

## THE EXCRETION OF SODIUM AND POTASSIUM BY THE MALPIGHIAN TUBULES OF *RHODNIUS*

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(With Four Text-figures)

### I. INTRODUCTION

About twenty years ago a very full account of the physiology of excretion in the blood-sucking insect *Rhodnius prolixus* Stål was given by Wigglesworth in three papers (Wigglesworth, 1931 *a*, *b* and *c*), and in order to place in proper perspective the problems which are the concern of the present investigation it is necessary to give a brief survey of the position as Wigglesworth left it.

First, in his investigation of the chemical composition of the urine he showed (among other things) that most of the fluid of the blood meal was excreted within 3 or 4 hr. as a clear urine, mainly a solution of sodium and potassium chlorides more or less isotonic with the ingested blood. In the first drops of urine passed after feeding the proportion of sodium to potassium was high, but during the next 2 or 3 hr. the proportion of sodium to potassium fell steadily until at the end of this 'diuresis period' the relative proportions were reversed. Thereafter the urine was generally retained in the rectum; it developed a yellow colour, its osmotic pressure increased and a white sediment of 'uratic spheres' accumulated.

Secondly, Wigglesworth described the whole system of excretory organs. There are four Malpighian tubules each about 4.35 cm. long. The upper or distal portion of each tubule, constituting about two-thirds of its whole length, is translucent and colourless, or faintly yellow; the striated inner border is of the honeycomb ('wabensaum') type. The cells of the lower or proximal portion are almost transparent, and the striated border is of the brush ('bürstensaum') type. Each Malpighian tubule enters the rectum through a small ampulla, the four ampullae being grouped around the opening of the midgut. The ampullae contain very large cells with long mobile processes which may extend into the rectum. The rectum is a muscular sac capable of considerable distension, and the glandular region of the epithelium is confined to the area around the opening of the midgut and Malpighian tubules.

Thirdly, he investigated the process of excretion, showing that fluid was secreted into the upper portion of the tubule and reabsorbed from the lower portion with the deposition therein of uratic spheres. He showed that the contents of the upper portion were weakly alkaline, those of the lower portion distinctly acid. These observations he assimilated to the following theory of the excretory mechanism. A solution of sodium and potassium urates is secreted into the upper portion of the

tubule; on passing down to the lower portion it is made acid with  $\text{CO}_2$ ; this causes precipitation of uric acid as uratic spheres; sodium and potassium are reabsorbed as a solution of bicarbonates.

That these interesting observations have remained so long without being followed up seems probably due to the inadequacy of standard chemical methods for dealing with the small quantities of fluid available. But the position has improved in recent years with the development of spectrometric and activation techniques; it has now become possible to determine sodium and potassium by flame photometry and osmotic pressure by freezing-point depression on samples of the order of one-thousandth of a cubic millimetre. The present paper gives an account of the application of these techniques to two of the questions which arise from the original work of Wigglesworth, namely,

(i) whether the variations of sodium/potassium ratio in the urine are associated with corresponding variations in the insect's haemolymph,

(ii) whether the histologically distinct portions of the Malpighian tubule have physiologically distinct roles in the excretion of sodium and potassium.

## II. MATERIAL AND METHODS

The stock of *Rhodnius* was reared in the laboratory according to the methods of Buxton (1930). For the experiments adult insects which had been without food for at least 3 weeks were used.

The insects were usually allowed to gorge themselves to repletion on rabbit's blood. The great distension of the stomach in a fully gorged insect crowds the other organs together making dissection difficult, and for this reason in some experiments involving operations upon the Malpighian tubules the insect was removed from the rabbit at the stage when the connexivum was just beginning to unfold. In other experiments the insects were allowed to feed through a piece of mouse skin upon ox blood with varying amounts of potassium chloride added. I am very grateful to Dr Wigglesworth for drawing my attention to this method of feeding which he has himself used with *Rhodnius* but never published.

For purposes of collecting the urine a piece of brass wire was fastened with soft wax to the bases of the forewings and the insect was held over a varnished watch-glass containing liquid paraffin. If samples of haemolymph were to be taken during the course of excretion the posterior portions of the wings were cut off and a thin layer of soft wax was applied to the abdominal tergites with a heated wire. With a fine needle a puncture was made between two tergites near the lateral margin and the drop of haemolymph exuded (about 0.25 cu.mm.) was collected in a pipette, the wound being then sealed over with wax. During the later stages of excretion collections of urine were made directly from the rectum by inserting a cannula through the anus. In some cases a wide cannula was permanently inserted and the urine removed with a pipette as it accumulated.

Operations upon the Malpighian tubules were carried out under a medium power binocular microscope, the preparation being illuminated through a glass rod light-guide. The insect was fastened with soft wax to a small table, about 2 by 0.5 cm.,

mounted on a ball-and-socket joint. The posterior abdominal tergites were completely removed. It was necessary to take precautions to avoid desiccation of the preparation during these operations which often took an hour for completion. Moist chamber methods have certain disadvantages; if the chamber is small, access to the preparation is difficult, if it is large it is difficult to ensure that the air is kept fully saturated. The method used in the present investigation was as follows. A stream of compressed air was bubbled through hot water and then passed through about 10 ft. of lead tubing laid out over the bench. The air stream left the tube saturated with water at room temperature and was then directed over the preparation through a cowl. Samples of haemolymph taken at the beginning and end of the operation showed practically no increase in osmotic pressure (see Table 5, serials 14, 15 and 16).

