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EXCRETION IN THE BLOOD-SUCKING BUG, *RHODNIUS PROLIXUS* STÅL.

I. THE CONTROL OF DIURESIS

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INTRODUCTION

The larvae of *Rhodnius* take large blood meals and a rapid diuresis follows. This diuresis represents a striking reversal of policy because in the long periods between meals these insects conserve water. This paper examines the way in which diuresis is achieved.

MATERIALS

A culture of *Rhodnius* was maintained by a system evolved from that of Buxton (1930). The larval stages take larger meals, relative to their weight, than do the adults and the subsequent diuresis is more extensive. Therefore, the large 5th-instar larvae were used in the experiments to be described.

RESULTS

The mechanism whereby excretion is accelerated

Diuresis might be caused by the appearance in the haemolymph of a substance that accelerates excretion. To test this hypothesis, a technique was developed by which it was possible to measure the rate of secretion of Malpighian tubules isolated in drops of haemolymph. To avoid the difficulties of making the necessary measurements on single tubules (Ramsay, 1954), the preparation consisted of all four Malpighian tubules attached to the part of the rectum through which they discharge. The preparation is dissected out from freshly fed insects under Ringer's solution in the following way. The attachments of the tubules with the tracheal system are cut, the mid-gut is severed a short distance from the rectum, and the preparation is freed by cutting round the anterior part of the rectum. The mass of tissue can then be transferred to a drop of haemolymph previously removed from the insect and kept under liquid paraffin in a wax-lined dish. The cuticular lining of the rectum is hydrophobic and spreads along the interface between the paraffin and the haemolymph so that the secretion from the Malpighian tubules is forced out into the paraffin. Plate 1 illustrates a completed preparation. The tubules are under no tension, are totally immersed in haemolymph and they discharge through the rectal cone as they do in the intact insect. With practice a preparation can be made in less than 4 min. so that the tubules are out of their natural environment only for this short time.

The main advantage of this preparation is that its activity can easily be studied by

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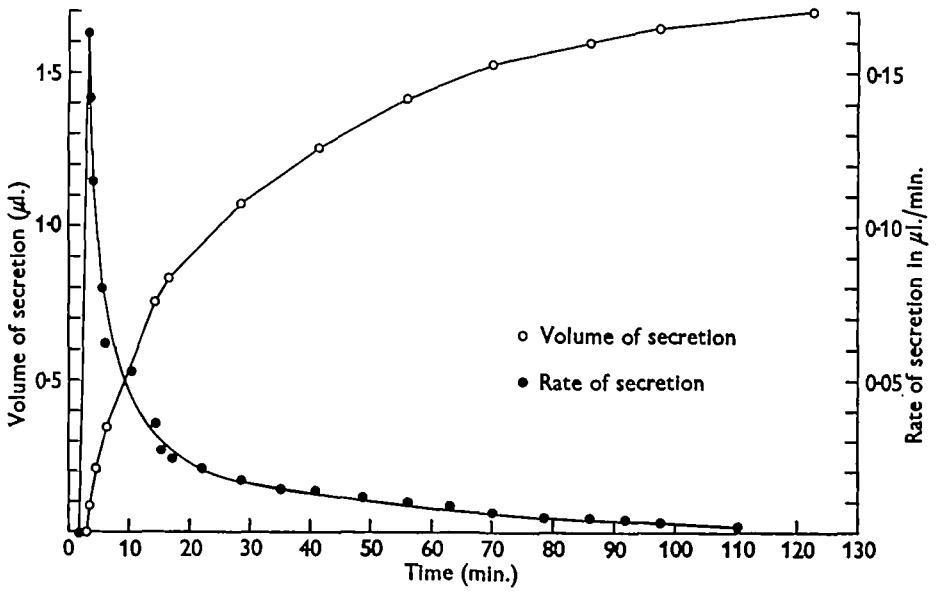
following the rate of growth of the drop of secretion. The volume of the drop was calculated from its diameter as measured with an ocular micrometer. This treatment assumes that the drops are spherical, which in fact they are not. However, the method was tested by estimating the volumes of drops delivered from a micrometer syringe and the errors were not found to exceed 2–3% for drops smaller than 10 μl. Since the relative volumes of drops rather than the absolute volumes are required for estimating rates of secretion, these errors are not important. Temperature has a considerable effect on secretion; therefore, each experiment involving the comparison of rates of secretion was carried out at a constant temperature and the light used to illuminate the preparations was passed through a piece of heat-absorbing glass.



