THE EXCRETION OF SODIUM, POTASSIUM AND WATER BY THE MALPIGHIAN TUBULES OF THE STICK INSECT, *DIXIPPUS MOROSUS* (ORTHOPTERA, PHASMIDAE)

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INTRODUCTION

Evidence has been published (Ramsay, 1953) that in a variety of insects potassium is actively secreted into the urine, in which it is present in very much higher concentration than in the haemolymph. In that paper the suggestion was made that the active secretion of potassium into the tubule might be an essential process in the formation of urine. 'It seems possible that the active secretion of potassium, accompanied by some anion, might produce a high osmotic pressure in the tubule which would cause water to pass inwards through the wall; and that this in its turn would promote a passive diffusion of sodium into the tubule.' If this simple hypothesis is true it follows that the urine can never be hypotonic to the haemolymph. This hypothesis was put to the test on the Malpighian tubules of the stick insect (Ramsay, 1954) and clearly shown to be untenable; the urine could be, and often was, slightly but definitely hypotonic to the haemolymph, showing that water as well as potassium could be actively secreted against a gradient. This disposes of the hypothesis in its simplest form, but does not negative the suggestion that active secretion of potassium is an essential process in the formation of urine. The experiments to be described in this paper were undertaken in the first place in order to establish the relation between the rate of urine formation and the concentrations of potassium in the urine and medium, and in the second place to study the movement of sodium in relation to active transport versus passive diffusion.

The design of such experiments is dominated by certain practical considerations. Wigglesworth (1931*a*) has commented upon the unsuitability of Ringer solution as a medium for the Malpighian tubules of *Rhodnius*, and up to the present the same applies in the case of the stick insect. The media used in experiments must therefore be based on haemolymph. The maximum volume of haemolymph which can be obtained from a single insect is about 0·05 ml. The life cycle is about 9 months. The fairly large culture maintained for this investigation yields about 1·5 ml. of haemolymph per month. Although it is a simple matter to make a preparation of the whole battery of Malpighian tubules as described in another paper (Ramsay, 1955) and thereby obtain urine in relatively large quantities, such a preparation requires about 0·5 ml. of medium to bathe it, and if the medium is largely haemolymph progress along these lines promises to be slow. For this reason it has been necessary to use single tubules isolated in drops of medium of about 50 mm.^{3} or less in volume.

MATERIAL AND METHODS

A description is given elsewhere (Ramsay, 1955) of the excretory system of the stick insect. The Malpighian tubules are of three kinds, of which only two, the superior and the inferior tubules, have been used in the present work. There is some slight gradation in the appearance of the superior tubules from one end to the other; the inferior tubules resemble the superior tubules over most of their length but end distally in a dilatation which is packed with white granules.

The fresh haemolymph of the stick insect is not a very convenient medium for physiological work since it almost invariably coagulates during the course of an experiment. It has been found that if the fresh haemolymph is heated to 100° C. for a few minutes and then centrifuged a clear fluid, which will be called 'serum', is separated from a compacted clot. As a physiological medium for Malpighian tubules serum does not appear to be in any way inferior to haemolymph (see later). An analysis of the main inorganic constituents of serum is described in the paper referred to above and is reproduced for convenience in Table 1, col. 1, of the present paper.

Table 1

	Serum (1)	Ringer (2)
Sodium	11 m.equiv./l.	17 m.equiv./l.
Potassium	18 m.equiv./l.	15 m.equiv./l.
Calcium	7 m.equiv./l.	6 m.equiv./l.
Magnesium	108 m.equiv./l.	132 m.equiv./l.
Chloride	87 m.equiv./l.	150 m.equiv./l.
Phosphate (as $PO_4^{\equiv}$)	39 m.equiv./l.	30 m.equiv./l.
Sucrose	0	100 mM./l.
Δ	171 mM./l. NaCl	172 mM./l. NaCl
pH	6·6	6·7

A Ringer solution approximating to serum in composition can be prepared in the following way: 20 ml. M-H_3PO_4, 20 ml. M-NaOH + M-KOH, 80 ml. M-$MgCl_2$ and 50 ml. 0·1 M-$CaCl_2$ are mixed, made up to 1000 ml. with distilled water and brought to pH 7·0. An extensive precipitate is formed; this is filtered off and the filtrate is brought to pH 6·7. Its osmotic pressure is roughly equivalent to 120 mM./l. NaCl. 34 g. sucrose are then added to raise the osmotic pressure to about 170 mM./l. NaCl. Analysis gives the figures of Table 1, col. 2. The Malpighian tubules do not survive well in this fluid unless serum is added to it, but it can be used as a dissecting fluid for preparation of the tubules.