Wigglesworth (1931*b*) has described how one tubule on each side has a forwardly directed loop in the course of which the change from upper to lower portion of the tubule is situated. In the present work the forwardly directed loop on the right-hand side was used, and in preliminary experiments in which liquid paraffin stained with Sudan III was injected it was ascertained that this loop connected with the more ventral of the two ampullae on the right-hand side. The region of the ampullae is concealed by coils of tubules, and it was first necessary to cut through the fine tracheae which connect the tubules to the rectum. When this was done the rectum could be drawn to the left and the ampullae with their entering tubules exposed. A sample was first collected from the tubule entering the ventral ampulla, representing the lower portion, and a further sample was then taken from the upper portion at its junction with the lower portion in the forwardly directed loop. In this way the composition of the fluid in the upper and lower portions could be compared on the same tubule with its tracheal supply intact.

Collections were made by grappling the tubule with a fine tungsten hook and thrusting a silica pipette into it where it passed over the hook. Both the hook and the pipette were mounted on a simple micromanipulator with independent movements. The pipette was drawn with a steep taper and was about  $20\mu$  in diameter at the tip; it was partly filled with liquid paraffin and this was kept under slight pressure to prevent fluid entering until penetration had been effected. Unfortunately, it cannot be said that this method worked well. When urine flow was vigorous and the tubule was well distended attempts were generally successful, but the resistance of the tubule to penetration was such that thrusting with the pipette often resulted merely in dragging the tubule over the hook. In some cases penetration was only achieved by tearing the tubule and slipping it over the end of the pipette with forceps held in the hand, the hook then serving merely to prevent it sliding off. Once the tip of the pipette was seen to have entered the tubule a droplet of liquid paraffin was injected and the tip was then thrust farther in beyond the droplet. Slight suction was often necessary to start the flow of fluid into the pipette. Collection took 5–20 min. according to the rate of urine flow, and the samples taken were 0.01–0.1 cu.mm. in volume. Special attention was given to the possibility of haemolymph being drawn into the pipette through the wound in the tubule. The

pipette was always thrust in as far as possible so that its increasing diameter at the point of penetration filled the lumen of the tubule. The pipette being slightly inclined to the horizontal, it was possible to raise it so that the point of penetration was exposed while the part of the tubule in which the tip lay remained below the surface of the haemolymph. In some experiments a crystal of aniline blue was placed upon the region of penetration, but no dye could be detected in the sample. When collection was completed the pipette was raised above the surface of the haemolymph before being withdrawn from the tubule, and the sample was ejected into a watch-glass filled with liquid paraffin.

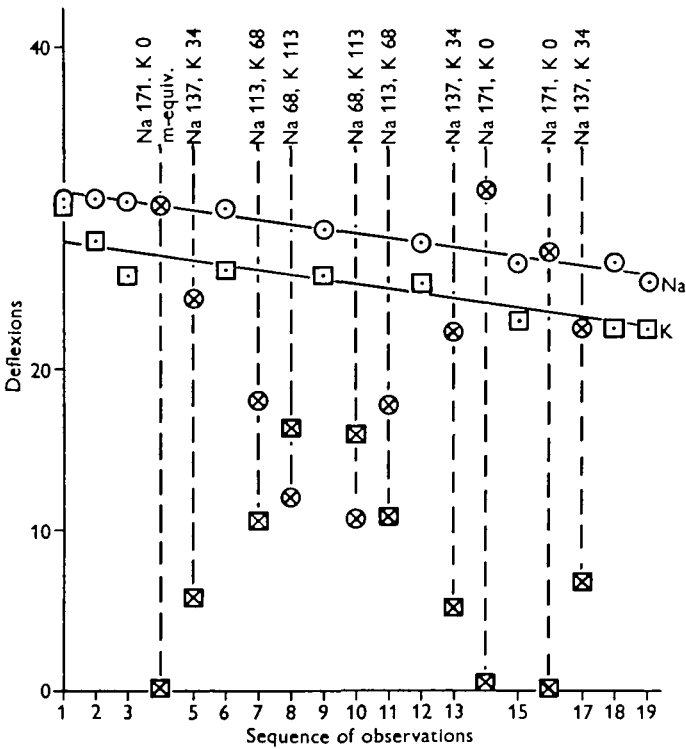


Fig. 1. A series of observations taken during the calibration of the flame photometer. The standard solution contained 171 mM. NaCl plus 171 mM. KCl/l. The concentrations of sodium and potassium in the calibrating solutions are given beside the observed deflexions. The symbols used are: for the standard solution Na  $\circ$ , K  $\square$ ; for the calibrating solutions Na  $\otimes$ , K  $\boxtimes$ . For further explanation see text.

