

EFFECT OF DIET VISCOSITY ON THE OPERATION OF THE PHARYNGEAL PUMP IN THE BLOOD-FEEDING BUG *RHODNIUS PROLIXUS*

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SUMMARY

1. The rate of pumping and total number of pump strokes was recorded whilst *Rhodnius prolixus* fed on measured amounts of artificial diets.
2. Increasing the viscosity of the diet caused a decline in both the frequency with which the pharyngeal pump operates and the average stroke volume of the pump.
3. In 5th-instar *Rhodnius*, the stroke volume tends toward a maximum value of about 60 nl at viscosities lower than about 3 cP.
4. Feeding rates (volume per unit time) agree with Poiseuille's law at high viscosities, but are less than predicted at low viscosities.
5. The maximum power output of the pump occurs at a viscosity between 1 and 3 cP, which is probably the range of the effective viscosity of blood in tubes with the dimensions of *Rhodnius* stylets.
6. These results are inconsistent with a hypothesis of a central nervous system 'oscillator' controlling the pump muscle, independent of feedback, but are consistent with a model involving peripheral feedback from stretch receptors, with an appropriate delay.

INTRODUCTION

One of the striking anatomical features of the blood-feeding bug *Rhodnius prolixus* (Stahl) is the highly developed pharyngeal (or cibarial) pump. Its basic features have been described by Bennet-Clark (1963*a, b*). The pump runs from just anterior to the base of the antennae to the posterior margin of the eyes. In this region the floor of the pharynx is a stiff U-shaped channel. The roof is attached to the large set of pump muscles (pharyngeal or cibarial dilators) whose origin is the dorsal cuticle of the head. Contraction of these muscles causes the roof of the pharynx to be raised, thus lowering the pressure in the lumen. The resulting pressure differential between pharynx and the blood at the end of maxillary food-canal forces the blood into the pharynx. Blood flows posteriorly into the oesophagus and stomach when the muscle relaxes, presumably as a result of energy stored in the elastic walls of the pump.

The large size of the pump and its muscle undoubtedly is related to the high pressure necessary to force the diet at a high rate through a food canal with a terminal opening of about 10 μm . Bennet-Clark (1963*a*) calculated that a pump capable of

developing between 2 and 9 atm of pressure is required for a 5th-instar *Rhodnius* to consume approximately 300 μ l of blood in 15 min or less.

Although something is known about the anatomy and general operation of the pump in *Rhodnius*, nothing is known of its control by the central nervous system. It appears to be innervated by the frontal nerve, the cutting of which renders the pump inoperative (Kraus, 1957). There appear to be no descriptions of sense organs such as stretch receptors associated with the pump. It may be that control involves a 'central oscillator', and not necessarily any peripheral feedback, as in many rhythmic patterns of behaviour in insects (Wilson, 1966). Alternatively, control might be described by a peripheral feedback model similar to that of Rice (1970), who has described three stretch receptors on the wall of the cibarium in tsetse (*Glossina austeni*) and the blowfly (*Phormia regina*) which increase their firing rate in response to movements of the cibarial wall similar to those produced by contraction of the cibarial dilator muscle. On the basis of this he proposed a model for the reciprocal action of the two parts of this pump which involved sensory feedback from the dorsal and ventral stretch receptors. When the ventral muscle contracts, firing from the ventral receptor inhibits the ventral muscle but excites the dorsal muscle. This then contracts, stretching the dorsal receptor; the discharge of the dorsal receptor inhibits the dorsal muscle but excites the now-relaxed ventral muscle, thus producing an alternating action, pumping food up the pharynx into the crop. There is, however, no direct evidence that the pump actually operates in this manner, maintaining its rhythm by sensory feedback.

One method of testing whether a 'central oscillator' or a 'peripheral feedback' model is applicable to the neural control of pumping in *Rhodnius* is to change the mechanical loading of the pump, which can be done simply by varying the viscosity of the food. If the pump rhythm depends on a central oscillator, then increasing the viscosity should not affect the pump frequency; assuming the pressure produced by the pump cannot increase substantially, a higher viscosity diet would mean a slower filling of the pump and thus a decreased stroke volume. In contrast, the peripheral feedback model should allow the stroke volume to be maintained as the viscosity increases, at the expense of pump frequency, which should decline.

The development of a method for monitoring feeding activity using changes of electrical resistance (Smith & Friend, 1970), and the ease with which *Rhodnius* can be induced to gorge on artificial diets containing the phagostimulant adenosine triphosphate (Friend & Smith, 1977) have allowed these alternatives to be investigated here.

