J. exp. Biol. **129**, 329–346 (1987)
Printed in Great Britain © *The Company of Biologists Limited 1987*

THE PHYSIOLOGY OF COMPENSATION BY LOCUSTS FOR CHANGES IN DIETARY PROTEIN

BY J. D. ABISGOLD AND S. J. SIMPSON

Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS

Accepted 26 January 1987

SUMMARY

1. Previous work has demonstrated that fifth-instar nymphs of *Locusta migratoria* L. respond to differences in levels of dietary protein by altering intermeal interval but not meal size (Simpson & Abisgold, 1985): insects fed a diet with 14% protein (p) eat the same sized meals more frequently than those fed a diet with 28% protein (P). The physiological basis for this compensatory response is investigated.

2. Insects fed the P-diet had a significantly larger increase in blood osmolality during and after a meal than did those fed the p-diet.

3. Unexpectedly, this difference in blood osmolality did not result in a variation in the rate at which the fore-, mid- and hindgut emptied. Therefore a change in the rate of decline in negative feedback from gut stretch receptors does not underlie the alteration in interfeed interval.

4. 40% of the difference in blood osmolality between p- and P-fed insects was attributable to changes in the blood concentration of free amino acids. Of the 16 free amino acids found, 11 occurred in significantly higher concentrations in the blood of P-fed insects.

5. There was no significant difference in the polypeptide and protein content of the blood of insects fed the p- or P-diet.

6. Increasing either blood osmolality or free amino acid concentration by injection delayed the next meal: injections that increased both had the greatest effect.

7. A mechanism is discussed whereby both blood osmolality and the concentration of various free amino acids regulate the time between meals, and thus compensatory feeding in response to changes in dietary protein.

INTRODUCTION

Insects are known to alter their feeding behaviour in response to changes in both dietary composition and their nutritional requirements (see reviews by Mattson, 1980; Scriber & Slansky, 1981; Bernays & Simpson, 1982). Simpson & Abisgold (1985) showed that fifth-instar nymphs of *Locusta migratoria* are able to compensate for a 50% dilution of their dietary protein by reducing the time between meals but keeping meal size constant.

Key words: locusts, protein intake, osmolality, free amino acids, compensatory feeding behaviour.

Various physiological changes which influence the timing of the next meal are known to occur when a locust feeds (Simpson & Bernays, 1983). Haemolymph osmolality increases during and after a meal, as a result of salivation and nutrient absorption (Bernays & Chapman, 1974*a*), as does the concentration of those nutrients absorbed. Such changes in the composition of the haemolymph have been shown to slow the rate of gut emptying (Baines, 1979) and this in turn, *via* input from gut stretch receptors, affects the time when the next meal is taken (Bernays & Chapman, 1973; Simpson, 1983). Whether haemolymph osmolality or the concentration of specific nutrients is important in regulating the rate of gut emptying in the locust is uncertain, although Gelperin & Dethier (1967) showed that in the blowfly the influence on gut emptying is a function of the osmolality of the diet rather than its specific nutrient content. It is also possible, but unproven, that changes in haemolymph osmolality and nutrient composition may influence interfeed interval by a direct effect on responsiveness of the animal to food stimuli (Bernays & Simpson, 1982). The aim of the present study was to determine which physiological processes are involved in the compensatory feeding behaviour of *Locusta migratoria* in response to changes in the proportion of protein in an artificial diet. In particular, are nutrient feedbacks involved?

MATERIALS AND METHODS

Artificial diets

Variations on the artificial diets of Dadd (1961) were used. The high-protein diet (P) contained 27·5% protein (a 3:1:1 mixture of low-fat, vitamin-free casein, bacteriological peptone and egg albumen), 27·5% digestible carbohydrate (a 1:1 mixture of sucrose and white dextrin), 41·1% cellulose, 2·4% Wesson's salts, 0·5% linoleic acid, 0·5% cholesterol, 0·3% ascorbic acid and 0·2% vitamin cocktail (see Dadd, 1961). The low-protein diet (p) was similar in all respects except that it contained half the protein and 54·8% cellulose. Cellulose, which is virtually indigestible to locusts (Morgan, 1976; Martin, 1983), was used in order to maintain the bulk of the diets. Samples of the protein component of the diets were also hydrolysed and analysed for amino acid content. Diets were presented dry and allowed to equilibrate to room relative humidity (RH). On testing, both diets had a water content of 4%. That the consistency of the two diets was similar is illustrated by the fact that meal lengths, meal sizes and ingestion rates did not differ among insects fed either diet *ad libitum* (Simpson & Abisgold, 1985).

Insects

Locusts were reared at the Department of Zoology, Oxford, in the standard manner (Hunter-Jones, 1961), using seedling wheat as the green food source. Males weighing between 440 and 550 mg were removed from stock cages during the first day of the fifth instar (termed day 0), and placed individually in 17×12×6 cm clear plastic boxes. Each box contained a strip of expanded aluminium running around its sides which acted as a perch. Adjacent boxes were screened from each other using

cardboard partitions. For the duration of the experiments locusts were maintained at 30 ± 1 °C under an L:D 12 h:12 h light regime. From day 0 until the beginning of day 3 in a 10-day instar, locusts were fed ample seedling wheat and a dry food in the form of bran given in a 5-cm diameter Petri dish. At the onset of day 3, the wheat and bran were removed and replaced with a Petri dish filled with 1·5–2 g of either the P- or p-diet. Insects were then left for 3–5 h before any recordings were made, during which time several meals of the artificial diets would have been eaten (Simpson & Abisgold, 1985).

Measurement of gut emptying and haemolymph osmolality

Insects were observed until they had completed a meal of a minimum of 3 min duration followed by a period of 4 min without feeding (see Simpson & Abisgold, 1985). The food was then removed and the insect left for 0, 15, 30, 45, 60, 75, 90, 105 or 120 min. After this, the osmolality of a 3-μl haemolymph sample obtained through a cut coxal membrane was measured using a Wescor 5100C vapour pressure osmometer. The osmometer produced highly consistent readings for standard solutions under the same conditions of temperature and RH as in the experiments (e.g. 290 mosmol kg^{-1} standard, $\bar{x} \pm$ S.E. 289·9 ± 0·2, $N = 10$). One blood sample per insect was taken. Estimates for the rate of gut emptying on the two diets were obtained by removing the whole gut from the insect at one of the above times and separating it into the foregut and the combined mid- and hindgut (including the midgut caeca). The two portions of the gut were then weighed to the nearest 0·1 mg.

In addition to the above times, haemolymph osmolality and gut masses were measured at the start of a meal (S), a meal being considered to have begun once a locust had moved to the food dish and ingested for 5 s.

