

THE EFFECT OF HAEMOLYMPH OSMOTIC PRESSURE ON THE MEAL SIZE OF NYMPHS OF *LOCUSTA MIGRATORIA* L.

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SUMMARY

Injection of trehalose into the haemolymph of *Locusta* nymphs reduced the amount of grass eaten in one meal. Greater effects were produced by higher concentrations and most reduction occurred when meals began 20 min after injection.

Sodium chloride, glycine, proline, sorbose, inulin or a mixture of solutes had similar effects.

The similarity of effects is explicable in terms of the increase in haemolymph osmotic pressure.

The increase in the osmotic pressure normally occurring during feeding is not great enough to affect the current meal.

INTRODUCTION

The haemolymph osmotic pressure of flies affects their responsiveness to water (Barton Browne & Dudzinski, 1968) and possibly their feeding behaviour (Bolwig, 1953). Regular changes in the haemolymph osmotic pressure occur in the course of a normal feeding cycle in *Locusta migratoria* (Bernays & Chapman, 1974) and the present study is an investigation of the possible effects of different osmotic pressures on meal size.

METHODS

The details of rearing and feeding of the insects are given elsewhere (Bernays & Chapman, 1972). All insects were kept well fed, and only males 2-3 days old in the 10 or 11 day-long fifth instar were used. They were fed on *Agropyron repens* both as normal routine and in the experiments. In most experiments the insects were deprived of food for 5-6 h before feeding to ensure that when they fed the meal size was unaffected by food already in the foregut.

Individual insects in 1 lb jam jars were observed feeding from behind a screen with peep holes. The jars were also screened from each other so that the insects were disturbed as little as possible. An insect was deemed to have finished feeding when it had stopped for two consecutive minutes.

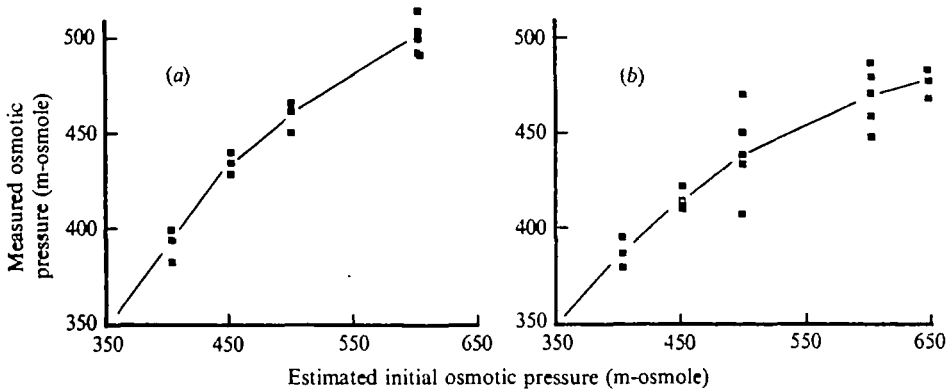


Fig. 1. Measured osmotic pressure of the haemolymph in relation to estimated initial values at (a) 10 and (b) 20 min after injection of trehalose.

Haemolymph osmotic pressure

Haemolymph osmotic pressure was measured by the cryoscopic method of Ramsay & Brown (1955) using apparatus with a thermocouple attachment and digital read-out in m-osmole. Small samples of haemolymph (5–15 μ l) were taken into micropipettes from cut coxal membranes and kept under liquid paraffin at 10 °C for 1–4 days.

The initial haemolymph osmotic pressure, immediately following injection, was calculated because the injected material probably took some minutes to disperse evenly to all parts of the body so that measured values would not be representative of the whole system. The calculation was based on the assumption of a uniform dispersal of the injected material in an initial blood volume of 140 μ l with an osmotic pressure of 350 m-osmoles (Bernays & Chapman, 1974).

Graphs were plotted of these calculated initial values against the measured values of osmotic pressure at 10, 20 or 30 min after injection, a different graph being required for each material at each time because of their different rates of regulation. Fig. 1 illustrates the curves for trehalose 10 and 20 min after injection. From such data the osmotic pressure at the start of feeding at different time intervals following the injection of known amounts of solute could be calculated. This was necessary because attempts to obtain blood samples at this time disturbed the insects.

The results are expressed in terms of the osmotic pressure prevailing at the start of feeding rather than the initial level following injection, because the former takes into account the different rates of regulation of the solutes, and a more coherent picture emerges.

Alteration of haemolymph osmotic pressure

Osmotic pressures were altered by injecting concentrated solutions of different materials in distilled water into the haemolymph between tergites 4 and 5 with an Aglar syringe. Concentrated solutions of trehalose, sorbose, inulin, glycine, proline and NaCl were injected in volumes of 0.1–10.0 μ l so as to produce calculated increases in haemolymph osmotic pressure of 50, 100, 150, 200 and 250 m-osmoles with each substance. In one experiment mixed solute injections were given. This solution contained trehalose, glycine, NaCl and KCl, in the proportions approximating to those found in the haemolymph (Dûchateau, Florkin & Lederman, 1952).