A method has been described (Ramsay, 1950; Ramsay, Falloon & Machin, 1951) for the determination of sodium in small volumes of fluid by flame photometry. This method has now been extended to deal with potassium as well as sodium in the same sample; it is still under development, and it is hoped that a full description of it will be published in due course. Its accuracy is still rather poor, but in view of the very large variations in the concentrations of sodium and potassium indicated in Wigglesworth's studies it was felt that the method, even in its present stage, could

be usefully applied to these problems. One of the first difficulties is that throughout the course of a series of measurements the sensitivity of the apparatus varies. (This is believed to be due to the effect of temperature changes upon the refractive index of the prism.) It is therefore necessary to make determinations upon a standard alternately with determinations upon an unknown. Some of the measurements made for purposes of calibration are reproduced in Fig. 1. The volume of fluid delivered by the burette was approximately  $0.7 \times 10^{-3}$  cu.mm. The standard used for most of this work was a solution containing 171 mM. NaCl plus 171 mM. KCl/litre. Determinations were made upon the standard and upon the calibrating solutions in the order shown, and the time taken for each determination was roughly the same,

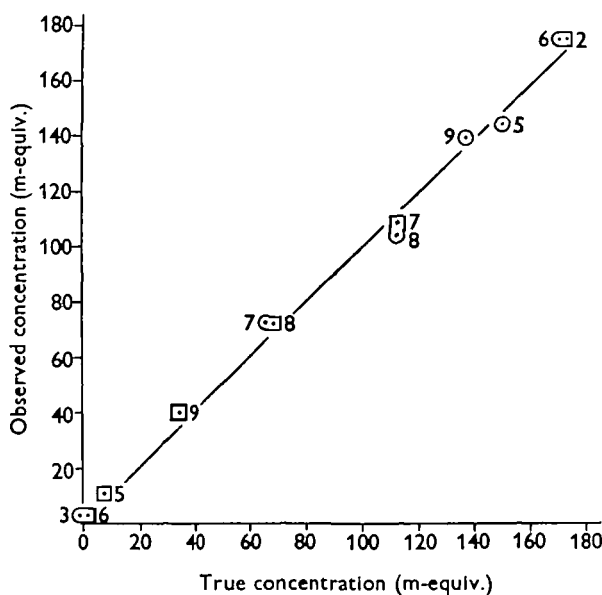


Fig. 2. The results of calibration tests with known solutions, showing the errors involved in the assumption of a linear relationship between deflexion and concentration. The symbols used are: Na ○, K □. The figure associated with each point on the graph gives the number of observations upon which the point is based. For further explanation see text.

so that the abscissa of Fig. 1 represents a time scale. The deflexions were plotted and smooth curves were fitted by eye to the deflexions of the standard, one for sodium and one for potassium. The deflexions given by the calibrating solutions were then compared with the appropriate portions of the standard curves. To take an example. The deflexions of observation 7 are Na 18.1, K 10.9; the corresponding standard deflexions from the curves are Na 29.3, K 26.3. Assuming that deflexion is directly proportional to concentration (see below) the observed concentrations of the calibrating solution are: Na  $171 \times \frac{18.1}{29.3} = 106$  m.equiv., K  $171 \times \frac{10.9}{26.3} = 71$  m.equiv.

The true concentrations were Na 113 m.equiv., K 68 m.equiv. so that the errors are Na -7 m.equiv., K +3 m.equiv. The average values of the observed concentrations are plotted against the true concentrations in Fig. 2, from which it emerges that

the error involved in the assumption of linearity is nowhere greater than 7.5 m.equiv. The scatter of the standard deflexions about the standard curves has been worked out for several series (forty-six observations in all for each element) and has an average value corresponding to  $\pm 5$  m.equiv. for both sodium and potassium. A single observation might be in error by (conventionally) three times the average random error plus the systematic error, i.e. by 22.5 m.equiv. In practice two determinations were made on each unknown, and if the two determinations differed by more than 20 m.equiv.—which was very seldom—two more determinations were made. It is difficult to define the accuracy of the method in simple statistical terms, but it is reasonable to suppose that in the results here presented a difference in concentration greater than 15 m.equiv. is real and not due to the errors of analysis.

In some experiments the total osmotic pressure was determined by freezing-point depression (Ramsay, 1949). Osmotic pressure is expressed in terms of the equivalent concentration of monovalent electrolyte (e.g. NaCl) in mm./litre. These measurements were made in duplicate and the probable error is of the order of  $\pm 2$ mm.

### III. RESULTS

#### (a) *Haemolymph of fasting Rhodnius*

The experiments on fed insects shortly to be described indicated that the concentration of potassium in the haemolymph was only about  $\frac{1}{10}$ – $\frac{1}{20}$  of the concentration of sodium. Determinations were subsequently made on the haemolymph of fasting insects with the apparatus adjusted to give maximum sensitivity to potassium and with a standard solution containing 150 mm. NaCl plus 7 mm. KCl, fairly closely approximating to the haemolymph in composition. Three insects were taken and the averages of four determinations upon each insect are given in Table 1, together with the averages of two determinations of freezing-point depression.

