THE CONTROL OF MEAL SIZE IN THE BLOOD SUCKING BUG, *RHODNIUS PROLIXUS*

By H. C. BENNET-CLARK*

The Zoological Laboratory, Cambridge

INTRODUCTION

It has already been shown that the abdominal cuticle of 5th-instar larval *Rhodnius* becomes plasticized and consequently less rigid during the early stages of the meal (Bennet-Clark, 1962); and that the epicuticle has become flattened by the end of the meal and probably acts as a limit to further stretching of the abdomen (Bennet-Clark, 1963 a).

The work described here is concerned with the mode of operation of the pharyngeal pump in determining the rate of feeding and the maximum size of the meal.

THE EXPERIMENTAL ANIMAL

Fifth-instar larvae of *Rhodnius*, of unfed weight ranging between 40 and 43 mg., were chosen for this work. Of a batch of 50 bugs, reared by the usual methods, about 20 were within the desired range. These bugs take a meal of about 310 mg. in about 15 min.

The rate of increase in volume of the isolated living abdomen when inflated at various pressures

Methods. The progress of the volume change of the abdomen was measured by applying a known inflating pressure to the inside of the decapitated bug through a calibrated capillary tube containing a column of liquid (Fig. 1). The walls of the capillary were coated with Hopkins & Williams's 'Repelcote' to prevent wetting and consequent uncertainties in the position of the meniscus. With a capillary 35 cm. long, it was possible to measure the passage of 450 mm.³ with an accuracy of 1·5 mm.³.

The pressure supply consisted of an air bottle of approximately 2 l. volume, connected to a mercury manometer. Pressures could be adjusted to an accuracy of about 1 mm. Hg. The change in pressure during the experiment was negligible, the volume of the bottle being far larger than the volume change of the abdomen.

All experiments were carried out at a constant temperature of 35° C., this being the temperature at which blood normally enters the feeding bug.

The experimental procedure was to decapitate the bug and fit it on a tapered glass tube, containing heparinized ox blood, connected through the measuring capillary to the pressure supply. The position of the meniscus in the capillary was adjusted to a convenient point on the scale and pressure was applied. The position of the meniscus was recorded every ½ min. for 10 min. and every minute thereafter. The experiment

* Present address: Department of Zoology, The University, Edinburgh.

was plotted against time in Fig. 4, which shows the course of feeding of four individuals. The skin temperature of the rabbit was found to be 34·7° C., as measured with a thermistor probe of small heat capacity.

In another experiment, as soon as each bug had finished feeding it was killed with
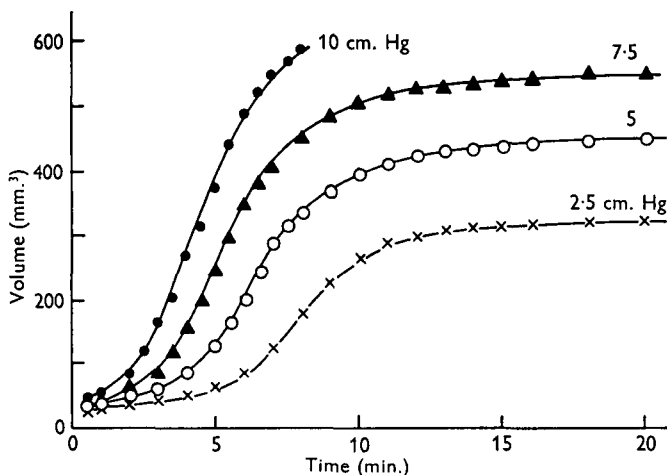