Measurement of haemolymph composition

Blood samples of 3–15 μl were taken from insects other than those used for blood osmolality determinations. Samples were removed at the start of the meal or at 0, 15, 30, 45, 60, 75 or 90 min after the end of the meal. Again only one sample per insect was used. The blood was spun for 3 min in a Beckman Model 150 Microfuge at 14 000 ***g*** to deposite the haemolymph cells. The supernatant was removed using a 50-μl Hamilton syringe and immediately frozen in liquid nitrogen, and later stored at −70 °C for 2–4 weeks. Polypeptides and proteins present in the blood were separated from free amino acids by precipitation with 20 % sulphosalicylic acid. The free amino acids were analysed using an LKB 4400 amino acid analyser. The protein and polypeptide fraction was hydrolysed to amino acids with 6 mol l^{-1} HCl and analysed on a Locarte Mark 4 amino acid analyser.

Injection experiment

The aim of the injection experiment was to distinguish between an effect of blood osmolality on intermeal interval and a more specific effect due to the levels of a group of particular amino acids in the blood. Insects were fed the low-protein diet from the beginning of day 3. Using information obtained from the free amino acids in the

haemolymph, four solutions were prepared and one of the four was injected into individual locusts in 10-μl volumes between tergites four and five using a 50-μl Hamilton syringe. Simpson (1982*a*,*b*) showed that insects of a similar mass range to those of the present study had an average haemolymph volume of $220 \pm 10\,\mu$l ($\bar{x} \pm$ S.E.) on the third day of the fifth instar. On this basis it was expected that an injection of $10\,\mu$l would represent a 4 % increase in blood volume. This information was used in estimating the composition of injected solutions. Pilot studies in which osmolality and amino acid concentration were measured directly showed that the expected effects of injection were realized. In addition, the effect of injection was clearly seen above any variation between individuals in blood volume or composition. Such variation was minimized by selecting insects of a standard mass, reared under controlled conditions. Injections were administered 40 min after the insect had taken a meal in the course of *ad libitum* feeding, which corresponded to the time taken for the blood amino acids to return to their pre-meal values for insects fed the low-protein diet. The compositions of the solutions injected were as follows.

Solution 1: amino acids in saline

Amino acids (Table 1) injected in saline (Mordue, 1969) isotonic with the blood of low-protein-fed insects 40 min after their last meal (see Fig. 1) raised the amino acid concentration of the blood up to the level of high-protein-fed insects, and also increased osmolality by 12 mosmol kg^{-1}, approximately a 3 % increase. Pilot studies had shown that the increases in blood amino acid content with injection were sustained after the injection and followed a time course similar to that demonstrated for high-protein-fed insects in Figs 1 and 3.

Tyrosine, although present in higher concentrations in the blood of high-protein-fed insects, was omitted from the injection as we found it impossible to get into solution in the amount required, although various methods were attempted including using tyrosine hydrochloride. An experiment to test the effect of omitting tyrosine

Table 1. *Concentration of each amino acid injected*

Amino acids	Amount injected (nmol)
Threonine	134
Glutamine	212
Serine	30
Methionine	267
Leucine	217
Phenylalanine	110
Isoleucine	133
Lysine	251
Valine	111
Alanine	283
(Tyrosine)	—

The amount of each amino acid made up in solution for the injection experiment was calculated as the amount needed to raise the levels found 40 min after the previous meal in the haemolymph of p-fed insects to those of P-fed insects. Tyrosine was omitted from the solution (see text).

was conducted. The protein component of the diets was hydrolysed and amino acid concentrations were measured. The low-protein diet was then enriched by the addition of the appropriate quantities needed to mimic levels in the P-diet for 10 of the 11 amino acids which were found to differ in concentration between the blood and P- and p-fed insects, tyrosine being excluded. The quantities eaten over 12 h by individual locusts of this enriched diet, or the unenriched p-diet or the P-diet were then measured. Insects ate the same amounts of the enriched p-diet and the P-diet ($40{\cdot}1 \pm 3{\cdot}1$ and $40{\cdot}9 \pm 4{\cdot}3$ mg, respectively, $\bar{x} \pm$ S.E.; $N = 10$), but ate significantly more ($62{\cdot}0 \pm 3{\cdot}1$ mg) of the low-protein diet. These amounts corresponded with figures from Simpson & Abisgold (1985) for quantities of low- and high-protein diets eaten during a 12-h period. Thus, when free amino acids were added to the low-protein diet, it was eaten in quantities similar to those of the P-diet. Tyrosine, although occurring in significant concentrations in the blood of P-fed insects, was not necessary to elicit this response. Its exclusion from the injection was therefore considered unimportant.

Solution 2: xylose in saline isotonic with blood

From pilot studies testing various sugars and sugar-alcohols (sorbose, xylose, mannitol, dulcitol and sorbitol) xylose was shown to be the compound which, when injected into the haemolymph of locusts, showed a time course of removal from the blood most similar to an injection of a mixture of amino acids (as in Table 1) with the same osmolality. Xylose, therefore, provided a compound which would act as an osmotic control for the amino acid injection. There was still the possibility, however, that xylose itself affects feeding. In order to test this, insects were injected with xylose in distilled water and their behaviour was compared with that of insects injected with isotonic saline. Locusts injected with xylose took approximately the same length of time to take a meal as did the saline-injected controls ($14{\cdot}8 \pm 2{\cdot}8$ and $16{\cdot}6 \pm 3{\cdot}7$, respectively, $\bar{x} \pm$ S.E.). We therefore concluded that the only noticeable effect of injecting xylose in saline on the insects' feeding behaviour was *via* a change in haemolymph osmolality.

Solution 3: amino acids (as in Table 1) in distilled water

This injection raised the blood amino acid concentration up to the level of the high-protein-fed insects, as did solution 1, but because the amino acids were in solution in water and not saline, decreased the blood osmolality by approximately 4 mosmol kg^{-1}. Thus, the injection altered the blood amino acid composition but produced no increase in blood osmolality.

Solution 4: saline isotonic with blood

This injection provided a control for the increase in blood volume of 10 μl and the trauma and effect on feeding behaviour caused by the injection.

After injection, the insects were left for 3 min before food was added to the container. During this time they almost invariably settled into the resting position on the side of the arena and became quiescent. In the majority of cases if the food

provided was an artificial diet (which is olfactorily relatively unstimulating), then the control as well as the test insects remained quiescent for at least 40 min and did not contact the food. Such a lowering of excitability is commonly observed even as a result of sham injections (S. J. Simpson, unpublished results; E. A. Bernays, personal communication). Since after 40 min the effect of the injections on blood composition would have declined significantly, it was necessary to provide a more stimulating food in order that any differential effect of the injections on behaviour could be seen. Freeze-dried seedling wheat proved to be sufficiently stimulating (olfactorily and/or visually) that 70% of the control insects commenced locomotion and then fed within 20 min. This was the sort of proportion that would have been expected to feed on the low-protein diet had the insects not been injected (Simpson & Abisgold, 1985).

