J. Exp. Biol. (1963), 40, 763–773
With 1 plate and 9 text-figures
Printed in Great Britain

STUDIES ON CROP FUNCTION IN THE COCKROACH (*PERIPLANETA AMERICANA* L.)

I. THE MECHANISM OF CROP-EMPTYING

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(*Received* 29 *May* 1963)

INTRODUCTION

The absorption of glucose in the midgut of the cockroach is known to be limited by the rate of release of fluid from the crop (Treherne, 1957). The rate of crop-emptying was shown to be linearly related to the concentration of the ingested solution, so that the amount of fluid leaving the crop decreased with increasing concentration. The release of material into the midgut appeared to be related to the osmotic pressure of the meal, for crop-emptying remained constant when widely different substances were tested at the same osmotic concentration. This state of affairs contrasts with that in the mammalian stomach, where the delay in gastric emptying with concentrated sugar solutions seemed to involve more than a simple osmotic effect (Reynell & Spray, 1956). The system in the cockroach crop appeared to operate to prevent the saturation of the absorptive mechanisms in the midgut (Treherne, 1957, 1958).

Nothing is known about the physiological mechanism responsible for controlling the release of fluid from the crop into the midgut. The present paper is the first of a series which attempts an analysis of some of the factors involved in the control of crop function in the cockroach.

METHODS AND MATERIALS

In the X-ray studies starved cockroaches were fed 0·1 ml. of a standard colloidal dispersion of barium sulphate ('Micropaque') containing 0·1 M/l. glucose. The insects were held on to a Perspex plate with short lengths of plasticine placed over the legs. The original radiographs were made at a $\times 2$ magnification, with the insects placed at a distance of 45·0 cm. from the anode. The standard conditions for exposure were: 40 mA., 20 mA. and 40 kV. Exposures were made at 30 min. intervals over an experimental period of 8 hr. In some cases the volumes of the meal and of the air space within the crop were estimated by measuring the areas in the radiographs. This was done by tracing the outlines on transparent paper and cutting out the appropriate areas which were determined by weighing the paper.

In the measurements on crop-emptying starved individuals were fed on an experimental solution containing 8·0 mM/l. of the dye Amaranth (Treherne, 1957). The relative volume of fluid contained in the crop was estimated by determining the

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distribution of dye between the crop and the remainder of the gut. The concentration of the dye was determined in solution at pH 10·0 using a Unicam absorptiometer at an absorption maximum of 510 mμ. As in the previous investigation the insects were killed by immersion in boiling water, a procedure which appeared to produce no violent movement of the gut. In some experiments the mouths of recently fed cockroaches were blocked by dipping the heads in molten polyester wax at 30° C. This procedure involved the entry of wax into the pharynx. In some experiments this entry was prevented by first plugging the mouth with plasticine.

The movements and mode of action of the proventriculus were observed through a small window placed in the dorsal abdominal cuticle. The window consisted of a small piece of cover-glass held in position with a cement composed of a mixture of bee's wax and resin (Krogh & Weis-Fogh, 1951).

The viscosities of the methyl cellulose solutions were determined by measuring the rate of fall of steel ball bearings down fluid-filled tubes 2·5 m. in length. The values used in this paper were calculated from Stokes' law.

The structure and mode of action of the proventriculus

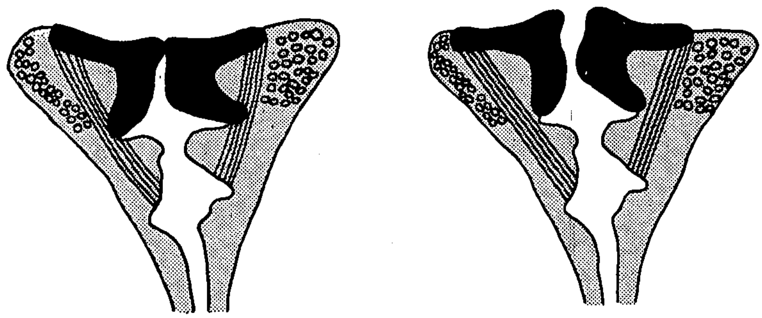
It is obvious that the release of material from the crop into the midgut is restricted by the action of the proventriculus. This function has been demonstrated, for example, in previous experiments on sugar absorption (Treherne, 1957) and in the X-ray studies described in this paper.