Fig. 2. Graph of the time course of the increase of volume of living 5th-instar *Rhodnius* abdomen when inflated with ox blood at various pressures. —●—, 10 cm. Hg.; —▲—, 7·5 cm. Hg; —○—, 5 cm. Hg; —×—, 2·5 cm. Hg. Each curve represents averaged results for five individuals.
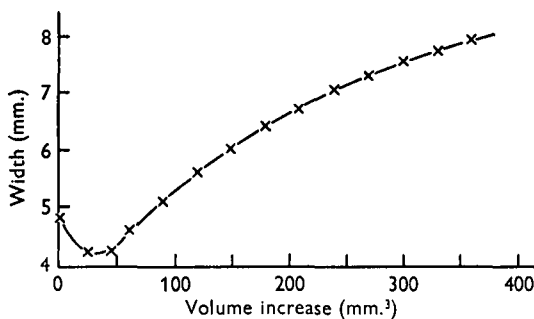

Fig. 3

Fig. 4

Fig. 3. The relation between width of the fourth abdominal segment and volume of blood injected into the abdomen of a 5th-instar larva, at a pressure of 5 cm. Hg.
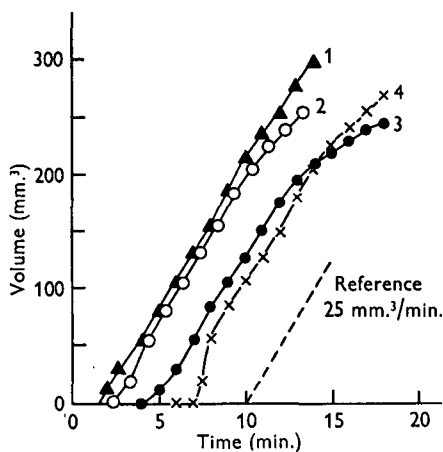
Fig. 4. Increase of volume during feeding plotted against time. Four larvae of 5th instar. Volume changes obtained from measurements of width converted by means of the relationship in Fig. 3. A line of slope 25 mm.³/min. is included for reference purposes.

chloroform and weighed. The estimated figure for the volume increase was multiplied by 1·06, the specific gravity of blood, and added to the original weight of the animal, 40–43 mg. The comparison between the true weight and the estimated weight is

shown in Table 1 and it can be seen that the average of the overall experimental error is under 5%. The method of following the course of feeding is clearly of sufficient precision to show that the rate of feeding is substantially constant throughout the meal.

Table 1

Individual	Width (mm.)	Real weight (mg.)	Estimated weight (mg.)	Error %
1	7·1	298	293	1·8
2	7·2	349	318	9·8
3	7·3	344	330	4·2
4	7·6	365	367	0·5
			Average	4·1

THE PHARYNGEAL PUMP

The anatomy of the pharyngeal pump has been described elsewhere (Bennet-Clark, 1963 b). It consists of a long U-shaped girder, running along the length of the head, with the open end of the U upwards. A piston of similar shape fits into the girder and is attached by its flexible margins to the ridges of the girder. A large muscle runs dorso-ventrally between the rigid central portion of the piston and the roof of the head. When the muscle contracts, the piston is pulled upwards and, on relaxation of the muscle, the piston returns under the elasticity of its lateral regions so as to fit closely inside the girder. The elastic regions of the piston stain strongly in dilute aqueous methylene blue, a staining reaction similar to that of the 'rubber' found in the wing mechanism of locusts, described by Jensen & Weis-Fogh (1962).

A model of the pump

Owing to the small size and 'unorthodox' design of the pump, it was difficult to measure or even to guess at its mechanical properties; a model was therefore made. The body was a U-shaped steel girder and a piston was made of elastic P.V.C. formed to the shape of the girder by heating the whole model to about 150° C. A pair of steel plates, attached to the centre of the piston, represented the region of muscle attachment (Fig. 5). The model had proportions similar to those of the real pump; the width of the base of the U-shaped girder was 6 cm., the arms of the U were 3 cm. high and the distance between the tips of the arms was 7·5 cm., 1·25 times the width of the base. It will be shown later that the latter ratio is of importance in the functioning of the model.

Mechanical properties of the model

The effective area of the piston was determined by drawing outlines of the cross-section of the lumen at unit intervals of movement of the centre of the piston from the girder. These drawings were cut out and weighed. The results are shown in Table 2; it will be seen that the effective area of the piston remains constant over the excursions shown here and thus that the pressure throughout the stroke will be proportional to the applied force. At an excursion of more than 4·4 cm., the girder became distorted and the piston began to stretch along its length.

