meal feeding

When a single meal of 15 μl of 250 mmol l^{-1} sucrose was administered to 3-day-old hungry flies, there was a marked elevation in both labellar and tarsal thresholds over the 6-h time course of the experiment (Fig. 4). Tarsal thresholds were significantly higher than labellar thresholds only at 4 and 6 h ($P < 0·05$, Duncan's test). Labellar MATs were highest 10 min after feeding, and declined at approximately the same rate as did tarsal MATs. The decline of labellar MATs after a single meal was best approximated by the equation: MAT = 2·25−0·127h, $r^2 = 0·5899$. Tarsal thresholds reached their highest value 30 min after completion of the meal and declined only slightly over the ensuing 6-h period. The decline of tarsal MATs over time (10 min to 6 h post-feeding) was best approximated by the equation: MAT = 2·469−0·077h; $r^2 = 0·3138$. The slopes of the labellar and tarsal MAT equations were not significantly different ($P > 0·05$, $F_{1,66} = 3·42$).

A single meal of 3·7 μl of 250 mmol l^{-1} sucrose caused only small rises in labellar and tarsal thresholds (Fig. 4). Tarsal thresholds were significantly higher than labellar thresholds at all times except 10 min and 3 h ($P < 0{\cdot}05$, Duncan's test). Labellar MATs were significantly different from pre-feeding levels only during the first hour after feeding and were highest at 10 min ($P < 0{\cdot}05$, Duncan's test). Tarsal MATs were significantly different from pre-feeding levels only at 30 min after feeding.

To determine if fly age and previous nutritional history affected labellar and tarsal thresholds following a single meal, hungry 3-day-old flies were allowed access to 62·5 mmol l^{-1} sucrose for 5 days. They were then starved for 48 h, after which each fly was fed a single meal of 15 μl of 250 mmol l^{-1} sucrose (fed–starved–refed flies). Labellar thresholds of fed–starved–refed flies (Fig. 5B) rose in the same way as they did in 3-day-old flies fed a single meal of the same concentration and volume. However, the labellar MATs of the fed–starved–refed flies fell at a slightly different rate from that of the 3-day-old flies. In both the 3-day-old flies and the fed–starved–refed flies, the maximal labellar MAT occurred 10 min after feeding (Fig. 5B). The decline of labellar MATs over time for the fed–starved–refed flies was best approximated by the equation: MAT = 1·95−0·114h; $r^2 = 0{\cdot}7418$; compare this with the equation for 3-day-old flies: MAT = 2·25−0·127h (Fig. 4). These slopes were significantly different ($P < 0{\cdot}05$, $F_{1,48} = 6{\cdot}13$). Tarsal thresholds in the fed–starved–refed flies after the 15 μl meal of sucrose (Fig. 5A) rose as they did in 3-day-old, previously unfed flies. Unlike the result with 3-day-old flies, there was no significant decline in the tarsal MATs for fed–starved–refed flies.

Effects of nerve transections on labellar and tarsal thresholds

Surgical transection of the MAN in long-term-fed flies had no significant effect on either tarsal or labellar thresholds in flies tested 2 or 5 h after surgery (Table 1). In contrast (Table 2), transection of the RN caused a drop of 1 log unit in tarsal MATs, but left labellar thresholds unchanged. After RN transection, tarsal thresholds did not return to the level of 0·6–0·9 log units seen in hungry flies, but remained more than 1 log unit above that level.

Table 1. *Effects of median abdominal nerve transection on tarsal and labellar mean acceptance thresholds (MAT) in flies fed 250 mmol l^{-1} sucrose for 4–5 days*

Time after operation (h)	Tarsal MAT		Labellar MAT	
	Sham-operated	MAN-transected	Sham-operated	MAN-transected
2	3·08 ± 0·05	2·85 ± 0·13	2·61 ± 0·13	2·68 ± 0·03
5	3·25 ± 0·04	3·01 ± 0·10	2·55 ± 0·09	2·61 ± 0·07

Values are the mean MAT ± S.E. of three replicate experiments, each consisting of 40 flies.
No sham-operated MAT was significantly different from its respective MAN-transected MAT at either time point ($P > 0{\cdot}05$, ANOVA).

