J. Exp. Biol. (1967), **46**, 35–41
With 2 text-figures
Printed in Great Britain

HORMONAL CONTROL OF EXCRETION IN THE AMERICAN COCKROACH

I. RELEASE OF A DIURETIC HORMONE FROM THE TERMINAL ABDOMINAL GANGLION

BY RICHARD R. MILLS
Department of Biology, Tulane University, New Orleans, La. 70118

(*Received* 1 *July* 1966)

INTRODUCTION

Recent investigations on the physiology of excretion in *Rhodnius prolixus* indicate that a diuretic hormone is released from the fused mesothoracic ganglion (Maddrell, 1962, 1963, 1964*a*, *b*). The hormone can be extracted from the posterior neurosecretory cells of the ganglion and it promotes an increased rate of diuresis by the Malpighian tubules.

In the American cockroach only an 'antidiuretic principle' has been found. Extracts from the brain, corpora cardiaca, corpora allata, and suboesophageal ganglion appear to inhibit diuresis (Wall & Ralph, 1964). Since it seemed possible that the American cockroach might have both types of hormones this study was initiated. The results obtained suggest that the terminal abdominal ganglion releases a diuretic hormone that increases tubule secretion.

METHODS AND MATERIALS

Adult male American cockroaches *Periplaneta americana* (L.) were used in all experiments and were maintained as previously described (Mills, 1966*a*).

Homogenates were made with the same saline-buffer as previously used for the terminal abdominal ganglion (Mills, Mathur & Guerra, 1965). Haemolymph was obtained by the capillary method of Mills (1966*b*) or as described.

RESULTS

Evidence for the release of a diuretic hormone into the haemolymph

Water was withheld from groups of adult male cockroaches for 3 days. They were then allowed to drink for approximately 10 min. (which was the experimentally determined length of time it took to satisfy the animals). If the insects were not allowed to drink their fill, no diuretic activity could be detected.

Haemolymph was collected and assayed for the presence of a diuretic hormone. Malpighian tubules *in vivo* (of normal animals) bathed in the 'active haemolymph' caused a greater secretion of fluid into the hind-gut than in similar experiments using equal volumes of saline. The results of the initial experiments were extremely variable, probably due to the dilution of the active principle. However, a more reliable assay

was later developed. Approximately 50 μl. of 'active' haemolymph were dropped on the mass of Malpighian tubules lying adjacent to the mid-gut. These tubules are usually yellow in appearance and can easily be distinguished.

To determine the diuretic hormone activity several different tubule preparations were made. Initially, sections of the mid-gut–hind-gut complex containing the Malpighian tubules were isolated. However, this proved to be tedious due to the number of tubules and their location within the fat body. In addition, 15–20 min. were

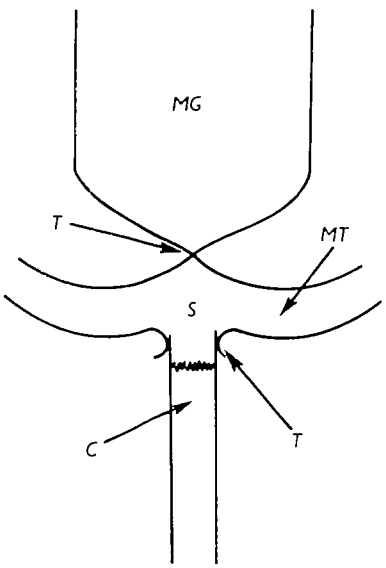

Fig. 1. Method used to determine the volume of urine secretion by the Malpighian tubules. The mid-gut (*MG*) is tied off at *T* with thread. The hind-gut is severed approximately 2 cm. from its junction with the Malpighian tubules (*MT*) and saline (*S*) is injected into the cavity. A precalibrated capillary tube (*C*) is inserted into the hind-gut forcing saline up into the tube (wavy line). The hind-gut is tied around the capillary tube at T. Increased diuresis forces fluid up into the capillary tube and this can be measured by use of an ocular micrometer in a dissecting microscope.

required, after which time the tubules were often inactive. Another method was devised which involved the insertion of a capillary tube (of a precalibrated volume) into the hind-gut. The mid-gut anterior to the insertion of the Malpighian tubules was ligated after filling the small space with saline. Secretion was determined by the movement of fluid along the capillary tube as measured with a dissecting microscope fitted with an ocular micrometer. Volume changes down to 0·1 μl. could be measured. This method is depicted in Fig. 1, and all assays were conducted with normal animals.