MATERIALS AND METHODS

Insects used were 5th-instar *Rhodnius prolixus*, taken from a laboratory colony, and kept in humid incubators. Experiments were not conducted until at least 15 days after ecdysis, during which time the animals were not fed.

Diets were made up in glass-distilled water. All diets had a base of 0.1 mM ATP in 0.15 M NaCl, which normally induces 90–95% of test insects to gorge (Friend & Smith, 1971*b*). To increase viscosity of the diet, dextran (mol. wt. 60000–90000) was dissolved in the saline in various amounts; it had previously been determined that even high concentrations of dextran did not affect the readiness of *Rhodnius* to feed. Diets were presented at 36 °C in artificial feeding chambers (Smith & Friend, 1977).

Diet viscosity was measured with a simple viscometer, consisting of a 20 cm length of capillary tubing attached to the tip of a burette. The burette and most of the capillary were supported vertically in a wide glass tube containing water at 36°C. The capillary passed through a rubber bung closing the end of the water bath. The diet under test was placed in the burette, the tap opened, and the time for a given volume of diet to flow out through the capillary was noted. The system was calibrated with solutions of known viscosity.

Action of the pharyngeal pump was observed using an electrical resistance technique, basically as previously described (Smith & Friend, 1970). One modification was to not use a voltage source in series with the animal; sufficient signal was available by simply connecting the platinum electrodes, one in the thorax and one in the diet, to the input of a Grass P15 high input-impedance preamplifier.

A further modification was to record from the brass mesh on which the animal stood during feeding, rather than from an electrode in the thorax. This produced a much noisier signal due to the additional resistive pathway through the animal and due to foot movements, but, by appropriately filtering the output of the preamplifier with a steep band-pass filter (General Radio Type 1952 Universal Filter), the pump strokes (though not the shape of the resistance changes) could usually be distinguished from the noise. A bandwidth of about 30–200 Hz was usually employed. Most records were obtained in this manner, since it involved no operative interference with the animals, which would therefore feed more readily, and was easier.

For observation of the shape and duration of the changes in signal associated with pumping, a storage oscilloscope and/or a Brush Mark 280 pen recorder were used. For recording the number of strokes of the pharyngeal pump, and the frequency of pumping, the signal triggered the sweep of a second oscilloscope. A signal derived from the sweep circuit was then used as the input to a ratemeter and a pulse counter. The output of the ratemeter was recorded on a chart recorder.

All insects were weighed before and immediately after feeding to determine quantity of diet ingested. Weights were converted to volumes ingested, using the density of the diet which was measured by weighing known volumes of fluid. Only a small fraction of the insects tested with implanted electrodes would feed, and not all of those tested without an electrode produced satisfactory signals. Results were sufficiently similar with the two methods of recording to suggest that normal feeding behaviour was occurring, and so the results were pooled. Ten satisfactory recordings were obtained for each viscosity tested; results were analyzed using standard statistical procedures.

RESULTS AND DISCUSSION

Fig. 1 shows a record of pump activity in a 5th-instar larva of *Rhodnius* feeding on ATP-NaCl. In this record, strokes last about 50 ms and occur at intervals of 160 ms (6.25 strokes per second). Typically, each pumping 'spike' had a double peak, the amplitudes of which were rather variable. The origin of the recorded voltage changes is unknown. They may represent impedance changes, in association with a voltage source such as a junction potential between electrodes and haemocoel or diet, or they may reflect the current due to activity of the pump muscle, in a manner analogous to an electrocardiogram. Without further insight into their origin, the pumping spikes

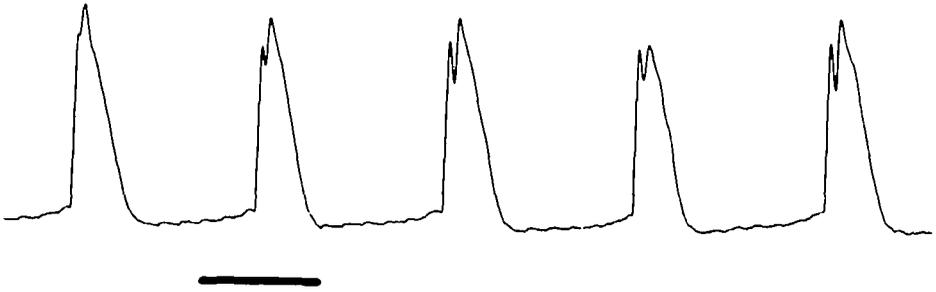


Fig. 1. Record of voltage changes between haemolymph and diet of a 5th-instar *Rhodnius* feeding on 0.15 M NaCl containing 10 mM ATP. Note the characteristic although somewhat variable double peak. Each 'wavelength' of this record corresponds to one cycle of the pharyngeal pump. The animal in this record was pumping at a frequency of just over 6 strokes per second. Scale bar = 0.1 s.

