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THE ACTION OF THE PROVENTRICULUS OF THE WORKER HONEYBEE, *APIS MELLIFERA* L.

By L. BAILEY

Bee Research Department, Rothamsted Experimental Station

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(With Plate 13 and Seven Text-figures)

CONTENTS

INTRODUCTION

During the investigation of the infection of the ventriculus of the honeybee by *Nosema apis*, information was needed of the passage of pollen grains through the intestine. Some of the work of Whitcomb & Wilson (1929) was repeated for this purpose and results contradictory to those of these workers were obtained, particularly those concerning the action of the proventriculus.

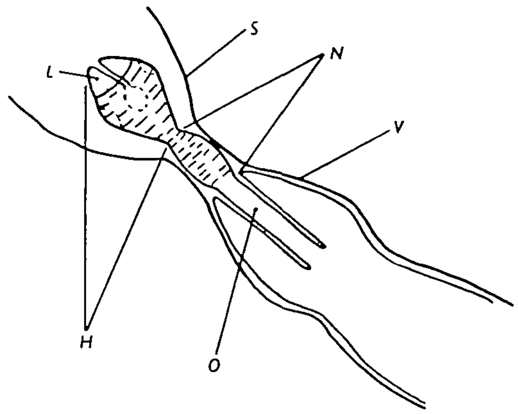
REVIEW OF LITERATURE

The mode of action of the proventriculus has been a subject of some controversy.

Trappmann (1923) gives a fairly detailed account of the histology of the organ. His brief account of its action simply states, however, that the four lips, by means of snapping and sucking movements, lead food back from the crop to the mid-gut. He also states that the proventriculus prevents nectar from flowing back from the crop to the ventriculus in the foraging bee and that it prevents the ventricular contents from being regurgitated into the crop. The latter action is effected by the oesophageal invagination (Text-fig. 1). The following discussion refers only to the part of the proventriculus within the honey stomach (Text-fig. 1—'Head') and the part connecting the honey stomach with the ventriculus ('Neck').

Trappmann's idea that the band of circular muscle around the neck prevents the evagination of the head into the ventriculus indicates that the true action of the proventriculus had escaped his observations.

Snodgrass (1925) does not agree that the proventriculus is a filtering organ. 'It has often been supposed that the proventricular mouth enables the worker to pick out pollen grains from the nectar in the honey stomach and pass them on into the stomach, leaving the nectar to be carried into the hive and stored in a cell. But the worker does not eat pollen while she is engaged in collecting nectar, and if pollen is found in the honey stomach it is only such as was contained in the honey taken from the cell.' This answer simply evades the question. He also states that the movements of the lips 'suggest their picking imaginary pollen grains out of nectar, but the action is probably merely the ordinary process by means of which the proventriculus passes to the ventriculus any kind of food in the honey stomach'. He does not indicate what this 'ordinary process' really is.



Text-fig. 1. Diagram of proventriculus. *L*, lip; *S*, honey stomach; *N*, 'Neck'; *V*, ventriculus; *O*, oesophageal invagination; *H*, 'Head'.

Whitcomb & Wilson (1929) show that the proventriculus is capable of separating pollen grains from fluid in the crop. However, their description of the whole organ thrusting forward into the crop and then withdrawing, combing out the pollen, which is immediately passed back into the ventriculus is undoubtedly erroneous, as will be seen later. Their description of pollen collecting locally around the lips of the proventriculus before being picked out of the crop may have been that of an artifact described later.

Imms (1934) states that the proventriculus serves to pump food from the crop to the ventriculus. This action, as will be seen later, takes place, but it is only part of the function. He speaks of an X-shaped lumen which, guarded by four lips, leads from the crop into the ventriculus. This interpretation of the lumen could be suggested by a study of transverse sections of the proventriculus (Pl. 13, fig. 2), but it is not the permanent shape.

Zander (1922) and Metzer (1910) correctly described the pumping action of the proventriculus and stated that the fringe of hairs on the edge of the lips (Text-fig. 7) acts as a rake, probably allowing nectar to *pass back to the crop* but holding the pollen grains in the cavity of the proventriculus. Their hypothesis is quite correct but they did not develop the idea at all, in spite of the detail which they observed. They were perhaps thinking of pollen being cleared from the nectar gathered by foragers which may have led Snodgrass to deny such filtering activity on the grounds quoted above. Zander describes the lips as being the means whereby the forager prevents nectar from entering the midgut and says that the sucking action of the proventriculus allows the contents of the crop to pass back to the ventriculus in a steady stream. Both these statements are incorrect.

Hejtmanek (1933) mentions that pollen is passed as a bolus by the proventriculus from the honey stomach into the ventriculus. However, his observations were based on the examination of the alimentary canal after it had been extracted from the bee, and so the action of the proventriculus was not seen. He simply states that the chitinous lips form the pollen into clumps.

His statement that the pollen remains in even suspension within the honey stomach and that pollen passes to the hinder end of the ventriculus within a few minutes were confirmed by the experiments described below.

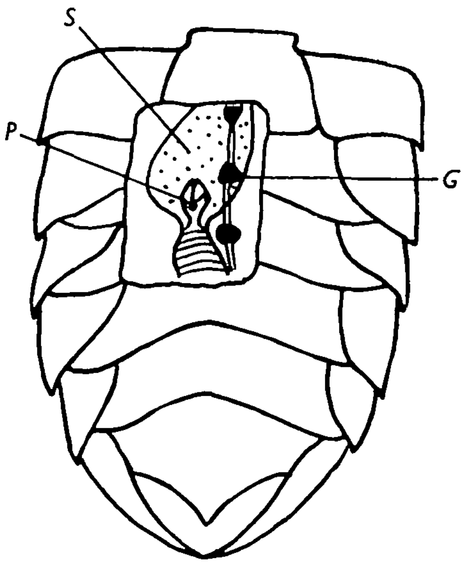
He denies that the concentration and volume of pollen suspension has any effect upon the speed of passage of pollen through the alimentary canal. This is only true for the passage of pollen through the ventriculus and hind-gut, but is not true for its passage through the honey stomach and proventriculus.

MATERIAL AND METHODS

Hazel pollen and pollen taken from the pollen baskets of bees were stained bright red with magenta red, suspended in clear syrup and fed to starved individual worker bees from a capillary pipette. For observations on the passage of pollen down the midgut the whole gut was pulled out of the abdomen of the anaesthetized bee by cutting off the head and pulling out the last abdominal segment with forceps. Careful slitting of the ventriculus with fine needles, or dehydration and clearing in cedar-wood oil, showed the state of the pollen.

For observation of the action of the proventriculus the starved bee was anaesthetized with chloroform or ether and secured ventral side uppermost to a soft wax block.