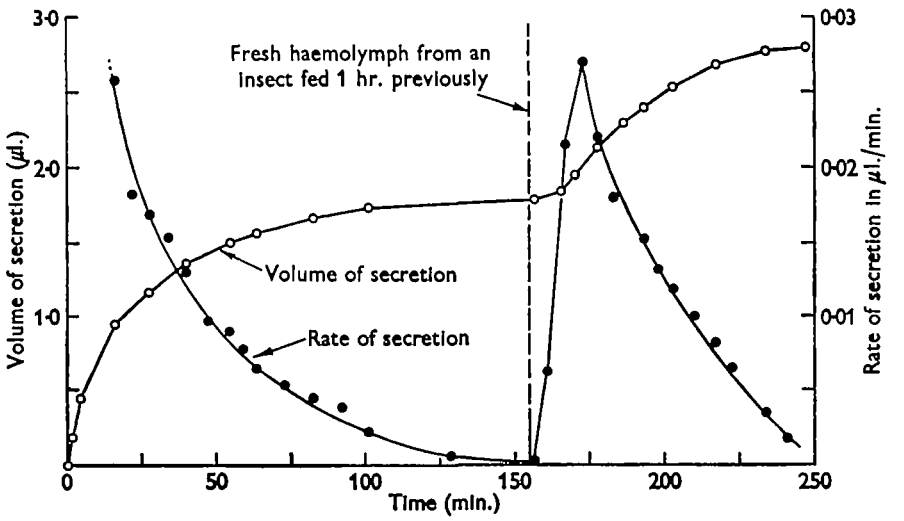
Text-fig. 1. The secretory behaviour of a set of isolated Malpighian tubules.

The rate of secretion of such a preparation is at first high, but soon falls to a very low level (Text-fig. 1). The preparation can then be used to test the ability of samples of haemolymph to accelerate secretion. More than 50 experiments showed that when haemolymph taken from a recently fed insect was added to that bathing the tubules, there was a marked temporary increase in the rate of secretion (Text-fig. 2). But if the sample of haemolymph was taken from an unfed insect (40 cases) or from an insect after the end of diuresis (10 cases), there was no response. In contrast, the haemolymph of insects that had only been feeding for 3 min. (5 cases) caused increased secretion by the tubules. The dissection of these insects showed that diuresis was already under way, for crystals of uric acid that are present in the lower parts of the tubules of unfed insects were being swept out at this time. Since it can be shown that the osmotic concentration of the haemolymph falls after feeding, these effects might have been caused by changes in concentration of the haemolymph bathing the tubules. However, the results were unaltered when the experiments were repeated with samples adjusted to the same concentration.

These experiments demonstrate that, during diuresis, the haemolymph contains

some substance that accelerates secretion by the Malpighian tubules, which it does not contain at other times.

In other insects, urine production has been attributed to the dual processes of secretion in the Malpighian tubules and resorption in the hind-gut (Ramsay, 1958), in particular the rectum (Phillips, 1961). Therefore, although the acceleration of secretion is largely responsible for diuresis in *Rhodnius*, a slowing of resorption from the rectum might also play some part. The high rate of excretion during diuresis and the fact that the rectum voids its contents every 2–3 min. suggest that any resorption from the rectum is negligible. But, since nothing is known of the rate of resorption at other times, it is impossible to say whether a change in the activity of the rectum contributes to diuresis.