Insects were observed for the next 45 min and their behaviour, (locomotion, feeding or quiescence) noted every 30 s. In this way it was possible to see whether osmolality, amino acid concentration or a combination of the two delayed feeding relative to the control and so mimicked the effect of the high-protein diet.

RESULTS

Haemolymph osmolality and the rate of gut emptying

Fig. 1 shows the change in blood osmolality with time since the last meal for insects fed the P- or p-diets. There are several points to be made from these data. First, the blood osmolality of insects fed the high-protein diet was higher than that of insects fed the low-protein diet ($P < 0{\cdot}0001$ ANOVA). Second, the difference in osmolality between insects fed different diets was produced largely during the course of the meal, with the P-fed insects incurring a mean increase of 37 mosmol kg^{-1} as compared with the significantly smaller increase of 10 mosmol kg^{-1} shown by the p-fed nymphs. Over the course of the next 60 min the blood osmolality of the P-fed nymphs remained high, with an indication of a further increase between 45 and 60 min. Such an increase could possibly be due to a secondary influx of absorbed amino acids into the blood as products of digestion. Between 60 and 75 min the blood osmolality of the P-fed insects fell markedly and continued to decrease over the next 45 min to its pre-meal value. The haemolymph osmolality of the p-fed insects, having increased by a comparatively smaller amount during the meal, fluctuated slightly about this level over the next 75 min after which it decreased slightly and returned to its pre-meal value. From earlier work (Simpson & Abisgold, 1985), it was known that the mean intermeal intervals for insects fed the P- and p-diets were 71 and 49 min, respectively, as represented by the arrows in Fig. 1. Comparing the mean osmolality of the haemolymph at these times shows that the values obtained for insects fed the two diets are very similar (P = 386 mosmol kg^{-1} and p = 382 mosmol kg^{-1}); that is when P-fed and p-fed insects initiate a meal their blood osmolality is more or less equal.

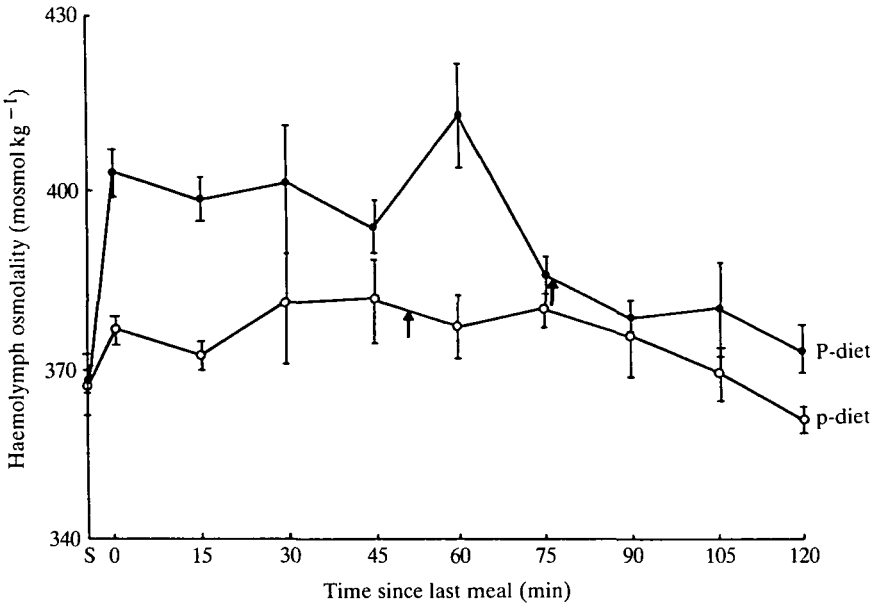

Fig. 1. The change in haemolymph osmolality with time since the last meal for insects fed the P- or p-diet. Each point represents the mean of 12–15 insects. Different insects were used for each point. Bars represent ±S.E.M. Arrows indicate the average time at which the next meal would have been taken had food been present.

Fig. 2A,B show the masses of the mid- and hindgut and the foregut at various times after the last meal. There was no significant difference between insects fed the two diets in the mass of the various portions of the gut, as is reflected in similar faecal mass productions on the two diets (Fig. 2C).

Haemolymph composition

Total free amino acids

The total concentration of free amino acids in the blood was significantly higher for insects fed the high-protein diet (Fig. 3). As with osmolality (Fig. 1), a large proportion of the increase in amino acid concentration occurred during the meal on the P-diet, with a possible further increase between 45 and 60 min after the meal. After 60 min, the amino acid level decreased to its pre-meal value for the high-protein diet. For the insects fed the low-protein diet, there was little, if any, increase in free amino acid levels during the meal. At subsequent times after the meal the amino acid concentration fluctuated to a small extent about a mean level of approximately 25 nmol μl^{-1}, increasing slightly 60 min after the meal and then returning to its pre-meal value. The time course for change in level of total free amino acids in the blood is broadly similar to that for blood osmolality on both the low- and high-protein diets. 40 % of the osmolality difference can be attributed to the difference in total free amino acid concentration between the diets.

Individual amino acids

Of the 16 amino acids commonly found in locust blood, 11 were found in significantly higher concentrations in the haemolymph of the high-protein-fed insects in an analysis of variance (Table 2). These were threonine, methionine, valine, isoleucine, leucine, phenylalanine, lysine, tyrosine, serine, glutamine and alanine. With the exception of valine, all the amino acids increased in concentration in the haemolymph of the high-protein-fed insects during the meal to some extent and continued to increase over the next 60 min, after which they returned to their pre-feed level, although the time courses differed from each other with some contributing more to the early high levels and others to the later high levels of total free amino acid (Fig. 4). Insects fed the low-protein diet showed significantly smaller increases in the haemolymph levels of these 11 amino acids. The rank order of amino acids in hydrolysed dietary protein did not correlate with blood concentrations at any