A window was carefully cut out of the sternites (Text-fig. 2) with a sharp razor. The film of fat body was carefully pushed aside with blunt needles, and the proventriculus was then easily visible when illuminated directly with a focussing lamp.



Text-fig. 2. Ventral view of abdomen showing window dissection; *S*, honey stomach; *G*, ganglion; *P*, proventriculus.

If necessary it could be brought nearer to the surface by hooking round the neck with a blunt, bent, entomological pin. Care was taken not to injure the ventral nerve cord and ganglia. These would usually slip over to one side to allow clear vision.

Occasionally the window was moistened with a drop of saline (0·75 % NaCl, 0·02 % KCl, 0·02 % $CaCl_2$ buffered to pH 6·7 with Na_2HPO_4 and HCl). If the drop of saline was large enough, the illumination of the proventriculus was aided by its additional focusing effect.

THE PASSAGE OF POLLEN DOWN THE VENTRICULUS

(*a*) *Brood-rearing bees*

Bees were collected from the top of the combs during March and April when brood-rearing was in progress and extensive foraging had not yet begun.

The bees were starved for 1 or 2 hr. in an incubator at 33° C. They were then fed with a drop of pollen suspension by holding them upside down by the wings and allowing a drop to fall on to their mouth-parts from the pipette. After feeding they were kept in separate containers in the incubator for definite periods of time, after which their alimentary canals were pulled out and examined.

Table 1. *Rate of progress of pollen down alimentary canal*

Bee	Period after feeding	Position of pollen package
		(*a*) Brood-rearing bees
1	5 min.	Half-way down ventriculus
2	45 min.	Half-way down ventriculus
3	1 hr.	Posterior end of ventriculus
4	3·5 hr.	Three-quarters way down ventriculus
5	3·5 hr.	Posterior end of ventriculus
6	5·5 hr.	Posterior end of ventriculus
7	14 hr.	Posterior end of ventriculus
		(*b*) Nectar-gathering bees
1	1 hr.	Posterior end of ventriculus
2	2·5 hr.	Entering hindgut
3	3 hr.	One-third way down hindgut
4	4 hr.	Three-quarters way down hindgut
5	6 hr.	In rectum

The pollen was usually gathered in the ventriculus in packages surrounded by a tough skin of peritrophic membranes, as described by Whitcomb & Wilson (1929). However, the number of packages and their positions in the ventriculus varied from bee to bee.

In some cases the package would be near the hindgut after about five minutes. In others it would only reach the hindgut after 2 or 3 hr. In one bee, dissected after 14 hr., the pollen was still in the ventriculus even though the rectum was relatively empty for the time of year. No red pollen at all was present in the hindgut or rectum. Many other bees were observed to retain the pollen in the ventriculus for many hours (Table 1 *a*).

On several occasions it was seen that the pollen packages at the hind end of the ventriculus were composed of two clearly demarcated halves. The anterior half was formed of red pollen and the posterior half was unstained. The unstained pollen was obviously that which had been eaten in the hive. (Stained pollen never lost the dye, even when it finally reached the rectum and had been there for a day or longer.) This indicated that the pollen had moved relative to the encircling peritrophic membranes and that the membranes move down the gut at a much slower pace.

(*b*) *Foraging bees*

Later, in May, foraging bees were taken from the flowers where they were working and experiments similar to the above were repeated.

The red pollen in these experiments reached the rectum fairly regularly and in a far shorter space of time (Table 1*b*). The bees were fed the weak suspension of pollen (see § (*c*)).

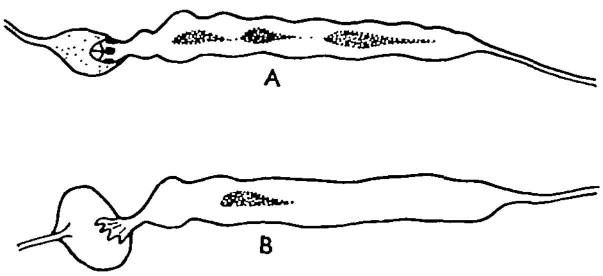
Very few scattered grains of unstained pollen were present and these were principally in the rectum.

(*c*) *Effect of concentration of pollen*

Bees were then fed with different concentrations of pollen, one concentration being much stronger than that used previously.

The more concentrated pollen produced a chain of packages in the ventriculus, some of which reached the hind end of the ventriculus after 4 or 5 min. (Text-fig. 3*a*).

The weaker concentration of pollen generally produced one packet about half-way down the ventriculus after about 20 min. (Text-fig. 3*b*).



Text-fig. 3. Pollen packets in the ventriculus. A. After feeding with concentrated pollen. B. After feeding with dilute pollen.

The bees fed on the weaker suspension had a clear or nearly clear honey stomach after about 20 min.

It seems, therefore, that the bee is capable of filtering pollen from the fluid in the crop. It may have been a weak suspension of pollen which Whitcomb & Wilson (1929) used, requiring but one packet of pollen to clear the crop.

Whitcomb & Wilson (1929) state that the peritrophic membranes in the ventriculus contract round the pollen grains to form the package of pollen. It seemed strange that this action, if it occurs, could form a chain of practically discrete packages. The only organ capable of producing such packages seemed to be the proventriculus itself. When weak suspensions of pollen were fed, it presumably gathered the whole amount of pollen together and then passed it back to the ventriculus. When strong pollen suspensions were fed, a package was passed back long before the crop was clear of pollen, several packages being necessary to achieve this end.

THE ACTION OF THE PROVENTRICULUS

If the gut is pulled out of the abdomen of the bee and laid on a warm slide, the proventriculus will continue to move its lips for some minutes. However, the action is usually weak and fitful.

If, however, a window is dissected out of the abdomen of the bee, as described above, the bee will live for many hours if kept warm and the window kept moist with buffered saline.

The action of the proventriculus is vigorous and regular for periods up to 30 min. or an hour when the dissection is good with minimum damage to nerve cord, tracheae, air sacs, etc. It will probably live for much greater periods, but this has not been necessary for the purpose of the following experiments.

When the bee was fed with pollen suspension the pollen was seen passing into the crop within a second or two and the crop distended rapidly. Pollen became distributed evenly throughout the crop and remained so disposed until only a few grains were left. The crop was seen to be continually writhing and pulsating vigorously. This action was so strong that the pollen grains could be seen moving about inside, and the action obviously kept the crop contents well stirred and the pollen grains evenly distributed. The proventriculus itself never moves as a whole relative to the honey stomach, although it may be moved to some extent by the powerful activity of the crop.

This activity of the crop was rarely seen when the alimentary canal was pulled out of the abdomen and was quickly destroyed if the window dissection was done badly. The organ is obviously unable to exert much, if any, pressure, but a contraction of the branching and anastomosing muscle fibres of the wall (Wigglesworth, 1947) in some areas and a corresponding relaxation in others creates no pressure resistance but provides an admirable stirring movement.