The proventriculus of the cockroach consists of an anterior and a posterior portion. The anterior portion bears on its inner surface a series of six large, radially arranged chitinous teeth which, under normal circumstances, entirely occlude the lumen; the posterior portion bears six smaller 'cushions', the intima of which is spinose. The musculature is well developed. Around the six teeth is a heavy ring of circular muscles which extend less prominently over the posterior portion of the proventriculus. Longitudinal muscle fibres extend from the upper and outer edges of each of the teeth to the chitinous surface of the 'cushions'. A diagrammatic representation of the proventriculus is shown in Text-fig. 1. A more detailed description of this structure can be found in the paper by Sanford (1918).

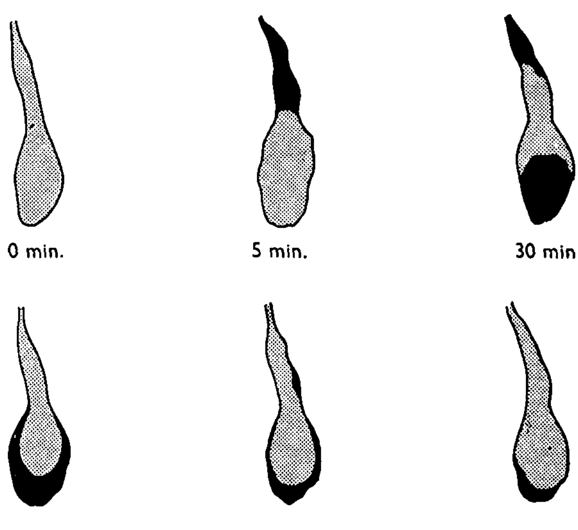
When the foregut and part of the midgut were removed from a recently fed cockroach it was found to be impossible to force the meal past the proventriculus by applying pressure to the crop. The presence of a pharyngeal sphincter can also be inferred from the fact that the meal was not forced through the mouth. When the lower part of the proventriculus was cut away the meal was still held within the crop. When, however, a fine needle was pushed against the teeth from a posterior direction, so as to tilt them forward into the lumen, the fluid ran freely out of the crop. It is suggested that the contraction of the longitudinal muscles of the proventriculus would have a similar action, thus producing the condition represented in Text-fig. 1*B*. In a few of the preparations in which the movements of the proventriculus were observed through a window placed in the abdomen of the insect it was possible to see the teeth of the proventriculus tilt up into the lumen of the crop. Usually this tilting movement was preceded by a distinct rapid contraction of the heavy circular muscle of the proventriculus. The movement of the teeth coincided with a slower, but quite distinct, relaxation of the circular muscles.

X-ray studies on crop-emptying

In these experiments the successive changes in volume both of the whole crop and of the ingested barium meal were followed in a series of X-ray photographs taken at intervals of 30 min. over a period of 8 hr. (Pl. 1, figs. 1, 2; Text-fig. 2). The standard barium meal contained 0·1 M/l. glucose in each case.

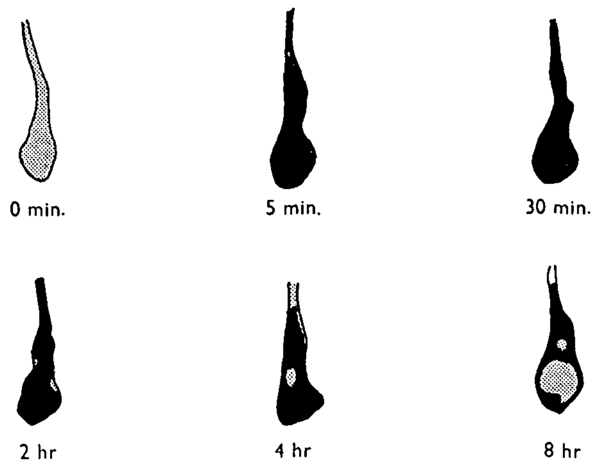


Text-fig. 1. Diagrammatic representation of the proventricular valve in the open and closed positions.