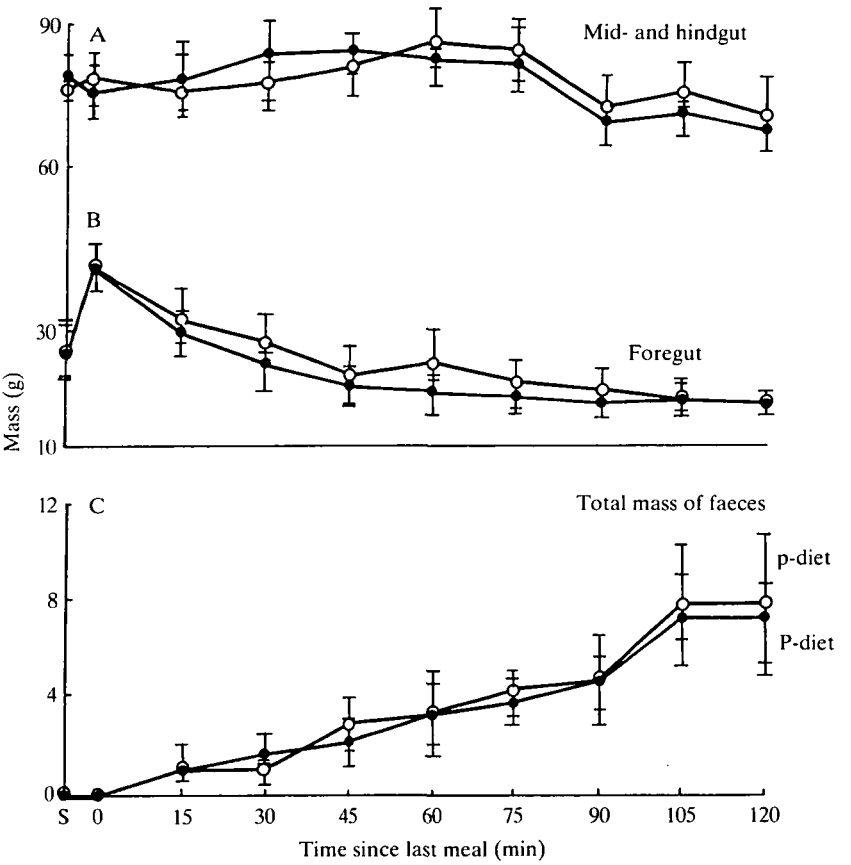

Fig. 2. The mean masses of the fore-, mid- and hindguts and faeces produced after a meal for insects fed either the P- (●) or p- (○) diet, $N = 12–15$. Bars represent ±S.E.M. Different insects were used for each point. Fore-, mid- and hindguts were wet-weighed whereas faeces were dried in an oven at 70°C for 3 days before weighing.

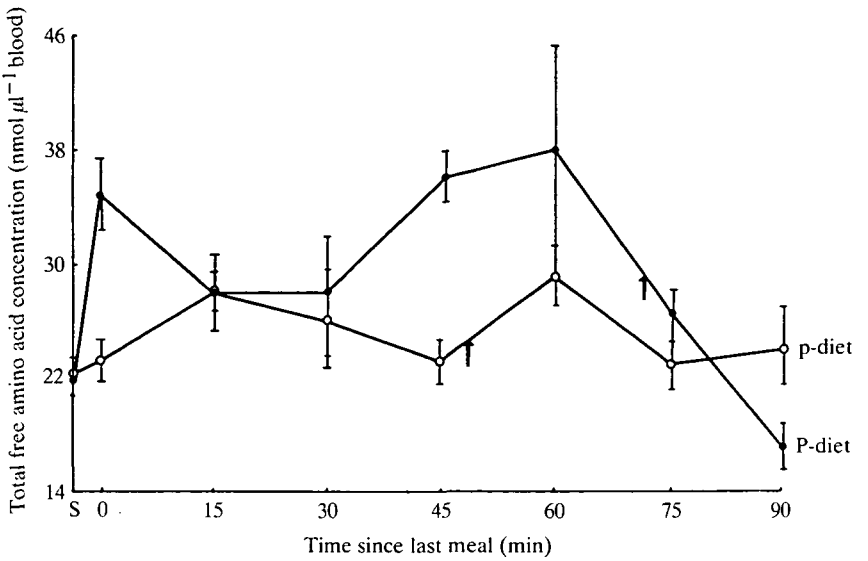

Fig. 3. The change in total free amino acid levels for insects fed either the P- or p-diet, $N = 5$. Bars represent ±S.E.M. Arrows indicate the average time when the next meal would have been taken had food been present.

of the times after the meal (Spearman's Rank Correlation; $P > 0{\cdot}05$ in all cases); see Table 2.

Proteins and polypeptides

An analysis of variance showed that there was a small but significant difference between the protein and polypeptide content of the blood of P- and p-fed insects ($P = 0{\cdot}04$), (Fig. 5). However, this difference is due to the large amount of protein and polypeptide present 45 min after the meal in the blood of the low-protein-fed insects. There was a very large variation associated with this data point and N is only 5. With the exception of the levels present 45 min after the meal, there were no significant differences between the amounts of protein present in the blood of insects fed the two diets; the protein levels fluctuated over the first 75 min and then increased between 75 and 90 min, possibly as protein was assimilated from free amino acids and stored in the blood.

Injection experiments

Locusts injected with a solution of amino acids in saline (solution 1), which caused an increase in both amino acid content and blood osmolality, took a significantly longer time to feed after injection than insects injected with a saline control (solution 4), ($P < 0{\cdot}01$, Kolmogorov–Smirnov two-sample test). Locusts injected with either xylose in saline (increasing osmolality only, solution 2) or amino acids in water (increasing amino acid concentration only, solution 3) also delayed feeding relative to the control after the injection ($P < 0{\cdot}05$), but to a lesser extent than insects injected with solution 1 (Fig. 6). Thus it is evident that both an osmotic effect and a specific

Table 2. *Concentrations of amino acids present in the dietary protein, and in the blood of high- and low-protein-fed insects at various times after a meal*

Amino acid	Dietary protein ($mg\,g^{-1}$)	Concentrations ($nmol\,\mu l^{-1}$) in blood: Insects fed on high-protein diet — Time since last meal (min) S	E	45	60	Insects fed on low-protein diet — Time since last meal (min) S	E	45	60	Significance of difference in blood amino acid concentration in insects fed high-protein *vs* low-protein diets
Aspartic acid	73	0	0·06	0·04	0·11	0·05	0·04	0·03	0·03	NS
Threonine	32	0·8	1·1	1·3	1·4	0·7	0·7	0·8	1·0	**
Serine	29	1·7	1·8	2·9	2·7	1·5	1·7	1·7	2·3	*
Glutamine	182	1·8	2·6	3·2	3·2	1·8	2·7	2·3	2·1	**
Proline	84	3·1	4·5	5·0	6·0	3·5	3·5	3·0	3·6	NS
Glycine	38	5·0	4·9	7·9	6·9	4·7	5·0	4·2	5·7	NS
Alanine	44	0·9	1·1	1·7	2·3	0·9	1·1	1·0	1·6	*
Valine	57	0·6	0·6	1·1	0·8	0·6	0·6	0·6	1·0	*
Methionine	24	1·3	1·7	2·7	2·7	1·0	1·3	1·5	1·9	**
Isoleucine	42	1·3	1·7	2·7	2·7	0·5	0·7	0·7	1·1	**
Leucine	78	0·7	1·0	1·3	1·7	0·5	0·7	0·7	1·0	**
Tyrosine	34	1·0	1·0	1·7	1·7	0·7	0·9	0·9	1·3	**
Phenylalanine	43	0·4	0·6	0·8	1·0	0·4	0·4	0·5	0·7	**
Histidine	25	2·0	2·2	2·2	2·9	1·9	2·1	2·2	1·8	NS
Lysine	67	2·0	2·3	3·1	2·9	2·1	2·0	2·0	2·4	*
Arginine	44	0·6	0·8	1·1	1·3	0·9	0·7	0·7	1·2	NS

The levels of amino acids in the dietary protein were obtained by hydrolysing the protein component of the artificial diet used in the experiments.