Pollen was seen to accumulate rapidly in the proventriculus. At first this accumulation took place in four 'cheeks' or pouches, and these rapidly filled with tightly packed pollen (Pl. 13, figs. 5, 6). These pouches seemed to separate and then to collapse together, with a gradually decreasing amplitude as the proventriculus filled with pollen. It appeared that the proventriculus was filling and emptying. However, the emptying was not into the ventriculus but back into the crop. (When syrup, which had been stained deeply with gentian violet, was fed to the bee, the filling and emptying of the crop could be seen quite clearly, but the dark ball of syrup in the proventriculus disappeared when the organ emptied and usually did not pass back to the ventriculus. Occasionally, however, a bolus of dark syrup moved down the neck into the ventriculus. On one occasion the proventriculus developed the shorter stroke as when straining pollen, but in this case it was found to be packed with crystals of gentian violet which had not dissolved completely in the syrup.)

After a while the proventriculus was packed with pollen, with a solid core as well as the full pouches, and eventually the whole mass passed as a bolus down the neck into the ventriculus, leaving but one or two grains behind in the pouches (Pl. 13).

The movements of the lips during these processes were asynchronous. They snapped open and closed very rapidly but individually. They appeared to be letting pollen suspension into the proventriculus during the expansion process by these movements and to be guarding against the ejection of grains back into the crop.

No accumulation of pollen in the crop near the lips of the proventriculus, as described by Whitcomb & Wilson (1929), was observed. However, in one or two cases when the alimentary canal was pulled out of the abdomen and laid on a slide, a proventriculus full of pollen would collapse and discharge its contents into the crop giving a local cloud of pollen grains. Sometimes the proventriculus would continue repeatedly to take in and expel the cloud of pollen as though the guarding action of the lips had been deranged. Rough handling of the gut during its extraction often caused this to happen, and the proventriculus quickly ceased to perform any significant action. Probably some damage had been done to the organ. In any case the action was weak and spasmodic and was undoubtedly an artifact. It was never observed in the 'Window dissections'.

Sometimes, if the bees had been starved for long periods (4 or 5 hr.), the first action of the proventriculus would be to pass back two or three boluses at once without any filtering action and then to start the normal filtering. This occurred whether the syrup contained pollen or not.

When pollen and syrup was taken in this way the pollen remained in a dispersed condition in the ventriculus and showed no signs of being collected into a packet.

THE EFFECT OF CONCENTRATION OF POLLEN ON THE ACTIVITY OF THE PROVENTRICULUS

(a) *Qualitative experiments*

Windows were dissected out of the abdomens and the bees were fed:

(1) Syrup heavily stained with gentian violet.

(2) Pollen suspension with 1 volume of pollen to 15 volumes syrup.

(3) Pollen suspension with 1 volume of pollen to 7 volumes syrup.

With stained syrup, nineteen boluses were passed to the ventriculus. The first bolus was passed after 3 min. and the rest followed at intervals varying between 15 and 40 sec. with an average interval of 21 sec.

With the weaker pollen suspension four boluses of pollen were observed with intervals, in seconds, of 380, 380, 200, 140.

Since the time intervals for the first two boluses occupied nearly 13 min. heat was applied by means of a hot spatula held reasonably close to the window. The activity was increased and the further two boluses were passed after much shorter intervals. (Apart from this particular application of heat all the experiments were carried out during the same afternoon at room temperature (about 20° C.).)

With the stronger suspension the intervals were, in seconds, 90, 50, 230, 60, 150. Thus it seems that boluses are passed back more quickly, the stronger the suspension of pollen in the crop. This is to be expected, assuming a uniform activity of the proventriculus at a fixed temperature and a uniform dispersion of pollen in the crop.

To try and gain more controlled conditions unaffected by unknown hazards of the dissection the bees were fed quantitatively and killed for examination after definite intervals of time. Bees were collected from the top of the combs and anaesthetized with ether. This caused vomiting of the crop contents. They were allowed to recover in the incubator at 33° C. and were kept there without food for 2 hr. After this time they begin to weaken very quickly and the crop was quite empty.

They were then stuck on glass rods with a piece of soft wax by the dorsal side of the thorax.

Each bee was fed 0·01 ml. of pollen suspension from a calibrated capillary pipette, and after a definite time interval in the incubator was killed, and the whole of its alimentary canal extracted.

(1) Six bees fed pollen suspension with 1 volume pollen to 7 volumes syrup. They were killed at 3 min. intervals. The crops were all about the same size but the gradation of strength of pollen suspension was obvious.

(2) (*a*) Six bees fed a suspension with 1 volume pollen to 3 volumes syrup killed over the same time intervals as in Exp. 1. They showed no obvious difference in pollen concentration. The crops were all the same size.

(*b*) Four bees were fed the 1 : 3 suspension and killed over intervals shown below:

(i) 30 min.: heavy concentration of pollen in crop.
(ii) 45 min.: concentration obviously lower.
(iii) 70 min.: one or two grains only, in the crop.
(iv) 85 min.: clear fluid in crop. This crop was markedly less in size than all the others, including Exps. 1 and 2(*a*).

(*b*) *Quantitative experiments*

Counts were then made of pollen grains in the honey stomach by means of a haemocytometer. The crops were burst on the calibrated grid and stirred gently with a wooden splint to ensure even distribution.

Each bee was fed 0·01 ml. of suspension containing 6500 grains/cu.mm. The results are given in Table 2. The figures under 'Minutes after feeding' refer to pollen concentration in grains per cu.mm.

Table 2. *Pollen concentration in the honey stomach*

Series	Minutes after feeding												
	1	5	7	10	12	15	17	20	23	25	27	30	35
1	—	500	—	420	—	—	—	—	—	260	—	100	0
2	1000	780	380	440	380	—	—	—	—	260	—	100	—
3	4000	—	3250	—	—	—	—	1350	—	—	—	—	—
4	—	—	5280	—	—	—	2500	—	3250	—	2400	—	—
5	—	2720	—	600	—	2160	—	130	—	30	—	40	0

Considerable variation in the performance of individual bees is still obvious, but in general it may be said that the concentration of pollen grains is reduced at a remarkable rate over the first few minutes, and then this rate of reduction is less marked when the concentration of pollen in the crop falls to a low level.

A more reliable method of starving the bees was developed by taking bees as they emerged from the hive entrance and starving them for 1 hr. These bees then invariably had an empty honey stomach.

The method of filtration by the proventriculus, together with the maintenance of the even distribution of pollen grains within the honey stomach by the vigorous movements of the walls of the latter, suggested that the concentration of pollen grains would follow a logarithmic curve when plotted against time.

There were four experiments in which individual bees were fed:

(1) 0·02 ml. suspension containing about 190,000 pollen grains/ml.