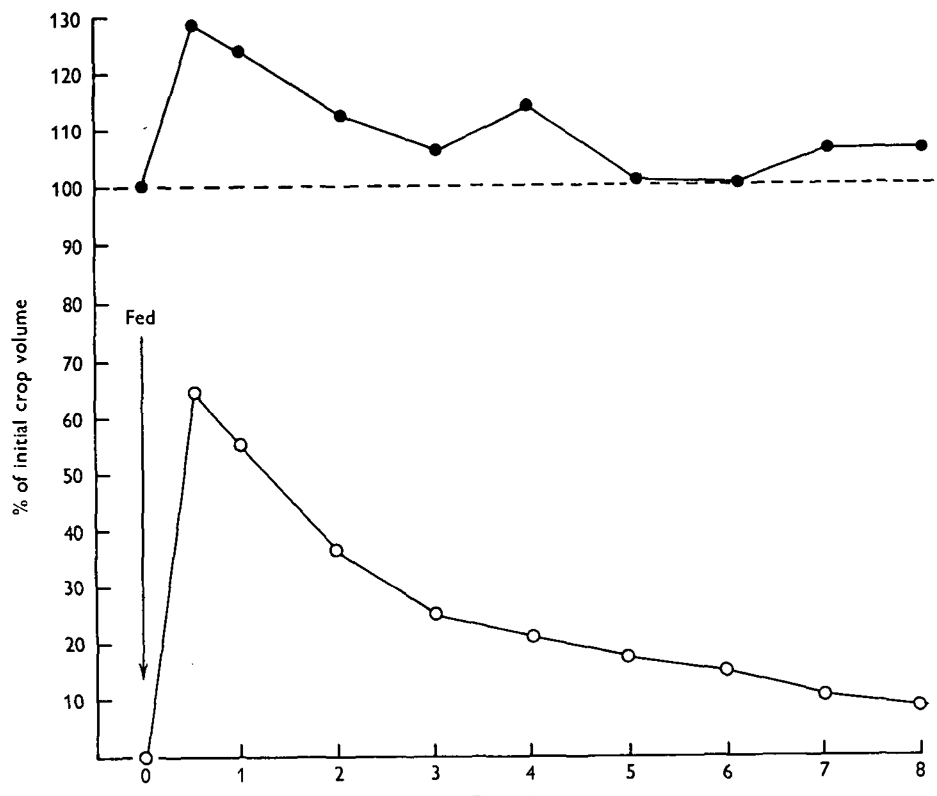


Text-fig. 2. Diagrams of successive stages during the ingestion and subsequent emptying of a barium meal from the crop. The outlines were traced from X-ray photographs. The dark portions represent the space occupied by the barium meal.

Text-figures 2 and 3 illustrate some of the stages during the ingestion and subsequent emptying of a barium meal from the crop. Text-figure 2 represents the typical condition in which the crop contained a relatively large volume of air even immediately after feeding. In both these experiments it is clear, however, that there was only slight change in the total volume of the crop during the whole of the experimental period. In particular it should be noted that the decline in volume of the barium meal as it emptied into the midgut was not paralleled by an appreciable decline in the volume of the whole crop.

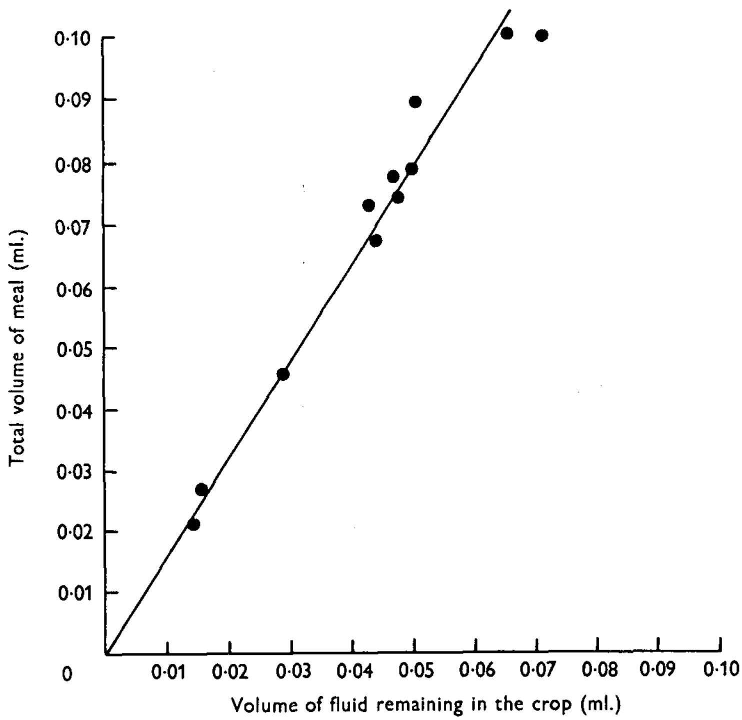


Text-fig. 3. The ingestion and emptying of a barium meal in which the crop contained relatively little air immediately after feeding.



