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

III. PRESSURE CHANGES DURING FEEDING AND CROP-EMPTYING

By K. G. DAVEY* AND J. E. TREHERNE†

Department of Zoology, University of Cambridge

INTRODUCTION

Previous studies have shown that there is a differential release of fluid from the crop at different osmotic concentrations (Treherne, 1957) which is partially controlled by alterations in the frequency of opening of the proventricular valve (Davey & Treherne, 1963a). Subsequent investigation has, in addition, demonstrated the existence of a receptor mechanism in the pharynx capable of detecting differences in osmotic concentration together with a nervous pathway involved in the control of the proventricular valve (Davey & Treherne, 1963b). These studies showed, however, that it was necessary to postulate the existence of additional complex changes in the behaviour of the valve or its associated hydrostatic pressure gradient. The present investigation is an attempt to determine more precisely the role of hydrostatic pressure in the passage of fluid from the crop into the midgut and to estimate indirectly the part played by changes in the effective dimensions of the proventricular orifice in these processes. These findings have been related to the possible mechanism of nervous control involved in crop-emptying in this insect.

METHODS

The hypodermic needle (S 28 G) which was connected to the pressure transducer was fastened into the crop by the following procedure. The crop was first exposed by removing a small area of cuticle from the anterior region of the dorsal abdominal surface on one side of the mid-line. A piece of dental wax, previously rolled under pressure on a warm surface to form a thin sheet, was cut to cover the hole in the cuticle. A round hole slightly larger than the diameter of the hypodermic needle, was made in the appropriate position in the wax and the sheet was then sealed over the wound with a warm cautery. By carefully pressing on the abdomen it was possible to manipulate the crop so that it bulged through the hole in the wax. After ascertaining that no nerves were involved, a small hole was cut in the crop and the cut edges were reflected in position by a warm cautery. This procedure left an opening from the surface of the cockroach directly into the crop lumen into which the hypodermic needle was thrust and sealed with wax. For measurements of the pressure de-

veloped in the haemocoel the same technique was used except that the crop was not opened.

The pressure transducer used in these experiments was a super-heterodyne modification, by Dr R. H. J. Brown, of the second capacitance transducer circuit described by Machin (1958). This apparatus was coupled to a Telequipment oscilloscope (D 31) which was used with a Cossor oscilloscope camera. The pressure transducer was calibrated against a water-filled manometer.
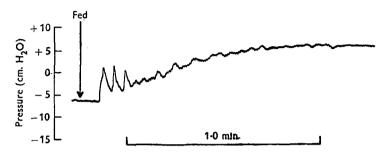

Fig. 1. Oscilloscope record showing the pressure changes in the crop of an insect fed with 1·0M glucose solution.
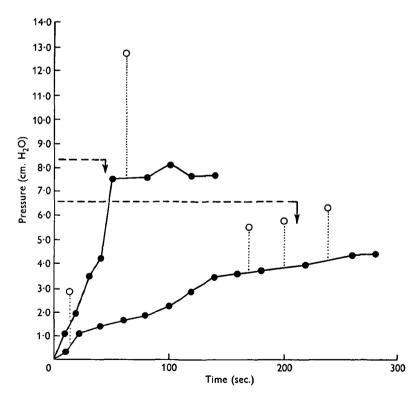

Fig. 2. Graphical representations of the pressure changes measured in the crops of two insects at varying times after feeding them with 0·1 M glucose solutions. The closed circles represent the general pressure level, the open circles the transient pressures attained within the crops. The horizontal broken lines represent the actual periods of feeding observed with these insects.

RESULTS

(1) *Pressure changes in the crop on feeding*

The hydrostatic pressure within the crops of operated starved cockroaches was found to be at, or slightly below, atmospheric pressure (Figs. 1 and 3). Immediately after individuals had been fed with 0·1 or 1·0M glucose the pressure within the crop increased rapidly (Figs. 1 and 2). Frequently this increase was accompanied by appreciable transient rises in pressure within the crop. The general increase in pressure appeared to be confined to the actual period of drinking by the insect (Fig. 2). This increase appeared to be of the same order of magnitude in insects fed with 0·1 or 1·0M glucose (Table 1).