(2) 0·02 ml. suspension containing about 20,000,000 spores of *Nosema apis*/ml. (Spores were obtained by grinding up the ventriculi of infected bees in a little water and filtering the fluid through a small, loosely packed plug of cotton wool. The spores passed through the filter leaving all other solid matter behind. The spores were then concentrated by centrifuging the filtrate.)

(3) 0·02 ml. suspension containing 1,450,000 pollen grains/ml.

(4) 0·04 ml. suspension containing 1,450,000 pollen grains/ml. The bees were then kept in small cages in an incubator at 34° C. for definite periods of time and the concentrations of particles within the honey stomach were determined as before.

The results are shown graphically in Text-fig. 4 A, B, C and D.

The curves were derived statistically on the assumption that they were exponential of the type $y = be^{-at}$, where y = pollen concentration at any particular time t, b = initial concentration of pollen, $e = 2{\cdot}71828$, $a = 1/T$, where T is the time constant.

The values of b and a were derived from the normal equations

$$5{\cdot}3020\Sigma y_i^2 \log b - 2{\cdot}3026\Sigma y_i^2\, t_i\, a = 5{\cdot}3020\Sigma y_i^2 \log y_i,$$

and
$$5{\cdot}3020\Sigma y_i^2\, t_i \log b - 2{\cdot}3026\Sigma y_i^2\, t_i^2\, a = 5{\cdot}3020\Sigma y_i^2\, t_i \log y_i,$$

and the variance of a was derived from the equations

$$\sigma^2 = \frac{5{\cdot}3020}{n-2}\left[\Sigma y_i^2(\log y_i)^2 - \log b\Sigma y_i^2 \log y_i + 0{\cdot}43429a\Sigma y_i^2\, t_i \log y_i\right],$$

$$\Delta = (5{\cdot}3020\Sigma y_i^2)(2{\cdot}3026\Sigma y_i^2\, t_i^2) - (5{\cdot}3020\Sigma y_i^2\, t_i)(2{\cdot}3026\Sigma y_i^2\, t_i),$$

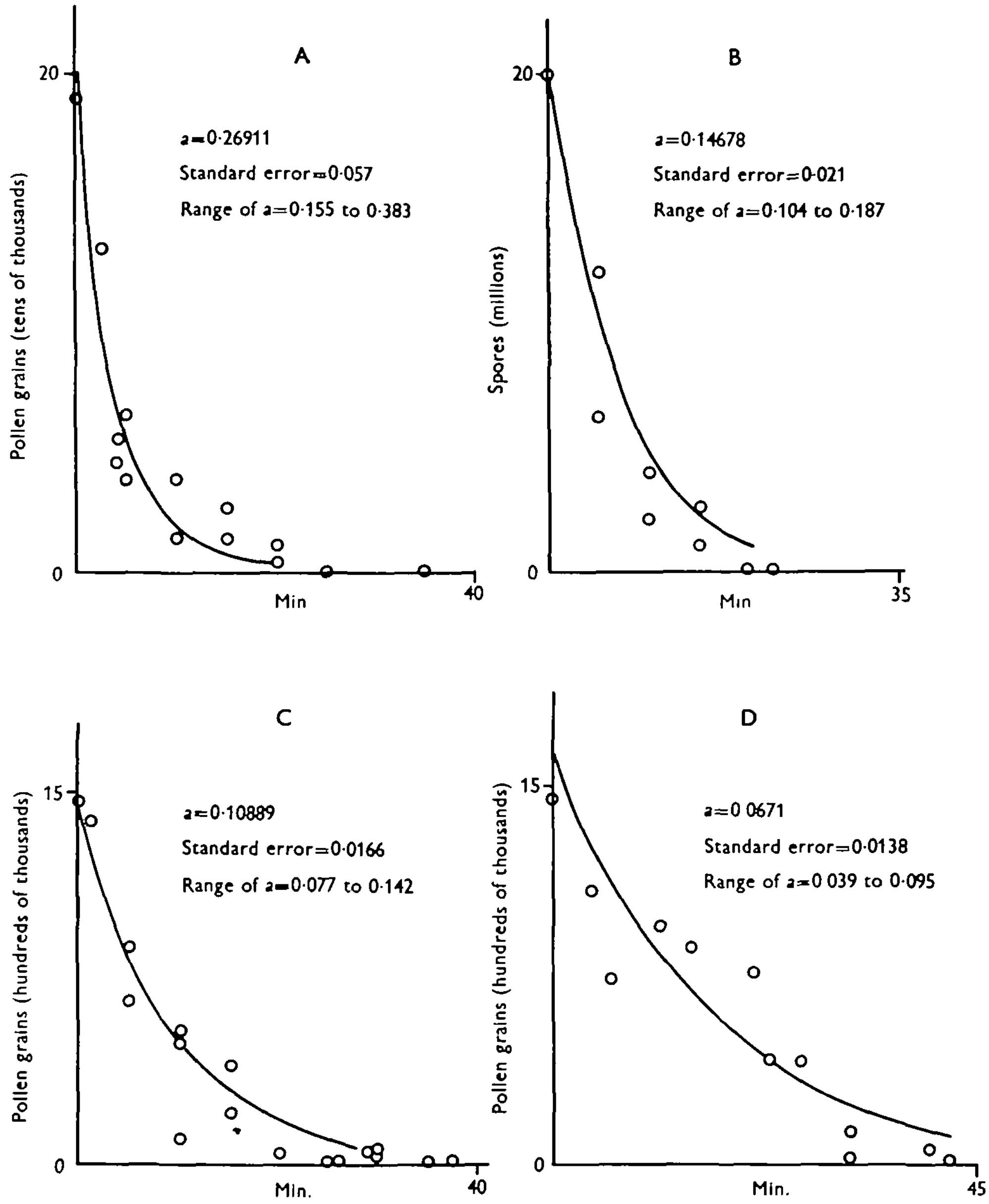
$$\text{Variance of } a = \frac{2{\cdot}3026\,\sigma^2(5{\cdot}3020\Sigma\, y_i^2)}{\Delta}.$$

The 5 % fiducial limits of a, $(a \pm 2\sigma_a)$, "define the range of a".

It is seen that Text-fig. 4A is significantly different from Text-fig. 4C and D and Text-fig. 4B is significantly different from Text-fig. 4D.

The high value of T in Text-fig. 4D is due to the high volume of fluid, since the proventriculus has a fixed maximum volume and so can only filter a certain fixed volume per unit time. It will therefore take longer to clear a crop distended with pollen suspension compared with a crop containing only a small volume of fluid even if the concentration of the pollen in each crop is the same.

The low value of T in Text-fig. 4B (spores) is due to the small size of the spore of *Nosema apis*. The spore is only about 6 by 3 μ, whereas the pollen grains were between about 16 and 32 μ. The spore therefore is considerably smaller in volume than the average pollen grain, and the proventriculus is able to maintain an expansion and contraction of high amplitude, since the spores are packed away into the pouches. This means that the high rate of filtration can continue over a long period of time with only a few boluses to pass back into the crop.