An earlier paper (Ramsay, 1954) describes the method of setting up a preparation of an isolated Malpighian tubule in a droplet of medium under liquid paraffin. This type of preparation lends itself not only to the collection of urine but also to the measurement of the electrical potential difference (p.d.) across the wall of the tubule, according to the method used in earlier work (Ramsay, 1953). A positive sign indicates that the lumen of the tubule is positive with respect to the medium.

Osmotic pressure was measured by freezing-point depression (Ramsay, 1949) and expressed as that concentration of NaCl, in mM./l., having the same osmotic

pressure. The average of two readings was taken, and the standard error is approximately ± 1 mM./l. NaCl. Concentrations of sodium and potassium were determined by flame photometry (Ramsay, Brown & Falloon, 1953). It is difficult to describe the accuracy of this method concisely. The great imponderable in flame photometry is interference error, arising from the presence of substances other than sodium and potassium in the fluid under investigation. In comparisons between different fluids, such as haemolymph and urine, having different backgrounds of possibly interfering substances, serious errors may arise. This difficulty has been met as far as possible by making up the standard solutions for haemolymph, serum and Ringer to contain 100 m.equiv./l. of magnesium and 10 m.equiv./l. of calcium (and correspondingly for urine 30 m.equiv./l. of magnesium and 10 m.equiv./l. of calcium) and by swamping variations in phosphate with excess of ammonium phosphate. It would be reasonable to assume that for purposes of comparison between haemolymph and urine the figures are accurate to ± 10%. Where two samples of haemolymph (or two samples of urine) are compared the method is very much more reliable, and indeed where the observations on the two samples are made alternately in the same 'run' interference errors can be disregarded and differences in concentration can be assessed as significant or otherwise by appropriate statistical treatment of the observations.

Volumes of medium, of the order 1–50 mm.^{3}, were measured in capillary pipettes. Volumes of urine, generally less than 1 mm.^{3}, were obtained by measuring with an eye-piece micrometer the diameter of the droplet as it was allowed to sink through liquid paraffin. These measurements are probably accurate to ± 10%, which in view of the variations in the rate of urine production is adequate for present purposes.

All experiments were carried out at room temperature, 14–17° C.

RESULTS

(1) *The preparation and testing of artificial media.* It has been stated above that serum is a satisfactory medium for the physiological study of Malpighian tubules. The evidence for this is presented in Fig. 1, which summarizes the results of an experiment with a superior tubule immersed in serum under liquid paraffin. It is shown that the tubule can continue to secrete urine of normal composition for over 24 hr. The performance of tubules in fresh haemolymph is not noticeably different.

On the other hand, the inferiority of Ringer as compared with serum is striking as is shown by the figures of Table 2.

It is therefore obvious that conclusions drawn from experiments carried out in a medium of pure Ringer would be open to the objection that the tubules were in an abnormal state. Satisfactory conditions, however, can be produced by using Ringer to which some serum has been added. In a medium containing 3 parts Ringer to 1 part serum the tubules survive for more than 8 hr. and produce urine at a rate which is of the same order as when the medium is pure serum. This

provides a basis for the preparation of media enriched or deficient in sodium and potassium, as will be described in subsection (4).