When the piston is pulled, it moves away from the girder. A plot of movement against load (Fig. 6) shows that, as the load is increased, the piston moves little until, at a load of about 45 gm., it 'clicks' outwards. If the load is then removed gradually,

the piston 'clicks' inwards at a load of about 20 gm. The readings used for Fig. 6 were taken 5 sec. after application of the load to allow a constant amount of plastic deformation of the rubber. To test the effect of altering the geometry of the pump on the load cycle, the arms of the steel girder were bent to various positions and the outwards and inwards 'clicking' forces were measured. Figure 7 shows that, as the width of the open end of the pump is decreased, so the load required to 'click' the piston outwards increases. When the pump girder is splayed open, the asymmetry decreases markedly. The inwards clicking force is scarcely altered by the shape of the girder.
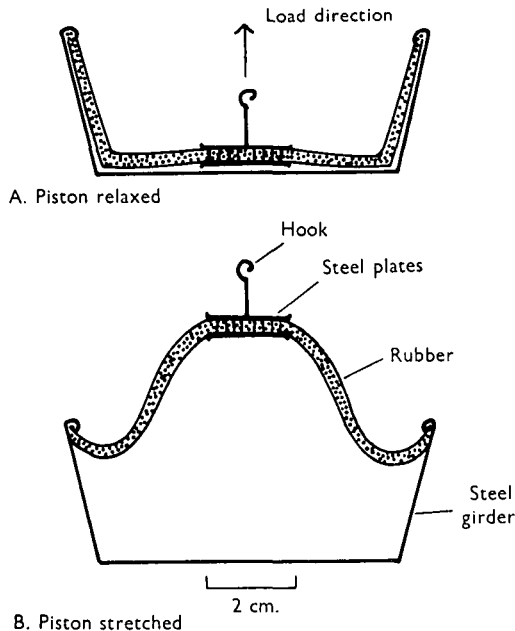

Fig 5. Diagram of a model of a section of the pharyngeal pump of *Rhodnius*. *A*. The piston is shown in the 'relaxed' position; the pump would be empty. *B*. By application of force to the hook the pump would be filled and energy would be stored in the rubber.

Table 2

Distance, piston to girder (cm.)	Weight of section of lumen (mg.)	Weight change per cm. movement of piston (mg.)
0·4	12·4	38
1·4	50·4	37
2·4	87·2	36
3·4	123·2	40
4·4	163·0	

The force required to hold the piston out is far less than that required to pull the piston outwards. The elastic forces opposing the outward movement were measured by finding the load that these forces could just overcome at different points in the stroke. It is clear, from Fig. 6, that at minimum extension the piston force is over 40 gm. and at maximum extension, only 20 gm. Figure 8, plotted by the method just

described, shows that the elastic forces (and hence the internal pressures which they could produce) rise throughout the inwards stroke, although this stroke cannot commence unless the piston load is less than 20 gm.
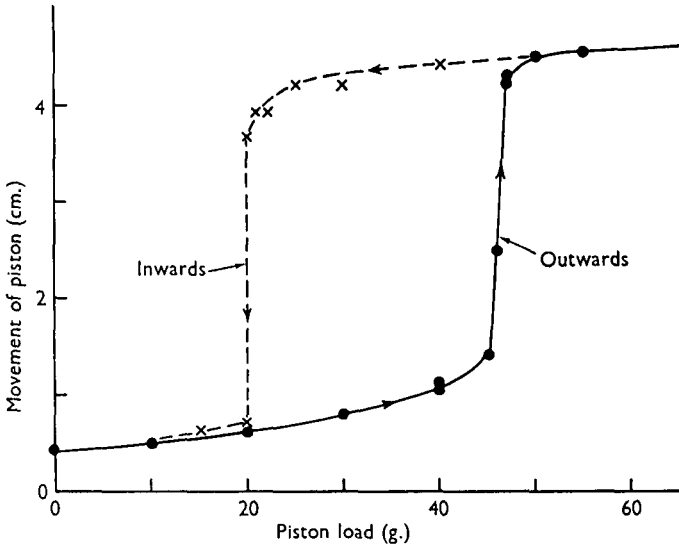