Our earlier studies indicated that RN transection reduces, but does not eliminate, the rise of tarsal threshold in starved flies fed single meals of sucrose (Edgecomb *et al.* 1987). MAN transection, however, had no effect on tarsal threshold rises after single meals. In the light of this, we asked whether RN transection would affect the

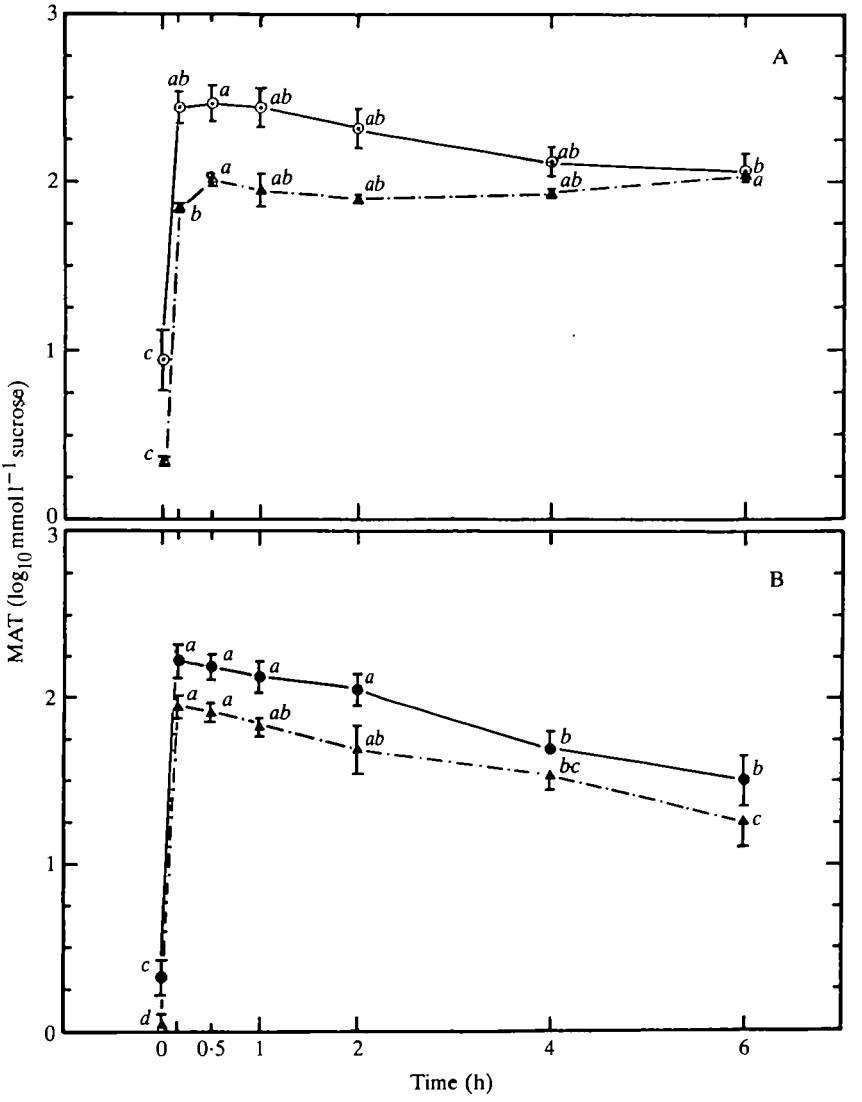

Fig. 5. Tarsal and labellar mean acceptance thresholds (MATs) for flies fed a single 15 μl meal after 48 h of starvation following 5 days of *ad libitum* feeding on 62·5 mmol l^{-1} sucrose. Each point is the mean ± S.E. of three MAT determinations, each consisting of 40 flies. (A) Tarsal MATs. ○——○, 3-day-old starved flies (data from Fig. 4). △–·–△, fed–starved–refed flies. (B) Labellar MATs. ●——●, 3-day-old starved flies (data from Fig. 4); ▲–·–▲, fed–starved–refed flies. Points followed by the same letters within each experimental group are not significantly different ($P > 0{\cdot}05$, ANOVA with Duncan's multiple range test).

Table 2. *Effects of recurrent nerve transection on tarsal and labellar mean acceptance thresholds (MATs) following* ad libitum *feeding on 250 mmol l^{-1} sucrose*

Time after operation (h)	Tarsal MAT		Labellar MAT	
	Sham-operated	RN-transected	Sham-operated	RN-transected
2	3·09 ± 0·11	2·01 ± 0·04*	2·56 ± 0·04	2·60 ± 0·12
5	3·04 ± 0·16	2·14 ± 0·13**	2·61 ± 0·14	2·59 ± 0·27

Values are the mean MAT ± S.E. of three replicate experiments, each consisting of 40 flies.
* RN-transected MAT is significantly different from the MAT of the paired sham-operated flies ($P < 0·008$, ANOVA).
** RN-transected MAT is significantly different from the MAT of the paired sham-operated flies ($P < 0·012$, ANOVA).

