

## INDUCTION OF TRANSPORT OF ORGANIC ANIONS IN MALPIGHIAN TUBULES OF *RHODNIUS*

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### SUMMARY

1. The ability of *Rhodnius* Malpighian tubules to transport organic anions such as p-aminohippuric acid (PAH) and amaranth depends on the physiological state of the insect. Unfed insects progressively lose this ability, but the transport mechanisms rapidly become activated after a blood meal.
2. This induction occurs in adults as well as larval instars and is not prevented by decapitation.
3. No increase in rates of excretion of organic anions follows a meal of Ringer's solution or a single injection of 0.1  $\mu$ moles of PAH into the haemolymph.
4. Accelerated PAH excretion is induced in insects fed (a) blood plasma alone or a suspension of red blood cells in saline, (b) solutions of serum albumin or casein, and (c) whole or diluted milk.
5. These results show that the ingestion of a protein-rich meal is sufficient to induce an accelerated transport of organic anions such as PAH and they suggest that this induction is not controlled by a hormone released in response to abdominal distension but depends instead on the continued presence in the haemolymph of some product of digestion of the meal.

### INTRODUCTION

It has recently been shown that the Malpighian tubules of several insects have transport mechanisms for removing organic anions of two kinds, acylamides and sulphonates, from the haemolymph (Maddrell, Gardiner, Pilcher & Reynolds, 1974). We now report that, in *Rhodnius*, the rate at which these mechanisms operate depends very much on the physiological state of the insect, being almost inoperative in starving insects, but rapidly activated after a blood meal.

### MATERIALS AND METHODS

Malpighian tubules were removed from larvae and adults of *Rhodnius prolixus* at various stages both before and after blood meals. In some experiments, the animals were artificially fed on warmed saline-based meals through a thin membrane (Gardiner & Maddrell, 1972). Preparations of the isolated tubules were made as described previously (Maddrell, 1969). They were usually stimulated to secrete by the inclusion

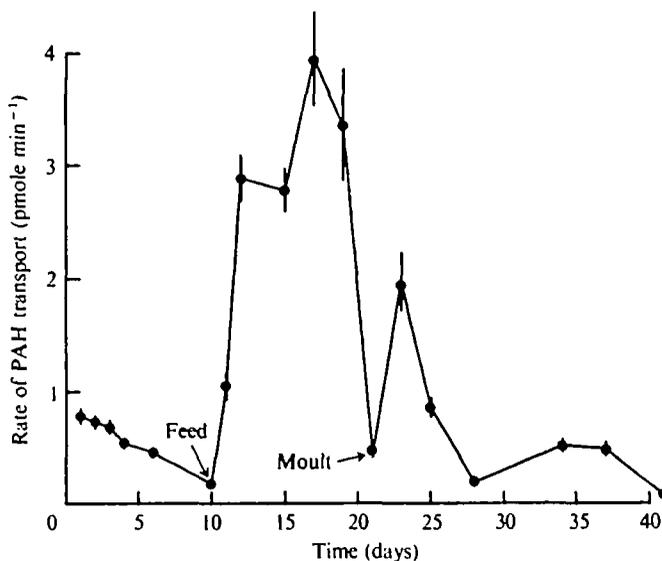


Fig. 1. Changes in rate of transport of PAH by Malpighian tubules isolated from 4th stage larvae of *Rhodnius* starved until day 10 then fed and allowed to moult to the 5th instar on day 21. Each point is based on an average of 20 determinations with no point representing fewer than 10 determinations. Vertical lines attached to the points represent the extent of the standard error of the mean.

of  $ca. 10^{-5}$  M 5-hydroxytryptamine (5-HT) in the bathing medium (Maddrell, Pilcher & Gardiner, 1969).

The ability of these tubules to secrete organic anions was tested by the addition to the bathing solution of either 0.05 mM tritium-labelled p-aminohippuric acid (PAH), supplied by the Radiochemical Centre, Amersham, or 0.04 mM amaranth. PAH in the secreted fluid was determined by conventional scintillation counting techniques using an Intertechnique ABAC SL40 scintillation counter. The concentration of amaranth in the secreted fluid was determined using a Beckman 151 spectrophotometer.