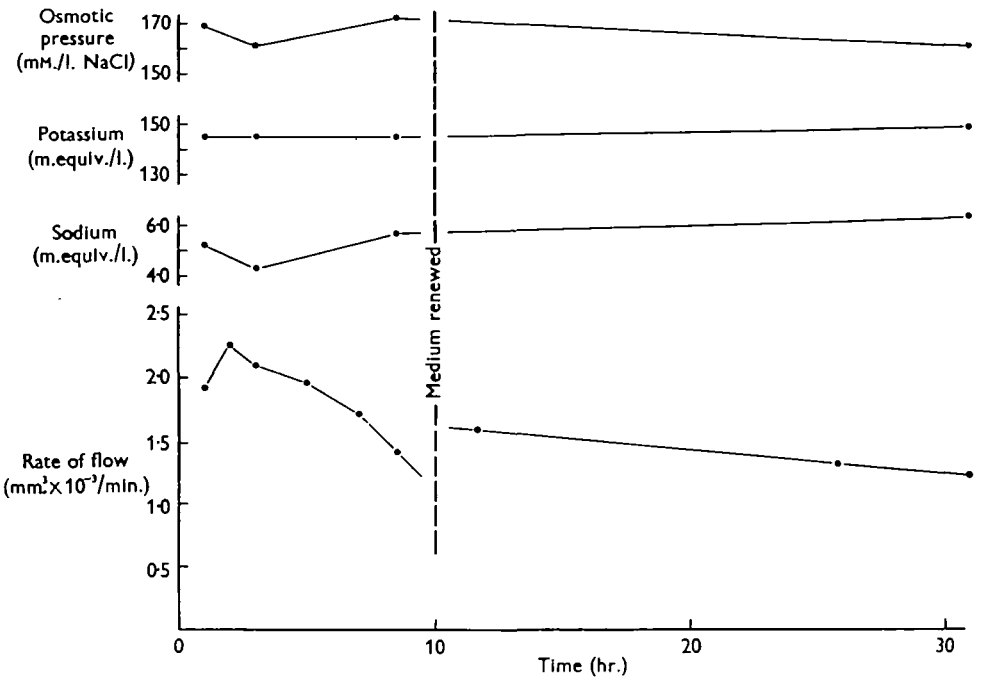

Fig. 1. Showing the ability of the tubule to maintain a flow of normal urine when bathed in serum. The points are plotted against the times at which the collections were made. Urine flow continued after 30 hr., further collections being made at 32 and 46 hr., but these were not analysed.

Table 2

Medium	Rate of urine flow during first 2 hr. (mm.³ × 10⁻³/min.)	Duration of urine flow (hr.)
Pure serum	1·24	>24
Pure serum	1·59	>24
Pure Ringer	0·28	<9
Pure Ringer	0·65	<5
1 pt. serum, 3 pts. Ringer	1·33	>8
1 pt. serum, 3 pts. Ringer	2·28	>8

(2) *Regional differentiation in the Malpighian tubules.* In the superior tubule of the stick insect there is a slight gradation in appearance from one end to the other, and it was considered necessary to investigate the possible existence of a corresponding gradation in physiological properties.

The tubule was cut into three approximately equal lengths. Each length was then prepared for collection of urine under liquid paraffin as if it were a whole tubule, and all three lengths were immersed in the same droplet of serum, of

about 25 mm.^{3} volume. After a suitable interval the urine produced by each length of tubule was collected for volume measurement and analysis. The p.d. across the wall of the tubule was then measured over each of the three lengths.

The results of four such experiments are presented in Table 3. It is at once clear that there is some gradation of physiological activity in that the sodium/potassium ratio of the urine is greater at the proximal end than at the distal end, consistently so in all four cases. The rate of urine production, measured per unit length, is consistently greatest in the middle region. The p.d., which in all cases is such that the inside is positive to the outside, shows a tendency to be greatest in the distal region, but this tendency is not statistically significant.

Table 3

Tubule	Region	Sodium (m.equiv./l.) (1)	Potassium (m.equiv./l.) (2)	Osmotic pressure (mM./l. NaCl) (3)	Rate of flow ($mm.^3 \times 10^{-3}$/min.) (4)	Length (cm.) (5)	Rate of flow per unit length (6)	$p.d._{sq.}$ (Na) (7)	$p.d._{mean}$ (8)	$p.d._{sq.} - p.d._{mean}$ (9)
1	Proximal	9·9	102	165	0·17	0·4	0·44	+ 5	+33	−28
	Middle	6·0	128	167	0·70	0·7	1·00	+18	+31	−13
	Distal	2·9	150	172	0·67	0·7	0·97	+36	+33	+ 3
2	Proximal	10·1	95	162	0·24	0·5	0·48	+ 5	+12	− 7
	Middle	6·8	113	157	0·70	0·7	1·00	+15	+15	0
	Distal	3·9	138	165	0·33	0·7	0·47	+29	+24	+ 5
3	Proximal	8·9	112	163	0·46	0·95	0·48	+ 7	+35	−28
	Middle	8·3	135	163	0·67	0·8	0·84	+ 9	+38	−29
	Distal	5·2	155	171	0·56	0·7	0·80	+21	+36	−15
4	Proximal	10·0	75	180	0·25	0·8	0·31	+ 5	+25	−20
	Middle	6·8	140	161	0·72	0·8	0·90	+14	+34	−20
	Distal	4·4	145	176	0·15	0·9	0·17	+25	+33	− 8

A large number of collections of urine from both superior and inferior tubules have been analysed for sodium, potassium and osmotic pressure, and no significant differences between the two types of tubule have been noted. The proximal and middle regions of the inferior tubule are closely similar in appearance to the corresponding regions of the superior tubule. It is therefore probable that the same gradation in physiological properties is present in these regions of the inferior tubule. On the other hand, the appearance of the distal dilatation of the inferior tubule suggests that its properties may be quite different.

There does not appear to be any measurable passage of water across the wall of the distal dilatation. If the dilatation is cut off and its cut end drawn out from a droplet of serum into the surrounding liquid paraffin no urine is secreted; and conversely, if the dilatation is drawn out into the liquid paraffin while still in connexion with the rest of the tubule in the serum no fluid accumulates around it. But there is some transfer of potassium from the lumen to the medium, as has been demonstrated in the following way.