Text-fig. 4. The volume changes following the ingestion of a barium meal, estimated from a series of X-ray photographs. The closed circles represent the volume of the crop, the open circles that of the barium meal.

Text-figure 4 is a graphical representation of the events occurring during the ingestion and emptying of a test meal, the volume of which was approximately estimated by measuring the area occupied by the barium in the X-ray photographs. It will be seen that there was a slight increase above the resting volume of the crop following the ingestion of the barium meal. This increase is, however, less than half the volume of the barium meal itself. Similarly, the decline in the total crop volume was appreciably less than the volume of the test meal. It is clear from these results that the air space in the crop showed a proportional decrease on ingestion followed by an increase as the barium meal was released into the midgut.



Text-fig. 5. The effect of the volume of the meal on the amount of fluid released from the crop during an experimental period of 2 hr. Test solution, 0·5 M glucose.

Crop-emptying of glucose solutions containing dye

Measurements were made of crop-emptying under various experimental conditions with a test solution of 0·5 M/l glucose and 8·0 mM/l. Amaranth. In each case the extent of crop-emptying was calculated from the amount of dye recovered from the crop relative to that contained in the midgut. Text-figure 5 illustrates the effects of various volumes of test meal on the amount of fluid released into the midgut. There was an approximately linear relation between the volume of the meal and the amount remaining in the crop after the experimental period. Thus the proportion of the test meal released through the proventriculus appears to be independent of its initial volume.

Experiments were performed to determine the effects on crop-emptying of blocking the mouth immediately after a test meal. The mouth was blocked in one of two ways, either by directly dipping the head of a freshly fed insect into molten polyester wax (at 30–35° C.) or by plugging the mouth with plasticine before dipping in the molten wax. The latter procedure prevented the entry of wax into the pharyngeal region which occurred with the first method. Table 1 shows the results obtained in experiments with cockroaches treated in this way. Both mouth-blocking techniques appeared to slow down the rate of crop-emptying significantly. The mean value for the individuals with mouths blocked with plasticine and wax was significantly different from those with mouths blocked with wax alone ($P < 0{\cdot}05$). The possible reasons for these differences will be considered in a later section of this paper.

Table 1. *The effects of blocking the mouth immediately after drinking on the rate of crop-emptying during an experimental period of 2 hr.*

Serial	Experimental treatment	% of crop-emptying	Mean ± S.E.
1	Normal	65·0	63·4 ± 1·9 %
2		66·6	
3		61·5	
4		69·1	
5		63·9	
6		54·6	
7	Mouth blocked with wax	83·3	88·4 ± 0·9 %
8		88·1	
9		86·1	
10		86·1	
11		90·7	
12		91·2	
13	Mouth plugged with plasticine then blocked with wax	85·7	79·2 ± 3·7 %
14		64·4	
15		82·9	
16		85·7	
17		77·4	
18		69·0	

Observations on the frequency of opening of the proventricular valve

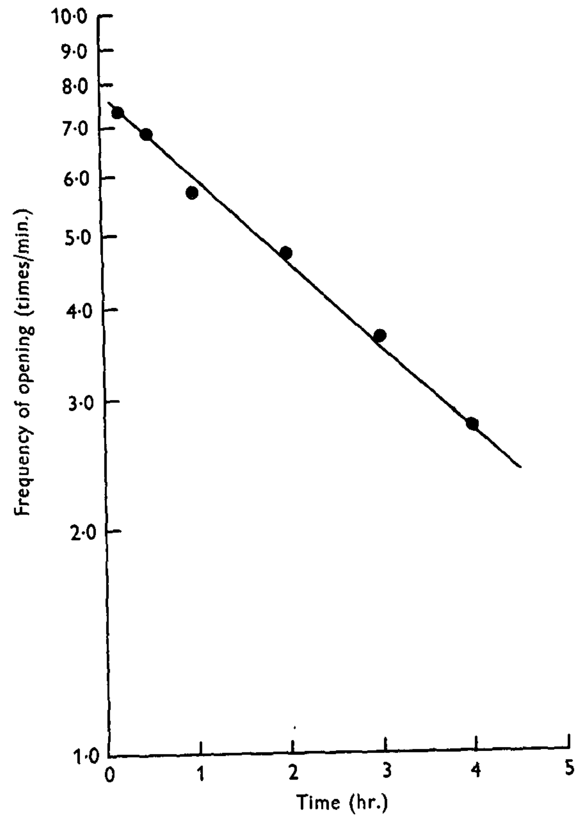
The movements resulting from the contractions of the longitudinal muscles of the proventriculus were observed through a small window placed in the abdomen of individuals which were allowed to feed on solutions of different concentrations of glucose. Text-figure 6 illustrates the changes in frequency of contraction with time in an operated insect which had been fed with a solution of 0·1 M/l. glucose. It will be seen that the frequency of contraction of the longitudinal muscles declined in an approximately exponential manner throughout the period of the experiment.