## RESULTS

### *Induction of PAH transport*

PAH excretion by Malpighian tubules was measured in fed and unfed 4th and 5th stage larvae. The results show that PAH transport declined gradually as the insects aged, but that within 1–2 days after a meal there was a large increase in the rate of PAH secretion (Figs. 1 and 2). After this upsurge, the rate declined with a half-time of about 10–15 days. During the later stages of moulting the rates of PAH transport were somewhat erratic (see Fig. 2, for example). This can reasonably be attributed to the reorganization of the Malpighian tubules which occurs at this time and which involves a growth in both length and diameter. The rate of fluid secretion elicited by 5-HT also varied during this period.

That the increase in PAH excretion is not linked to the changes involved in and accompanying moulting was shown by two types of experiments. In the first, fed 5th stage insects were decapitated directly after feeding, which effectively prevents the

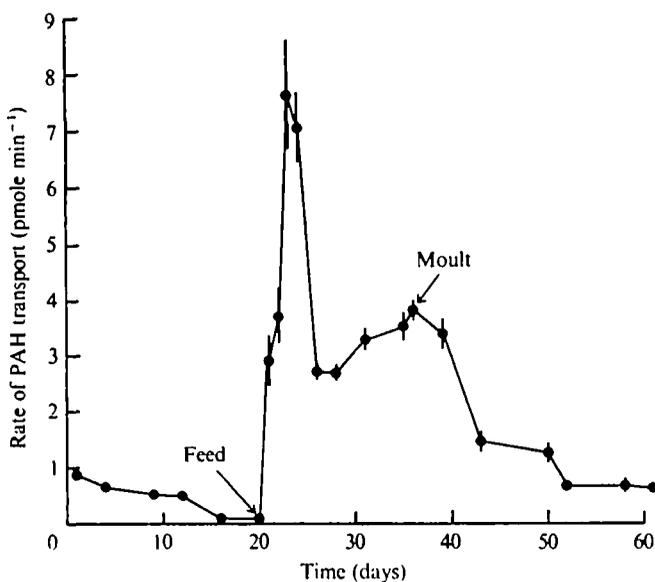


Fig. 2. Changes in rate of transport of PAH by Malpighian tubules isolated from 5th stage larvae of *Rhodnius* starved until day 20 then fed and allowed to moult to the adult state on day 37. Each point is based on an average of 20 determinations with no point representing fewer than 10 determinations. Vertical lines attached to the points represent the extent of the standard error of the mean.

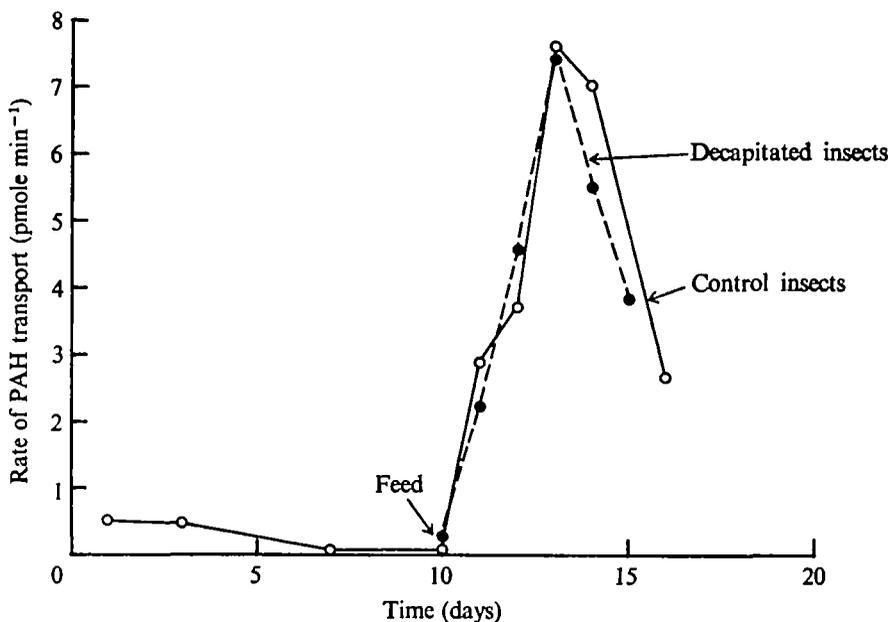


Fig. 3. Changes in rate of transport of PAH by Malpighian tubules isolated from 5th stage larvae of *Rhodnius* starved until day 10 then fed and the experimental insects (●-●) decapitated. Data for control insects (○-○) from Fig. 2. Values for the decapitated insects are each based on an average of six determinations.