Table 1. *The initial hydrostatic pressure developed in the crop*
after feeding with 0·1 and with 1·0M solutions of glucose

Serial	Glucose concentration	Pressure (cm. water)	Mean ± S.E.
1		7·6	
2		4·6	
3	0·1 M	4·3	5·5 ± 0·7
4		4·3	
5		6·7	
6		7·1	
7		9·5	
8	1·0M	3·8	6·5 ± 1·2
9		8·6	
10		3·6	

(2) *Transient pressure changes in the crop*

At varying times after feeding regular bursts of transient pressure increase were observed (Fig. 3). These increases were not associated with any obvious body movements, which were found to cause large and irregular increase in pressure in these insects. Cutting of the nervous pathway from the pharyngeal region to the proventriculus, demonstrated in a previous investigation (Davey & Treherne, 1963*b*), resulted in complete cessation of the transient pressure increases. There was consider-
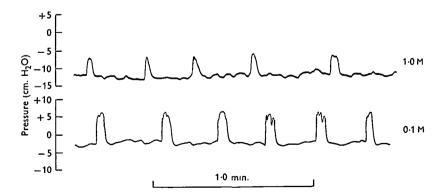
Fig. 3. Oscilloscope records of the transient pressure changes in the crops of insects fed with 0·1 and with 1·0M glucose solutions.

able variation in the wave form of the transient pressure changes, but in general those observed in insects which had ingested solutions of 0·1 M glucose appeared to be essentially similar to those in insects which had been allowed to drink 1·0 M glucose (Fig. 3).

(3) *The hydrostatic pressure in the haemolymph of operated insects*

Fig. 4 illustrates the appreciable negative pressure which was demonstrated in the haemolymph of starved cockroaches. It will be seen that on feeding there was no obvious increase in pressure paralleling that in the crop.
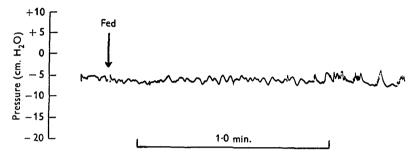

Fig. 4. The hydrostatic pressure measured in the haemolymph of a starved insect subsequently fed with 0·1 M glucose solution.

(4) *The decay of pressure within the crop*

Fig. 5 illustrates the decline in pressure within the crop at various times after feeding operated insects with 1·0 M glucose solution. In this experiment the general
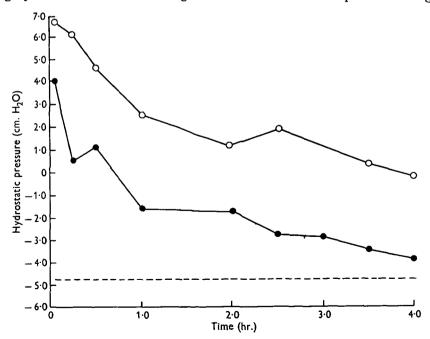

Fig. 5. Graphical representation of the decay in the general pressure level (closed circles) and the transient pulses (open circles) in an insect fed with 1·0 M glucose solution. The broken line represents the pressure level within the crop immediately before feeding.

level of hydrostatic pressure fell below atmospheric pressure after about 1 hr. and eventually approached the initial resting pressure of $-4 \cdot 7$ cm. water. The transient pressure maxima showed a progressive decline but did not fall below atmospheric pressure during the period of this experiment.