When the extent of diuresis is followed at 2 min. intervals (Fig. 2) it can be seen that the activity rises rapidly after drinking and then quickly declines to a rather low level. It should be noted here that the absolute values illustrated in the figure are open to some doubt since the quantitative assay was accurate to only 0·1 μl. Smaller values were estimated. However, the general shape of the curve is real and it indicates that the hormone has a short-term effect on diuresis.

Experiments designed to show the site of hormone release

Since it appeared that a diuretic hormone was released into the blood, experiments were initiated in an attempt to discover which part of the body released the hormone. Preliminary studies involved the placing of ligatures on the neck and between the thorax and abdomen 10 min. after giving the cockroaches water. Approximately 100 μl. of haemolymph were obtained from each region by the use of a microsyringe, accomplished by gently squeezing the animals on one side and inserting the needle on the other. Many times the needle had to be re-inserted and occasionally the fluid

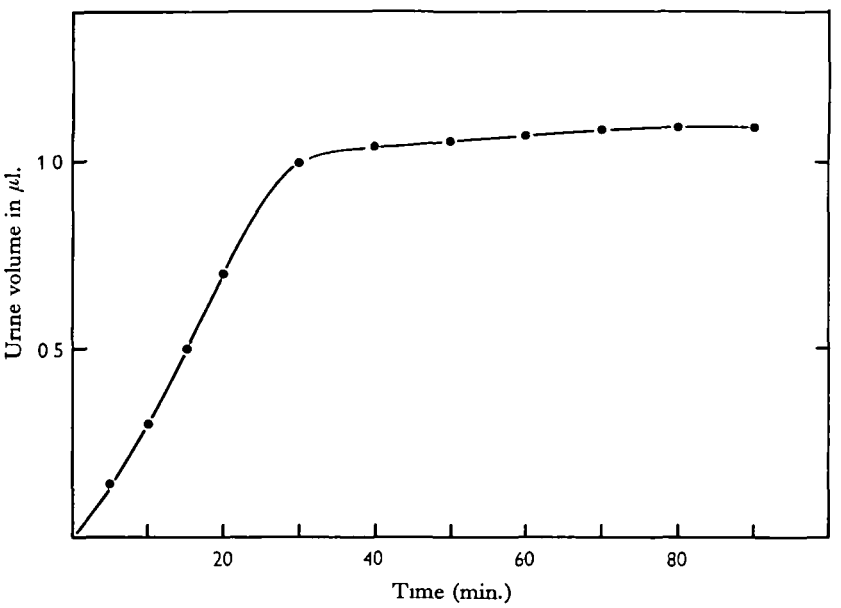

Fig. 2. Changes in total urine volume during a given period. The method of measurement is shown in Fig. 1. After 30 min. the increase in urine is so small that the added volume is only estimated.

Table 1. *The release of a diuretic hormone into the haemolymph after isolating various body regions by ligation*

Experiment	Body region isolated	No. of animals	Evidence of diuretic activity
(1) Ligations between the head, thorax and abdomen	Head	19	0
	Thorax	19	1
	Abdomen	19	11
(2) Ligations between the various abdominal segments	Abdominal segments		
Between: 2, 3	1, 2	16	0
4, 5	3, 4	16	0
6, 7	5, 6	16	7
8	7, 8	16	9
	Terminal portion	16	2
Between: 5, 6	1–5	18	0
8	6–8	18	12
	Terminal portion	18	2

contained particles of tissue. However, it appeared from the results that the active principle was taken up by the syringe.

The results of the experiments suggested that the hormone was liberated in the abdomen. Further experiments that involved ligation between various abdominal segments revealed that some organ located in the sixth or eighth segment was responsible for the release. Table 1 summarizes these data.

The next series of experiments was designed to determine what specific organ or tissue was involved. Various tissues within these segments were extirpated, washed, homogenized and the resulting brei was centrifuged. The supernatant was dropped on a fresh preparation and the activity determined. Only the terminal abdominal ganglion was found to possess diuretic hormone. Thus, it was concluded that the terminal ganglion of the central nervous system released the hormone.