Text-figure 7 presents the combined data from experiments carried out on operated individuals which had been fed on solutions of 0·1, 1·0 and 2·0 M/l. glucose. These data showed that although the frequencies of contraction were greater in the more dilute solutions they declined at approximately the same rates in the three solutions. These rates of decline of frequency are summarized in Table 2, together with the rate obtained from data on insects fed with distilled water (Text-fig. 8), and are compared with the calculated rates of crop-emptying obtained at these concentrations (data from Treherne, 1957).

Observations on the effects of viscosity on crop-emptying

Some effects of viscosity on the release of fluid from the crop were investigated by feeding starved insects with solutions of glucose containing 2·0 or 4·0% methyl cellulose. The viscosities of the various solutions used in these experiments are shown in Table 3.

Table 4 summarizes the results of experiments on the rate of crop-emptying in



Text-fig. 6. The decline in frequency of opening of the proventriculus in an insect fed with 0·1 M glucose.

Table 2. *Comparison of rate of decline in volume of crop contents with rate of decline in frequency of opening of the proventricular valve at three concentrations of glucose*

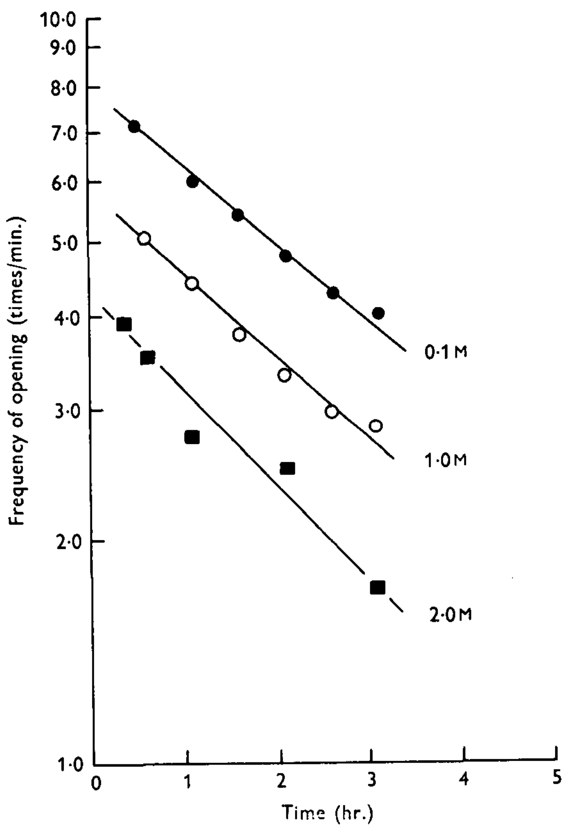
Glucose concentration	Rate of decline of volume* of crop contents (half-time) (hr.)	Rate of decline of frequency of opening of proventricular valve (half-time) (hr.)
0	1·94	2·80
0·1 M	2·50	2·80
1·0 M	4·54	3·00
2·0 M	13·03	2·60

* Data from Treherne (1957).

insects fed with distilled water, 0·1, and 0·5 M/l. glucose containing 0, 2·0 and 4·0% methylcellulose. These figures show the percentage of crop-emptying together with the half-time of the process, calculated from the relation

$$t_{0\cdot 5} = \frac{t \times 0\cdot 3010}{\log_{10} v/v_1},$$

where v is the initial volume of the meal and v_1 that remaining in the crop after time t (Treherne, 1957). It will be seen that in solutions of normal viscosity and in solutions containing 2% methyl cellulose the rate of crop emptying decreases as the concentration of glucose increases; but this decrease is less apparent in solutions containing



Text-fig. 7. Combined data showing the decline in frequency of opening of the proventriculus in insects fed with 0·1, 1·0 and 2·0 M glucose.