Table 1. *Haemolymph of fasting Rhodnius*

Serial	Na	K	O.P.
20	191	22	238
21	162	11	207
22	157	9	221

In the case of serial 20 there was so little haemolymph in the body that it was not possible to collect more than a few hundredths of a cu.mm., and this insect may have been in a state of partial desiccation. But it is clear from these measurements that the composition of the haemolymph just before feeding is not widely different from its composition just after feeding on normal blood.

#### (b) *Haemolymph and urine of naturally fed Rhodnius*

All the data under this head are assembled in Table 2, and the figures for serial 5 are plotted in Fig. 3. These results confirm the findings of Wigglesworth in that (i) the potassium concentration rises during the course of excretion, and (ii) the

occurrence of sediment and of the yellow colour are associated with a considerable rise in osmotic pressure. They also show clearly that while these extensive changes in the sodium/potassium ratio of the urine are taking place the changes in the sodium/potassium ratio of the haemolymph are relatively small. This settles the first of our questions; the Malpighian tubules and rectum are able to keep the composition of the haemolymph constant within fairly narrow limits.

(c) *Haemolymph and urine of artificially fed Rhodnius*

The figures in Table 2 indicate that there is a decrease in sodium concentration and an increase in potassium concentration in the haemolymph during the course of

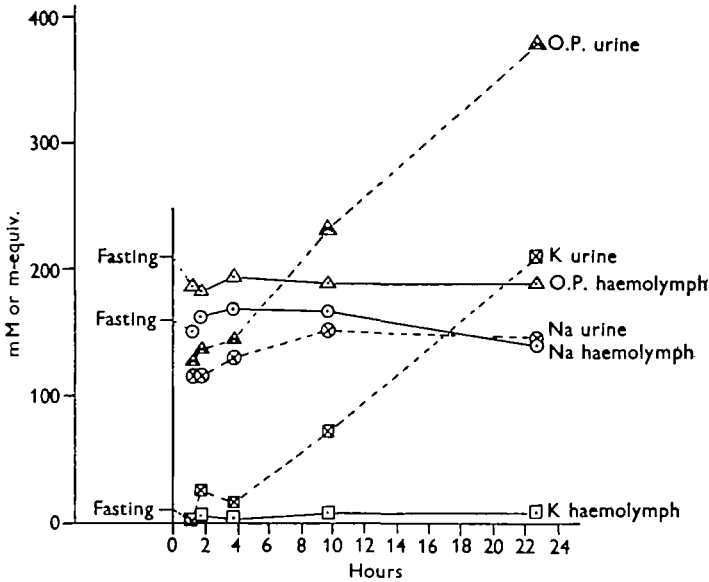


Fig. 3. For explanation see text. The 'fasting' values are based on the data in Table 1.

excretion, but the changes are small. It is therefore of some interest to ascertain what happens when the excretory mechanism is submitted to greater stress, and this was easily done by allowing the insect to feed upon blood in which the concentration of potassium had been artificially increased. The results of these experiments are shown in Table 3. The first experiments with artificial blood mixtures were carried out with approximately 3 parts of ox blood to 1 part of 171 mM. KCl. The insects fed on this quite readily and showed no ill effects, in spite of the fact that in one case (serial 3) there was appreciable increase in the potassium concentration of the haemolymph. A mixture of 1 part of ox blood to 3 parts of 171 mM. KCl was then tried, and since the method of mounting the insect for collection of urine made it difficult to observe its behaviour, some insects were fed but not mounted. Serials 17 and 18 stopped feeding before becoming fully distended and did not pass any urine, although there was a considerable accumulation of clear fluid in the rectum. The potassium concentration in the haemolymph was raised

Table 2. *Haemolymph and urine of naturally fed Rhodnius*

Serial	Temp. °C	Time after feeding	Haemolymph			Urine			Appearance of urine	How obtained
			Na	K	O.P.	Na	K	O.P.		
1	24	(a) 0:30	—	—	—	126	22	—	Colourless, no sediment	Passed into watch-glass
		(b) 2:00	—	—	—	129	25	—	Colourless, no sediment	Passed into watch-glass
		(c) 19:00	175	5	—	169	58	—	Colourless, some sediment	By cannulation
2	24	(a) 0:20	147	4	—	140	16	—	Colourless, no sediment	Passed into watch-glass
		(b) 0:45	180	6	—	132	10	—	Colourless, no sediment	Passed into watch-glass
		(c) 1:35	162	8	—	152	7	—	Colourless, no sediment	Passed into watch-glass
5	18-20	(a) 1:20	151	1	187	116	1	127	Colourless, no sediment	Permanently cannulated
		(b) 1:50	162	5	182	116	25	137*	Colourless, no sediment	Permanently cannulated
		(c) 3:45	169	3	194	131	16	145*	Colourless, no sediment	Permanently cannulated
		(d) 9:45	167	7	189	152	72	232	Colourless, no sediment	Permanently cannulated
		(e) 22:45	141	8	189	146	210	379	Yellow, much sediment	Permanently cannulated
6	18-20	(a) 0:30	156	5	180	101	8	114	Colourless, no sediment	Permanently cannulated
		(b) 21:30	149	0	180	220	163	392	Yellow, much sediment	Permanently cannulated
7	18-20	24:00	173	4	209	101	256	428	Yellow, much sediment	Passed into watch-glass
9	18-20	29:00	167	4	202	116	200	302*	Yellowish, some sediment	Passed into watch-glass
10	18-20	44:00	164	7	209	192	137	362	Yellow, much sediment	By cannulation
11	18-20	17:00	163	9	201	100	53	154	Colourless, little sediment	By cannulation

\* See text, p. 125, l. 26.