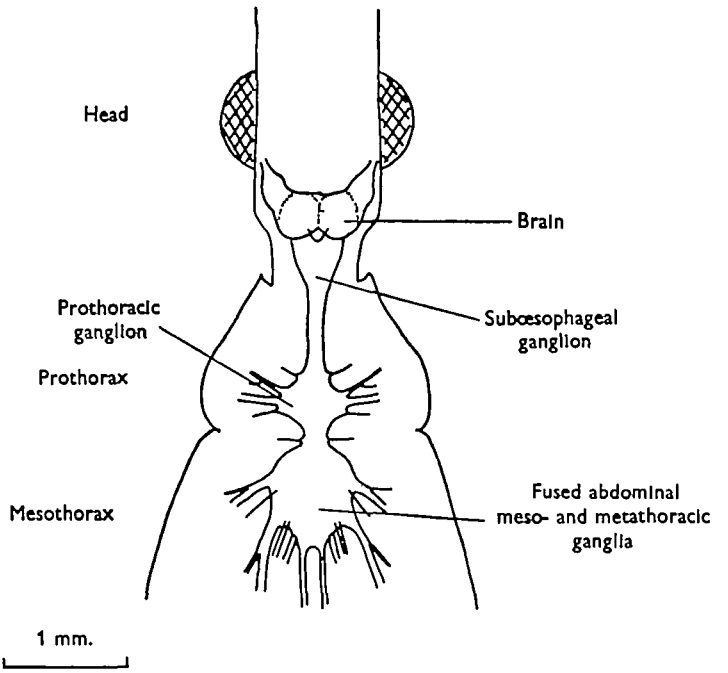


Text-fig. 2. The effect of adding haemolymph taken from an insect in diuresis to that bathing a resting set of isolated tubules.

The source of the diuretic factor

The possibility that the factor was derived from the blood meal itself was eliminated in experiments in which twenty 5th-stage insects were fed on warm Ringer's solution through a stretched rubber membrane as described by Harington (1960). All these insects subsequently excreted normally. It follows from this that the diuretic factor has its source within the tissues of the insect. The tissues were, therefore, assayed for diuretic activity using preparations of isolated tubules. Each piece of tissue to be tested was ground up in Ringer's solution and the resulting brei was kept under liquid paraffin. Portions of such breis were added to preparations of tubules which were secreting at a low rate and any that caused an increase in the rate of secretion were judged to have diuretic activity. The corpus allatum, the corpus cardiacum, and approximately equal amounts of salivary gland, fat body, trachea, muscle, gut wall and cuticle were tested and none possessed any activity. In contrast, all parts of the central nervous system were highly active, especially the large fused ganglionic mass situated in the mesothorax, which comprises the meso- and metathoracic and all the abdominal ganglia (Text-fig. 3).

The concentration of diuretic activity in each of the parts of the central nervous system was compared in the following way. Breis of each part were diluted until they produced comparable effects on secretion by sets of tubules bathed in the same amounts of haemolymph. In order to obtain a more meaningful comparison, the disparity in size of the parts of the central nervous system was taken into account. The dry weight of each part was estimated by measuring with a travelling microscope the deflexion produced when the piece of tissue was hung on the end of a horizontal glass fibre. Table 1 sets out the results of these experiments. Clearly, the most likely source of the diuretic factor is the ganglionic mass in the mesothorax where diuretic activity is nearly ten times more concentrated than it is in the next most rich source.



Text-fig. 3. The central nervous system of *Rhodnius*.