rises in labellar thresholds seen following a single meal of sucrose. Accordingly, RN transections and sham operations were performed on 3-day-old starved flies. The flies were then given a 15 μl meal of 250 mmol l^{-1} sucrose. Labellar MATs were measured before, 10 min, 30 min, and 1, 2, 4 and 6 h after the meal. As a check of the effectiveness of the surgery, tarsal MATs were determined before and 3 h after feeding. Labellar MATs of both RN-transected and sham-operated flies were maximal 10–30 min after the meal and declined thereafter (Fig. 6). There was no significant difference in labellar thresholds between RN-transected and sham-operated flies at any time after feeding. The thresholds for sham-operated and RN-transected flies fell at similar rates after the meal ($P > 0·05$, $F_{1,32} = 0·24$). The decline in labellar MATs with time for sham-operated flies was best approximated by the equation: $MAT = 2·145 - 0·120h$, $r^2 = 0·447$, and for RN-transected flies the equation was: $MAT = 1·945 - 0·144h$; $r^2 = 0·4945$. As expected, tarsal MATs of RN-transected flies 3 h after feeding were significantly lower than the tarsal MATs of the respective sham-operated flies (Table 3), in agreement with our earlier study.

DISCUSSION

Our results show that long-term feeding of aqueous sucrose to *P. regina* leads to elevated labellar taste thresholds and to elevated tarsal taste thresholds. Further, they demonstrate that both labellar and tarsal thresholds are higher when the concentration of aqueous sucrose fed is higher (cf. Fig. 1A and 1B). These results are in general agreement with the observations of Kawabata & Shiraishi (1977), Shiraishi & Yano (1984) and Bowdan & Dethier (1986). Our experiments with hungry flies fed single meals of aqueous sucrose provide complementary evidence that (i) labellar thresholds rise after single meals, (ii) the threshold rise occurs within minutes of the meal, and (iii) the rise is greater when the meal volume is greater. Thus, not only the concentration of the meals in long-term feeding experiments, but also the volume of a single meal, can cause proportional rises in labellar threshold.

After single meals (Fig. 4), or following the removal of food in *ad libitum* feeding experiments (Fig. 1A,B), labellar and tarsal thresholds declined following the initial

rise. The rates at which thresholds declined in *ad libitum*-fed flies subjected to food deprivation depended upon the concentration of aqueous sucrose they had been fed. Higher sucrose concentrations led to slower rates of decline. In a similar study, Bowdan & Dethier (1986) fed flies *ad libitum* on 100 mmol l^{-1} sucrose, removed the food, and observed drops in threshold as food deprivation continued. They