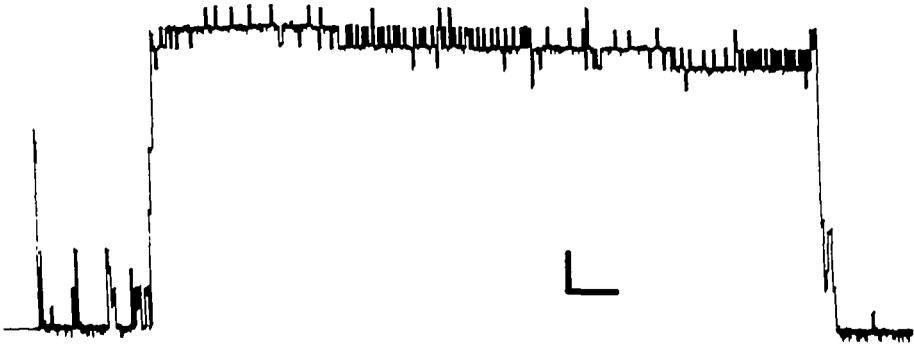


Fig. 2. Record of pumping frequency through a whole meal for a 5th-instar *Rhodnius*. The signal is the output of a ratemeter counting the number of pump strokes in 2 s 'bins' and thus appears as steps with a 0.5 s resolution. Note the early component of the record consisting of about four distinct 'tasting' periods, followed by steady pumping for a further 11.5 min. The pumping frequency declines from about 7.5 strokes per second to just under 7.0 strokes per second over the meal duration. Vertical scale bar = 1 stroke s⁻¹. Horizontal scale bar = 50 s.

will only be used as indicators of pump frequency: a one-to-one correspondence with pump strokes has been established earlier (Friend & Smith, 1971*a*).

The frequency of pumping remained fairly constant throughout the meal although typically a 15–20% decline was seen for a short period towards the end of feeding (Fig. 2). Such a decline was seen by Bennet-Clark (1963*a*), who observed pump activity as a 'pulsation' through the top of the head. The decline may possibly represent an increase in the time to empty the pump as the back-pressure due to stretching of the abdominal cuticle increases; the pressure for emptying will be the difference between the pressure generated by elastic forces in the pump wall, which should remain constant, and the pressure in the abdomen, which increases as the volume ingested increases (Bennet-Clark, 1963*b*). Such an explanation would support the peripheral-feedback model of pump control.

The results of varying viscosity are shown in Table 1. The time taken for a complete meal – that is until the insect voluntarily stops – increased with increasing viscosity

Table 1. Time of feeding, number of pump strokes and volumes of diet consumed by 5th-instar *Rhodnius prolixus* feeding on saline/ATP diets of different viscosities.

(Values are ± 1 S.E., $n = 10$.)

| Viscosity (cP) | Feeding time (s) | Pump strokes | Intake (μ l) |
|----------------|------------------|----------------|-------------------|
| 0.8 | 743 \pm 25 | 5412 \pm 173 | 325 \pm 13 |
| 2.2 | 898 \pm 93 | 5584 \pm 250 | 323 \pm 19 |
| 3.6 | 1073 \pm 33 | 5075 \pm 201 | 267 \pm 13 |
| 4.5 | 1259 \pm 90 | 5742 \pm 284 | 270 \pm 15 |
| 6.5 | 1533 \pm 107 | 5777 \pm 435 | 217 \pm 13 |

of diet, presumably because the power output of the pump muscle is limited. Meal size might be expected to be independent of viscosity, since according to Maddrell (1963), the cessation of feeding is a result of input to the CNS from abdominal stretch receptors. However, the results here show a significant although rather variable trend toward lower intakes at higher viscosities. In contrast, there was no significant change in the number of strokes of the pump for a complete meal as viscosity was increased, although the variability increased somewhat. Although this might suggest a control of meal size by counting pump strokes, animals with the nerve supply to the abdomen cut or with a punctured abdomen continue to feed for longer than normal (Maddrell, 1963), which would necessitate more pump strokes than seen here. The explanation for the decrease in meal size must lie elsewhere, perhaps in a greater readiness to respond to abdominal stretch at the prolonged times seen at higher viscosities.