Table 2. *The effect of ganglionic extracts on diuresis*

Extract	No. of animals	Extent of diuresis (μl) 30 min. after watering: without A_T extract	Extent of diuresis (μl) 30 min. after watering: with A_T extract* from dehydrated animals
B (dehydrated)	10	0	1·0
CC	8	0	0·9
CA	10	0·25 (±0·15)	1·2
SEG	10	0	0 8
T_1	8	0	1 0
T_2	8	0	1 0
T_3	8	0·1 (±0·1)	1·0
A_1	6	0·1 (±0·1)	1 0
A_2	6	0	1·0
A_3	6	0·1 (±0·1)	0·9
A_4	6	0	1·0
A_5	6	0	1·0
A_T (Control)	6	—	1·0
B (Normal)	6	0	0·4
CC	6	0	0·4
CA	10	0 (±0 1)	1·0
SEG	6	0	0·2
T_3	8	0	0·7
A_1	6	0	0·7
A_3	6	0	0·7
A_T	8	0	0·4

* Variation was ±0·15 μl.
For explanation of symbols see the text.

DIURETIC ACTIVITY OF OTHER TISSUE EXTRACTS

Since it has been shown that various other ganglia of the American cockroach contain an antidiuretic principle (Wall & Ralph, 1964), homogenates of the brain (*B*), corpora allata (*CA*), corpora cardiaca (*CC*), suboesophageal ganglion (SEG), thoracic ganglia (T_1, T_2, T_3) and abdominal ganglia (A_1–A_T) from dehydrated animals were tested for diuretic activity. The *CA* was the only other tissue to cause an increase in fluid secretion. This was limited and was equal to only one-fourth that of the A_T.

These studies do not eliminate the possibility that diuretic hormone is present in the

tissues listed above. If the tissues contain an antidiuretic hormone as indicated by Wall & Ralph (1964) then the two hormones could counteract each other. In an effort to determine whether an antidiuretic principle was present, extracts of all the ganglia in the central nervous system were tested individually and in combination with extracts of the A_T. The results of these experiments (Table 2) suggest that such a principle is present, which confirms the work of Wall & Ralph (1964).

DISCUSSION

The results of this investigation suggest that a diuretic hormone is liberated into the blood by the terminal abdominal ganglion, and that this hormone promotes fluid secretion by the Malpighian tubules. The extent and duration of diuresis is not extensive, which indicates the hormone is inactivated or in some way removed from the haemolymph. A similar phenomenon occurs in *Rhodnius* (Maddrell, 1963), and the tanning hormone bursicon, which is released from the same terminal abdominal ganglion in *Periplaneta* (Mills *et al.* 1965) is inactivated after being discharged into the blood (Mills, 1965, 1966*b*). In the cockroach a prote aseis present in the blood after ecdysis which may destroy bursicon and it is possible that a similar reaction may also break down the diuretic hormone. Purification studies on these hormones have shown that both are proteins which would tend to support this view (Mills & Lake, 1966).

When 'active' ganglion extracts from dehydrated animals are combined with ganglionic extracts from normal animals diuresis is inhibited. This suggests that an inhibitor is present or that an 'antidiuretic principle' is acting in an antagonistic manner to cancel the effect of the diuretic hormone. The latter appears to be more feasible since Wall & Ralph (1964) have found that various extracts cause an inhibition of dye uptake by the Malpighian tubules in *Periplaneta*. In addition, saline extracts from the terminal abdominal ganglion of normal animals enhance water reabsorption by the rectum while similar preparations from dehydrated animals have no effect (Wall & Ralph, 1965). This supports the assumption that an antidiuretic principle is present and suggests that this ganglion may contain and release both a diuretic and an antidiuretic hormone.

Saline homogenates of the corpora allata also appear to promote diuresis. The extent of diuresis is limited and diuresis can be demonstrated only after prior dehydration of the animal. Stutinsky (1953) inhibited diuresis in the rat by injecting extracts of the corpora allata of *Blabera fusca*. This tends to indicate that the *CA* contains an antidiuretic principle, although the possibility of a diuretic hormone (of lesser concentration) being present cannot be eliminated. On the other hand, Altmann (1956) found that in the honeybee, *CA* extracts increased the excretory rate of the Malpighian tubules. Thus, it appears that in insects both types of hormones can occur in the *CA*. The results of the present investigation support this contention.

Comparison between the diuretic hormones of *Periplaneta* and *Rhodnius* reveal several similarities. It appears that both animals release the hormone after ingestion of a liquid. The hormone is liberated from the most terminal posterior ganglion of the central nervous sytem in both *Rhodnius* (Maddrell, 1962, 1963) and *Periplaneta*. During the normal course of diuresis the rate of urine production increases rapidly but then declines (Maddrell, 1963, 1964*a*) indicating a short life of the hormone.

