

NEGATIVE PRESSURES PRODUCED IN THE PHARYNGEAL PUMP OF THE BLOOD-SUCKING BUG, *RHODNIUS PROLIXUS*

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INTRODUCTION

The blood-sucking insect *Rhodnius prolixus* Stål is remarkable for its ability to ingest enormous meals in a brief period of time. In addition it is rarely noticed by the host and this appears partly to be a consequence of the narrowness of the feeding apparatus, which makes very delicate perforations in the skin of the host. In view of the narrowness of the feeding canal and the relatively high viscosity of blood it was of interest to study the hydraulics of the system, with special reference to the forces exerted by the pump musculature.

THE ANIMAL AND THE PROBLEM

For the purpose of this study fifth instar *Rhodnius* larvae were used. The fifth instar bug is capable of taking a meal of 300 mg. of blood in 15 min., a weight increase of over six times the original weight (Buxton, 1930). This represents an average rate of feeding of 20 mg. (approx. 20 mm.³ of blood) per minute.

The stylet canal of adult *Rhodnius* has been described by Lavoipierre, Dickerson & Gordon (1959) who found that it has an apical diameter of less than 10 μ and a proximal diameter of about 60 μ . Confirmatory measurements of freezing-microtome sections, by the author, have suggested that the apical diameter in the fifth instar larva is about 8 μ , that the canal tapers at the rate of 1 μ increase of diameter per 100 μ of length, and that both these figures are valid for all instars.

Regarding the stylet canal as a pipe of diameter 10 μ and length 200 μ (the proximal 5 mm. do not signify in this context) and considering a rate of flow of 0.33 mm.³/sec. (observed) the speed of the current at the apex of the stylet canal must be around 4.4 m./sec. Calculation of the pressure required to force a liquid along a tube at a known rate can be made from Poiseuille's formula:

$$Q = \frac{\pi p r^4 t}{8 l \eta},$$

where Q is the volume moved in cm.³, π is a constant 3.142, p is the pressure difference in dynes/cm.², r is the tube radius in cm., l is the tube length in cm., t is the time in seconds, and η is the viscosity of the fluid in poises. In the present case the following values have been used: $Q/t = 3.3 \times 10^{-4}$ cm.³/sec, $r = 5 \times 10^{-4}$ cm., $l = 2 \times 10^{-2}$ cm., $\eta = 7.2 \times 10^{-3}$ poises (that of water at 35° C.). This gives $p = 1.96 \times 10^6$ dynes/cm.²

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and as $981 \text{ dynes} = 1 \text{ g.}$ the force required to move water at the observed rate is about 2 kg./cm.^2 or about 2 atmospheres.

This figure of 2 atmospheres can be taken as a likely minimum as observation suggests that a more typical diameter for the tube apex is 8μ , the pressure then required being about 4.5 atmospheres. It has been assumed for convenience that blood has the same viscosity as water; no study of the viscosity of blood has been made at pressures and flow rates approaching those found here but the viscosity is likely to be between 1.5 and 2 times that of water (Bayliss, 1952, 1959). It will be seen that the pressure required for *Rhodnius* to feed at the observed rate could be as high as 9 atmospheres and is unlikely to be lower than 2 atmospheres; the interesting feature of the system is that it is a sucking pump that is required to produce this pressure.

Thus the problem is twofold: firstly, whether the structure of the pump and its associated musculature is likely to be capable of producing the forces suggested and secondly whether the supposition that the pump is capable of producing negative pressures is capable of experimental verification.

The anatomy of the pump

The feeding pump of *Rhodnius* and its musculature occupy a large part of the head. In the fifth instar larva, the head is about 5 mm. long and 0.8 mm. in diameter, the eyes being only about 1 mm. in front of the neck. A sagittal dissection displays the body of the pump. It consists of a U shaped girder, about 3.5 mm. long and 0.28 mm. wide, attached along its base to the floor of the head by interstitial tissue, with the open side facing upwards. The moving member of the pump is a piston which is attached by a rubbery ligament along either edge of the girder, and travels from a position at which it fits closely into the inside of the U of the girder to a position about level with the top of the girder. The filling stroke is performed by muscles running from the dorsal wall of the head to the piston, emptying being achieved by the return of the piston under the elastic force of the rubbery ligament (Bennet-Clark, 1962*b*). The vertical travel of the piston is about 0.16 mm. and the calculated swept volume of the pump about 0.13 mm.^3 which agrees satisfactorily with the observation that the pump beats at 3 beats/sec. (Bennet-Clark, 1962*b*) and that the rate of feeding is $0.33 \text{ mm.}^3/\text{sec.}$

The pump musculature runs practically the full length of the head and in transverse section is seen to be about 0.5 mm. wide and 0.5 mm. in effective length, being composed of two blocks, in which the fibres run along a plane about 30° either side of the plane of movement of the piston (Fig. 1). From these data, and the foregoing data on the dimensions of the pump, it is possible to calculate the distance through which the muscle contracts and its rate of contraction.