Table 1. *The occurrence of diuretic activity in the various parts of the central nervous system*

	Brain	Sub-oesophageal ganglion	Pro-thoracic ganglion	Meso-thoracic ganglionic mass
The concentration of each part necessary to cause a resting set of tubules in 6 μl. of haemolymph to produce a further 0·5 μl. of secretion (ganglia/100 μl. of haemolymph—average of 10 trials) (*A*)	0·7	5·0	10·0	0·07
The dry weight of each part (μg. $\pm$ 1·0 μg.) (*B*)	11	3	4	13
Therefore the relative concentration of diuretic activity in each part ($1/A \times B$ expressed as a ratio)	5·2 :	2·7 :	1 :	44

In an attempt to identify the source of the diuretic factor, freshly fed *Rhodnius* were constricted at various positions either with ligatures of fine cotton or by pinching the insect with fine forceps held shut with clamps. After about an hour equal volumes of haemolymph were taken from either side of the constriction and tested on preparations of isolated tubules. The results of these experiments are summarized in Table 2. They show that active haemolymph can only be found in a part of the insect which includes the ganglionic mass in the mesothorax. This strongly suggests that this part of the central nervous system is the source of the diuretic factor.

Similar experiments have led to the same conclusion. The flow of urine was normal

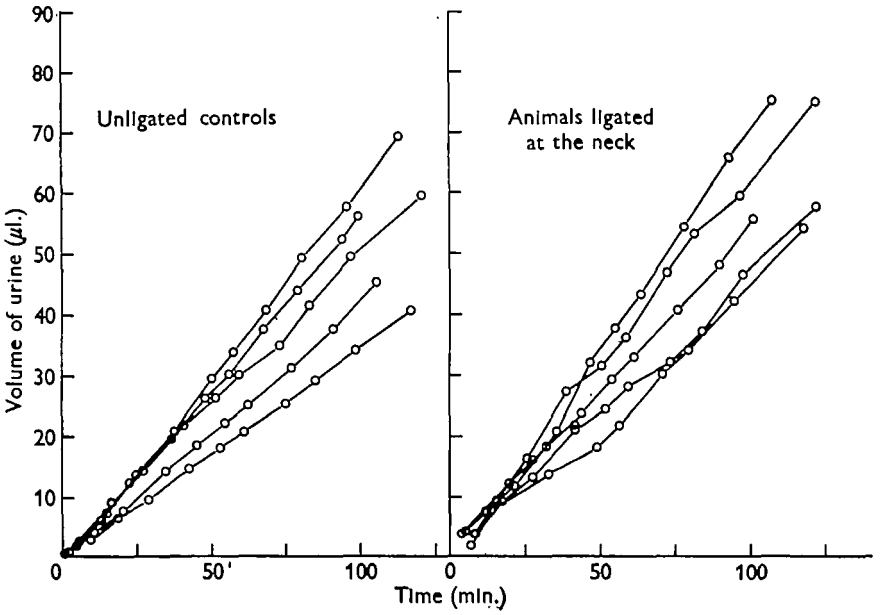
Table 2. *The diuretic activity of samples of haemolymph removed from recently fed insects constricted at various positions*

Position of the constriction	Method of constriction	Activity of haemolymph taken from in front of the constriction	Activity of haemolymph taken from behind the constriction	Effect of constriction as revealed by subsequent dissection
At the neck	Ligature	Not tested	+ + (14 animals)	Ventral nerve cord intact
Between the thorax and abdomen	Ligature	+ + + + + (9 animals)	Nil (5 animals)	Nervous supply to the abdomen was still intact
At the prothorax	Pinched with fine forceps	Nil (3 animals)	+ + (14 animals)	This treatment crushed the prothoracic ganglion and usually broke the ventral nerve cord
At the mesothorax	Pinched with fine forceps	Nil (5 animals)	Nil (9 animals)	This treatment crushed the mesothoracic ganglionic mass and usually broke the nerve cord
Between the pro- and mesothorax	Ligature	Nil (6 animals)	Nil (6 animals)	In these cases all the ganglionic mass had been included in the constricted part of the insect
			+ (5 animals)	In these cases a part of the ganglionic mass was still protruding into the haemocoele behind the constriction