From the number of pump strokes for a meal and the meal's duration, the mean stroke interval (inverse of pump frequency) was readily calculated and was found to increase linearly with viscosity (Fig. 3). This result cannot be directly interpreted in terms of Poiseuille's law since stroke volume also changed with viscosity (Fig. 4), the relationship being nearly linear above about 3 cP. Values shown in Fig. 4 are mean volumes, calculated for each insect from meal weight and number of pump strokes: it is not known whether stroke volume remained constant throughout each meal. It would appear that just over 60 nl is an average maximum attainable stroke volume, and that this is approached at the two lowest viscosity diets. This value may represent a maximum filling capacity of the pump.

According to Poiseuille's formula, the feeding rate, i.e. the average amount ingested per unit time, should be directly proportional to the reciprocal of viscosity at constant mean pressure. From Fig. 5, it can be seen that Poiseuille's law is followed closely at the three higher viscosities, but that there is a rapidly increasing departure at the two lowest viscosities; the apparent limit on stroke volume seen in Fig. 4 has not been adequately compensated for by an appropriate increase in stroke frequency.

Bennet-Clark (1963*a*) calculated that at a pumping frequency of about 3 strokes per second, the pump muscle may be shortening at a rate of approximately its own length per second. The present results show a stroke volume only about one-half of the 130 nl estimated by Bennet-Clark but a pumping frequency more than double (7 *v.* 3 strokes per second). Since these differences have approximately equal but opposite effects on the calculation of shortening rate, Bennet-Clark's figures may still apply. At rates of shortening in this order, muscle generally produces significantly less force than its isometric tension (Close, 1972), which could provide an explanation of the departure

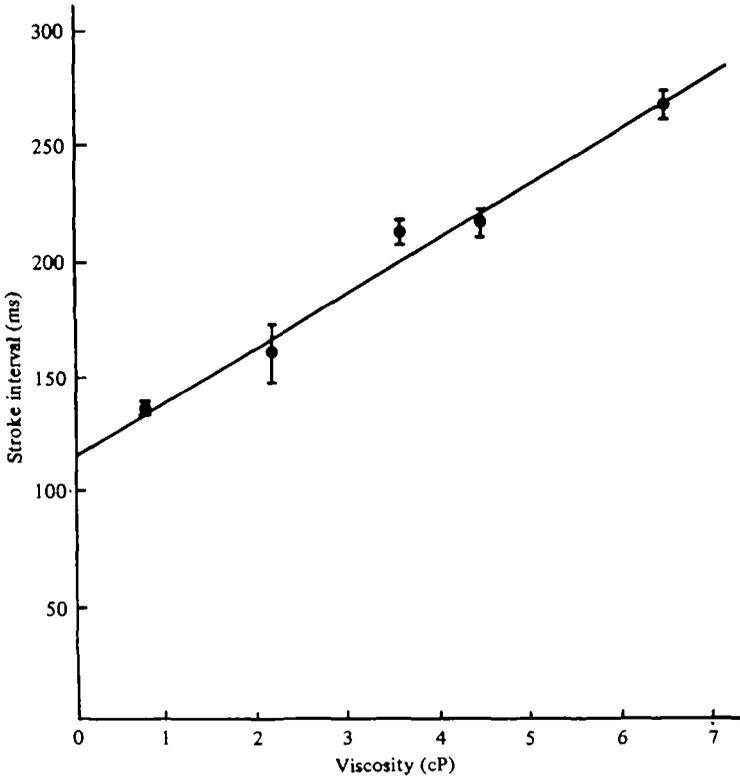


Fig. 3. Interval between consecutive strokes of the pharyngeal pump versus viscosity. Error bars in this and following figures are ± 1 s.e.