Fig. 6. The strain in the piston of the model pump, expressed as displacement of the piston from the girder, plotted against stress, expressed as the load on the hook on the piston (see Fig. 5). —●—, Outwards travel (the filling stroke); —×—, inwards travel (the emptying stroke).
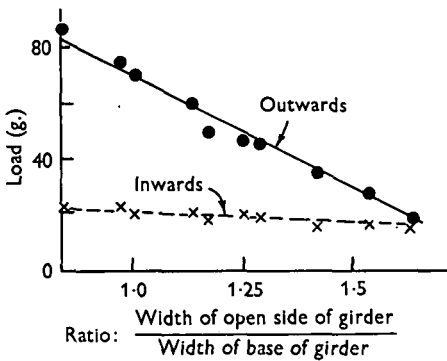

Fig. 7

Fig. 8

Fig. 7. The value of the critical load that will just bring about the 'click' in the model pump plotted against the shape of the girder, expressed as the ratio of width of open side of girder to width of base of girder. —●—, Click in the outwards direction; —×—, click in the inwards direction.
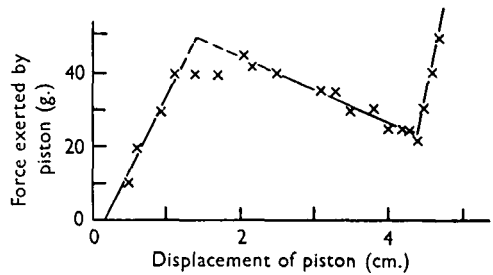
Fig. 8. The force exerted by the elasticity of the piston of the model pump during the inward stroke plotted against the displacement of the piston expressed as the distance from the 'relaxed' position.

The rate of pulsation of the pump in the feeding bug

It is possible to observe the pulsation of the pharyngeal pump through the top of the head of the feeding bug and to record its rate. Figure 9 shows the rate of pulsation against time in one individual. The record is typical in that the rate does not vary by

more than 20% during the major part of the meal but falls suddenly to less than half the maximum rate during the last 10 sec. of feeding, after which the animal withdraws its stylets and walks away.
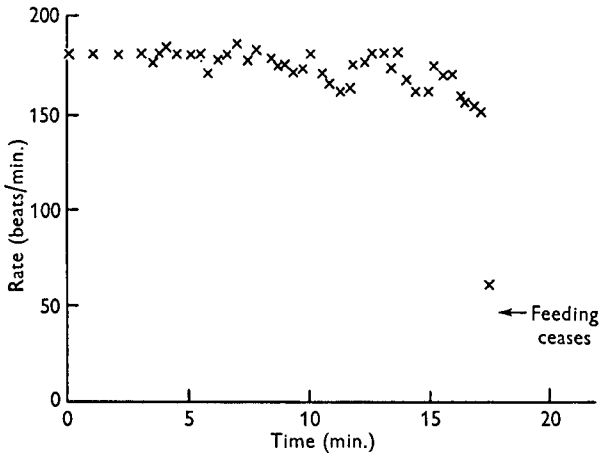

Fig. 9. The rate of pulsation of the pharyngeal pump plotted against time in a naturally feeding 5th-instar larva.

DISCUSSION

It is clear from the observations on feeding bugs (Fig. 4) that the rate of feeding is substantially constant. On the other hand, the observations with isolated living abdomens show that, for a constant pressure, the rate of volume change is not constant (Fig. 2).