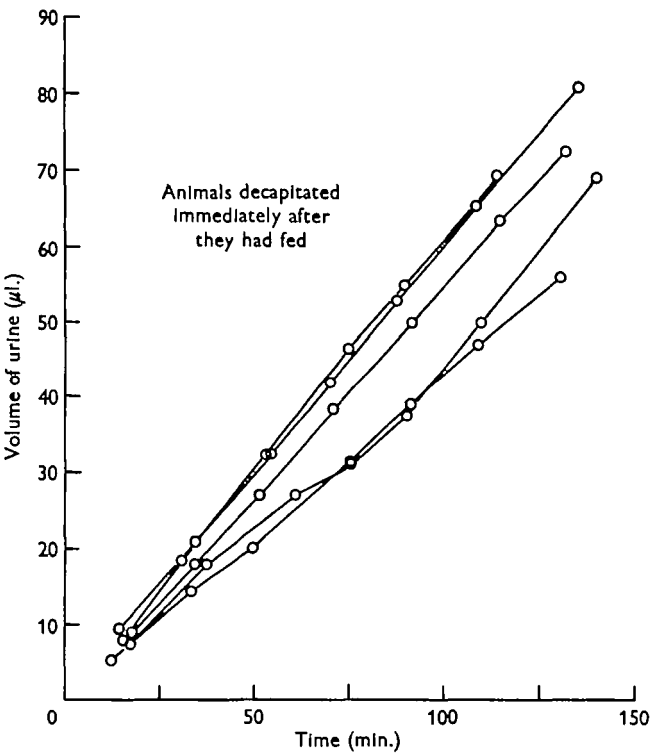
The activity of haemolymph from unconstricted controls can be represented by + + (5 animals).

in fed insects ligated at the neck (Text-fig. 4) or decapitated (Text-fig. 5). Similarly, constriction of the prothorax with fine forceps did not affect diuresis, but when the mesothorax was pinched the flow of urine soon ceased (Text-fig. 6).

In another series of experiments parts of the nervous system were severed or removed before the insects were fed. For surgical operations on the nervous system, the insect was held on its back by pushing its legs into pieces of modelling clay. The longitudinal fold of cuticle running between the legs could then be cut open with a fine scalpel to reveal the ganglionic mass and its nervous connexions. When the required operation had been performed, the sides of the fold were held together and the incision was sealed with an adhesive mixture of resin and beeswax. In ten insects the ganglionic mass was removed and when they later took a meal of blood there was no subsequent diuresis. This suggested either that the mass was the source of the diuretic factor or that the continuity of the nerve cord was essential to its release. To decide between these alternatives, the nerve cord was cut through between the



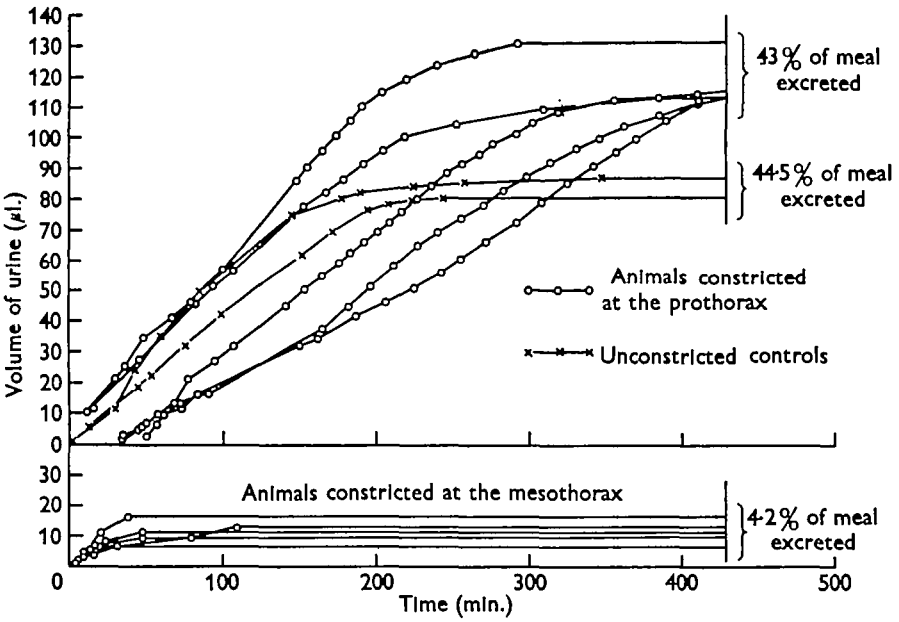
Text-fig. 4. The course of diuresis in fed insects ligated at the neck.