The levels of significance are based on the difference between insects fed the two diets in an ANOVA with time as another factor.

Each data point is based on amino acid analyses from five individuals. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; S, start of the meal; E, end of the meal.

amino acid effect contribute to the lengthening of interfeed interval shown by insects fed the high-protein diet.

DISCUSSION

Haemolymph osmolality and the rate of gut emptying

The increase in blood osmolality of approximately 10% ($37\,mosmol\,kg^{-1}$) during the meal for locusts fed the high-protein diet corresponds to increases in osmolality found previously for *Locusta migratoria* fed seedling wheat (Bernays & Chapman, 1974*a*,*b*). Insects fed the low-protein diet incurred a much smaller increase in haemolymph osmolality ($10\,mosmol\,kg^{-1}$, approximately 3%) during the meal and consequently experienced lower osmolality levels in the blood over the next 60 min than did the high-protein-fed insects. Such differences in blood osmolality between the two experimental groups of insects were expected to produce a difference in the rate of gut emptying, as has been previously demonstrated in the locust (Baines,

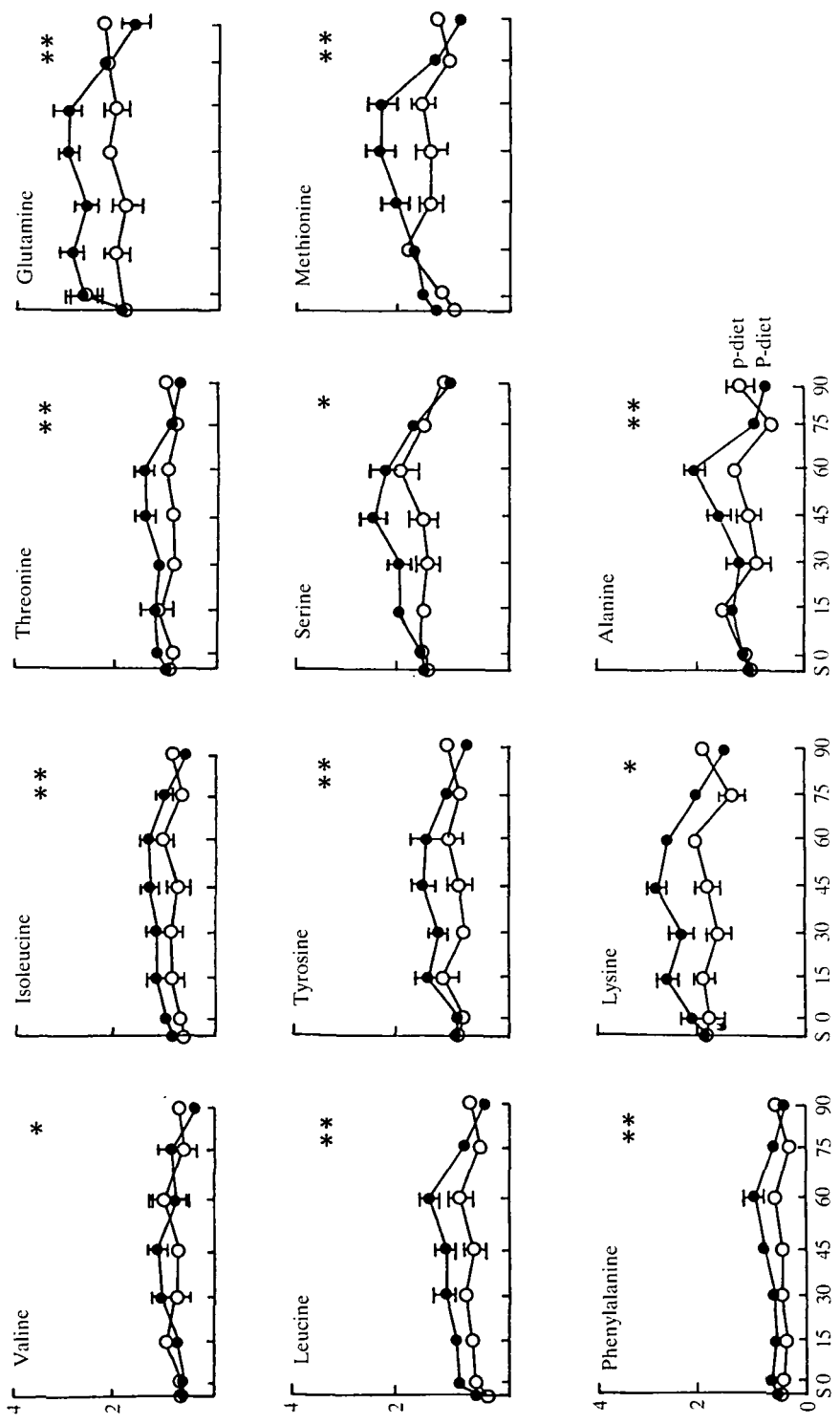

Fig. 4. The change in haemolymph concentration with time since the last meal of those amino acids found in higher concentrations in P-fed than in p-fed insects, $N = 5$. Bars represent ±S.E.M.; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

1979) and the blowfly (Gelperin & Dethier, 1967). However, this was not the case; the guts emptied at the same rates on the two diets.

This result raises an apparent paradox. How can insects on the low-protein diet begin a meal with the same amount of food in their gut from previous meals as do high-protein-fed insects (Fig. 2A,B), yet eat the same sized meals more frequently