The combined data for the decline in pressure within the crop showed that there was no significant difference as between insects fed with 0·1 and with 1·0M glucose (Fig. 6). Semi-logarithmic plots of these data demonstrated that the decay in the transient and general pressure levels within the crop followed an approximately exponential course with both concentrations of glucose (Fig. 7).
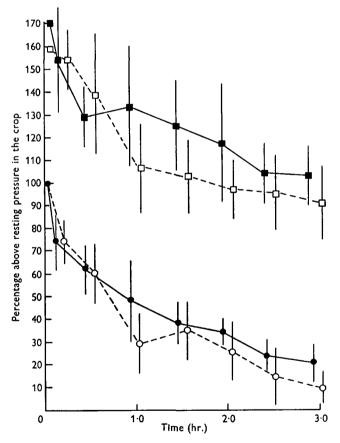

Fig. 6. Combined data for the decay in general and transient pressure levels in the crop of insects fed with 0·1 and 1·0M glucose solutions. The square symbols represent the transient pressures, the circles the general pressure levels in the crop (closed, 0·1 M; open, 1·0M glucose). The vertical lines represent the extent of twice the standard error of the mean.

The release of fluid from the crop into the midgut due to the pressure gradient across the proventricular valve can be described by a modification of the Poiseuille equation:

$$\frac{dv}{dt} = -\frac{\pi}{8\eta}\frac{p}{l}r^4 Tf,$$

where v is the volume of fluid released in time t, r and l the effective radius and length of the orifice of the valve, p the pressure difference across the valve, η the viscosity and T and f the duration and frequency of opening of the valve respectively. The frequency of opening of the valve has been measured in a previous investigation (Davey & Treherne, 1963a) while data have been given in the present study of the decline of pressure within the crop at different concentrations of ingested glucose solution. The
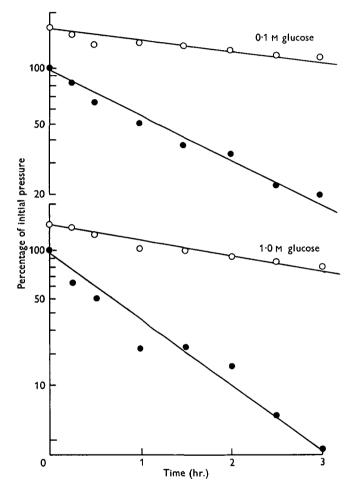

Fig. 7. Semi-logarithmic plots of the data illustrated in Fig. 6 showing the decay in general (closed circles) and transient (open circles) pressure levels within the crops of insects fed with o·1 and with 1·0M glucose solutions.

remaining factors in the above equation give a description of the behaviour of the valve at any given viscosity. A valve parameter, S, can thus be represented:

$$S = \frac{\pi}{8\eta} \frac{r^4 T}{l}.$$

Figs. 8 and 9 are graphical representations of the data contained in the above equations from experiments on insects fed with o·1 and with 1·0M glucose solutions.
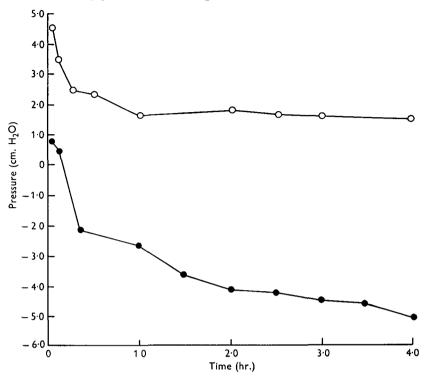
Fig. 8. Decay in general (closed circles) and transient (open circles) pressure levels within the crop of an insect fed with 0·1 M glucose containing 4·0 % methyl cellulose.

Fig. 9. Graphical representation of the data contained in the modified Poiseuille equation, for insects fed with 0·1 M glucose solution. The values for the change of volume of the crop contents are taken from Treherne (1957), those for the frequency of opening of the proventricular valve are from Davey & Treherne (1963 a).

The above results have been calculated from the maximum possible hydrostatic pressure gradient across the valve, that is, at the peak of the transient pressure rises. If the general pressure level is used instead of the transient ones in the above equation then the values of S become much higher and their rate of increase is also greater (8.8×10^{-9} at o sec. rising to 1.4×10^{-7} after 10^4 sec. with the 1·0M glucose solution). It seems clear, therefore, that in all circumstances encountered in these experiments the valve parameter, S, showed an appreciable exponential increase with time.