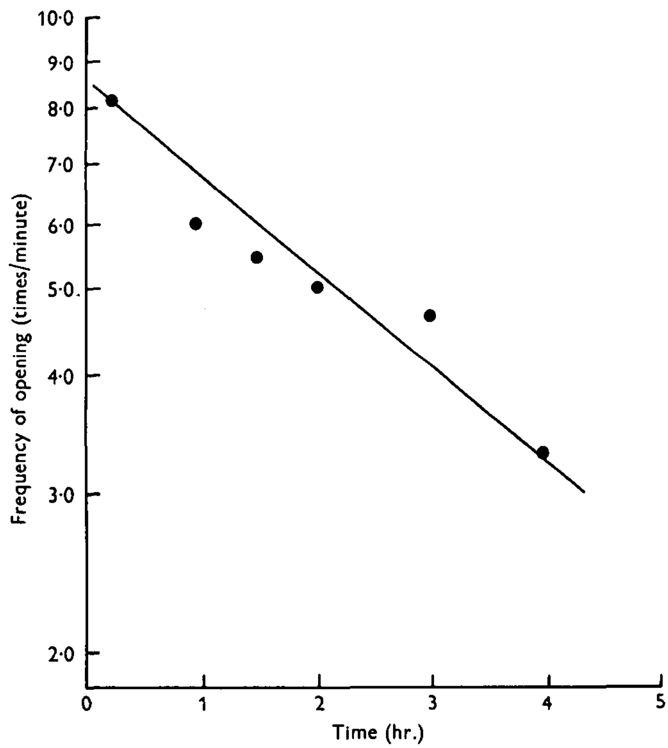
Table 3. *Viscosities of the various experimental solutions used in this investigation*

Solution	Viscosity
0·1 M Glucose	1·03 centipoises*
0·5 M Glucose	1·29*
2·0% Methyl cellulose	58·6
4·0% Methyl cellulose	563·7

* International critical tables.

4 % methyl cellulose. The rate of crop-emptying does not appear to be directly related to the viscosity of the ingested solution. With 4·0 % methyl cellulose for example, the half-time of crop-emptying was 3·8 times greater than with distilled water alone, whereas the viscosities of these solutions differed by a factor of more than 500.

Text-figure 9 illustrates a comparison between the decline in the volume of the crop contents and the frequency of contraction of the proventriculus, both plotted against time in 0·1 M/l. glucose containing 4·0 % methyl cellulose. As with solutions of

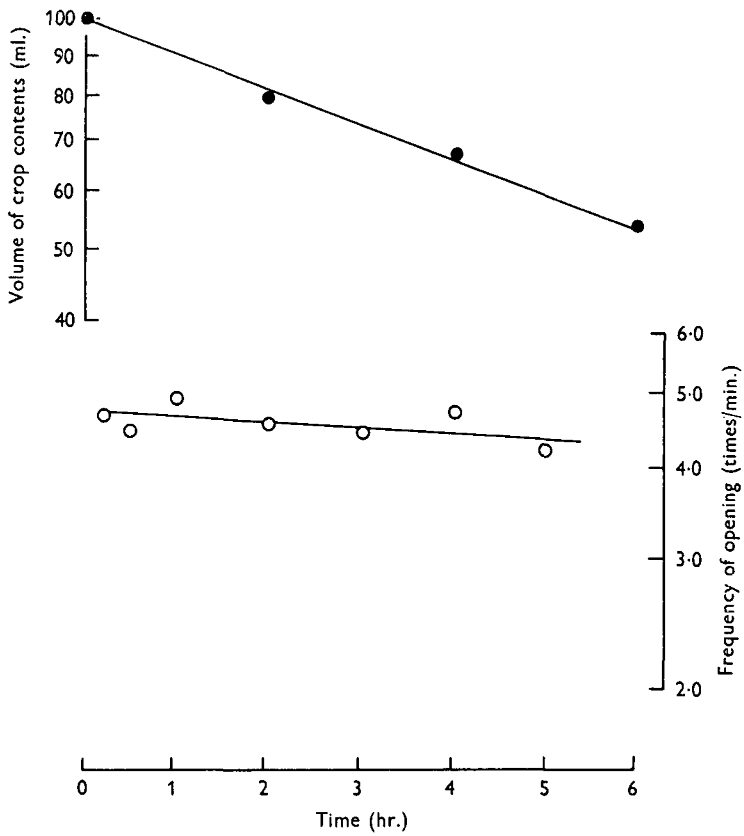


Text-fig. 8. Decline in frequency of opening of the proventriculus in an insect fed with distilled water.