It is necessary to consider the various types of limitation that could affect the rate of filling of the abdomen by a pump. If the pump is limited in its rate solely by the pressure against which it is emptying, progress of feeding will be described by a curve of the type seen in Fig. 2. If the pump is limited solely by the maximum rate at which it can fill, the rate of feeding will be constant, but there will be no mechanical limit to the size of the meal. If the pump has a certain maximum power, its rate will depend on the back pressure and hence the rate of pumping into a load such as the abdomen will not be constant. A fourth type of limitation that can be envisaged is where the pump is capable of pumping a constant rate independent of any back pressure below a critical value at which the pump is incapable of emptying.

It is proposed to consider this fourth type of limitation in greater detail, assigning values to the rate of pumping and the critical back pressure. The normal 5th-instar larva takes a meal of about 310 mg. in about 15 min. This is a rate of uptake of about 20 mm.^{3} of blood per minute. It will be seen from Fig. 2 that, at a pressure of 2·5 cm. Hg, an abdomen stretches to a maximum volume of about 320 mm.^{3}. The properties proposed for the pump are that it should pump at a rate of 20 mm.^{3} per minute at any pressure up to 2·5 cm. Hg, at which pressure pumping ceases.

If such a pump is operated against a load such as that provided by the abdomen the course of fluid uptake can be predicted from Fig. 2. Figure 10 shows the way in which this prediction is made. From the origin at *A* to the point *B* at which the

constant rate line cuts the 2·5 cm. Hg constant pressure line (from Fig. 2), the rate of pumping will be rate-limited. Until the slope of the 2·5 cm. Hg line exceeds 20 mm.[3] per minute at C, the rate will be pressure-limited. From point C the rate will again be rate-limited until the 2·5 cm. Hg pressure line is met at D. Thereafter, the rate of pumping will again be pressure-limited and will be very slow. Figure 11 shows how the rate of pumping of the postulated pump varies with time and it will be seen that the rate falls briefly from about the second to the fifth minute and again, more markedly, after about 18 min.
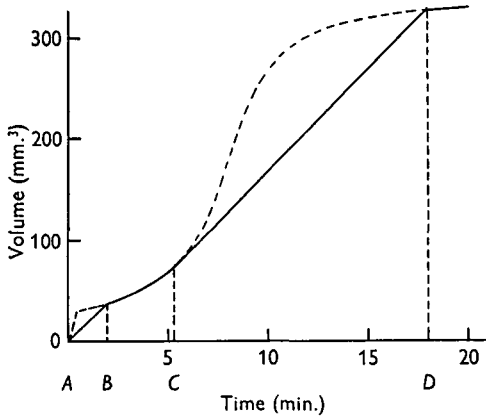

Fig. 10   Fig. 11

Fig. 10. Diagram showing the time course of fluid uptake via a hypothetical pump into a 5th-instar abdomen. The dotted curve is the 2·5 cm. Hg line from Fig. 2. The full line is the curve for a pump assumed to be capable of pumping at 20 mm.³/min. at any pressure up to 2·5 cm. Hg. For further explanation see text.
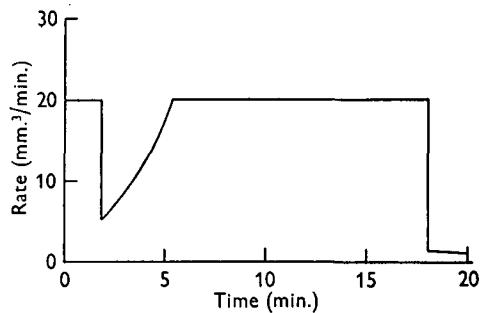
Fig. 11. The rate of fluid uptake plotted against time via a hypothetical pump into a 5th-instar abdomen. The curve is derived from Fig. 10.

The fall in rate between the 2nd and 5th min. is the only major discrepancy between the predicted performance of the pump and its observed performance in naturally feeding bugs (Figs. 4, 9). It is suggested that plasticization is less rapid and less complete in the artificially inflated abdomen than in the feeding bug and this is borne out, in part, by earlier observations (Bennet-Clark, 1962) which showed that free circulation of fluid within the haemocoel was of great importance in ensuring even plasticization of the abdominal cuticle.