Temperature had a profound effect on diuresis (Maddrell, 1964*a*) and preliminary studies with the cockroach have produced similar results.

Maddrell (1964*b*) found that the central nervous sytem anterior to the mesothoracic ganglionic mass did not play a role in the control of the hormone release but that stretch receptors sensitive to vertical distention of the abdomen were involved. Preliminary experiments with the cockroach show that cutting the nerve chord between the fifth and terminal ganglia does impair diuresis. However, severing the chord between various ganglia has progressively less effect the more anterior the operation. Decapitation and operations performed on the thorax do not appear to influence diuresis, which indicates that the control of hormone release resides within the abdomen. Núñez (1956) observed that in beetle larvae the hormone was released by sensory information received in the abdomen, which agrees with the work of Maddrell (1964*b*).

In summary, it appears that both an antidiuretic and a diuretic hormone are released from the terminal ganglion with the former being in the greater concentration.

SUMMARY

1. When adult male cockroaches are deprived of water for 3 days the subsequent overcompensation of water ingestion causes the release of a diuretic hormone into the blood.

2. Ligation experiments indicate that the hormone was released from the posterior part of the abdomen.

3. The testing of various tissues extirpated from within this region revealed that only extracts of the terminal abdominal ganglion could promote diuresis.

4. Homogenates of ganglia from normal animals fail to elicit the diuretic response; this and other evidence suggests the presence of an antidiuretic hormone.

Appreciation is extended to Dr S. H. P. Maddrell, Cambridge University, for reading the manuscript. This work was indirectly supported by a grant, GB 4773, from the National Science Foundation.

REFERENCES

ALTMANN, G. (1956). Die regulation des Wasserhaushalte der Honigbiene. *Insectes Sociaux* **3**, 33–40.

MADDRELL, S. H. P. (1962). A diuretic hormone in *Rhodnius prolixus* Stål. *Nature, Lond.* **194**, 605–6.

MADDRELL, S. H. P. (1963). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. I. The control of diuresis. *J. Exp. Biol.* **40**, 247–56.

MADDRELL, S. H. P. (1964*a*) Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. II. The normal course of diuresis and the effect of temperature. *J. Exp. Biol.* **41**, 163–76.

MADDRELL, S. H. P. (1964*b*). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. III. The control of the release of the diuretic hormone. *J. Exp. Biol.* **41**, 459–72.

MILLS, R. R. (1965). Hormonal control of tanning in the American cockroach. II. Assay for the hormone and the effect of wound healing. *J. insect Physiol.* **11**, 1269–75.

MILLS, R. R. (1966*a*). A cockroach rearing cage designed for the collection of oothecae. *J. Econ. Entomol.* **59**, 490.

MILLS, R. R. (1966*b*) Hormonal control of tanning in the American cockroach. III. Hormone stability and postecdysial changes in hormone titre. *J. insect Physiol.* **12**, 275–80.

MILLS, R. R. & LAKE, C. R. (1966). Hormonal control of tanning in the American cockroach. IV. Preliminary purification of the hormone. *J. Insect Physiol.* (in the Press).

MILLS, R. R., MATHUR, R. B. & GUERRA, A. A. (1965). Studies on the hormonal contro lof tanning in the American cockroach. I. Release of an activation factor from the terminal abdominal ganglion. *J. Insect Physiol.* 11, 1047–53.

NÚÑEZ, J. A. (1956). Untersuchungen über die Regelung des Wasserhaushaltes bei *Anisotarsus cupripennis*. *Z. vergl. Physiol.* **38**, 341–54.

STUTINSKY, F. (1953). Mise en evidence d'une substance antidiurétique dans le cerveau et le complexe rétrocérébral d'une Blatte (*Blabera fusca* Brunn.). *Bull. Soc. Zool. Fr.* **78**, 202–4.

WALL, B. J. & RALPH, C. L. (1964). Evidence for hormonal regulation of Malpighian tubule excretion in the insect *Periplaneta americana* (L.). *Gen. Comp. Endocr.* **4**, 452–6.

WALL, B. J. & RALPH, C. L. (1965). Possible regulation of rectal water reabsorption in the insect, *Periplaneta americana* (L.). *Am. Zool.* **5**, 211.