In order to effect changes in the osmotic pressure during feeding, a wide flexible plastic cannula (external diameter 1.5 mm), with the distal end sealed with parafilm, was inserted into the haemolymph through the pronotum dorsally, to the left of the median carina. It was sealed into position with a beeswax resin mixture. The operation was carried out on newly moulted insects, and 2 days were allowed with normal access to food to enable them to accommodate to the encumbrance before they were used in any experiment. The cannula, which was long enough to allow the insect to move about, was passed through the wall of the cage and then through a hole in an outer metal box which was illuminated by a 4 watt fluorescent strip light. Three minutes after feeding began, a long teflon needle (0.3 mm diameter) was inserted through the length of the cannula piercing the parafilm seal so that chemicals could be injected directly into the haemolymph during the meal. There was no interruption of feeding during the process.

The control insects were usually just pierced with the needle, but in the experiment on the reduction of haemolymph osmotic pressure, insect saline (Hoyle, 1953) was injected, while untouched controls were employed to check the effect of piercing.

Measurement of meal size

Meal sizes taken by insects previously deprived of food for 5–6 h were determined from the full foregut weights, less the mean empty foregut weight. The foreguts were weighed within 1 h of their removal from the test insects. When weighing was not immediate, dissected foreguts were sealed in containers to prevent water loss by evaporation. Apart from those insects with a cannula, test meals were given 5, 10, 20, 30 or 60 min after injections. The size of the meal taken by each of ten experimental insects was expressed as a percentage of the mean of the meal size of ten control insects.

RESULTS

Changes in meal size after injecting trehalose

Twenty insects tethered by wide cannulae, and deprived of food for 5 h, were allowed to commence feeding. After three minutes of continuous feeding, half of them were injected with 25 μ l distilled water to decrease the osmotic pressure by 60 m-osmoles, and half with 2 μ l of trehalose solution to increase the osmotic pressure by 100 m-osmoles. There was no apparent change in feeding behaviour, and the meal sizes (mean \pm S.E.) were 107 ± 6 mg and 104 ± 4 mg respectively.

If feeding was not permitted until some time after the injections, however, the amounts of grass eaten in one meal decreased. The extent of the reduction increased as the haemolymph osmotic pressure at the start of the meal increased (Fig. 2), but the effect of any particular osmotic pressure varied with the time after injection at which the meal started. Thus an osmotic pressure of 450 m-osmoles at the start of a meal led to a 5% reduction of a meal started 5 min after injection, a 20% reduction if it started after 10 min and a 65% reduction if it started after 20 min. After longer intervals, however, the effect became less marked so that 30 min after injection an osmotic pressure of 450 m-osmoles was associated with only a 15% reduction in meal size. Sixty minutes after injection, haemolymph osmotic pressure was never

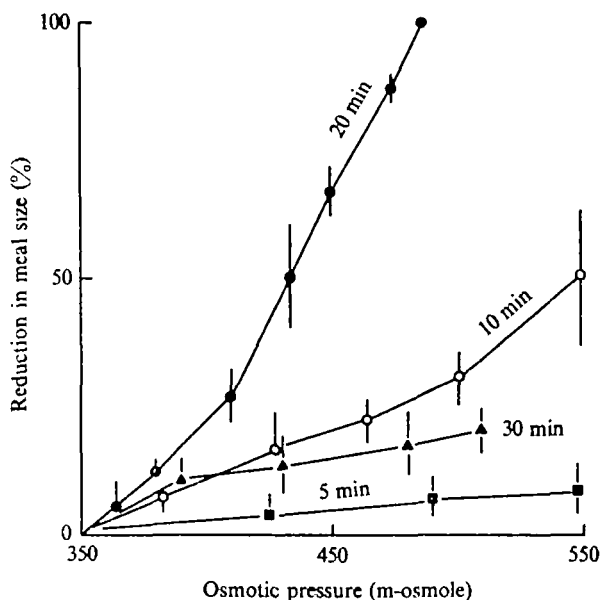


Fig. 2. The relationship between estimated haemolymph osmotic pressure at the start of feeding and reduction in meal size after the injection of trehalose. Each curve represents the results obtained when feeding started at the times stated after injection. Vertical bars in this and other figures indicate standard errors, with each mean value based on 10-15 insects.