Text-fig. 4.

Thus the time constant of Text-fig. 4B is lower than that of Text-fig. 4C, in spite of the higher concentration of particles represented by Text-fig. 4B.

Text-fig. 4A represents a pollen concentration whose total volume of solid matter was probably less than that in the spore suspension, and so the time constant of Text-fig. 4A is less than that of Text-fig. 4B.

Text-fig. 4C also shows the effect of the choking of the proventriculus. The volume was the same as in Text-fig. 4A, but the volume of solid matter was higher and so the time constant is higher than the latter.

It should be noted that if the proventriculus remained at a constant efficiency, regardless of the amount of solid matter within its lumen, then the rate of fall of concentration of any suspension of particles, however large, or small the concentration or size of particle, would be the same, provided the honey stomach contained the same volume in all cases.

The significant difference between the time constant of Text-fig. 4A and C is therefore due to the choking effect of the higher volume of solid matter on the proventriculus and the larger number of boluses which had to be passed, and not simply due to the larger concentration of particles, represented by Text-fig. 4C, which had to be filtered off. As described above, and as suggested by the short time constant of Text-fig. 4B, the actual concentration of particles is not the deciding factor. Indeed, Text-fig. 4C represents the filtration of more pollen grains in actual numbers per unit time than that represented in Text-fig. 4A, but the real efficiency of filtration was less.

The complication of the proventriculus being filled by solid matter, and so being reduced in efficiency, affects the curves, which cannot therefore be strictly exponential in character. They will be less steep than exponential curves at first and will gradually approach a curve of exponential nature because the efficiency of the proventriculus becomes less affected by the remaining smaller volume of solid matter. Also the proventriculus gradually becomes relatively more efficient, especially where the volume of solid matter was initially great compared with the total volume, because the total volume is gradually reduced by the removal of the solid matter. The curves also all fall to zero values in actual fact, whereas the derived curves do not. This means of course that the lower range of a, as derived statistically, is too low in all four cases. This may mean that all four curves really are different from each other (cf. lowest values of a in all four curves).

It may be said therefore that with the same volume of fluid and particles of the same size, but different concentrations, the lower concentration will be filtered more efficiently than the higher concentration (cf. Text-fig. 4A, C).

Smaller particles will be filtered more efficiently than larger ones (Text-fig. 4B).

With different volumes of the same concentration of particles of the same size the higher volume will be filtered more slowly (Text-fig. 4D).

There is, of course, no discrimination by the proventriculus of different-sized particles contained within the same suspension.

Maurizio (1949) found that the pollen in the honey stomach was reduced in concentration over a period of time and found that the rate of reduction was highest during the first minute—the concentration falling to half that which was fed. Thereafter the concentration fell only slowly. She could not account for this initial rapid fall in concentration because no pollen could be found within the ventriculus in more than half the bees examined. However, the bees had been kept in cages prior to the experiment and were starved for only half an hour. When bees are kept in cages they always keep their honey stomachs very full indeed, and 2 or 3 hr. (even at 34° C.) must elapse before the honey stomach is empty. Consequently her bees must have contained an appreciable amount of syrup when they were fed the pollen suspension and so the latter being diluted immediately it entered the honey stomach would account for the initial rapid reduction in concentration without any pollen leaving the honey stomach.

Maurizio found that the concentration was reduced by one-half or one-third during the first 16–20 min. discounting the initial reduction during the first minute or two. However, the honey stomach of the bees probably contained a greater volume than the 0·03 ml. which they were fed, and so the time constant was quite large, giving the more gentle slope of her curves relative to those described in this paper.

Maurizio was trying to find whether the concentration of pollen grains or their size had any effect on the rate of fall of concentration within the honey stomach, and, from conflicting statistical analyses, came to the conclusion that neither had any effect. For the practical purpose of honey analysis, it may be that the relatively slight variations in size of pollen grains, which the bee collects accidently whilst gathering nectar, produce little significant effect upon the pollen content of the fluid deposited in the cell of the comb, but the volume of nectar carried back to the hive will determine the quantity of pollen which actually arrives.

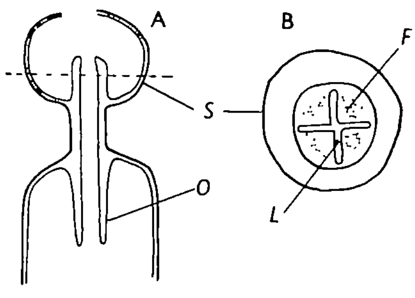
Only a study of the structure of the proventriculus and its action can give a clear picture of all these phenomena.

THE STRUCTURE OF THE PROVENTRICULUS AND THE MECHANISM OF FILTRATION

For the purpose of description the proventriculus may be regarded as a thickened inflected part of the crop where the latter leads to the ventriculus (Text-fig. 5). It has four folds which are continuations of the four major folds of the crop (Snodgrass, 1935).

Both crop and proventriculus have an outer layer of transverse musculature and an inner layer of longitudinal musculature. The longitudinal musculature of the proventriculus is powerful and by contraction causes the lumen of the organ to enlarge.

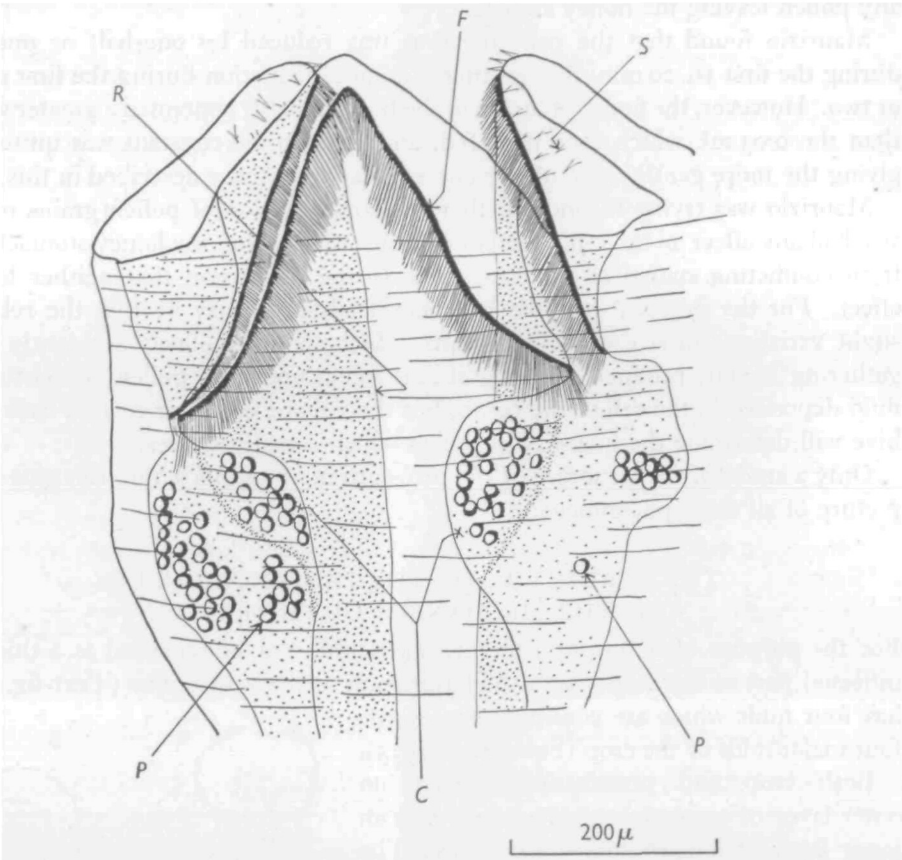
The 'lips' are extensions of the folds beyond the encircling band of transverse musculature, and in addition to the longitudinal muscles have short muscles connecting their tips to the outer wall. The contraction of the latter would cause the lips to open.