Table 3. *Haemolymph and urine of artificially fed Rhodnius*

Serial	Temp. °C.	Mixture of blood:KCl	Time after feeding	Haemolymph			Urine			Appearance of urine	Behaviour
				Na	K	O.P.	Na	K	O.P.		
3	24	3:1	(a)	162	16	—	99	89	—	Colourless, no sediment	Normal response
			(b)	150	33	—	96	115	—		
4	18-20	3:1	(a)	152	9	—	101	74	—	Colourless, little sediment	Normal response
			(b)	154	5	—	97	74	—		
17	18-20	1:3	(c)	24.00	155	9	—	57	137	Colourless, no sediment	Normal response
			(a)	0.35	121	69	—	—	—		
18	18-20	1:3	(b)	4.40	114	96	—	—	—	Yellowish, some sediment	Normal response
			(a)	0.45	98	92	—	—	—		
19	18-20	1:3	(b)	4.30	87	124	—	—	—	Colourless, no sediment	Normal response
			(c)	10.30	87	95	—	—	—		
			(a)	0.25	144	13	112	49	—	Colourless, no sediment	Normal response
			(b)	1.00	146	12	116	59	—		
			(c)	4.00	149	14	99	62	—	Colourless, no sediment	Normal response
			(d)	10.00	142	15	56	161	—		
			(e)	22.00	140	15	22	232	—	Yellow, much sediment	Normal response
			(f)	30.00	116	80	—	60	143		
			(g)	54.00	129	41	60	142	—	Colourless, some sediment	Weak response
			(h)	70.00	126	40	—	53	153		

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Some 10 times, yet the insects continued for many hours to respond with apparently normal questing movements of the legs when their antennae were touched. Later, however, the response weakened and failed. The following observations were made on another insect which had taken a full meal but which had not been mounted for the collection of urine.

Time after feeding		Behaviour
hr.	min.	
0	30	Thrown upon back, succeeded in righting itself.
1	15	Thrown upon back, tried to right itself but failed.
2	45	Thrown upon back, did not try to right itself; in normal posture attempted to walk with the front pair of legs only.
3	45	Relaxed its grip on a vertical paper surface and lay motionless. No recovery.

More interest attaches to the results of serial 19, shown plotted in Fig. 4. In this case the insect was fully distended after its meal. Urine flow began almost at once and continued at a brisk rate for 4 hr. During this period the concentration of potassium in the urine was relatively high but never exceeded the concentration of sodium. The last drop of urine, passed sometime between 4 and 10 hr., was colour-

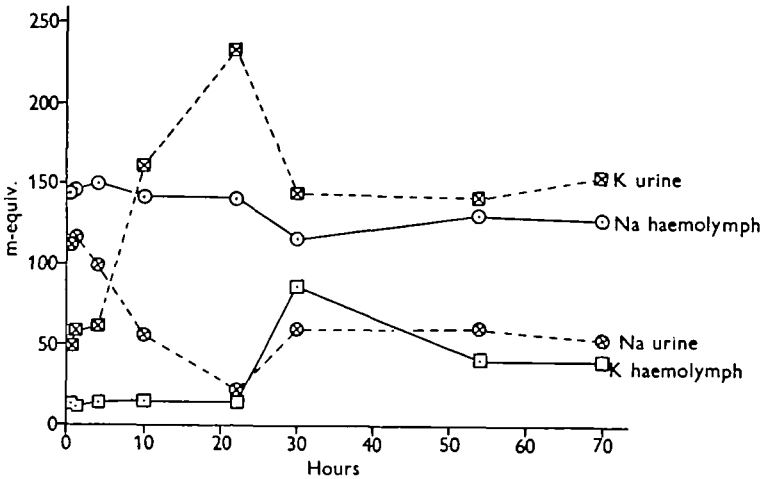


Fig. 4. For explanation see text.

less with a little sediment, and the concentration of potassium was now greater than that of sodium. At 22 hr. the rectum was cannulated, and the urine obtained was yellow with much sediment and a concentration of potassium about 10 times that of sodium. These changes in the urine normally indicate the end of the diuresis period, and up to this time the concentration of potassium in the haemolymph was still only 50% above normal and the insect was fully responsive. But sometime between 22 and 30 hr. events took a different course. The urine collected in the cannula was colourless with a moderate amount of sediment and a higher concentration of potassium than sodium, but the concentration of potassium in the haemolymph had risen to about 8 times normal and the insect showed very little response to stimulation. At 54 hr. the potassium in the haemolymph had fallen to

about 4 times normal, but the insect showed no response to stimulation although its heart could be seen beating and was still beating rather irregularly at 70 hr.