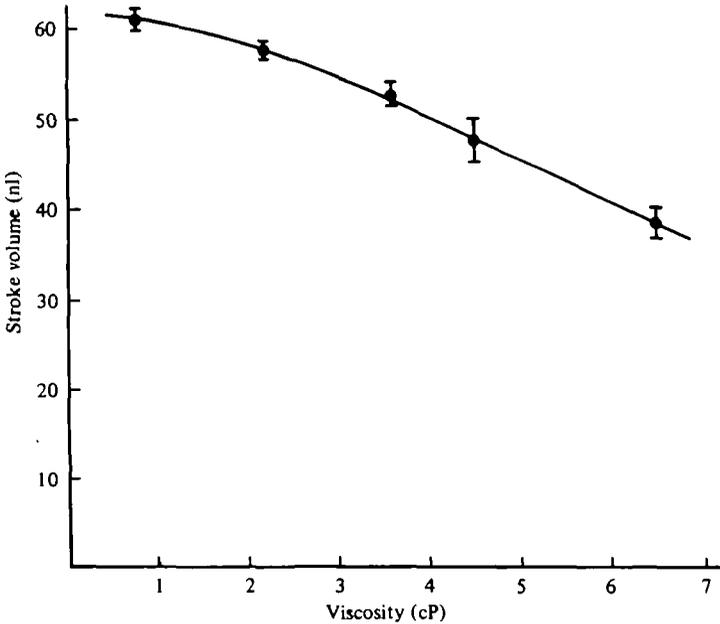


Fig. 4. Stroke volume versus viscosity for the pharyngeal pump. Stroke volumes are means of values calculated for each insect from weight gained and number of pump strokes. The curve is fitted by eye.

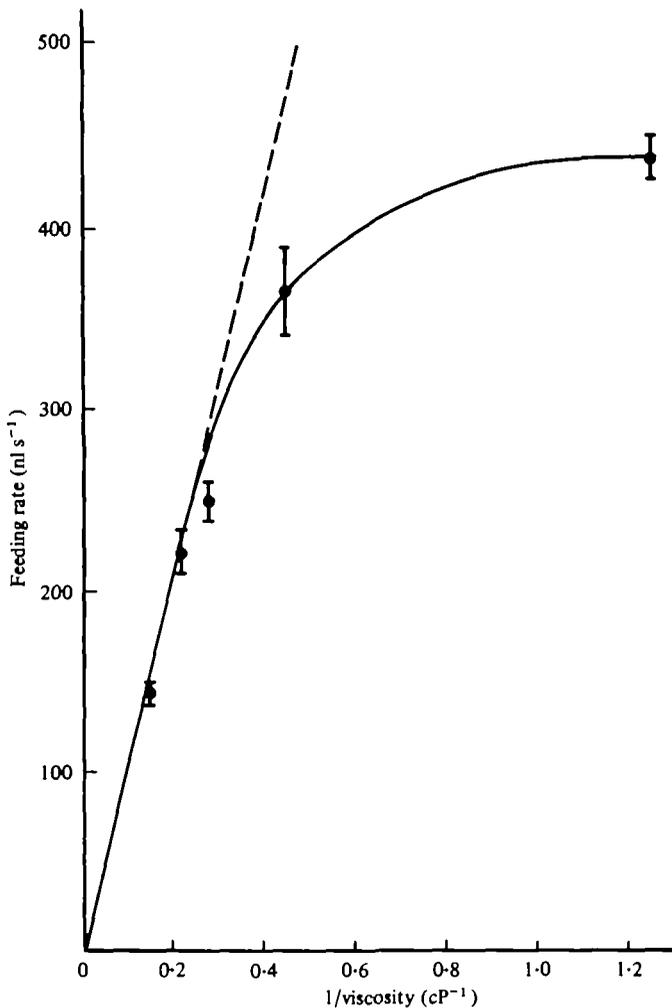


Fig. 5. Feeding rate versus reciprocal of viscosity. Note the increasing departure from the straight line predicted by Poiseuille's law at lower viscosities. The curve is fitted by eye. The line, extended dashed, is fitted through the points at the two highest viscosities tested and through the origin as demanded by Poiseuille's equation.

from the ideal curve in Fig. 5. At the higher pumping frequencies, the load on the pump muscle would be less than ideal, the speed of shortening too great, the tension would decline, and the rate of feeding would be less than predicted because of a reduction in the pressure term in Poiseuille's law.

The useful power output (energy per unit time) of the pump will be proportional to the feeding rate times the pressure produced by the pump. Since pressure is proportional to feeding rate \times viscosity, power is proportional to (feeding rate)² \times viscosity. Calculated values for the power, in arbitrary units, are plotted in Fig. 6. Although too few points are available to fit a curve, it can be seen that the maximum power output is probably at a viscosity between 1 and 3 cP.