Text-fig. 5. Diagram of proventriculus. A. Longitudinal section. B. Transverse section. *L*, lumen; *F*, fold or ridge of longitudinal musculature; *S*, honey stomach; *O*, oesophageal invagination.

The circular muscles are quite powerful but are somewhat thinner over the pouches (Pl. 13, fig. 3). This probably allows the pouches to expand as they fill with pollen. The chitin over the pouches seems to be endocuticle with its layer of epicuticle (Pl. 13, fig. 4), and so, without the exocuticle, will be quite elastic (Wigglesworth, 1947). The circular muscles round the neck are thicker than the rest and probably act as the sphincter described below.

The combs of hairs on the edge of the folds appear to be capable of being folded in upon the surface of the fold or to be opened away from the surface. Perhaps the contraction of the longitudinal muscles exerts a pressure, as they swell, against the



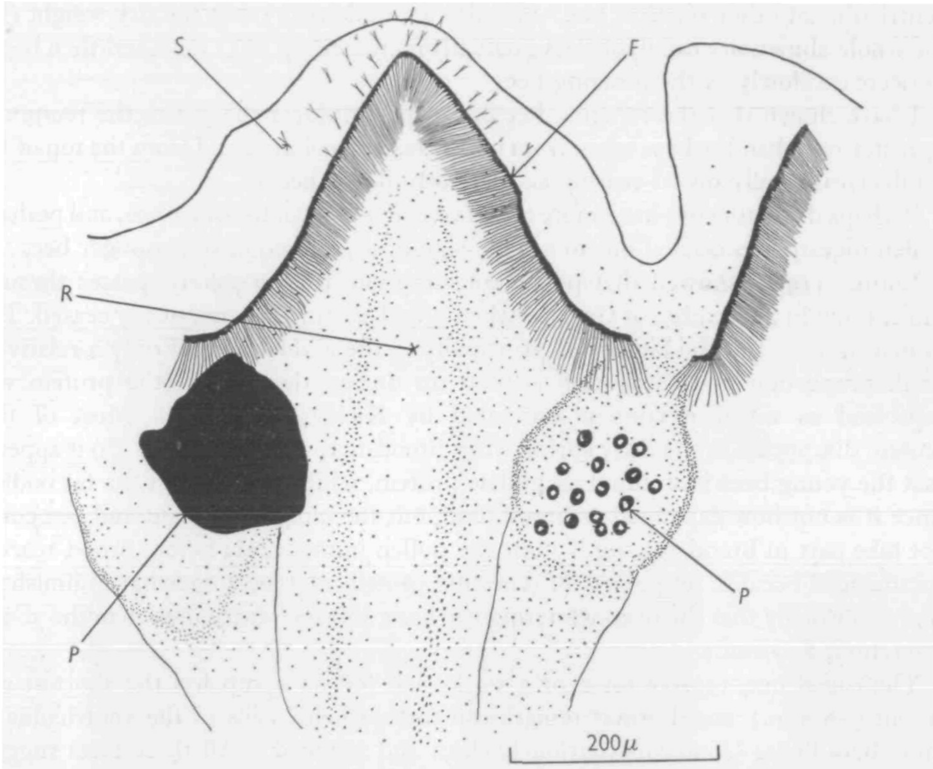
Text-fig. 6. Outer view of proventriculus. Specimen fixed in Bouin, dehydrated, cleared and mounted whole. The fourth lip and the two associated pouches behind are omitted to avoid confusion. *F*, fringe of hairs; *R*, internal ridge of longitudinal muscle; *P*, pollen grains in the pouches; *C*, crypts of the pouches between the encircling band of circular musculature and the internal longitudinal musculature; *S*, short spines.

sides of the folds, which are less heavily chitinized than their inner surface, and thereby close up the hairs against the surface. This will give unrestricted entry to pollen grains rushing in to the expanding lumen of the proventriculus.

When the longitudinal muscles relax, the hairs will open—perhaps automatically by the release of tension on the strained chitin. When the proventriculus is examined by carefully prising the lips apart with needles, the hairs are seen to be raised from the surface of the fold and are pointing inwards to the lumen.

The contraction of the circular muscles will cause the expulsion of the fluid contents of the proventriculus back into the crop, the thicker sphincter layer preventing entry into the ventriculus.

Pollen grains will be sieved off by the comb and forced into the pouches as the folds collapse upon each other. Repeated intake and expulsion of contents in this manner will gradually cause a mass of pollen grains to accumulate in each pouch (Text-figs. 6, 7; Pl. 13, fig. 6).



Text-fig. 7. Inner view of proventriculus. Showing one fold and associated parts with the two adjoining pouches. Proventriculus slit and opened out after immersion in Canada Balsam. The pollen mass to the left was dislodged from the pouch. Lettering as in Text-fig. 5.

Finally, a large mass of pollen grains is collected, and then the contraction of the circular muscles forces this large bulk against the hairs of the combs. Perhaps this back pressure on the sphincter causes it to open suddenly. A bolus of pollen passes through the neck into the ventriculus leaving but a few grains of pollen behind in the collapsed pouches (Pl. 13, fig. 5).

Whitcomb & Wilson (1929) show that the shells of the pollen grains are not broken at any stage, yet their contents are completely digested in the ventriculus.

The structure and operation of the proventriculus in the drone appear to be exactly like those of the worker bee.

The structure of the proventriculus in the queen bee, from an examination of preserved material, appears to be exactly like that of the worker.

DISCUSSION

Beutler & Opfinger (1949) and others have shown that pollen feeding influences the length of life of the adult bee. They point out that it is only the young bee (10 days old or less) whose life is significantly increased by pollen feeding.