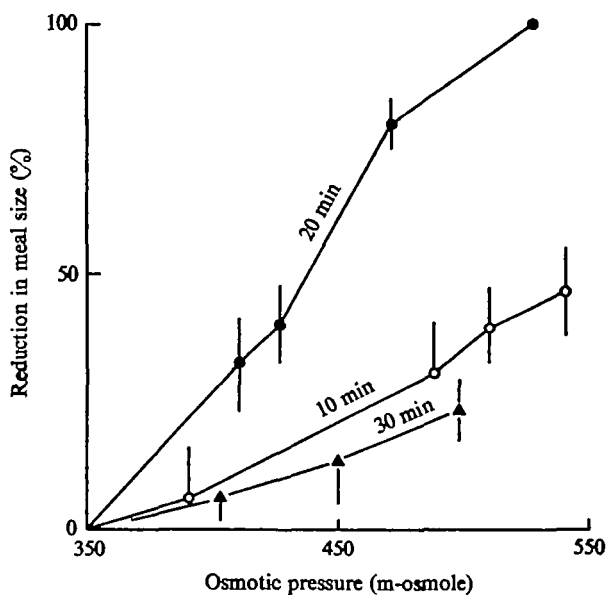


Fig. 3. The relationship between estimated haemolymph osmotic pressure at the start of feeding and reduction in meal size after the injection of NaCl. Each curve represents the results obtained when feeding started at the times stated after injection.

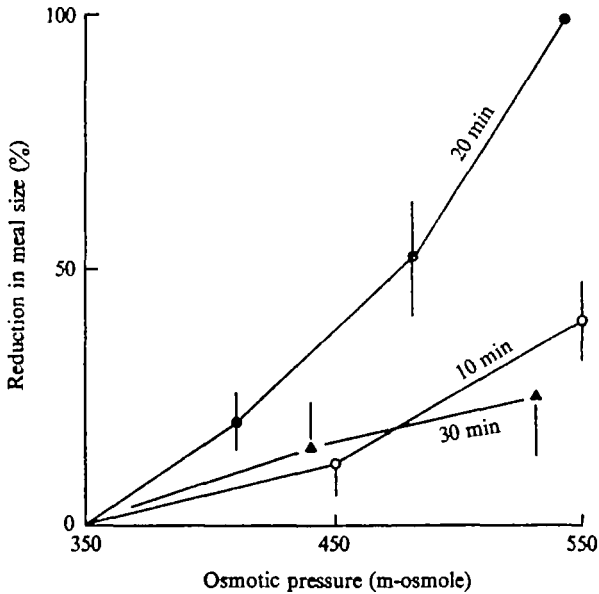


Fig. 4. The relationship between estimated haemolymph osmotic pressure at the start of feeding and reduction in meal size after the injection of glycine. Each curve represents the results obtained when feeding started at the times stated after injection.

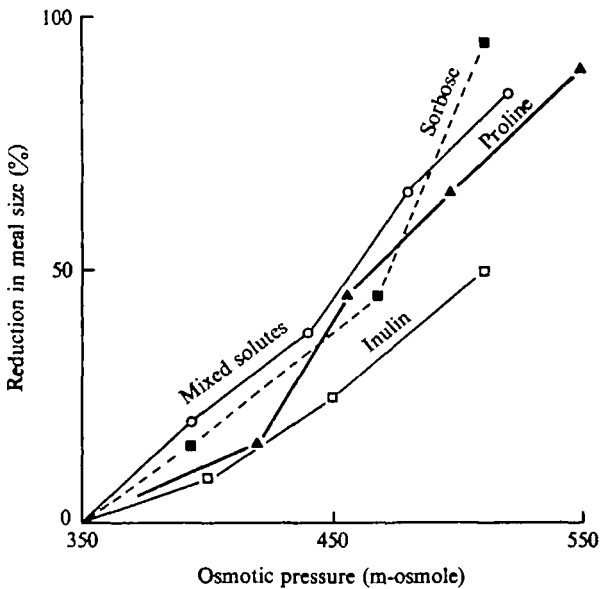


Fig. 5. The relationship between estimated haemolymph osmotic pressure at the start of feeding and reduction in meal size 20 min after the injection of different materials. Haemolymph osmotic pressures after inulin injections are calculated since difficulties were encountered in measurement.

more than 20 m-osmoles above the baseline level of 350 m-osmoles irrespective of how much was initially injected. The effect on meal size was then almost negligible.

Changes of meal size after injection of other substances

Injections of sodium chloride or glycine were also associated with reductions in meal sizes, and, as with trehalose, there was a maximum effect 20 min after injection (Figs. 3, 4). In these cases, as well as with sorbose, inulin, proline or a mixture of solutes tested at twenty minutes after injection, the reduction in meal size was proportional to the haemolymph osmotic pressure at the start of the meal (Figs. 3-5).