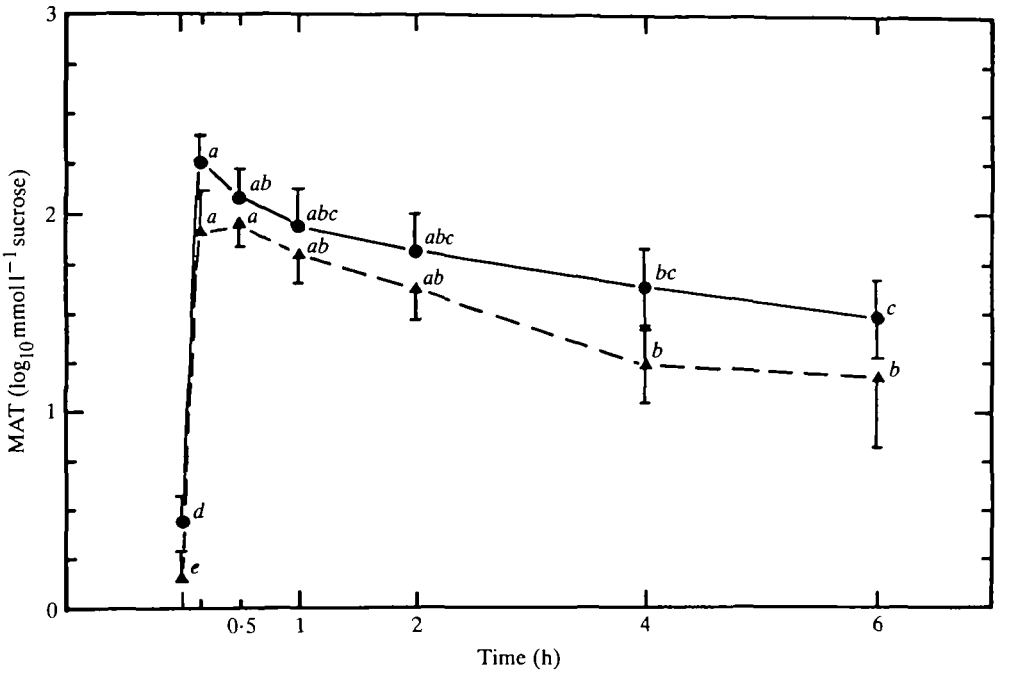

Fig. 6. Effect of a single meal of 15 μl of 250 mmol l^{-1} sucrose on labellar mean acceptance thresholds (MATs) following recurrent nerve (RN) transection in starved flies. Each point is the mean ± S.E. of MAT determinations of three replicate experiments, each consisting of 40 flies. Flies were tested before, 0·17, 0·5, 1, 2, 4 and 6 h after feeding. ●——●, labellar MATs of sham-operated flies; △---△, labellar MATs of RN-transected flies. There was no significant difference between the RN-transected and sham-operated labellar MATs at any time. Data points followed by the same letters within each experimental group are not significantly different ($P > 0·05$, ANOVA with Duncan's multiple range test).

Table 3. *Effects of recurrent nerve transection on tarsal mean acceptance thresholds (MATs) in starved flies prior to and after consuming a single meal of sucrose*

Time after feeding (h)	Tarsal MAT*	
	Sham-operated	RN-transected
Pre-test	0·48 ± 0·09[a]	0·54 ± 0·13[a]
3	1·69 ± 0·08[a]	0·84 ± 0·04[b]

Values are the mean MAT ± S.E. of three replicate experiments.
*Mean MATs followed by the same superscript in a given row are not significantly different ($P > 0·05$, ANOVA, Duncan's test).

estimated tarsal thresholds 6, 24 and 48 h after food removal and noted that 'tarsal thresholds of 24-h hungry flies were substantially lower than those of 6-h deprived flies. Labellar thresholds also dropped, but by smaller amounts. Both tarsal and labellar thresholds of 48-h deprived flies were lower than they were for 6- and 24-h deprived flies'. The relevant data from Bowdan & Dethier (1986) are plotted in Fig. 1A,B to facilitate comparison with our results. In discussing their observations, Bowdan & Dethier stated that 'tarsal and labellar thresholds do not change in the same way as the duration of deprivation increases'. It should be noted that Bowdan & Dethier (1986) based this statement on studies in which flies were fed only one concentration of sucrose (100 mmol l^{-1}) and tested at only three times subsequent to food removal. Our results (Fig. 1A,B), which involved feeding groups of flies either of two concentrations of sucrose (62·5 or 250 mmol l^{-1} *ad libitum*) and testing them at seven (250 mmol l^{-1}) or five (62·5 mmol l^{-1}) times after food deprivation began, strongly suggest the opposite conclusion: that tarsal and labellar thresholds decline in much the same way during food deprivation. Consistent with this is our observation that the rates of decline in labellar and tarsal thresholds were not significantly different after single 15 μl meals of 250 mmol l^{-1} sucrose.

During the first few hours of food deprivation, labellar and tarsal thresholds in long-term-fed flies (which were 8–12 days old at the time of testing, and which had been fed 250 mmol l^{-1} sucrose for 4–5 days preceding removal of food) were always higher than they were in 3-day-old hungry flies fed single 15 μl meals of 250 mmol l^{-1} sucrose. This difference in attained threshold levels might have been due to comparing younger, unfed flies with older ones having prior feeding experience. The possible role of age and feeding history was examined by comparing the rise in threshold after a single meal of 250 mmol l^{-1} sucrose administered to 3-day-old unfed flies with the rise in flies that had been starved for the first 3 days of adult life, subsequently fed 62·5 mmol l^{-1} sucrose *ad libitum* for 5 days, then starved for 2 days before the meal was administered. If age or previous feeding experience were responsible for the difference in thresholds observed in the single-meal and long-term-feeding experiments, then the threshold attained after a single meal administered to fed–starved–refed flies should approach that of the long-term-fed flies. Flies were fed 15 μl of 250 mmol l^{-1} sucrose, which increased the crop mass to 9·6 mg at 30 min after the end of the meal (Edgecomb *et al.* 1987). Using the crop mass and the regression equation relating crop mass to MAT for flies fed *ad libitum* on 250 mmol l^{-1} sucrose (cf. Fig. 5A and 5B), we calculated the labellar and tarsal MAT values expected at this crop mass to be 3·04 and 3·41 log units, respectively. The actual measured MATs in the fed–starved–refed flies were 1·91 and 2·01 log units for labellar and tarsal thresholds, respectively. The threshold elevations in fed–starved–refed flies were thus very similar to those in 3-day-old hungry flies given the identical meal and tested after the same time interval. Clearly, previous feeding history or age in hungry flies did not affect the immediate post-prandial rises in labellar and tarsal thresholds. The discrepancy between MATs observed in hungry flies fed single 15 μl meals *vs* MATs in long-term-fed flies (both groups having similar crop masses) indicates that thresholds in long-term-fed flies are influenced by factors