Lotmar (1939) showed that the length of the ventriculus of the brood-rearing bee (about 11–12 mm.) was appreciably longer, on the average, than the length of the ventriculus of other types of bee. She also showed (1951) that the dry weight (*less* the whole alimentary canal) of bees gradually increased up to 11 days and then began to decrease slowly in the foraging bee.

I have shown that the foraging bee passes pollen down the gut to the rectum at a greater rate than the bees taken from brood-rearing colonies and from the top of the combs (principally brood-rearing and comb-building bees).

Perhaps the young bee has a more active ventriculus than the older bee, and perhaps pollen digestion is carried out to a more significant degree in the younger bee.

Lotmar (1939) showed that pollen consumption, by the colony, passes through a maximum in September or October when brood-rearing has practically ceased. The protein is stored in the fat bodies principally in the abdomen, and only a relatively small proportion of this protein is used up during the winter (the protein was expressed as nitrogen content estimated by Kjeldahl analyses). Most of this protein disappears in the early spring when brood-rearing starts again. So it appears that the young bees in autumn assimilate protein, which is stored in the fat bodies, since it is not now expended as brood food, but the older, over-wintered bee could not take part in brood-rearing if it ate the pollen immediately before brood-rearing commenced because its powers of digesting protein may be somewhat diminished. It is noteworthy that the over-wintering bee has a shorter ventriculus than the young bee (about 8–9 mm.).

The caged bee, captive for 2 or 3 weeks and fed on syrup has the shortest gut (about 7–8 mm.), and Lotmar remarks on the epithelial cells of the ventriculus of these bees being 'somewhat curiously short and reduced'. All these facts suggest a diminished activity of the ventriculus in the older bee and, perhaps, atrophy of the organ.

Consideration of the source of invertase within the worker bee suggests a possible function of the proventriculus which may be of importance to the physiology of digestion. Evenius (1926) refers to the 'Speicheldrüsen' as the source of invertase, but used the whole head for his experiments and so his glands were really mostly pharyngeal. Ingelscent (1940) found invertase only in the thoracic salivary glands and none in the pharyngeals. Kosmin & Komarow (1932) and others (Wigglesworth, 1947) refer to the pharyngeals as the source. However, it is likely that sucrose is digested principally in the crop by invertase from these glands. Langer (1909) indicated by serological technique that the invertase in honey was identical with that found in the glands.

Pavlowsky & Zarin (1922) and Kosmin & Komarow (1932) found invertase in the ventriculus, but they used the whole organ, including contents for analyses, and their results therefore do not preclude the supply of this enzyme from a more anteriorly situated source. Evenius states that the concentration of invertase in the ventriculus decreases down its length, suggesting its supply from the crop and its gradual dilution or destruction within the ventriculus.

It may be that the ventriculus of the bee is an organ primarily producing proteolytic enzymes and is not greatly concerned with the digestion of sucrose.

In any case, the proventriculus filters off the pollen as compact masses and leaves the syrup or honey behind. This alone probably facilitates the digestion of pollen, since the proteolytic enzymes are not diluted by an excess of fluid. The proventriculus therefore serves the purpose of dividing the two principal items of food for separate treatment. (Butler (1949) indicates the doubts expressed of the capability of the bee to digest polysaccharides and lipoids.) A few experiments on the effect of honey upon the proteolytic activity of the ventriculus showed that this separation of the two food constituents must be advantageous to their efficient digestion.

Ventriculi of pollen-eating bees were ground up in measured quantities of the buffered saline described above, and drops of the fluid, serially diluted with the saline, were incubated on the gelatin emulsion on strips of photographic plate. The strips were placed in Petri dishes lined with wet filter-paper and were incubated at 34° C. The gelatin was completely digested into water-soluble products after 1 hr., leaving a clear hole when the plate was washed in cold water, and only dilutions equivalent to one ventriculus in 2 or more ml. of buffer showed incomplete digestion.

Parallel experiments, using portions of the same stock of ground-up ventriculi but using fresh honey as the diluting fluid, showed that concentrations equivalent to one ventriculus in 0·5 ml. or less of a solution equivalent to 25% honey, were needed to effect complete digestion of the gelatin. Both honey buffered to pH 6·7 and pure honey gave the same effect.

The effect of the honey is probably a competitive action by the sugar molecules with the proteolytic enzymes for water, as shown by Nelson & Schubert (1928).

Besides avoiding dilution of the enzyme therefore this efficient separation of pollen must reduce the inhibitory action of honey upon proteolytic activity to a considerable extent.

SUMMARY

1. The proventriculus of the worker honeybee is an organ which effects a highly efficient separation of pollen grains from the medium in which they are suspended.

2. The pollen grains are packed tightly together by the proventriculus and are passed as a bolus down to the ventriculus.

3. The boluses pass quite quickly towards the posterior end of the ventriculus (5–20 min.), depending on the concentration and amount of pollen suspension which is fed.

4. The peritrophic membranes do not move down the ventriculus with these packages but pass down at a slower rate.

5. At the posterior end of the ventriculus the pollen may be held up for a considerable time. In the brood-rearing bee it stays there for many hours (up to 12 or more).

In the forager it begins to pass down the hindgut to the rectum after 3 hr. or less.

6. The volume of fluid within the honey stomach, the size of particles in suspension and their concentration have significant effects on the rate and efficiency of filtration by the proventriculus.

7. Some reflexions are made on the possible physiological significance of the mechanism of the proventriculus to the worker honeybee.

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REFERENCES

BEUTLER, R. & OPFINGER, E. (1949). Pollenernährung und Nosema-befall der Honigbiene. *Z. vergl. Physiol.* **32**, 383–421.

BUTLER, C. G. (1949). *The Honeybee. An Introduction to her Sense-Physiology and Behaviour.* Oxford: Clarendon Press.

EVENIUS, J. (1926). Die Fermente im Darmkanal der Honigbiene. *Ardh. Bienenk.* **7**, 229–44.

HEJTMANEK, J. (1933). Ein Beitrag zur Feststellung der Durchgangszeit des Pollens durch den Verdauungstrakt der Honigbiene. *Českého Včelaře*, **3**, 1–5.

IMMS, A. D. (1934). *A General Textbook of Entomology.* London: Methuen.

INGLESCENT, H. (1940). Zymotic function of the pharyngeal, thoracic and post-cerebral glands of *Apis mellifica*. *Biochem. J.* **34**, 1415–18.

KOSMIN, N. P. & KOMAROV, P. M. (1932). Über das Invertierungsvermögen der Speicheldrüsen und des Mitteldarmes von Bienen verschiedenen Alters. *Z. vergl. Physiol.* **17**, 267–78.

LANGER, J. (1909). Beurteilung des Bienenhonigs und seiner Verfalschung mittels biologischer Eiweissdifferenzierung. *Arch. Hyg., Berl.*, **71**, 308–330.