Reduction of haemolymph osmotic pressure

The osmotic pressure was reduced by injection of 10 or 50 μ l distilled water. The resultant meal sizes started 10 min after injection (20 insects in each case) were, respectively, 102 ± 5 mg and 100 ± 7 mg, and did not differ significantly from those taken by control insects which were injected with similar volumes of insect saline or just pricked with the needle (92 ± 4 mg and 94 ± 3 mg).

DISCUSSION

The experiments described in this paper indicate that the injection of materials into the haemolymph of *Locusta migratoria* affects feeding behaviour. This may be due to specific effects of the individual chemicals, but the similarity of the results with such different substances suggests that a more general explanation is possible in terms of osmotic pressure.

Meal size is related to the osmotic pressure of the haemolymph at the start of the meal, but meals started 20 min after injection of materials are very much smaller than meals started after longer or shorter intervals. This suggests that the amount eaten is affected not just by the osmotic pressure prevailing at the time of the meal, but rather by the total osmotic effect over a period before feeding.

This has been tested by plotting the meal size reduction against the osmotic excess, expressed as the area under the curve relating osmotic pressure to time over the 20 min prior to the start of feeding. Twenty minutes was chosen because maximum reduction in meal size following injections occurred after this interval. Treated in this way, all the results follow the same linear trend (Fig. 6) irrespective of whether the test meal began 10, 20, 30 or 60 min after the injection, and irrespective of whether the substance injected was a carbohydrate, a salt, an amino acid, or a mixture of these. This strongly suggests a common effect of all the different materials used irrespective of their chemical nature.

Haemolymph osmotic pressure increases during feeding in these insects by about 40 m-osmoles (Bernays & Chapman, 1974), but since the artificial increase of haemolymph osmotic pressure by 100 m-osmoles during the course of a meal did not affect meal size, it is unlikely that immediate feedback as a result of absorption or other haemolymph changes during the meal can normally be concerned in regulating meal size. Reduction in osmotic pressure also had no effect in increasing meal size, possibly because the normal limit is set by the foregut stretch receptors irrespective of other conditions (Bernays & Chapman, 1973).

In some situations, however, haemolymph osmotic pressure may exceed the normal

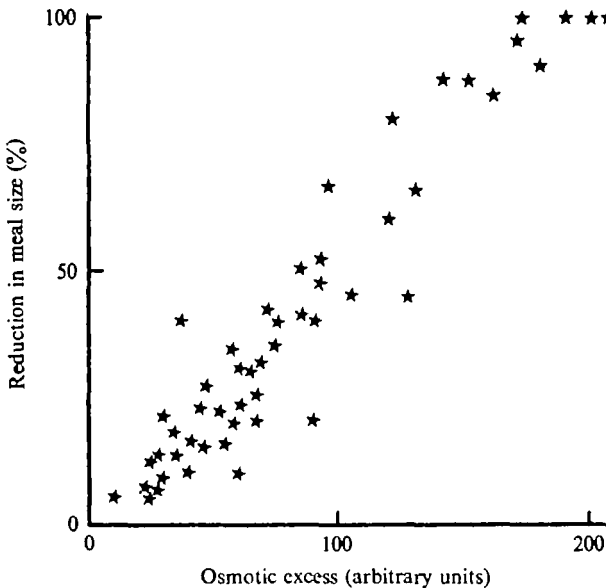


Fig. 6. The reduction in meal size in relation to the osmotic excess over 20 min prior to the meal. The osmotic excess expressed in arbitrary units is derived from the areas under the curves relating osmotic pressure to time over the 20 min prior to the start of feeding.

baseline level for more extended periods, and in such cases a reduction in meal size would be expected if osmotic pressure has a controlling influence. It has been shown (Bernays & Chapman, 1974) that osmotic pressure does not return to the baseline of 350 m-osmoles until about 2 h after feeding, and it is also known that if an insect feeds within this period the meal size is smaller than would be anticipated in terms of crop distension (Bernays & Chapman, 1972). The extent of the reduction corresponds with what would be expected from the osmotic excess on the basis of the results given in Fig. 6.

Haemolymph osmotic pressure is also high after 3 days without food (400 ± 11 m-osmoles compared with 351 ± 6 m-osmoles; Bernays, unpublished results) and again the size of the first meal taken after such an extended period of deprivation is 20% less than normal (Bernays & Chapman, 1972). A similar situation may prevail when locusts are given dry food, especially at low humidities when they are known to eat less (Sinoir, 1966).

Finally, in insects approaching ecdysis the haemolymph osmotic pressure is about 60 m-osmoles higher than in mid-instar (Bernays, unpublished results). This may partly account for the reduction in meal size observed at this time (Bernays & Chapman, 1972), although the extent of the reduction, amounting ultimately to a total cessation of feeding, is much greater than would be expected in these terms alone, and other factors are almost certainly important.

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