not present in flies fed a single meal. In other words, crop volume alone does not determine thresholds. The nature of the unknown factor(s) remains to be determined. The rates of decline differed slightly for labellar thresholds and markedly for tarsal thresholds in the previously-fed *vs* the 3-day-old starved flies. The basis for these differences is not known.

One possible mechanism to explain the post-prandial rise in labellar threshold would be a reduction in the sensitivity of sensory receptors as a result of feeding. Such a phenomenon has been observed in the chemosensilla of locust maxillary palpi (Bernays, Blaney & Chapman, 1972). With the exception of Omand (1971) and Omand & Zabara (1981), however, evidence indicates that the sensitivity of fly labellar chemosensory hairs does not change with age, nutritional history or nutritional status (Shiraishi & Yano, 1984; Kawabata & Shiraishi, 1977; Rachman, 1979; Getting & Steinhardt, 1972; Bowdan & Dethier, 1986; Hall, 1980).

Numerous studies have provided evidence that stretch receptors associated with the anterior foregut and with the crop in the abdomen are vital sources of information serving to limit meal size (Dethier & Bodenstein, 1958; Dethier & Gelperin, 1967; Gelperin, 1971). Axons of stretch receptors running in the recurrent nerve carry information about peristaltic activity in the foregut. Axons of stretch receptors running in the median abdominal nerve monitor crop volume and crop contractions. Surgical transection of either of these nerves causes hyperphagia. The volume of the crop and the degree of peristaltic and antiperistaltic activity in the crop duct appear to be key factors in determining meal size. In view of the similarities in changes in labellar and tarsal thresholds after meals and during deprivation, we re-examined crop size as a function of food deprivation after long-term *ad libitum* feeding. In good agreement with our earlier study (Edgecomb *et al.* 1987), we observed that crop masses declined with deprivation and that they declined faster in flies fed more dilute sucrose (see Fig. 2). There was a positive logarithmic relationship between crop mass and labellar MATs when flies were fed either 62·5 or 250 mmol l^{-1} sucrose. This relationship of crop mass to labellar MATs was similar to that between crop mass and tarsal MATs, suggesting that post-ingestive physiological changes related to crop filling and emptying (whether peristaltic activity, absorption of ingested materials, stimulation of crop or other stretch receptors) may affect labellar and tarsal thresholds in a similar manner.

Since the high tarsal thresholds in replete flies are at least partly mediated by the recurrent nerve (Edgecomb *et al.* 1987), we expected that labellar thresholds might be regulated by the same nerve. To our surprise, labellar thresholds in long-term-fed flies remained unchanged after recurrent nerve or median abdominal nerve transection (Tables 1, 2). Further, in hungry flies given a single meal, transection of the recurrent nerve did not prevent the normal post-prandial rise in labellar threshold. Transection of the recurrent nerve did, however, have the expected attenuating effect on tarsal thresholds, both in long-term-fed and single-meal-fed flies (Edgecomb *et al.* 1987). As in the earlier study, tarsal MATs were unaffected by transection of the median abdominal nerve. These observations suggest that the neural circuitry regulating labellar threshold is not subject to direct inhibition from

stretch receptors running in either the recurrent or median abdominal nerve, while the circuitry controlling tarsal responsiveness is affected by inputs from the recurrent nerve but not from the median abdominal nerve.

If inhibitory inputs running in nerves known to affect meal size do not affect labellar thresholds, what is the mechanism by which labellar thresholds rise (and later fall) after a meal? We have earlier recognized that there is an unknown factor(s) in addition to the recurrent nerve accounting for part of the rise in tarsal threshold after a meal (Edgecomb *et al.* 1987). This unknown factor(s) may also be responsible for post-prandial rises in labellar thresholds. Were this the case, the similarity in the rise and fall of labellar and tarsal thresholds after a meal could be easily understood.

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