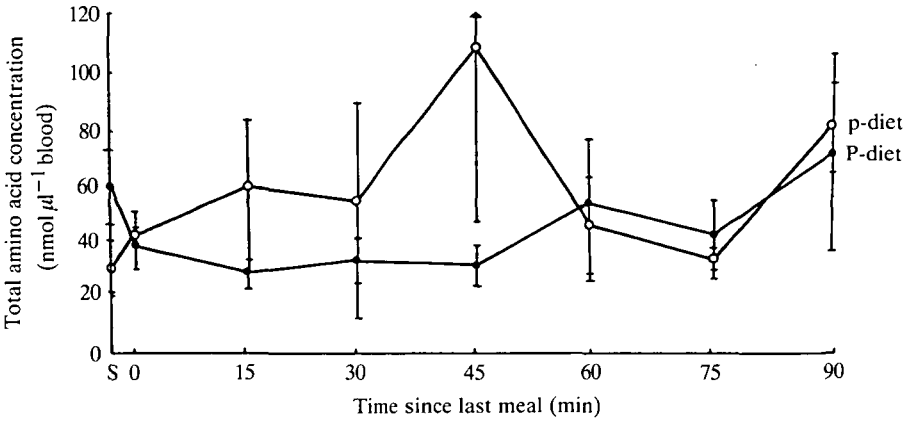

Fig. 5. The haemolymph polypeptide and protein content at different times since the last meal for insects fed either the p- or P-diet, as measured by the total number of nmol of amino acids produced upon hydrolysis (see text), $N = 5$. Bars represent ±S.E.M. ANOVA showed diet (P or p) to be significant as a main effect: $F = 4{\cdot}40$; df 1,64; $P = 0{\cdot}04$. Time since the meal was not significant either as a main effect or as an interaction with diet ($F = 0{\cdot}94$; df 7,64; $P = 0{\cdot}5$ and $F = 1{\cdot}27$; df 7,64; $P = 0{\cdot}3$ for time and diet × time, respectively).

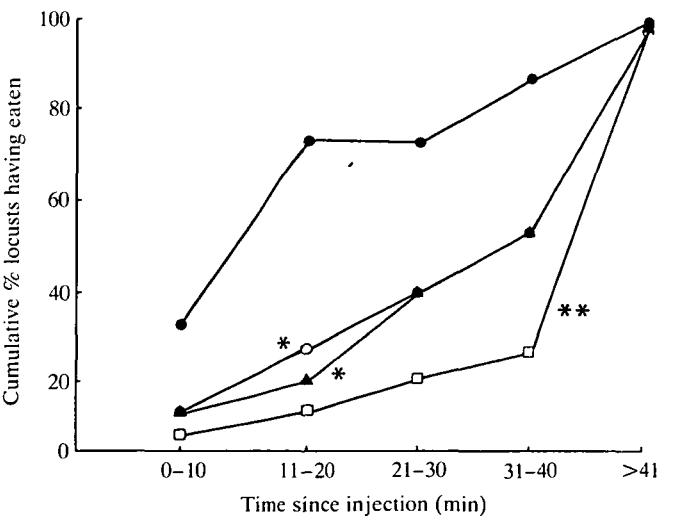

Fig. 6. The effect of injecting insects with one of four solutions upon subsequent feeding behaviour. □, amino acids in saline solution; ▲, xylose in saline; ○, amino acids in water; ●, saline only, $N = 15$. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$ relative to control injection (●) in a Kolmogorov–Smirnov two-sample test.

(Simpson & Abisgold, 1985) and have the same rate of passage of food through the gut (Fig. 2A–C)? The answer lies in the time taken for a meal to clear the gut for insects fed the two diets. Fig. 2 shows that after approximately 45 min since the meal the mass of food eaten has virtually cleared the foregut as well as the mid- and hindgut. Therefore, although low-protein-fed insects eat more often, an amount of food equivalent to the mass of each meal has passed through the gut before the next meal is taken, and so both groups of insects start the next meal with the same amount of food in their gut. Whatever delays feeding on P- as compared to p-diet is apparently not related to differences in the rate of passage of food through the gut and it's effect on inhibitory volumetric feedback.

Osmolality versus *specific amino acid effect*

A protein-rich meal produces a larger increase in haemolymph osmolality and amino acid content than does a low-protein meal. The time course of these effects following a meal correlates well with the time until the insects would have next fed if feeding *ad libitum* (Simpson & Abisgold, 1985). Injection experiments provide evidence that both haemolymph osmolality and amino acid content have a role in the regulation of interfeed interval. Altering the blood free amino acid profile of p-fed insects to that of P-fed insects without increasing blood osmolality resulted in an increase in the time to the next meal. Changing blood osmolality alone had a similar effect, while the combination of altered free amino acid profile and osmolality increased the latency to feeding by the greatest amount. This is the first report that we know of in any animal of a specific group of nutrients being directly involved in behavioural regulation of macronutrient ingestion.

Haemolymph osmolality has previously been shown to influence feeding in locusts (Bernays & Chapman, 1974*b*). Using insects deprived of food they found that changes of 50–250 mosmol kg^{-1} reduced the size of a subsequent meal, the largest reduction corresponding to the greatest increase in haemolymph osmolality produced by injection. The magnitude of the effect of increased osmolality upon meal size was also found to be related to the time between injection and the next meal. Bernays & Chapman (1974*b*) concluded that the amount eaten is affected not only by the haemolymph osmolality at the time the meal is taken but also by the total osmotic excess before feeding. The present study does not comply with those results. If osmotic excess did indeed affect subsequent meal size then it would be expected that the difference in osmolality between p- and P-fed insects (28 mosmol kg^{-1}), sustained over approximately a 75-min interval, would result in a difference in the meal size taken by the insects, the P-fed insects taking a smaller meal. This was not the case (Simpson & Abisgold, 1985). However, it is possible that the osmolality difference found in the present study was not large enough to evoke the response noted by Bernays & Chapman (1974*b*). In addition, in our experiments insects were not deprived of food. The present study indicates that, under the experimental conditions used, osmolality is involved in the *initiation* of feeding. Prevailing osmolality does not influence the *termination* of feeding, i.e. meal size, in insects

feeding *ad libitum* as, at the time each meal begins, osmolality levels are similar for insects fed the two diets.

Phifer & Prior (1985) showed an effect of haemolymph osmolality on both the initiation and termination of feeding in the slug. Increased osmolality decreased the probability that a slug would initiate feeding when presented with food and also decreased the duration of the output from the central nervous feeding motor pattern generator in a dissected preparation. The latter would presumably lead to shorter meals in an intact animal. How these effects relate to the patterning of feeding behaviour was not studied. Bernays (1977) demonstrated that a reduction in blood osmolality influences the termination of drinking in *Locusta migratoria*. She found no effect of increased osmolality on the initiation of drinking.

The cocktail of amino acids which was injected into the low-protein-fed insects to raise their blood amino acid profile to that of high-protein-fed insects contained 11 of the 16 amino acids commonly found in locust blood. Bernays (1980) found that injecting proline into the haemolymph of *Locusta migratoria* had no effect on locomotor activity and was therefore unlikely to affect feeding. However, proline is one of the six amino acids that were present at the same concentrations in the blood of P-fed and p-fed insects in the current experiments and, therefore, is probably not involved in the amino-acid-controlled regulation of compensatory feeding behaviour. Of those amino acids that are involved, all except serine, glutamine and alanine are essential to most insect groups, including the Orthoptera (see Dadd, 1985). Which amino acids are specifically essential to *Locusta migratoria* is unknown. The question remains as to whether all 11 amino acids injected in the current experiments are important in the regulation of interfeed interval or whether some of those 11 are more important than others. For example, the differing time courses of individual amino acids may possibly lead to differential effects on interfeed interval (Fig. 3). Alternatively, the absolute amounts of each amino acid present may be less critical than the overall ratio. Such questions are currently being investigated.