Table 4. *The effects of increased viscosity, produced by the addition of methyl cellulose, on the rate of crop-emptying with ingested solutions of various glucose concentrations*

% Methyl cellulose	Glucose concentration	*n*	Half-time of crop-emptying ±S.E. (hr.)
0	0	4	1·94 ± 0·43
	0·1 M	20	2·50 ± 0·10
	0·5 M	6	3·05 ± 0·35
2·0	0	8	2·67 ± 0·21
2·0	0·1 M	6	4·86 ± 0·72
	0·5 M	11	6·72 ± 0·54
4·0	0	6	7·30 ± 1·31
	0·1 M	6	8·13 ± 0·77
	0·5 M	6	7·78 ± 1·33

normal viscosity, the crop appeared to empty in an exponential manner with a half-time of approximately 8·0 hr.; the frequency of contraction on the other hand tended to remain relatively constant throughout the period of the experiment. This relatively constant frequency of contraction contrasts with that obtained with 0·1 M/l. glucose at normal viscosity where the frequency declined with a half-time of approximately 2·80 hr. (Text-fig. 7). The frequency for the more viscous solution (just over 4·0/min.) was much lower than the initial value of around 7·0–8·0/min. for the solution of normal viscosity.



Text-fig. 9. A comparison of the decline in volume of the crop contents and the decline in frequency of opening of the proventricular valve. The viscosity of the 0·1 M glucose solution was increased by the addition of 4·0 % methyl cellulose.

DISCUSSION

The factors which seem likely to affect the rate of crop-emptying are: (1) the effective dimensions of the valve orifice, (2) the frequency of opening of the valve, (3) its duration of opening, (4) the hydrostatic pressure gradient between the lumen of the crop and the midgut, and (5) the viscosity of the fluid contents. These factors in turn are influenced by the osmotic concentration of the fluid contents (Treherne, 1957). The present paper represents an attempt to elucidate the part played by two of these factors (2 and 5) in the controlled release of fluid of different osmotic concentrations from the midgut.