LOTMAR, R. (1939). Der Eiweiss-stoffwechsel im Bienenvolke während der Ueberwinterung. *Landw. Jb. Schweiz.* **53**, 34–70.

LOTMAR, R. (1951). Gewichtsbestimmungen bei Gesunden und Nosemakranken Bienen. *Z. vergl. Physiol.* **33**, 195–206.

MAURIZIO, A. (1949). Pollenanalytische Untersuchungen an Honig und Pollenhöschen. *Beih. Schweiz. Bienenztg*, **2**, 320–455.

METZER, C. (1910). Die Verbindung zwischen Vorder- und Mitteldarm bei der Biene. *Z. wiss. Zool.* **96**, 1.

NELSON, J. M. & SCHUBERT, M. P. (1928). Water concentration and the rate of hydrolysis of sucrose by invertase. *J. Amer. Chem. Soc.* **50**, 2188.

PAWLOWSKY, E. N. & ZARIN, E. J. (1922). Histology and digestive enzymes in the gut of the honeybee. *Quart. J. Micr. Sci.* **66**, 509–56.

SNODGRASS, R. E. (1925). *Anatomy and Physiology of the Honey Bee.* New York: McGraw-Hill.

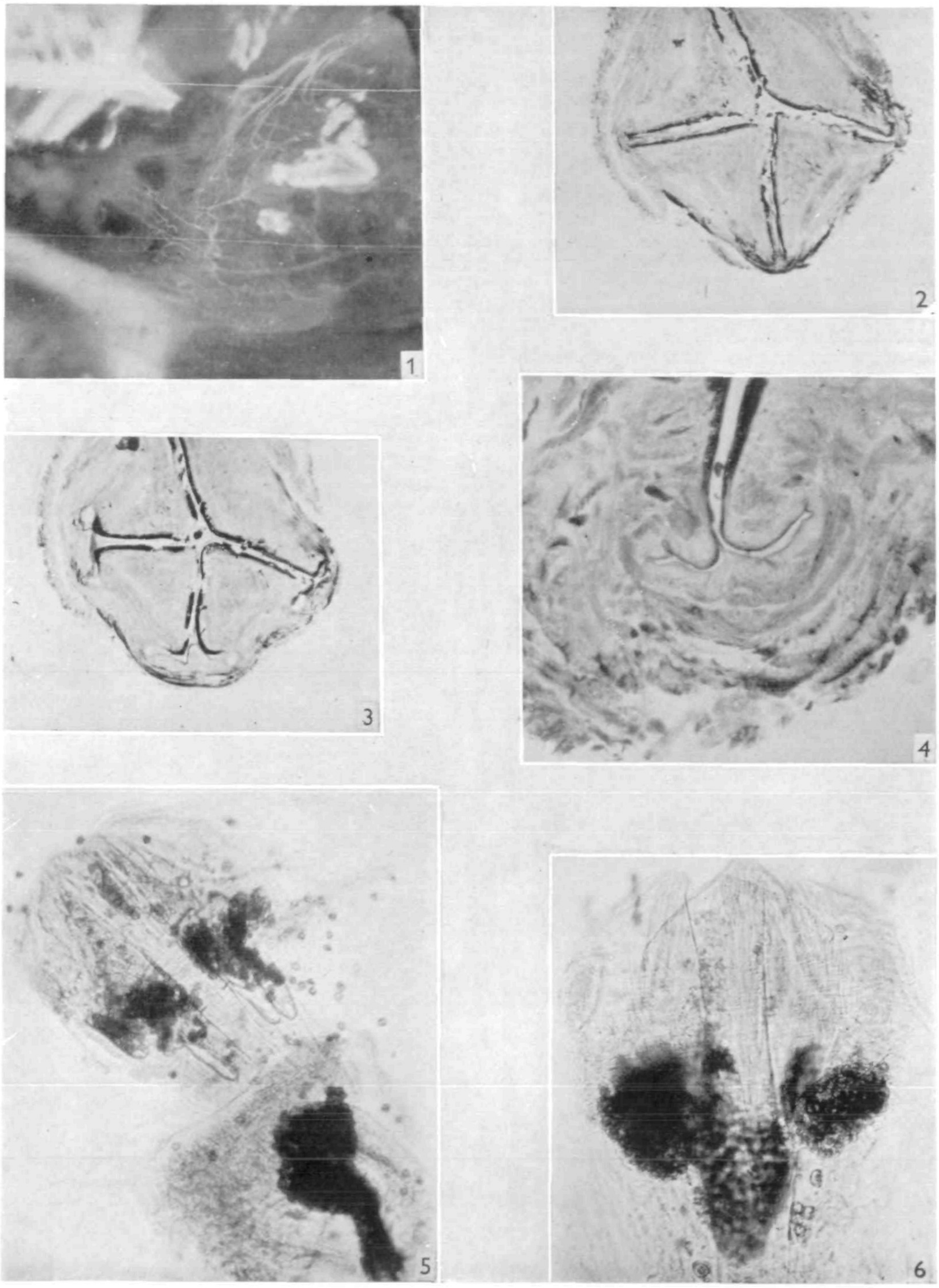
SNODGRASS, R. E. (1935). *Principles of Insect Morphology.* New York: McGraw-Hill.

TRAPPMANN, W. (1923). Anatomie und Physiologie des Zwischendarmes von *Apis mellifica L. Arch. Bienenk.* **5**, 190–203.

WHITCOMB, W. & WILSON, H. F. (1929). Mechanics of digestion of pollen by the adult honeybee and the relation of undigested parts to dysentry of bees. *Agric. Exp. Sta. Wis. Res. Bull.* **92**, 1–27.

WIGGLESWORTH, V. B. (1947). *The Principles of Insect Physiology.* London: Methuen.

ZANDER, E. (1922). *Der Bau der Biene.* Stuttgart: Eugen Ulmer.



BAILEY—THE ACTION OF THE PROVENTRICULUS OF THE WORKER HONEYBEE

EXPLANATION OF PLATE 13

Fig. 1. An electronic flash photograph of a window dissection showing two of the four pouches distended with stained pollen. (× 15.)

Fig. 2. Cross-section of the proventriculus where the comb of hairs ceases and before the pouches begin, giving the well known X-shaped lumen. (× 70.)

Fig. 3. Cross-section of the proventriculus showing pouches in the empty, collapsed state. (× 70.)

Fig. 4. Enlarged cross-section of a pouch showing the thick, hyaline, elastic, endocuticular layer with its thin epicuticular layer. (× 308.)

Fig. 5. Whole mount of a proventriculus which was killed as it was passing a bolus back to the ventriculus. (× 70.)

Fig. 6. Whole mount of a proventriculus showing the accumulation of stained pollen within the pouches. (× 70.)