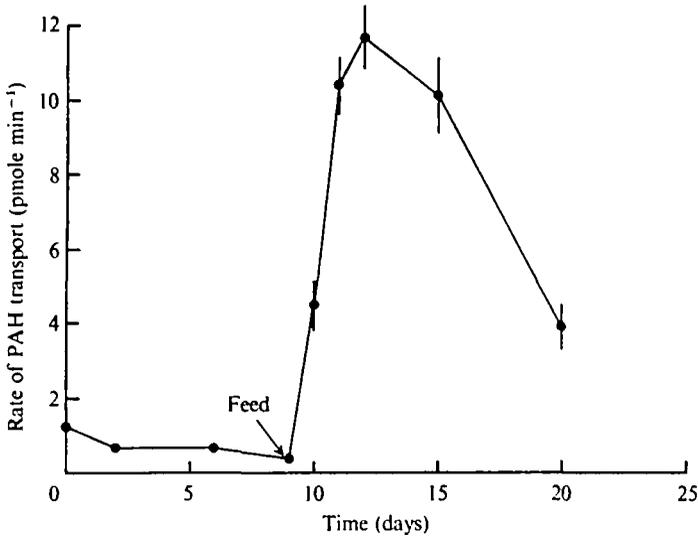


Fig. 4. Changes in rate of transport of PAH by Malpighian tubules isolated from adult *Rhodnius* starved until day 9, when they were given a blood meal. Each point is based on an average of 24 determinations with no point representing fewer than 10 determinations. The vertical lines attached to the points represent the extent of the standard error of the mean.

onset of moulting (Wigglesworth, 1934). This treatment did not, however, reduce the increase in the ability of the insects' Malpighian tubules to transport PAH (Fig. 3). In the second series of experiments adult *Rhodnius* were fed and the subsequent effects on the Malpighian tubules observed. Fig. 4 shows that a meal caused the same increase in the ability of adult tubules to transport PAH as it did in those from younger stages. As the Malpighian tubules do not grow in adults it is not surprising that there were no erratic values for tubular transport of PAH such as was observed following the feeding of 4th and 5th stage larvae.

Since decapitation does not prevent an increase in ability to excrete PAH, the question arises as to whether the process is hormonally controlled or initiated by the appearance in the haemolymph of products of digestion of the meal. *Rhodnius* fed Ringer's solution undergo normal diuresis (Maddrell, 1963); they also initiate moulting (Beckel & Friend, 1964), indicating that the hormonal control of this process is normal for at least the first few days after such a meal. It was, therefore, of interest to test the effect of a meal of glucose-free Ringer's solution on the ability of Malpighian tubules to transport PAH. It was found that such meals cause very little change in the rate of PAH transport, which suggests that the normal induction of transport is not controlled by a hormone released in response to the same stimuli that elicit release of the diuretic and prothoracicotrophic hormones. It seems more likely that the necessary stimulus is associated with the digestion of the meal. That the induction takes about 3 days to reach its maximum would accord with this suggestion.

To discover whether a more nutritious meal stimulated PAH transport, adult and 5th stage insects were fed Ringer's solution containing 34 mM glucose. A total of 110 tubules taken from these insects at various stages after the meal showed that they were no more capable of transporting PAH than before the meal. It appears therefore

Table 1. Induction of PAH transport in Malpighian tubules of 4th and 5th stage larvae of *Rhodnius* fed different meals