The model pump, in so far as it may justly be compared with that of the bug, could not start to empty if the back pressure exceeded a certain value; but once the emptying stroke commences it will be completed, since the elastic emptying force increases throughout the stroke. It has been shown elsewhere (Bennet-Clark, 1963 b) that the impedance of the stylet canal is considerable and it is therefore likely that the process of filling would take up the major part of the cycle. If this is the case relatively large variations in the duration of the emptying stroke will have a relatively small effect on the duration of each cycle of the pump. Thus it seems reasonable to regard the pharyngeal pump of *Rhodnius* as capable of pumping at a constant rate up to a certain pressure.

The foregoing argument can be checked at two points. First, if there is no rise in

back pressure the pump should continue to operate indefinitely. Secondly, if the abdominal stretching is restricted the meal should be both shorter and smaller.

The first point is easily tested by puncturing the abdomen of a bug and then allowing it to feed. Such a bug will continue to feed for many times the normal duration of the meal, while a pool of blood accumulates around it.

The second point can be tested in bugs that have been kept in the incubator for over two months. Such bugs have very unstretchable cuticles and it is observed that they will only take meals of 50–100 mg., feeding for only about 5 min.; but a more direct test can be achieved by providing bugs with a false epicuticle which resists stretching. For lack of suitable 5th-instar larvae, 4th instars were used, with an unfed weight of 15–16 mg. Two bugs were painted across the abdominal tergites with cellulose paint, two were painted across the sternites and three were left unpainted. The bugs were re-weighed after the paint had dried. The average meal size of the painted bugs was 98 mg. and of the controls 126 mg., a ratio of 1 : 1·28. The bugs were all feeding within one minute of the start of the experiment, the painted bugs feeding on average for 16 min. and the controls for 21 min., a ratio of 1 : 1·31. The correlation between meal size and feeding time was high.

In these three observations, there is no evidence that feeding ceases in the absence of abdominal turgor or that attempts were made to continue feeding after a certain degree of turgor had been reached, whether the constraint was naturally or artificially imposed. The stretch receptors described by Wigglesworth (1934) do not appear to exert any control on the size of the meal.

The mechanical control of meal size is not unexpected in view of the close correspondence between the stretching of the endocuticle during feeding and the degree of folding of the epicuticle (Bennet-Clark, 1963*a*). It will have been noted that during the inflation experiments volumes of 500 mm.^{3} were obtained at the higher pressures and that this is not in complete agreement with earlier findings. In fact, although this volume is nearly double that of the typical meal, the amount of cuticular stretching required is only $\sqrt[3]{\dfrac{500}{300}}$ or about 1·2 times greater. It is quite possible that this amount of stretching of the epicuticle could occur after it had become fully unfolded.

In conclusion, it is suggested that the mechanical properties of the cuticle may be important in determining the extent of expansion of insects in general. Cottrell (1962) has shown that in *Calliphora* the pharyngeal pump ceases to pulsate just after expansion has been completed. The correlation between internal pressure and the state of the cuticle in insects is a problem worthy of further investigation.

SUMMARY

1. The object of this investigation was to examine the mode of operation of the pharyngeal pump of *Rhodnius* and its possible role in determining rate of feeding and maximum size of meal.

2. The rate of pulsation of the pump remains constant throughout the meal, falling only in the last few seconds.

3. The pump is filled by muscular withdrawal of the piston and empties by elastic return of the piston.

4. The properties of the pump, which is very small, were studied on a working model. The model displayed marked 'click' properties, the force behind the emptying stroke increasing as the stroke progressed; thus the emptying stroke, once initiated, was always completed.

5. The pressure volume relations of the isolated abdomen were explored as a function of time.

6. It is concluded that the pump is stopped at a critical abdominal pressure of 2·5 cm. Hg., which prevents the initiation of the emptying stroke, that the rate of feeding is mainly limited by the rate of filling of the pump and that the size of the meal taken depends upon the ease with which the cuticle can be stretched.

7. Confirmatory evidence is adduced and discussed.

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