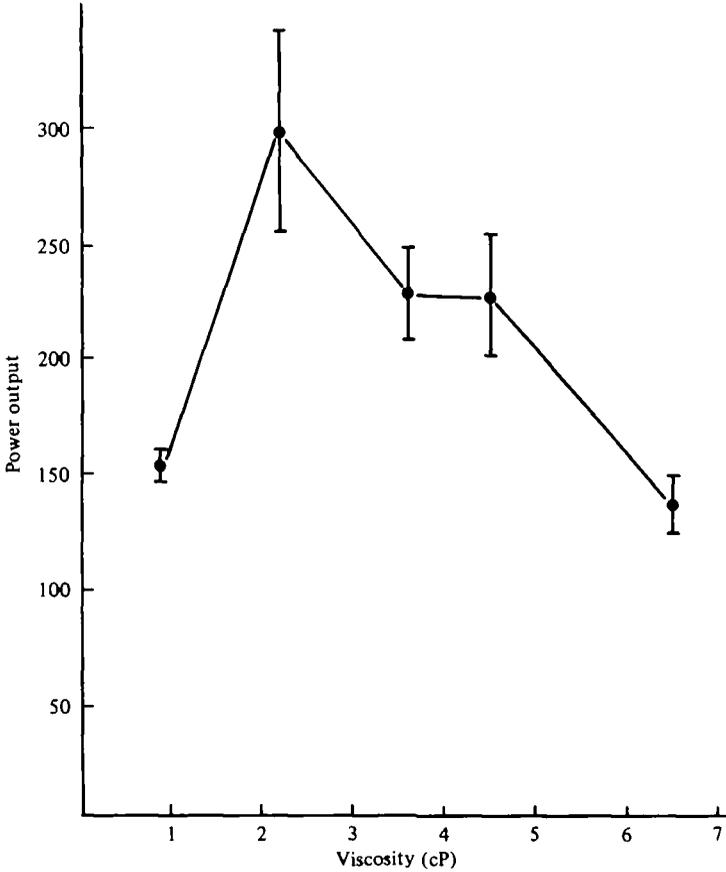


Fig. 6. Power output (in arbitrary units) of *Rhodnius* pharyngeal pump versus viscosity of diet. The trend suggests a maximum value for the power somewhere between 1 and 3 cP.

A highly relevant question here is what is the feeding rate during feeding on blood, the natural fluid? This cannot be accurately predicted from the above results because blood behaves as a non-Newtonian fluid, its apparent viscosity declining when flowing through very small diameter tubes (McDonald, 1960). Unfortunately, when insects were fed on diets of whole blood, it did not prove possible to obtain satisfactory records of pumping whilst maintaining adequate concentrations of cells in suspension. Haematocrits of blood extracted from the stomachs of insects giving adequate electrical records showed less than 10% RBCs. However, the effective viscosity of blood cannot be less than that of plasma, although it may approach this value in tubes less than 50 μm in diameter (McDonald, 1960). The viscosity of plasma has been measured to be between 1.5 and 2 cP by various workers (McDonald, 1960). It is likely then that the viscosity of whole human blood is effectively about 2 cP to a feeding *Rhodnius*. This value coincides with the approximate peak in power output seen in Fig. 6. Not surprisingly, it would appear that the pump of *Rhodnius* is operating close to optimally for fluids the viscosity of blood: the pump just fills on each stroke, and the feeding rate is such that the pump is performing at its maximum power output.

It remains to explore the implications of these results for the proposed model.

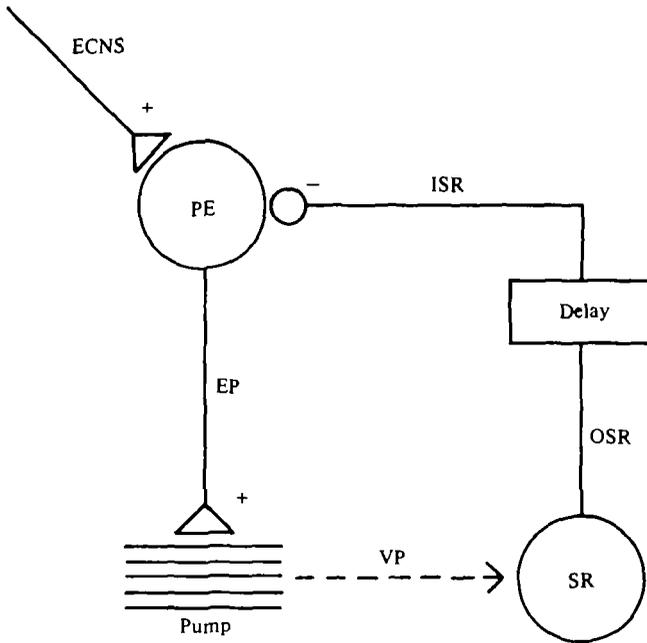


Fig. 7. Model for control of the pharyngeal pump of *Rhodnius* via feedback from a hypothetical stretch receptor. The pump muscle is excited via motor neurones (PE) which receive excitatory input from the brain (ECNS), and inhibitory input (ISR) from stretch receptors (SR) responding to pump volume (VP). The stretch receptor output (OSR) provides the inhibitory input ISR after a delay. For further details see text.