It appears that there were two distinct phases in the process of excretion in this insect, the first from 0 to 22 hr. and the second from 22 to 70 hr. The drops of urine collected during each phase were pooled and analysed.

0-22 hr., vol. 31 cu.mm.	Na 97 m.equiv.	K 79 m.equiv.
22-70 hr., vol. 16 cu.mm.	54 m.equiv.	144 m.equiv.

A sample of the blood mixture was laked and analysed and was found to contain Na 34 m.equiv., K 132 m.equiv. This case is further discussed in the next section.

#### (d) *Collection from the Malpighian tubules*

What is usually called 'urine' in *Rhodnius* would be more correctly called 'rectal fluid'. The fact that the concentration of potassium in the rectal fluid is greater than in the haemolymph does not automatically mean that the difference in concentration is established by the activity of the Malpighian tubules. Nevertheless, the relatively rapid rate of excretion and the relatively small surface exposed by the ampullae and rectal glands make it probable *a priori* that the Malpighian tubules play an important part.

In order to investigate the role of the Malpighian tubules it is natural that one should choose to work under conditions in which the composition of the urine differs substantially from that of the haemolymph, that is to say, at some time towards the end of the diuresis period or later. This was the first line of approach made to the problem. The insect was fixed to the operating table and an attempt was made (not always successful) to obtain a sample of urine by cannulation of the rectum. The abdominal tergites were then removed, a sample of haemolymph was taken and then collections were made first from the lower portion and then from the upper portion of the right-hand ventral tubule as already described. The results of five more or less successful experiments are given in Table 4.

The first point which clearly emerges is that in all cases except that of serial 12 the fluid in the upper portion of the tubule contains less sodium and much more potassium than the haemolymph. The differences are so great that the selective activity of the upper portion can hardly be in doubt. The samples taken from the lower portion all show a greater concentration of potassium and most show a smaller concentration of sodium than the haemolymph, but the differences are much less than in the case of the upper portion. This suggests that the differences in concentration established by the activity of the upper portion are degraded as the fluid passes through the lower portion, a somewhat surprising result if true.

These experiments were not altogether satisfactory for a variety of reasons. The operations were on the whole carried out too late in the course of excretion. Ideally one should choose a moment near the end of the diuresis period for the operation, but owing to the great variation in the duration of this period from one insect to another, the correct timing is a little difficult to achieve. In serial 12, for example, no urine had been passed at 7 hr. after feeding when the operation was begun; the

Table 4. Collections from Malpighian tubules of naturally fed *Rhodnius*

Serial	Temp. °C.	Time after feeding	Haemolymph			Urine			Upper tubule			Lower tubule		
			Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.
5	18-20	24.00	177	5	218	146	210	379	137	72	222	168	53	207*
6	18-20	24.00	188	7	221	220	163	392	86	122	227	140	67	218
8	18-20	26.00	164	11	182	—	—	—	98	133	212*	182	17	189*
9	18-20	29.00	167	4	202	116	200	302*	134	90	251	144	63	182*
12	18-20	7.00	149	6	179	—	—	—	188	12	251	202	13	262

Table 5. Collections from Malpighian tubules of artificially fed *Rhodnius*

Serial	Temp. °C.	Time after feeding	Haemolymph 1			Haemolymph 2			Upper tubule			Lower tubule		
			Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.
13	18-20	1.00	158	22	178*	—	—	—	106	124	235	95	95	178*
14	18-20	0.30	153	12	184	156	11	185	57	147	204	82	109	168*
15	18-20	0.30	147	14	179	149	23	182	97	145	261	98	105	196*
16	18-20	1.15	140	23	182	140	33	184	55	162	198*	93	113	188*

The second sample of haemolymph belonging to serial 13 was lost through an accident.

\* See text, p. 125, l. 26.

rectum was found to be highly distended with colourless urine in which there was hardly any sediment at all. When diuresis slackens and the tubules are no longer distended the difficulties of successful penetration are increased and the amounts of fluid obtained are less. There is also the possibility, envisaged by Wigglesworth, that fluid from the rectum may be drawn back into the lower portion of the tubule. In view of these disadvantages a second approach was made, taking advantage of the method of artificial feeding on ox blood with potassium chloride added. Under these conditions, even during the early part of the diuresis period, the urine had been found to contain substantially more potassium than the haemolymph. In this second series of experiments the insects were allowed to feed on a mixture of approximately 3 parts ox blood to 1 part of 171 mM. KCl, and two samples of haemolymph were taken, the first immediately after the insect had been opened up and the second at the end of the operation; this provided a check upon the possibility of changes taking place in the haemolymph during the operation. The results are presented in Table 5.

From the figures in Table 5 the following points may be noted:

- (i) The changes in the haemolymph during the operation are negligible.
- (ii) The fluid in the upper portion of the tubule contains less sodium (av. - 69 m.equiv.) and more potassium (av. + 127 m.equiv.) than the haemolymph; its osmotic pressure is greater (av. + 44 mM).
- (iii) The fluid in the lower portion of the tubule contains less sodium (av. - 56 m.equiv.) and more potassium (av. + 88 m.equiv.) than the haemolymph; its osmotic pressure is practically identical with that of the haemolymph (av. + 2mM.).
- (iv) The fluid in the lower portion of the tubule contains more sodium (av. + 13 m.equiv.) and less potassium (av. - 39 m.equiv.) than the fluid in the upper portion; its osmotic pressure is lower (av. - 42 mM.).