Stage	Meal	Days after meal	Rate of PAH transport (pmole min <sup>-1</sup> , mean $\pm$ S.E.(n))
5th	Washed red blood cells in 150 mM NaCl	2	8.88 $\pm$ 0.81 (11)
4th	Washed red blood cells in Ringer's solution	4	5.69 $\pm$ 0.41 (11)
5th	Plasma	2	11.79 $\pm$ 1.00 (8)
		5	6.83 $\pm$ 0.82 (8)
5th	2% bovine serum albumin in Ringer's solution*	2	6.01 $\pm$ 0.68 (8)
		3	6.51 $\pm$ 0.48 (8)
5th	2% casein in Ringer's solution*	2	2.18 $\pm$ 0.32 (8)
		5	3.57 $\pm$ 0.58 (8)
5th	Milk*	2	4.97 $\pm$ 0.68 (8)
5th	Milk diluted 1:2 with Ringer's solution*	5	1.11 $\pm$ 0.13 (8)
5th	Unfed controls		0.25 $\pm$ 0.03 (20)
4th	Unfed controls		0.13 $\pm$ 0.03 (4)

\* Insects were induced to feed by including small amounts of ATP in the meal.

that the ingestion of something more similar to the normal blood meal is necessary to stimulate PAH transport.

To analyse what it is in the blood meal that is essential to this induction we fed 4th and 5th stage insects on washed human red cells suspended either in 150 mM NaCl or in Ringer's solution. Malpighian tubules taken from such insects between 2 and 5 days after their meal all showed a greatly increased ability to transport PAH (Table 1). Somewhat surprisingly, tubules from 5th stage insects fed on the plasma from the same blood samples also showed a similar induction of PAH transport (Table 1). However, human plasma contains considerable amounts of albumin and globulins, proteins amounting to 6% by weight of the plasma (Altman & Dittmer, 1974), so it seemed possible that the presence of protein in the meal might be sufficient to cause an induction of PAH transport. To test this idea we fed insects on saline solutions containing serum albumin or casein and also fed some insects whole or diluted cow's milk. As Table 1 shows, Malpighian tubules from all these insects all showed an increased ability to transport PAH, though the increase was least marked in the insects fed the most dilute meal. Clearly the ingestion of protein is a sufficient stimulus for an induction of PAH transport.

To discover whether direct exposure to PAH could induce an increased rate of transport, 10  $\mu$ l of a 10 mM solution of PAH in Ringer's solution was injected into each of a group of ten 5th stage insects. Tubules taken from these insects at daily intervals during the next 3 days showed no increase in the rate of PAH transport which normally follows feeding. Unfortunately the tubules are so permeable to compounds of similar size to PAH (Maddrell & Gardiner, 1974) that they can rapidly excrete such small molecules by diffusive loss through the Malpighian tubule wall. As the induction of active PAH transport after a blood meal is a relatively slow process, it may be that a single injection of PAH does not maintain a sufficient concentration long enough for induction to occur.

Table 2. *Rates of transport of amaranth by Malpighian tubules of fed and unfed 5th stage larvae of Rhodnius*

Age of insect (days after moult)	Fed or unfed	Rate of amaranth transport ( $\mu\text{mole min}^{-1}$ , mean $\pm$ s.e.(n))
7-8	Unfed	0.85 $\pm$ 0.06 (6)
36	Unfed	0.15 $\pm$ 0.02 (6)
36	Fed 3 days previously	3.23 $\pm$ 0.25 (6)
36	Fed 7 days previously	3.46 $\pm$ 0.30 (6)

#### *Induction of amaranth transport*

The rates of amaranth transport were measured in Malpighian tubules from recently moulted 5th stage larvae (taken 6 days after the moult), starved 5ths (taken 36 days after the moult) and fed 5ths (taken 36 days after the moult but fed on day 29 or on day 33). As with PAH transport, the rate of amaranth transport declined after the moult but it increased greatly after the insect had taken a blood meal (Table 2).

#### DISCUSSION

The ability of Malpighian tubules of *Rhodnius* to transport organic anions rises dramatically in the first few days after a protein-containing meal. The stimulus for this does not appear to be the abdominal distension which is known to trigger the release of other hormones. Instead it seems likely that the stimulus is the appearance in the haemolymph of some product of digestion or of active intermediary metabolism following the meal. A similar induction of organic acid transport is known to occur in the kidneys of vertebrates. It is known, for example, that administration of penicillin or PAH to rats causes an acceleration of renal PAH excretion (Hirsch & Hook, 1970). Interestingly, in view of the failure of a single large dose of PAH to stimulate transport in *Rhodnius*, it has been found that frequently repeated administration of PAH is needed to induce an acceleration of renal PAH transport in rats; even daily application has only a limited effect (Bernhardt, Bräunlich, Dietze, Lungershausen & Schade, 1973). A further parallel is found in new born humans where the ability to excrete PAH is enhanced by a protein-rich diet (Calcagno & Lowe, 1963); in *Rhodnius* it is the ingestion of protein which seems chiefly to be responsible for the induction of PAH transport by a meal.