pump control. In each cycle of the pump, there must be contraction (filling) and relaxation (emptying) phases. In addition, there could be 'dead' time, with neither action occurring, although this would seem unlikely at viscosities close to that of blood since it would represent a wasteful inefficiency and prolongation of the act of feeding.

The observation that the frequency of pumping varies over the entire range of viscosities tested is inconsistent with the simple model of a central oscillator, independent of feedback. Although the variation in stroke volume would seem to contradict the peripheral-feedback model, I propose to show that all observed properties are consistent with such a model given a *delay* in the feedback loop. Indeed, delay in the error-correcting signal is one important way in which negative-feedback control loops can oscillate rather than maintain a set value. In the peripheral feedback model, it would be essential to delay inhibitory input to an 'excitation centre' until an appreciable volume increase in the pump had occurred; a rapidly increasing inhibition from a stretch receptor would then turn off excitation, allowing the pump to empty.

The main features of the proposed model are depicted in Fig. 7. Operation of the model can be described by a set of equations for the values of variables at time t . During pumping, the pump excitors (PE in Fig. 7) are activated by an excitatory input, ECNS, assumed to be constant. The output of the pump excitor, EP, is the algebraic sum of ECNS and an inhibitory input ISR:

$$EP = ECNS - ISR.$$

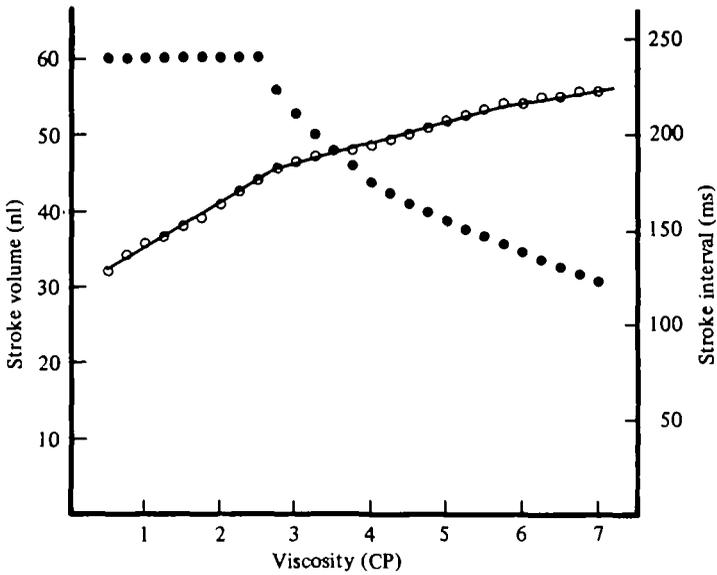


Fig. 8. Stroke volume (closed circles) and stroke interval (open circles) versus viscosity for the model pump. Each data point is the output of a computer program using an algorithm based on the description of the model in the text. Note the correspondence with the experimental values in Figs. 3 and 4. The slight fluctuations seen in the values for stroke interval (open circles) represent the resolution of the program which executes one loop for each time interval; the uncertainty for each point is approximately the diameter of the open circles. The curve is fitted within these limits to emphasize the trend rather than the fluctuations.

With EP greater than zero, the pump muscle contracts with a force assumed to be proportional to EP. Over a small time interval, the volume of the pump changes by an amount ΔVP proportional to EP and inversely proportional to the diet viscosity, VISC:

$$\Delta VP = EP/VISC, \quad \text{for } EP > 0.$$

When EP is zero or less, the pump muscle is relaxed and the pump is emptying; in this case ΔVP is negative and depends on an emptying force proportional to the cube root of the pump volume (VP) as well as VISC:

$$\Delta VP = -F \cdot VP_t^{1/3} / VISC, \quad \text{for } EP \leq 0.$$

The cube root of volume was chosen to represent elastic forces proportional to linear strain on some elements of the pump structure. A variable F allows the emptying rate to be adjusted. The pump volume at time $t + 1$, VP_{t+1} , becomes its value at time t incremented by ΔVP , up to a limit VP_{max} :

$$VP_{t+1} = \text{lesser of } \begin{cases} VP_t + \Delta VP, \\ VP_{max}. \end{cases}$$