Fig. 10 illustrates the decline in transient pressure maxima and the general pressure level within the crop in an insect which had been fed with 0·1M glucose solution
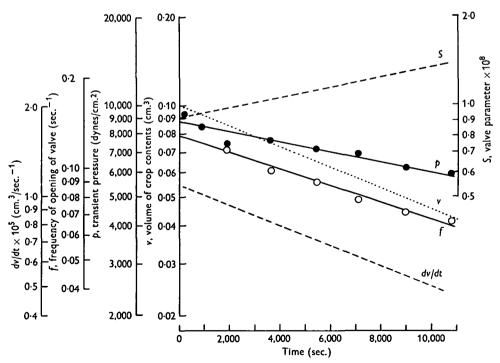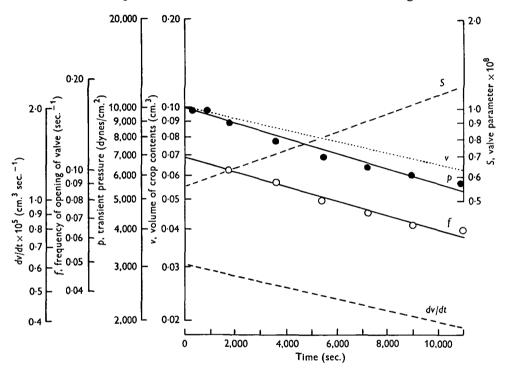

Fig. 10. Graphical representation of the data contained in the modified Poiseuille equation, for insects fed with 1·0M glucose solution. The values for the change in volume of the crop contents are taken from Treherne (1957), those for the frequency of opening of the proventricular valve are from (Davey & Treherne, 1963a).

containing 4·0% methyl cellulose. This concentration of methyl cellulose has a viscosity of approximately 563·7 centipoises (Davey & Treherne, 1963a). The pressure levels attained with this viscous solution were similar to those with the normal experimental solutions. The decay in the general pressure level also took place at a rate similar to that with solutions of normal viscosity; the transient pressure maxima, on the other hand, showed relatively little decline after an initial rapid fall.

DISCUSSION

These results have demonstrated that an increase in pressure within the crop occurs on feeding and that subsequently irregular bursts of transient pressure increases are developed. The initial increase on feeding appeared to be independent of the molarity

of the test meal, for there was no significant difference in the pressure developed on feeding with 0·1 and with 1·0M glucose solutions. Previous X-ray studies have shown that during the whole of the feeding cycle the total volume of the crop remained relatively constant, with only a slight increase on feeding (Davey & Treherne, 1963*a*). It is clear, however, that the pressure increase at feeding did not result from a simple compression of the air contained in the crop. An experiment in the previous investigation showed, for example, that the air space in the crop immediately after feeding was 65% of that in the unfed animal. Thus from Boyle's law:

$$\frac{p'}{p} = \frac{v}{v'} = \frac{100}{65} = 1·53,$$

where p, v, p' and v' are the pressures and volumes of the air space before, and immediately after, feeding. With a crop pressure close to that of the atmosphere this would correspond to an increase of about 547·6 cm. water as compared with the measured values of 5·5–6·5 cm. water. The feeding process must, therefore, involve a significant release of air as the fluid enters the crop through the pharyngeal pumps. Such a release of air can, in fact, be seen in the bubbles of air which appear from the mouths of drinking cockroaches. These results indicate that the general pressure level achieved in the crop immediately after feeding might be controlled in a fairly precise way by the insect.