With the exception of the difference in concentrations of sodium between the upper and lower portions of the tubule these differences may be judged significant in relation to the errors of analysis. The difference in osmotic pressure between the fluid in the lower portion and the haemolymph is not statistically significant in relation to variations between individual insects.

In answer to the second question posed in the introduction it is therefore concluded that the upper portion of the tubule is able to elaborate a fluid containing less sodium and more potassium than the haemolymph, and that these differences in concentration become degraded during the passage of the fluid through the lower portion. These conclusions and other points arising from the experimental results are further discussed in the next section.

#### IV. DISCUSSION

The results presented in this paper are substantially in agreement with the work of Wigglesworth, but certain differences are to be noted.

First, the duration of the diuresis period in the present experiments was in general much longer than in Wigglesworth's experiments. The insects showed a tendency to retain the urine in the rectum, which was often considerably distended.

In many cases, both for naturally fed and artificially fed insects, a considerable amount of air was observed to be present in the stomach after feeding, and it seemed that the elimination of urine was more rapid when the amount of air was less. The arrangements for allowing the experimental insects to feed from a rabbit were the same as those used in rearing the whole culture, which was thoroughly healthy. There is no reason to believe that the slower rate of elimination was due to impaired physiological condition of the insect.

Secondly, Wigglesworth found that at the end of the diuresis period the urine contained only traces of sodium. In the present work the concentration of sodium in the urine of naturally fed insects was never less than half its concentration in the haemolymph. It has already been pointed out that the fluid in the lower portion of the tubule has generally the same osmotic pressure as the blood. This implies that the very much greater osmotic pressure of the urine in the later stages of excretion (when the yellow colour appears) is due to the activity of the ampullae and/or the rectal glands, and is in keeping with the observation that the urine must be retained in the rectum for several hours before these changes are manifested. In the three cases in which direct comparison is possible, serials 5, 6 and 9, Table 4, the potassium concentration in the rectal fluid was always very much higher than in the fluid taken from the lower portion of the tubule, whereas the sodium concentration was higher in one case and lower in the other two. The high osmotic pressure and high concentration of potassium in the yellow urine might be due to the secretion of potassium into it, but in view of the ability of the rectal glands of *Rhodnius*, and of insects in general (Wigglesworth, 1932), to remove water from the faeces and urine, it is probable that there is reabsorption of water from the rectum during the period in which the yellow colour develops. If this is granted then sodium must be reabsorbed at the same time, since the sodium concentration of the urine does not rise parallel with the osmotic pressure and potassium concentration; but there is no need to postulate the secretion of potassium into the rectum. On this view the reabsorption of water and sodium would in time produce a urine of higher osmotic pressure than the haemolymph and containing only traces of sodium. But there is no obvious explanation of why this seems to have happened in Wigglesworth's experiments and not in the present experiments.

The variation of the sodium/potassium ratio in the haemolymph of insects is very much greater than in other classes of animals as yet studied. Boné (1944) has shown that a high sodium/potassium ratio is characteristic of carnivorous and omnivorous insects, and a low ratio of herbivorous insects. Insects also seem able to tolerate very considerable alteration of their normal ratio. Tobias (1948), working on the cockroach, caused the ratio to fall from 6.2 to 2.7 by administration of potassium chloride through the mouth without apparent harm to the insect, and Roeder (1948) likewise found that the nervous system of the cockroach, bathed in simple salt solutions, could tolerate variations of the same order without impairment of function. In *Rhodnius* the value of the ratio found for fasting insects is about 16. After a normal meal it may rise to 50 or over. After a meal of blood with potassium chloride added it may fall to 1 or 2, and yet for several hours the insect continues to make

co-ordinated movements. The heart can continue to beat for 48 hr. with the ratio at 3.

In considering the ability of the Malpighian tubules and rectum to keep down the potassium concentration of the haemolymph after a normal meal, one must bear in mind that at the time when high concentrations of potassium appears in the urine the rate of urine flow is slackening and the rate of doing secretory work is not perhaps so great as appears at first sight. It is otherwise when the insect has fed on blood with artificially increased potassium content, and under these conditions the potassium concentration in the haemolymph is seen to rise. Further examination of the case of serial 19 (Fig. 4) brings to light some interesting points. In the laked blood upon which the insect had fed the sodium/potassium ratio was 0.26. During the first phase of excretion the sodium/potassium ratio of the urine was 1.23. Even if one assumes that all the potassium in the ox blood was contained in the corpuscles and that these remained intact, it is quite clear that during the first phase the excretion of potassium relative to sodium was not adequate to maintain the normal proportion of these elements in the haemolymph and tissues. Yet during this phase the ratio in the haemolymph was maintained at about the relatively high level of 10. Conceivably the tissues might regulate the haemolymph by taking up potassium as they were shown to do by Tobias, but in view of the relatively large amount of fluid ingested and the relatively small bulk of the tissues this is improbable. It is more likely that the potassium is retained in the stomach. Wigglesworth suggested that the increased excretion of potassium towards the end of the diuresis period was due to the fact that it was present mainly in the corpuscles and was only liberated as digestion proceeded. This may indeed be true, but one should bear in mind the possibility that the gut epithelium may exert a selective action upon the passage of ions through it. Certainly, the flooding of the haemolymph with potassium at the beginning of the second phase strongly suggests the breakdown of some form of restraint.