Whether the induction of PAH transport in *Rhodnius* is caused by a direct action of some metabolite on the Malpighian tubules or, indirectly, say through the action of a hormone, is not known. In larvae of the mosquito, *Aedes aegypti*, accelerated uptake of sodium ions through the anal papillae is stimulated by a fall in the concentration of sodium in the haemolymph. This is thought to act indirectly on the papillae through a hormone which activates sodium transport (Stobbs, 1971).

We have recently discovered that Malpighian tubules of some insects will transport alkaloids such as nicotine and atropine at high rates against steep concentration gradients (Maddrell & Gardiner, 1975). It is interesting that in contrast to the dependence of PAH transport on the physiological state of the insect, tubules of *Rhodnius* continue to transport nicotine and atropine at a high rate regardless of

nutritional state. It is not clear why *Rhodnius* should need to maintain an ability rapidly to excrete organic cations like nicotine but should regulate its ability to excrete organic anions in phase with digestion of a blood meal.

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## REFERENCES

- ALTMAN, P. L. & DITTMER, D. S. (1974). *Biology Data Book*, vol. III. Bethesda, Maryland: Federation of American Societies for Experimental Biology.
- BECKEL, W. E. & FRIEND, W. G. (1964). The relation of abdominal distension and nutrition to molting in *Rhodnius prolixus* (Stål) (Hemiptera). *Can. J. Zool.* **42**, 71-8.
- BERNHARDT, G., BRAUNLICH, H., DIETZE, C., LUNGERSHANSEN, W. & SCHADE, R. (1973). Acceleration of renal excretion of p-aminohippuric acid in rats of different age by repeated administration of foreign substances. *Acta biol. med. germ.* **31**, 423-34.
- CALCAGNO, P. L. & LOWE, C. V. (1963). Substrate induced renal tubular maturation. *J. Pediat.* **63**, 851.
- GARDINER, B. O. C. & MADDRELL, S. H. P. (1972). Techniques for routine and large-scale rearing of *Rhodnius prolixus* Stål (Hemiptera, Reduviidae). *Bull. ent. Res.* **61**, 505-15.
- HIRSCH, G. H. & HOOK, J. B. (1970). Maturation of renal organic acid transport: substrate stimulation by penicillin and p-aminohippurate (PAH). *J. Pharmac. exp. Ther.* **171**, 103-8.
- 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. (1969). Secretion by the Malpighian tubules of *Rhodnius*. The movements of ions and water. *J. exp. Biol.* **51**, 71-97.
- MADDRELL, S. H. P. & GARDINER, B. O. C. (1974). The passive permeability of insect Malpighian tubules to organic solutes. *J. exp. Biol.* **60**, 641-52.
- MADDRELL, S. H. P. & GARDINER, B. O. C. (1975). Excretion of alkaloids by Malpighian tubules of insects. *J. exp. Biol.* (In Press.)
- MADDRELL, S. H. P., GARDINER, B. O. C., PILCHER, D. E. M. & REYNOLDS, S. E. (1974). Active transport by insect Malpighian tubules of acidic dyes and acylamides. *J. exp. Biol.* **61**, 357-77.
- MADDRELL, S. H. P., PILCHER, D. E. M. & GARDINER, B. O. C. (1969). Stimulatory effect of 5-hydroxytryptamine (serotonin) on secretion by Malpighian tubules of insects. *Nature, Lond.* **222**, 784-5.
- STOBBART, R. H. (1971). The control of sodium uptake by the larva of the mosquito *Aedes aegypti* (L.). *J. exp. Biol.* **54**, 29-66.
- WIGGLESWORTH, V. B. (1934). The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and 'metamorphosis'. *Q. Jl microsc. Sci.* **77**, 191-222.