It is now relevant to consider the nature of the hydrostatic pressure gradient between the lumen of the crop and the anterior part of the midgut. This is obviously affected by the appreciable negative pressure in the haemolymph which, if transmitted to the lumen of the midgut, would tend to increase the gradient across the proventricular valve. In addition the transient pressure changes would also increase this gradient if they corresponded with the opening of the valve. There is no direct evidence of the relationship between the pressure changes and the opening of the proventricular valve. The frequencies of the transient pressure increases were, however, of the same order as those for the opening of the valve measured in a previous study and they also decayed at similar rates (Davey & Treherne, 1963*a*). Cutting of the nervous pathway between the pharynx and the proventriculus, which is known to be involved in the control of opening of the valve (Davey & Treherne, 1963*b*), also resulted in a cessation of the transient pressure changes. On the basis of this circumstantial evidence, therefore, it might be tentatively supposed that the transient pressure changes were related to the opening of the proventricular valve.

The data summarized in Figs. 8 and 9 show that the frequency of opening of the proventricular valve, the transient pressures in the crop, the volume and the rate of change of volume of the crop contents all declined in an exponential manner. The valve parameter, S, on the other hand, increased during the period of the experiments, the initial value being much lower with 1·0M than at 0·1 M glucose. Thus, in addition to changes in frequency of opening of the valve (Davey & Treherne, 1963*a*), variation of the valve parameter can also be reasonably supposed to contribute to the demonstrated control of crop-emptying at different osmotic concentrations (Treherne, 1957).

The actual rate of increase of the valve parameter occurred more rapidly with the more concentrated solution. Such an increase in value of S with time could be due to a longer period of opening of the valve or to an increase in the dimensions of the orifice.

There is no direct evidence to distinguish between these two possibilities—which are, of course, not mutually exclusive.

The present results indicate that there is a fairly standard pressure cycle within the crops of recently fed cockroaches. The general pressure level attained immediately after feeding, and its subsequent decay, together with that of the transient pressure pulses, were not significantly different as between ingested solutions of 0.1 or of 1.0 M glucose. It has already been suggested that the initial pressure may be controlled in a fairly precise way. The fact that the decay in the general pressure level appeared to be fairly constant and not directly related to the rate of decrease in the amount of fluid in the crop suggests that this process may be controlled by some nervous mechanism. The total volume of the crop is also known to remain relatively constant throughout the experimental period despite the fact that fluid is being continuously released into the midgut (Davey & Treherne, 1963 b).

One explanation of the relatively constant decline in general pressure in such a system could be that this was achieved by a differential oral uptake of air. Such an uptake would, according to this hypothesis, be greater with dilute solutions so that the rate of fall in pressure would be similar to that with more concentrated ones in which the decline in fluid volume occurred more slowly. This postulate would serve to explain the earlier observation, which is otherwise difficult to account for, that blocking of the mouth immediately after feeding reduced the rate of release of fluid from the crop into the midgut (Davey & Treherne, 1963 a). Such a system capable of maintaining a relatively constant volume of the crop would most probably involve the action of stretch receptors which have been demonstrated in this organ in other species (Clarke & Langley, 1962).

In a previous study it was shown that opening of the proventricular valve was effectively prevented by severing the nervous pathway between the pharyngeal region and the proventriculus (Davey & Treherne, 1963 b). The demonstration of the approximately constant rate of decay of transient pressure pulses within the crop, which tended to parallel the decline in frequency of opening of the proventriculus, suggests that these two processes may be linked and possibly controlled by a common nervous mechanism. This supposition accords with the observation that cutting of the nervous pathway, besides causing the proventriculus to remain closed, also caused a cessation of the transient pressure pulses within the crop.