For a piston movement of 0.16 mm. the muscle must contract $1/\cos 30^\circ$ this amount, 0.185 mm., which is 0.27 of the length, or, at 3 beats/sec. it must move $1.6 \times$ its length/sec. Of this, a half is a passive return to its original position, but if the majority of the cycle is spent in the filling stroke (as seems likely from the nature of the stylet canal and from the nature of muscular contraction, which will be considered shortly) the rate of contraction of the muscle will be 0.8 (or rather more) of its length per second.

The isometric force of locust fibrillar flight muscle is 4 kg./cm.^2 at 35° C. (Weis-Fogh, 1956). Hill (1938) has shown that frog muscle will produce about $1/2$ its

isometric force when contracting at 0.27 its length/sec, 1/4 at 0.35 its length/sec, and about 1/10 the isometric force at 1 length/sec. These figures are for 0° C.; at 20° C., the rate of contraction for a given force is approximately trebled and it seems likely that it will not be lower at 35° C., the temperature of the bug's head when feeding. No figures are available for the dynamic forces of insect muscle but it seems not unjust to assume that the tension at a rate of contraction of 1 length/sec. will not be less than one-quarter of the isometric tension, or about 1 kg./cm.² and that it could be far higher. Clearly the greater the proportion of the pump cycle occupied by the filling stroke, the higher will be the force that can be exerted.

The mean width of the pump muscle is about 0.5 mm. The effective width of the piston is less than the width of the girder, owing to the geometry of movement of the

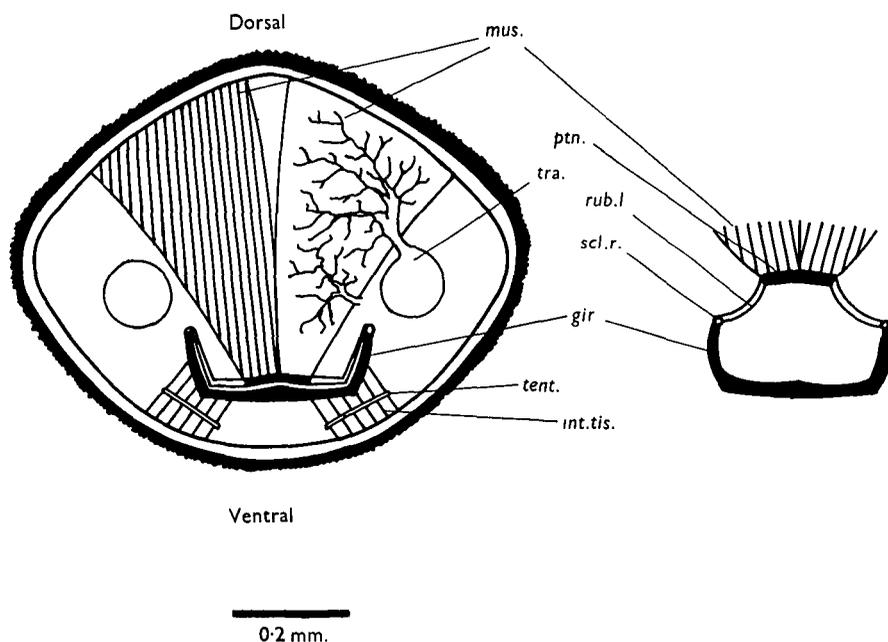


Fig. 1. Diagrammatic cross-section of the head of fifth instar larval *Rhodnius*, cut 1 mm. in front of the eyes, showing the relation between the pump muscles and the structure of the pump body. The mode of operation of the pump is also shown; on the left, the pump muscle is shown relaxed and on the right the muscle is shown relaxed. The diagram is to scale but is considerably simplified. Key: mus., the pump muscle; ptn., the central part of the piston to which the muscle is attached; tra., the lateral trachea of the head; rub.l., the rubbery ligament of the piston; scl.r., the sclerotized ridge running along either edge of the girder; gir., the girder of the pump; tent., the tentorium; int.tis., interstitial tissue running between the girder and the floor of the head.

piston and the rubbery ligaments. The effective width of the piston was calculated by measuring the change in the area of the cross-section of the pump lumen for a known excursion of the piston by the aid of camera lucida drawings. The effective width of the piston is calculated as 0.17 mm. The effective width of the pump muscle is the true cross-section times $\cos 30^\circ$, i. e. 0.43 mm.