Text-fig. 5. The course of diuresis in insects decapitated immediately after feeding.

prothoracic ganglion and the ganglionic mass. Twenty-two such insects were fed and in nineteen of them the extent of diuresis was normal. These results demonstrate that the presence of the ganglionic mass is necessary for the release of the factor but the continuity of the nerve cord anterior to the mass is not. It was still possible that the mass was a part of an essential nervous pathway to the source behind the mass. However, since no structure behind the ganglionic mass has been found to contain diuretic activity and since the diuretic factor is released into the haemolymph of the mesothorax (Table 2), this possibility may be disregarded.

It seems reasonable to conclude that the diuretic factor is released from the ganglionic mass in the mesothorax.



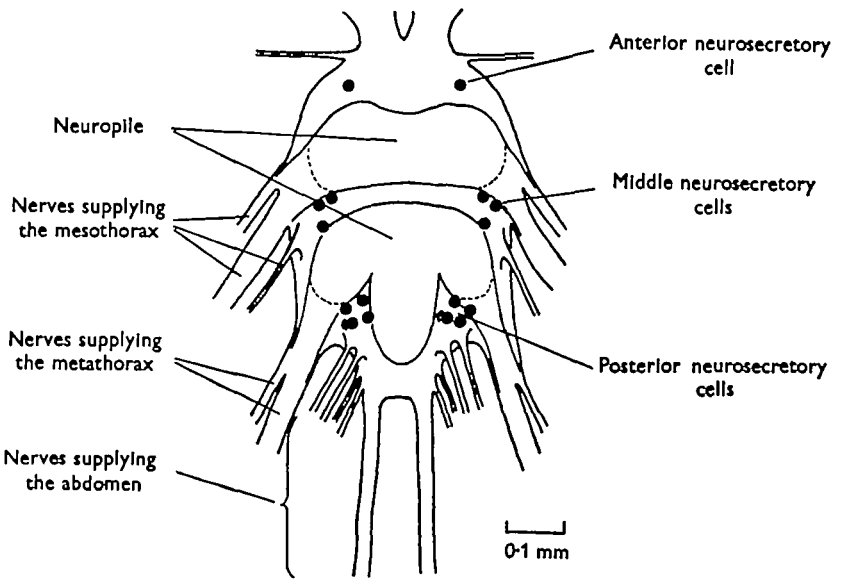
Text-fig. 6. The course of diuresis in fed insects constricted either at the prothorax or at the mesothorax.

The location of diuretic activity within the ganglionic mass

In an attempt to decide which part of the ganglionic mass was the ultimate source of the factor, the mass was cut into pieces and the pieces were tested for diuretic activity. This showed that all the activity was to be found in the posterior half of the mass (3 cases) and was divided equally between the lateral halves (5 cases). Further, it was discovered that activity was confined to the back one-third of the mass (3 cases). The significance of these results became apparent when the distribution of the neurosecretory cells in the mass was examined.