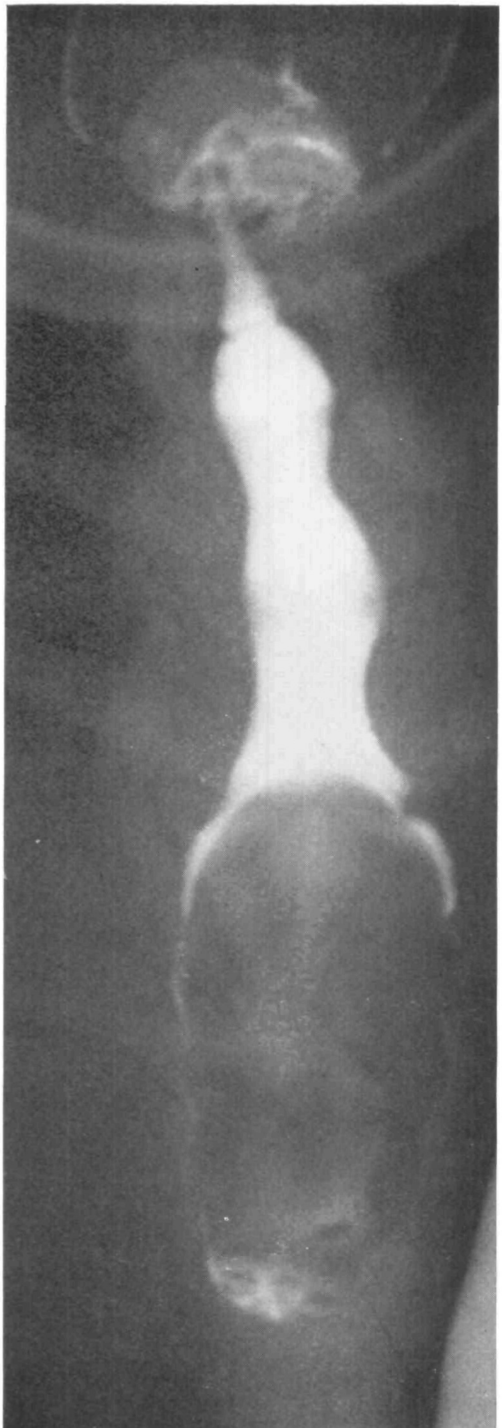


Fig. 1

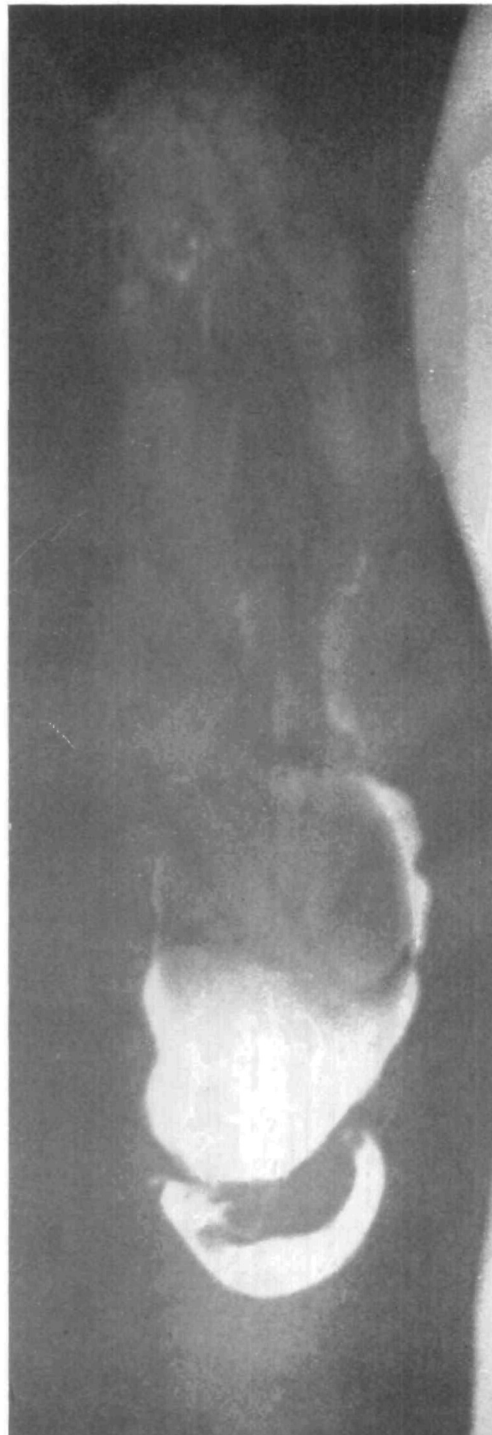


Fig. 2

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The possible effect of viscosity is most easily disposed of. On the basis of flow through a simple orifice obeying the Poiseuille equation an increase of viscosity by a factor of 537·2 (the difference between distilled water and a 4% solution of methyl cellulose) would be expected to decrease the rate of crop-emptying by a factor of $2{\cdot}18 \times 10^{-4}$; in fact the observed decrease was by a factor of only 0·36. It therefore seems unlikely that viscosity has an important effect on the rate of crop-emptying at different osmotic concentrations. There must be changes in some of the limiting factors listed above whereby the changes in viscosity are accommodated.

The frequency of opening of the proventriculus altered in response to changes in the glucose concentration of the ingested fluid so that there is every reason to believe that this factor is of importance in controlling the rate of crop-emptying. However, only at concentrations of about 0·1 M/l. glucose did the frequency of opening of the valve decline at the same rate as did the volume of fluid in the crop. It is clear, therefore, that at other concentrations some additional factors must exert an effect. It is hoped that these other factors may be elucidated in a later study.

SUMMARY

1. The structure and mode of action of the proventriculus are described.

2. X-ray photographs have shown that as the crop empties the decrease in volume of the fluid is partially compensated for by the swallowing of air.

3. The effects of various factors upon the rate of crop-emptying have been studied using solutions of different osmotic pressures. Changes in viscosity, effected by the addition of methyl cellulose, produce only a minor reduction in crop-emptying. The frequency of opening of the proventricular valve is not proportional to the rate of crop-emptying over the whole range of concentrations used, and it is assumed that changes in other parameters must affect the process.

Our thanks are due to Mr R. Akester for his kindness in allowing us to use his X-ray apparatus and to Dr K. E. Machin for several helpful discussions during the course of this work.

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EXPLANATION OF PLATE

Fig. 1. X-ray photograph of a starved cockroach feeding on a barium meal.

Fig. 2. X-ray photograph of same insect 8 hr. after ingesting the test meal. Barium can be seen in the loop of the midgut which passes beneath the crop.