With a possible dynamic tension of not less than 1 kg./cm.² it follows that as the pump muscle is around 2.5 times as wide as the piston so the force that it can exert

on the piston will be at least 2.5 kg./cm.^2 , a figure similar to that calculated as being the minimum necessary to cause the observed rate of blood flow in the stylet canal.

Experiments on feeding

Using an electrically heated feeder, similar to that described in a previous paper (Bennet-Clark, 1962*a*), it was possible to control various of the environmental conditions in which the bugs were feeding, while the temperature of the blood was kept constant.

A series of three experiments was devised to test the contention that the pump was capable of producing negative pressures. The rate of feeding was measured under three different conditions, first with the bugs and the feeder at atmospheric pressure, secondly with the bugs and the feeder at reduced pressure, and thirdly with the bugs at atmospheric pressure and the blood in the feeder at reduced pressure. These experiments are represented in Fig. 2.

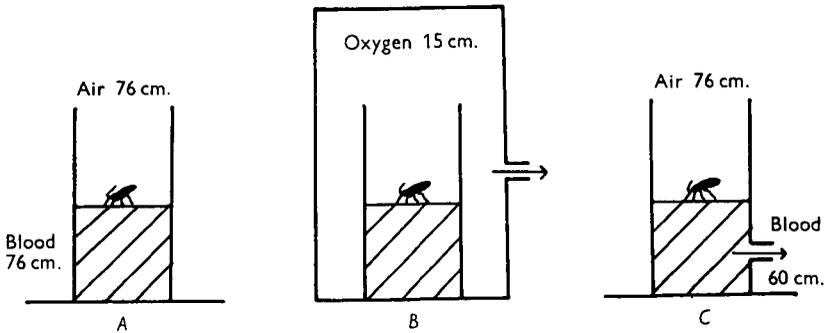


Fig. 2. Diagram of the conditions in the experiments on the rate of feeding of *Rhodnius*. *A*, Bugs and feeder at atmospheric pressure. *B*, Bugs and feeder at 15 cm. Hg. *C*, Bugs at atmospheric pressure and blood in feeder at 60 cm. Hg.

In the first experiment, the feeder was filled with ox blood and five bugs from a homogeneous batch were allowed to feed at atmospheric pressure; the average time of feeding was 20 min. and the average rate of feeding 15.2 mg./min.

In the second experiment, a further batch was placed on the feeder, in a vacuum desiccator, the desiccator was closed and pumped down to about $1/3$ atmosphere, brought back to atmospheric pressure by filling with oxygen and then pumped down to a final pressure of 15 cm. Hg. by which time the bugs had been feeding for 8 min. The pressure was kept at this value until feeding was completed, when the bugs were removed and weighed. The average time taken to feed was 23 min. and the average rate of feeding was 12.5 mg./min. It will be seen that the rate of feeding differs little from that found with bugs at atmospheric pressure; this finding is all the more surprising when the vapour pressure of the blood, around 4 cm. Hg., 35° C. , is taken into account as it then appears that if the pressure reduction in the pump is more than 11 cm. Hg, about 0.14 atmospheres, the liquid in the pump should boil.

If, however, the calculations made earlier on the pressure required for feeding are grossly in error, bugs should find difficulty in feeding from blood at a lower pressure than themselves. In the third experiment a tube was attached to the feeder so that the

pressure in the blood reservoir could be lowered without affecting the pressure around the bugs. Great difficulty was experienced in obtaining a good seal between the feeding membrane and the walls of the feeder, although this did not prove as important a source of leakage as did the holes made by the bugs themselves.

Bugs were unable to feed continuously, as the top of the feeder was apt to fill with air and the air bubbles had to be removed every few minutes. However, with the blood at 60 cm. Hg and the bugs at 76 cm. Hg, a batch of five bugs were allowed to feed for a total of 26 min. in 7 successive periods. They did not commence probing for 2-3 min. at the start of the experiment, and assuming that they did not start feeding after each disturbance for 1 min., the total feeding time becomes 17 min., in which time the average meal taken was 125 mg. The average rate of feeding is 7.4 mg./min., against a pressure gradient of 16 cm. Hg. It is clear, despite the shortcomings of the technique, that bugs are capable of sucking against a considerable pressure gradient.

The fact that bugs are capable of feeding both at reduced pressures and against a pressure gradient suggests that the mode of operation of the pump is anomalous and that the column of liquid within the pump does not break down at its vapour pressure.