Thomsen (1952) describes the median neurosecretory cells in the brain of living *Calliphora* as 'bluish white, which makes them distinguishable from the surrounding brain tissue'. Large cells of exactly similar appearance occur in the ventral nerve cord of *Rhodnius* and it is assumed that they, too, are neurosecretory, an assumption which appears to be justified by the evidence which follows. The distribution of these cells in the ganglionic mass is shown in Text-fig. 7. By tearing the sheath off the mass

it was possible to expose the neurosecretory cells and they could then be teased out with fine glass needles. Five experiments showed that breis of the two hindmost groups of cells contained more than 97% of the diuretic activity of the whole mass. The other neurosecretory cells were found to contain no detectable activity, while the rest of the mass possessed only a small amount of activity, comparable in concentration to that found in the suboesophageal and prothoracic ganglia. The neurosecretory cells at the back of the mass are 15–20 μ in diameter. It can be calculated from this that diuretic activity is about 50,000 times more concentrated in these cells than it is in the rest of the ganglionic mass and that this activity would still be detectable at a dilution of $1:10^8$.



Text-fig. 7. A diagrammatic representation of the fused ganglionic mass in the mesothorax to show the distribution of the neurosecretory cells.

DISCUSSION

The presence of neurosecretory cells in the various ventral ganglia of insects has been known for some time (Van der Kloot, 1960). So far, relatively little evidence has appeared on the function of these cells. The suboesophageal ganglion has been shown to be the likely source of a hormone in *Leucophaea* (Scharrer, 1955), in the silkworm (Fukuda, 1952, 1953*a–c*; Hasegawa, 1952, 1957) and in the cockroach, *Periplaneta* (Harker, 1956), while the work of Hidaka (1956) suggests that the prothoracic ganglion is the source of a hormone in two species of *Papilio*. These cases seem to be the only ones known of hormones produced from parts of the central nervous system other than the brain. It is of some interest, therefore, to find in *Rhodnius* neuroendocrine cells—neurosecretory cells that release hormones (Van der Kloot, 1960)—that are closely applied to ganglionic material of metathoracic and abdominal origin, even though this is located in the mesothorax.

There is a considerable literature on the effects of extracts of insect nervous tissue on the movements of the heart, chromatophores and muscles (see Gersch, 1957;

Gersch & Unger, 1957; Ralph, 1962; review by Van der Kloot, 1960). In many of these cases, however, it is not clear which of the substances are involved in the normal functioning of the insect nor from which part or parts of the nervous system they are released. For example, the present investigation has shown that although a substance possessing diuretic activity can be extracted from all parts of the central nervous system of *Rhodnius*, it is only released in physiological quantities from one part, the mesothoracic ganglionic mass. The posterior neurosecretory cells in the mass contain a very high concentration of the diuretic activity that is to be found in all parts of the central nervous system. If, as seems possible, neurosecretory cells have evolved from a more widespread type of nerve cell by the exaggeration of synthetic mechanisms already present, then this would explain the occurrence of pharmacologically active substances at a low concentration throughout the nervous system.

SUMMARY

1. The mechanism underlying diuresis in *Rhodnius* has been investigated.

2. An isolated preparation of the Malpighian tubules of a 5th-instar larva is described.

3. The rate of secretion by such a preparation, isolated in a drop of haemolymph, is at first high but soon falls to a low level. It can be restored by the addition of haemolymph taken from an insect during diuresis.

4. It has been shown that diuresis is promoted by some substance, presumably a neurohormone, which can be extracted from the posterior neurosecretory cells of the fused ganglionic mass situated in the mesothorax.

This paper represents part of a thesis submitted to the University of Cambridge for the degree of Ph.D. I owe a debt of gratitude to Prof. V. B. Wigglesworth for his advice and supervision and to Dr J. Noble-Nesbitt who weighed parts of the nervous system for me. I thank the Department of Scientific and Industrial Research for generous financial support.