In the upper portion of the tubule the fluid contains less sodium and more potassium and has a higher osmotic pressure than the haemolymph. Since there is a net movement of water and solutes from the haemolymph into the lumen of the tubule we are bound to admit that the movement of potassium against the concentration gradient must be brought about by some active process involving the expenditure of energy. Sodium and water, on the other hand, move with their respective concentration gradients and these movements may be due to passive diffusion. In order to account for the secretion of urine by the upper portion of the tubule all we have to postulate is that potassium (together with some anion) is actively secreted into the lumen; this will raise the osmotic pressure of the tubule fluid, causing water to diffuse in, and the inward diffusion of water will set up a concentration gradient of sodium which will result in the passive inward diffusion of this ion.

In the lower portion of the tubule the composition of the fluid is more or less intermediate between that of the upper portion and that of the haemolymph. If we regard the cells of the lower portion as freely permeable to all the substances in question than all the changes can be accounted for on the basis of passive diffusion alone. But there is one very good reason why this simple explanation cannot be

accepted. Since the fluid coming from the upper portion of the tubule has an higher osmotic pressure than the haemolymph, passive diffusion of water would result in an increase in volume of the urine as it passed through the lower portion, but Wigglesworth's work suggests strongly that the reverse is true, that there is reabsorption of fluid into the haemolymph from the lower portion of the tubule. It is more likely that the cells of the lower portion are actively concerned in the reabsorption of water from the urine into the haemolymph; the osmotic forces involved, however, appear to be small. Whether or not there is reabsorption of water, it is at least certain that water does not pass from the haemolymph into the urine in the lower portion; and therefore if the potassium concentration of the urine falls in the lower portion, it follows that potassium must move from the urine into the haemolymph. Whether sodium moves inward or outwards cannot be decided until something is known about the changes in volume of the urine as it passes through the lower portion. One would also like to know more about the formation of hypotonic urine during the early stages of diuresis after a normal meal; unfortunately no collections were made from the Malpighian tubules of naturally fed insects during the early stages of diuresis.

The foregoing suggestions as to the mechanism of urine formation are regarded as no more than an attempt to apply the principle of economy of hypothesis to the available data.

The bearing of the present experiments upon Wigglesworth's theory of uric acid excretion is quickly dealt with. Wigglesworth's theory demands that sodium and/or potassium be secreted into the upper portion of the tubule and reabsorbed from the lower portion. This is precisely what has been found in the case of potassium; the question of reabsorption of sodium is still undecided.

There is just one other small matter which may be relevant. If a fluid contains only sodium and potassium ions with the equivalent number of monovalent anions, fully dissociated, then according to the conventions used in this paper  $\text{Na} + \text{K} = \text{O.P.}$  If  $\text{Na} + \text{K} < \text{O.P.}$ , other osmotically active substances must be present, if  $\text{Na} + \text{K} > \text{O.P.}$  then the cations must be in balance with polyvalent anions or dissociation must be incomplete. In Tables 2, 4 and 5 certain figures for O.P. are marked with an asterisk; these are cases in which  $\text{Na} + \text{K} > \text{O.P.}$  There are 13 such cases altogether, one under haemolymph, three under urine, two under upper tubule and under lower tubule there are seven cases out of the total of nine samples of fluid taken from the lower tubule. Some of the discrepancies are well within the limits of experimental error. But taking the four cases in Table 5 the average value of  $\text{Na} + \text{K} - \text{O.P.}$  for the lower tubule is 15 which is statistically significant ( $P = 0.05 - 0.01$ ) in relation to variation between individuals and which, being based upon at least eight observations for each element, is probably just significant in relation to the errors of analysis. What this means is hardly worth speculating upon at present; but it is an observation which may one day fit into place in a more detailed account of the mechanism of excretion.

Other work relating to the excretion of salts and water by Malpighian tubules was discussed in a recent paper (Ramsay, 1951).



## SUMMARY

1. The very considerable changes in the concentrations of sodium and potassium in the urine of *Rhodnius* during the normal course of excretion are not reflected in the haemolymph which remains relatively constant in composition.
2. The concentration of potassium in the haemolymph can be increased some tenfold by adding potassium chloride to the blood on which the insect feeds. Such increase in the concentration of potassium can be tolerated for several hours without loss of nervous co-ordination, but it is ultimately fatal.
3. The upper portion of the Malpighian tubule can secrete a urine which contains more potassium and less sodium than the haemolymph.
4. During the passage of this urine through the lower portion of the tubule the differences in concentration between urine and haemolymph became reduced.
5. It is probable that water and sodium, but not potassium, are reabsorbed from the urine in the rectum during the later stages of excretion.

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