The contact angle of cuticle to a blood-air interface

The reason for the failure of the blood to boil in the pump is not clear. Wigglesworth (1953) has shown that bubbles form readily from a supersaturated solution of air in water if the vessel is waxed, though there is little or no bubble formation on the walls of a clean glass vessel. The likely explanation of this is that the wax is not readily wetted and that, as a consequence of the large contact angle of water to the wax in a water-air-paraffin wax system, any bubble that starts tends to expand. Where the surface is readily wetted and the contact angle is low, any bubble will tend to be reduced.

In view of these effects it seemed desirable to measure the contact angles of insect cuticle and paraffin wax to ox blood plasma and to water at about the temperature of blood, 35° C.

The method adopted was derived from that of Beament (1945). A thermoregulated chamber was arranged so that while fresh liquid entered at its base, liquid was being continually sucked away from the surface. The specimen was mounted on a cork which could be swivelled. The advancing contact angle was measured by allowing the liquid level to rise up the side of the specimen, noting whether the meniscus appeared as a level continuation of the main liquid surface. If this was not the case, the angle of the specimen was adjusted and the liquid level raised again. After the specimen had been set, the angle was measured with a microscope with a protractor eyepiece. A similar procedure was adopted, as the level was lowered, to measure the retreating contact angle.

Paraffin wax specimens were prepared by dipping a sliver of glass cover slip in melted 56° paraffin. A flame was played over each specimen immediately prior to use and each specimen was discarded after one pair of readings. Specimens of cockroach cuticle were prepared by washing the fine surface grease away until only the bound basal monolayer remained (Beament, 1959). Specimens were washed until required and discarded after one pair of readings. It was not possible, owing to their minute size, to measure the contact angles of cuticle of the *Rhodnius* pump but the properties

of the pump cuticle appeared, from the imprecise experiments that were possible, to be similar to those of cockroach cuticle.

The contact angles between the different materials and media, at 35° C., were as follows:

	Advancing	Retreating
(a) Water to paraffin wax	112°	102°
(b) Water to cuticle	110°	85°
(c) Ox plasma to paraffin wax	95°	Very low
(d) Ox plasma to cuticle	100°	Very low

A very low contact angle is taken to be one of less than 30° and hence difficult to measure with certainty.

The results indicate that at the temperature at which the bug feeds, the retreating contact angle of cuticle to plasma (and hence of a CH₃ group to plasma) is exceedingly low and suggests that the interior of the pump becomes highly wettable as a consequence of the intake of blood.

DISCUSSION

The experiments described above present evidence for the hypothesis that the sucking pump of *Rhodnius* is capable of exerting a very high tension on the blood on which the bug feeds. The ability to feed at a high rate through a minute tube is clearly of advantage to the bug, which depends for its survival on failing to inconvenience the host. On the other hand, if the fluid within the pump boils as a consequence of the suction the effective pumping pressure cannot exceed one atmosphere.

If the pump is capable of producing the negative pressures postulated, it is desirable to compare its properties with those of other systems where such pressures are encountered. Preston (1952) has considered similar problems in trees, where the rate of flow in xylem vessels is from 0.1 to 0.6 m./sec. and where the tension in the water column appears to be of the order of atmospheres. It appears that so long as the xylem vessels are wetted, the water column does not break down, though once broken it cannot be reformed. This suggests that the nature of the surface and its consequent wettability are of great importance in maintaining the continuity of the column. Measurements of the tensile strength of water have yielded a variety of results but it appears to be at least as high as 40 atmospheres in the static case and somewhat less under dynamic conditions. There does not, however, appear to be any satisfactory physical explanation for the failure of the liquid column to break either in trees or in the sucking pump of *Rhodnius*.

SUMMARY

1. The sucking pump of *Rhodnius* drives blood from the host into the abdomen at a rate of up to 20 mm.³/min. The stylet canal is 8–10 μ diameter at the apex and the rate of taper is not more than 1 μ increase in diameter/100 μ length.
2. The pressure required to force water through such a tube at that rate is about 2 atmospheres. The pressure required with blood would be higher.
3. The musculature of the pump is theoretically capable of exerting a force on the pump piston of at least 2.5 atmospheres, while contracting at the required speed.
4. The bug is capable of feeding at near to the normal rate when the surrounding pressure is 0.2 atmospheres and also against a pressure gradient of 0.2 atmospheres.

5. The retreating contact angle of insect cuticle to a blood-air interface is very low.
6. It is suggested that the high tensile strength of the blood and the low contact angle of the pump walls to the blood are the reasons why cavitation does not occur in the feeding pump of *Rhodnius* when the internal pressure drops below the vapour pressure of blood.

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