In the previous study pharyngeal sense organs were described which, it was suggested, functioned as osmoreceptors forming part of a sensory pathway involved in the control of the frequency of opening of the proventricular valve (Davey & Treherne, 1963 b). Increases in the osmotic concentration of the ingested solution has been shown to produce corresponding decrease in the frequency of opening of the valve, although its rate of decay remained constant throughout the experimental period (Davey & Treherne, 1963 a). The remaining control of crop-emptying at different osmotic pressures appears, from the analyses illustrated in Figs. 8 and 9, to be achieved by variation of the effective dimensions of the orifice of the proventricular valve during the course of the release of fluid from the crop. The increase in the valve parameter, S, with time occurred more rapidly with the more concentrated solution. It is clear that such a complex variation cannot be related directly to the simple exponential decay in frequency of opening of the proventricular valve or the transient pressure pulses

resulting from the output of the pharyngeal sense organs. This complex behaviour must, therefore, be achieved by some further integration of the sensory information derived from the pharyngeal sense organs. The demonstrated nervous pathway from the pharynx to the proventriculus involved both the frontal and the ingluvial ganglia so that it is possible that the postulated integrative processes could take place in these structures.

The experiment with the viscous methyl cellulose solution showed that both the general pressure level and the transient pulses within the crop immediately after feeding were of the same order as those developed in solutions of normal viscosity. The rate of decay in general level of pressure was also essentially similar to that obtained with the normal glucose solutions, although, after an initial rapid fall, the transient pulses tended to remain relatively constant. This again demonstrates a linkage between the pressures attained in the transient pulses and the behaviour of the proventricular valve, in which the frequency of opening has been shown to remain relatively constant with this methyl cellulose solution (Davey & Treherne, 1963 *a*). The general similarity in pressure levels attained with the viscous solution when compared with normal ones indicates that the flow of the methyl cellulose solution through the valve (which occurred very much more rapidly than would be expected with parameters similar to those for normal solutions) must have been facilitated by increase in the effective dimensions of the orifice of the proventricular valve.

SUMMARY

1. The initial hydrostatic pressure developed in the crop on feeding was independent of the osmotic concentration of the ingested glucose solution. It is calculated that the pressure increase was not the result of a simple compression of the air contained in the crop, but that the feeding process must have involved a significant release of air from this structure.

2. The pressure pulses which developed after feeding appeared to be dependent on the same nervous pathway as that determining the frequency of opening of the proventricular valve. They also decayed at the same rate as the frequency of opening of the valve. Such a linkage between the pressure pulses and the opening of the valve might ensure a maximum pressure gradient between the lumen of the crop and the midgut.

3. An appreciable negative hydrostatic pressure was demonstrated in the haemolymph, which, if transmitted to the lumen of the midgut, would facilitate the maintenance of the hydrostatic pressure gradient across the proventricular valve.

4. Both the general and transient pressures within the crop decayed exponentially, there being no significant difference when solutions of different osmotic pressure were used.

5. With a viscous solution (containing 4% methyl cellulose) the pressure changes were of the same order as with normal solutions, except that the transient pressure pulses tended to remain constant after an initial relatively rapid fall.

6. A quantitative analysis, based on a modified Poiseuille relation, showed that the effective dimensions of the proventricular valve increased with time, the initial value of the 'valve parameter' being smaller and the rate of increase more rapid with the

more concentrated solution. It is concluded that variations in the effective dimensions of the valve contribute to the demonstrated control of crop-emptying at different osmotic concentrations.

7. The possible nervous mechanism capable of controlling the release of fluid from the crop is considered.

REFERENCES

CLARKE, K. U. & LANGLEY, P. (1962). Factors concerned in the initiation of growth and moulting in *Locusta migratoria* L. *Nature, Lond.* **194**, 160–2.

DAVEY, K. G. & TREHERNE, J. E. (1963a). Studies on crop function in the cockroach, *Periplaneta americana* L. I. The mechanism of crop emptying. *J. Exp. Biol.* **40**, 763–73.

DAVEY, K. G. & TREHERNE, J. E. (1963b). Studies on crop function in the cockroach, *Periplaneta americana* L. II. The nervous control of crop emptying. *J. Exp. Biol.* **40**, 775–80.

MACHIN, K. E. (1958). Two capacitance transducer circuits. *J. Sci. Instrum.* **35**, 424–5.

TREHERNE, J. E. (1957). Glucose absorption in the cockroach. *J. Exp. Biol.* **35**, 862–70.