Simpson & Abisgold (1985) found that locusts did not compensate for the dilution of the carbohydrate component of the diet. Insects were given one of four diets, either high- or low-protein (28 or 14 %) in combination with either high- or low-carbohydrate (28 or 14 %; i.e. PC, Pc pC or pc) and it was found that compensation was elicited in response to the dilution of protein only. It might be expected that increasing the digestible carbohydrate content of the diet from 14 to 28 % would lead to an increased blood osmolality and, on the results presented in the present paper, a lengthening of interfeed interval; yet interfeed interval on pC and pc did not differ. Nor did interfeed intervals on PC and Pc.

Diets PC and Pc were used in the present experiments. As an adjunct to the experiments reported so far, blood osmolality was measured for insects fed PC- or Pc-diets in order to determine whether the digestible carbohydrate was having an effect. Blood samples were taken in the same manner as previously described from insects fed either the PC- or Pc-diet.

As can be seen in Fig. 7, the osmolality during and after the meal was the same on the two diets, even though insects eating the high-carbohydrate diet received twice as

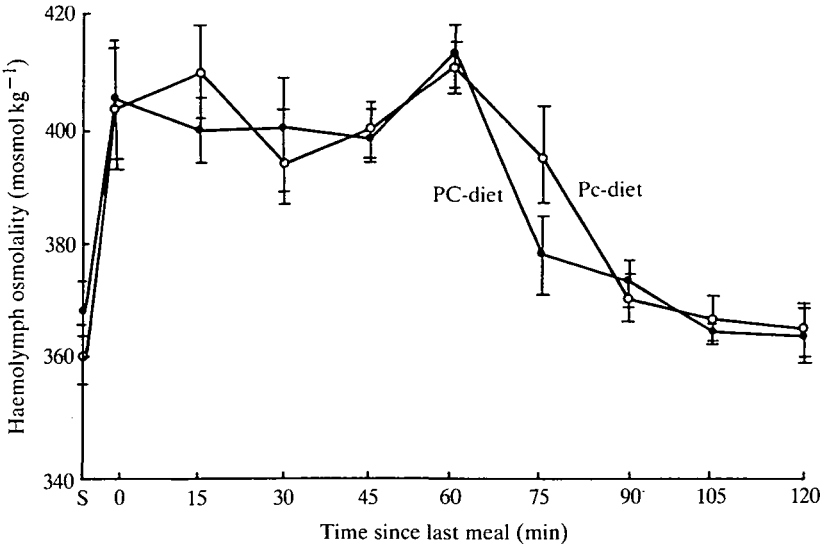

Fig. 7. The change in haemolymph osmolality for insects fed either the PC (●) or Pc (○) diet, $N = 5–7$. Bars represent ±S.E.M.

much carbohydrate per meal as the insects fed on the low-carbohydrate diet. It appears, therefore, that carbohydrate absorbed from the gut was removed from the haemolymph extremely rapidly, probably to the fat body. Rapid removal of absorbed carbohydrate from the blood has previously been demonstrated by Treherne (1958) in *Schistocerca gregaria*. Here, absorbed glucose was rapidly transported to the fat body. It seems that in the present case, trehalose was not subsequently released into the blood from the fat body in quantities sufficient to produce an osmotic difference between insects fed the Pc- or PC-diets.

The fact that carbohydrate differences in the diet produced no change in blood osmolality may explain why compensatory feeding behaviour was not observed. This is not to say that locusts do not regulate carbohydrate intake. Such compensation has been demonstrated for other insects (Gelperin & Dethier, 1967; Gordon, 1968; Nayer & Sauerman, 1974; Bignell, 1978; Nestel, Galun & Friedman, 1985) and locusts which have been fed for 4, 8 or 12 h on a protein-containing but carbohydrate-free diet will select a carbohydrate-containing but protein-free diet when it is offered in a choice test (S. J. Simpson, in preparation). The physiological mechanisms underlying this response are unknown.

There are several possible mechanisms by which a change in haemolymph osmolality and free amino acid content may influence the initiation of feeding. First, they may act directly upon the CNS by altering the output from excitatory centres, as is apparently the case in the slug, where the effect of changes in haemolymph osmolality associated with dehydration act directly upon the central nervous system, producing early termination of feeding bouts (Phifer & Prior, 1985). Second, they may act *via* internal taste receptors, the information being further integrated with output from gut stretch receptors and other changes associated with feeding.

Although osmolality has often been shown to be important in the regulation of feeding, internal receptors monitoring osmolality of key nutrients have never been found in insects. However, there are examples of external taste receptors that are specifically responsive to amino acids, for example on the palps of the cockroach (Sugarman & Jakinovich, 1986) and on the galea of the adult Colorado beetle (Mitchell, 1985), and intestinal chemoreceptors are found in mammals (Mei, 1985). A possibility is that osmolality or amino acid content of the blood influences the sensitivity of external taste receptors, and thus alters the responsiveness of the insect to food stimuli. Such an effect could be mediated *via* the accessory cells or receptor lymph. This mechanism has been proposed for the humoral factor which is released from the storage lobes of the corpora cardiaca after feeding and causes the pores at the end of the terminal sensilla on the palps to close (Bernays, Blaney & Chapman, 1972; Bernays & Mordue, 1973). Recently, Schoonhoven, Blaney & Simmonds (1987) have demonstrated that mouthpart sensilla of lepidoterous larvae vary in sensitivity with rearing diet, deprivation and time of day. Whether haemolymph nutrient levels or osmolality are responsible for this effect is not known. That proteins can cross the accessory cells and enter the receptor lymph has been demonstrated in the blowfly (Phillips & Vande Berg, 1976). However, in the present study it is difficult to see how a change in the sensitivity of external contact chemoreceptors could alter the insect's readiness to feed, since during interfeed periods locusts tend to perch some distance away from the food. Unless olfactory receptors are also affected, an additional mechanism by which the insect is stimulated to move must be involved.

Any of the above mechanisms of action of osmolality and free amino acids upon interfeed interval may be mediated *via* a hormone or another secondary messenger. Described mechanisms of release of humoral factors involved in the postprandial quiescence in locusts (Bernays & Mordue, 1973; Bernays, 1980) could not explain the present results, however, since release from the storage lobes of the corpora cardiaca occurs as a result of gut stretch, and, in the current experiments, meal sizes and gut emptying did not differ with dietary protein.

It is not surprising that levels of blood nutrients are involved in the regulation of feeding behaviour. The blood serves as a repository for stored nutrients, as well as those newly arrived from digested food in the gut. Hence levels of blood nutrients provide an ideal indicator not only of long-term nutrient status, but also of time since, and quality of, the previous meal.