The inhibitory feedback is provided by a stretch receptor (SR in Fig. 7) whose output OSR is proportional to the logarithm of the degree of stretch; the logarithmic relationship conforms to the usual transfer function of sensory receptors:

$$OSR_t = G \times \log \text{ greater of } \begin{cases} VP_t - VP_{thresh}, \\ 1. \end{cases}$$

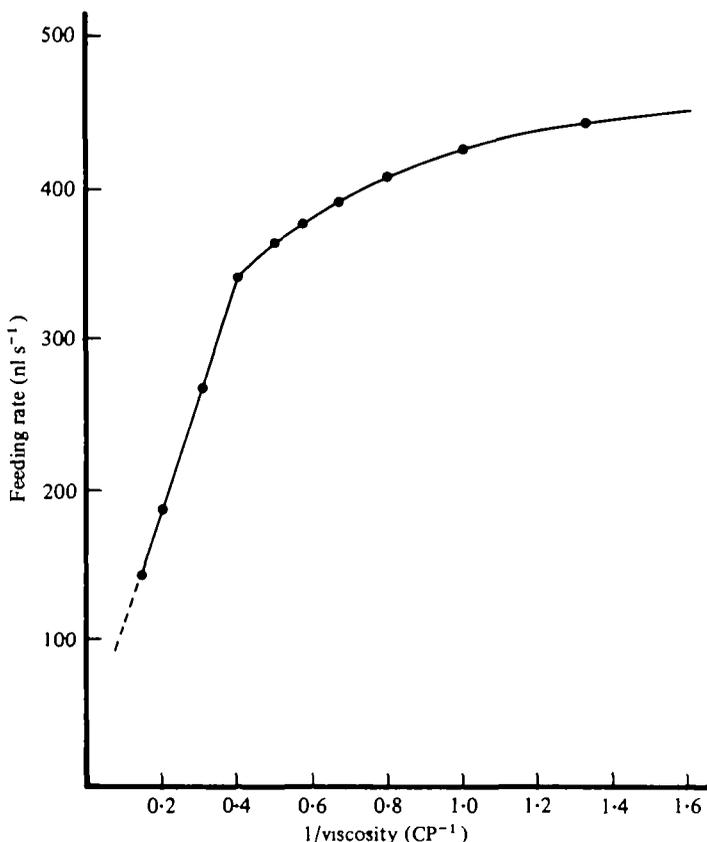


Fig. 9. The 'feeding rate' of the computer-simulated pump model as a function of viscosity. The curve matches that of Fig. 5 in trend, although it does not appear to go through the origin at infinite viscosity.

The model assumes the stretch receptor does not start to fire until a threshold value of the pump volume (VP_{thresh}) is reached. Finally, the inhibitory input to the pump exciters, ISR, is the stretch receptor output at time $t-\Delta t$ where Δt represents a constant delay:

$$\text{ISR} = \text{OSR}_{t-\Delta t}.$$

This description of the proposed model allowed a simple computer program to be written to test the effect of varying viscosity. The program allowed several variables to be altered; these were VISC, the factors F and G, the delay Δt , the level of central excitation, ECNS, and the threshold for stretch receptor firing, VP_{thresh} . The output of the program was a plot of pump volume against time, with an analysis of stroke volumes and intervals.

By appropriate choice of values, the model pump was readily found to oscillate, as expected. Increasing the value for the viscosity increased the stroke interval and decreased the stroke volume. Since the stroke volume of the real pump appears to approach a limit at viscosities less than about 3 cP (Fig. 4), the variables were adjusted until the maximum volume was just reached at $\text{VISC} = 2.5$. The assumption that the

stretch receptor has a threshold means that a variable component to the delay is introduced. At high viscosities, it takes longer for the pump to reach the threshold volume. Setting of a threshold value of 25% of maximum stroke volume for stretch receptor gave a model which behaved very similarly to the real pump (Figs 8, 9).

No attempt was made to model any loss of force at high contraction rates, nor to include the relationship between volume and emptying force proposed by Bennet-Clark (1963*a*), but these features would not have changed the qualitative properties of the model. Indeed, the model matches the observed properties of the pump in *Rhodnius* sufficiently well to explain the pump as an oscillator activated by a constant drive from the CNS with inhibitory feedback from stretch receptors.

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