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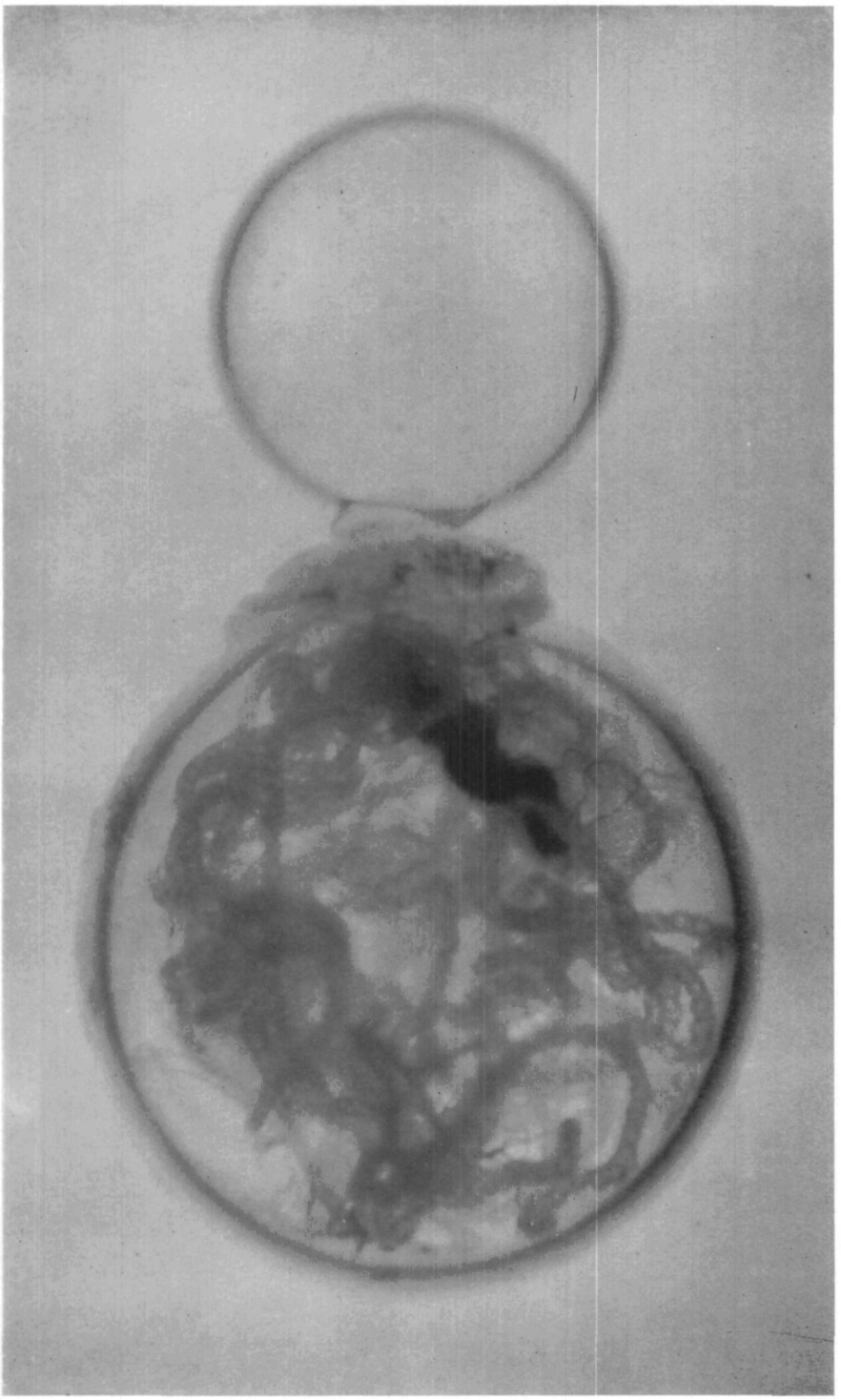
EXPLANATION OF PLATE

A preparation of the Malpighian tubules isolated in a drop of haemolymph.



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