We would like to thank Mr T. Gascoine for his help with the amino acid analysis, and Dr P. Willmer and Professor J. Kennedy for their criticism and discussion.

REFERENCES

BAINES, D. M. (1979). Studies of weight changes and movements of dyes in the midgut and caeca of fifth-instar *Locusta migratoria migratorioides* (R. & F.), in relation to feeding and food deprivation. *Acrida* **8**, 95–105.

BERNAYS, E. A. (1977). The physiological control of drinking behaviour in nymphs of *Locusta migratoria*. *Physiol. Entomol.* **2**, 261–273.

BERNAYS, E. A. (1980). The post-prandial rest in *Locusta migratoria* nymphs and its hormonal regulation. *J. Insect Physiol.* **26**, 119–123.

BERNAYS, E. A., BLANEY, W. M. & CHAPMAN, R. F. (1972). Changes in chemoreceptor sensilla on the maxillary palps of *Locusta migratoria* in relation to feeding. *J. exp. Biol.* **57**, 745–753.

BERNAYS, E. A. & CHAPMAN, R. F. (1973). The regulation of feeding in *Locusta migratoria*: internal inhibitory mechanisms. *Entomologia exp. appl.* **16**, 329–342.

BERNAYS, E. A. & CHAPMAN, R. F. (1974*a*). Changes in haemolymph osmotic pressure in *Locusta migratoria* larvae in relation to feeding. *J. Entomol.* **48**, 149–155.

BERNAYS, E. A. & CHAPMAN, R. F. (1974*b*). The effect of haemolymph osmotic pressure on the meal size of nymphs of *Locusta migratoria* L. *J. exp. Biol.* **61**, 473–480.

BERNAYS, E. A. & MORDUE (LUNTZ), A. J. (1973). Changes in the palp tip sensilla of *Locusta migratoria* in relation to feeding: the effects of different levels of hormone. *J. comp. Biochem. Physiol.* **45**A, 451–454.

BERNAYS, E. A. & SIMPSON, S. J. (1982). Control of food intake. *Adv. Insect Physiol.* **16**, 59–119.

BIGNELL, D. E. (1978). Effects of cellulose in the diets of cockroaches. *Entomol. exp. appl.* **24**, 54–57.

DADD, R. H. (1961). The nutritional requirements of locusts. IV. Requirements for vitamins of the b complex. *J. Insect Physiol.* **6**, 1–12.

DADD, R. H. (1985). Nutrition: Organisms. In *Comprehensive Insect Physiology, Biochemistry & Pharmacology*, vol. 4 (ed. G. A. Kerkut & L. I. Gilbert), pp. 313–390. New York, London: Pergamon Press.

GELPERIN, A. & DETHIER, V. G. (1967). Long-term regulation of sugar intake by the blowfly. *Physiol. Zool.* **40**, 218–228.

GORDON, H. T. (1968). Intake rates of various solid carbohydrates by male German cockroaches. *J. Insect Physiol.* **14**, 41–52.

HUNTER-JONES, P. (1961). *Rearing and Breeding Locusts in the Laboratory*. London: Anti-Locust Research Centre.

MARTIN, M. M. (1983). Cellulose digestion in insects. *Comp. Biochem. Physiol.* **75**A, 313–324.

MATTSON, W. J., JR (1980). Herbivory in relation to plant nitrogen content. *A. Rev. ecol. Syst.* **11**, 119–161.

MEI, N. (1985). Intestinal sensitivity. *Physiol. Rev.* **65**, 211–237.

MITCHELL, B. K. (1985). Specificity of an amino acid-sensitive cell in the adult Colorado beetle, *Leptinotarsa decemlineata*. *Physiol. Entomol.* **10**, 421–429.

MORDUE, W. (1969). Hormonal control of Malpighian tube and rectal function in the desert locust, *Schistocerca gregaria*. *J. Insect Physiol.* **15**, 273–285.

MORGAN, M. R. J. (1976). Gut carbohydrases in locusts and grasshoppers. *Acrida* **5**, 45–58.

NAYER, J. K. & SAUERMAN, D. M. (1974). Long-term regulation of sucrose intake by the female mosquito, *Aedes taeniorhynchus*. *J. Insect Physiol.* **20**, 1203–1208.

NESTEL, D., GALUN, R. & FRIEDMAN, S. (1985). Long-term regulation of sucrose intake by the adult Mediterranean fruitfly, *Ceratitus capitata* (Wiedemann). *J. Insect Physiol.* **31**, 533–536.

PHIFER, C. B. & PRIOR, D. J. (1985). Body hydration and haemolymph osmolality affect feeding and its neural correlate in the terrestrial gastropod, *Limax maximus*. *J. exp. Biol.* **118**, 405–421.

PHILLIPS, C. E. & VANDE BERG, J. S. (1976). Directional flow of sensillum liquor in blowfly (*Phormia regina*) labellar chemoreceptors. *J. Insect Physiol.* **22**, 425–429.

SCHOONHOVEN, L. M., BLANEY, W. M. & SIMMONDS, M. S. J. (1987). Inconstancies of chemoreceptor sensitivity. In *Proceedings of the Sixth International Symposium of Insect–Plant Relationships, Pau, France, 1986* (ed. V. Labeyrie). Amsterdam: Ned Entomol. Ver.

SCRIBER, J. M. & SLANSKY, F., JR (1981). The nutritional ecology of immature insects. *A. Rev. Entomol.* **26**, 183–211.

SIMPSON, S. J. (1982*a*). Changes in the efficiency of utilisation of food throughout the fifth-instar nymphs of *Locusta migratoria*. *Entomol. exp. appl.* **31**, 265–275.

SIMPSON, S. J. (1982*b*). Control of food intake in *Locusta migratoria* L. nymphs. Ph.D. thesis, University of London.

SIMPSON, S. J. (1983). The role of volumetric feedback from the hindgut in the regulation of meal size in fifth-instar *Locusta migratoria* nymphs. *Physiol. Entomol.* **8**, 451–467.

SIMPSON, S. J. & ABISGOLD, J. D. (1985). Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiol. Entomol.* **10**, 443–452.

SIMPSON, S. J. & BERNAYS, E. A. (1983). The regulation of feeding: locusts and blowflies are not so different from mammals. *Appetite: J. intake Res.* **4**, 313–346.

SUGARMAN, D. & JAKINOVICH, W., JR (1986). Behavioural gustatory responses of adult cockroaches, *Periplaneta americana* to D and L amino acids. *J. Insect Physiol.* **32**, 35–41.

TREHERNE, J. E. (1958). The absorption of glucose from the alimentary canal of the locust *Schistocerca gregaria* (Forsk.). *J. exp. Biol.* **35**, 297–306.