Two droplets of serum were placed under liquid paraffin about 1 mm. apart. The proximal end of the tubule was closed by a ligature and the tubule was arranged so as to bridge across the gap between the two droplets, the proximal and middle regions being in one droplet (about 25 mm.^{3}) and the distal dilatation in the other (generally < 1 mm.^{3}). The short length of tubule between the droplets was supported on a fine platinum hook. Samples were taken from the distal droplet at the beginning and end of the experiment, and, as Table 4 shows, an increase in potassium concentration was always detected.

Table 4

Initial concentration (m.equiv./l.)		Final concentration (m.equiv./l.)		Duration of exp. (min.)
Na	K	Na	K	
12·2	19·5	12·5	24·7	450
12·1	19·5	12·5	20·9	450
12·7	18·9	11·8	23·4	360
12·8	17·9	11·1	24·2	360

Table 5

	Initial concentration (m.equiv./l.)		Final concentration (m.equiv./l.)		Duration of exp. (min.)
	Na	K	Na	K	
Proximal droplet	20·2	17·8	21·4	7·4	200
Distal droplet	20·4	25·9	20·4	36·8	200

In one experiment where the two droplets were both small and of about the same size it was possible to demonstrate the transfer of potassium from one droplet to the other (Table 5).

Since there is no movement of fluid this transfer of potassium presumably occurs by diffusion along the lumen of the tubule, aided by convection produced by the writhing movements.

It seems likely that the amount of potassium passed back into the haemolymph via the distal dilatation is small compared with the amount of potassium which is passed into the gut with the urine. In this respect the experiments just described are open to the objection that the ligature applied to the proximal end, by preventing the escape of urine, undoubtedly leads to distension of the tubule which in its turn may facilitate the escape of potassium through the distal dilatation. An experiment was therefore carried out in which the proximal end was left open and the urine allowed to escape. The results of this experiment are presented in Table 6, from which it can be seen that the amount of potassium returned to the medium through the distal dilatation is insignificant compared with the amount leaving the proximal end as urine.

Notwithstanding the gradation in physiological properties revealed in the experiments described above, it was decided that the whole of the superior tubule

could be regarded as a single physiological unit for purposes of investigating the effects of variation in the composition of the medium. This decision is further examined in the Discussion.

Table 6

Tubule	Distal droplet					Urine		
	K initial (m.equiv./l.)	K final (m.equiv./l.)	K difference (m.equiv./l.)	Volume (mm.^{3} × 10^{-3})	K transported (μequiv. × 10^{-3})	K (m.equiv./l.)	Volume (mm.^{3} × 10^{-3})	K transported (μequiv. × 10^{-3})
1	19·4	22·4	3·0	257	0·77	139	310	43
2	19·2	20·2	1·0	148	0·15	129	310	40

(3) *Active transport of sodium.* In order to decide whether an ion is actively transported or whether its movements can be accounted for by passive diffusion it is necessary to know not only the concentration gradient but also the p.d. Following the procedure adopted in earlier work (Ramsay, 1953), $p.d._{eq.}$ is calculated from the sodium concentrations in the medium and in the urine according to the formula

$$p.d._{eq.} = -58 \log \frac{Na_{urine}}{Na_{medium}}.$$

The measured value of p.d., $p.d._{meas.}$, is then subtracted algebraically from $p.d._{eq.}$ and if the difference is negative then active transport of sodium must be assumed.

The relevant information is assembled in Table 3, cols. 7–9, from which it is seen that $p.d._{eq.} - p.d._{meas.}$ is negative in all except three cases. This shows that the tubule is capable of transporting sodium against an electrochemical gradient, a fact which was not established in the earlier investigation referred to above. It may also be noted here, in anticipation of what is to be described in the next subsection, that in certain circumstances the sodium concentration may be greater in the urine than in the medium (see Fig. 5*b*).

(4) *Rate of flow and composition of urine in relation to sodium and potassium concentrations in the medium.* For the experiments to be described in this subsection only superior tubules have been used unless otherwise stated. The single isolated tubule is treated as a functional unit, the slight gradation in physiological properties (subsection 2) being disregarded.

Variants of Ringer, enriched or deficient in sodium and potassium, were prepared, 1 part of serum being added to 3 parts of Ringer, with further addition of sucrose, if necessary, to bring the osmotic pressure to about 170 mM./l. NaCl. The 'very high' concentrations of sodium and potassium could not be reached without exceeding this value for osmotic pressure, and it was therefore necessary in these cases to reduce the magnesium concentration to 40 m.equiv./l. For this

reason the observations made in these media are not quite on a level with the others.

The media, prepared as described above, were analysed for sodium and potassium at the end of each experiment, and the concentrations in m.equiv./l. were found to be of the orders given in Table 7.

Table 7

	'Normal'	'Low Na'	'Low K'	'High Na'	'High K'	'Very high Na'	'Very high K'
Na (m.equiv./l.)	17	4	17	54	17	91	17
K (m.equiv./l.)	16	16	6	16	53	16	89

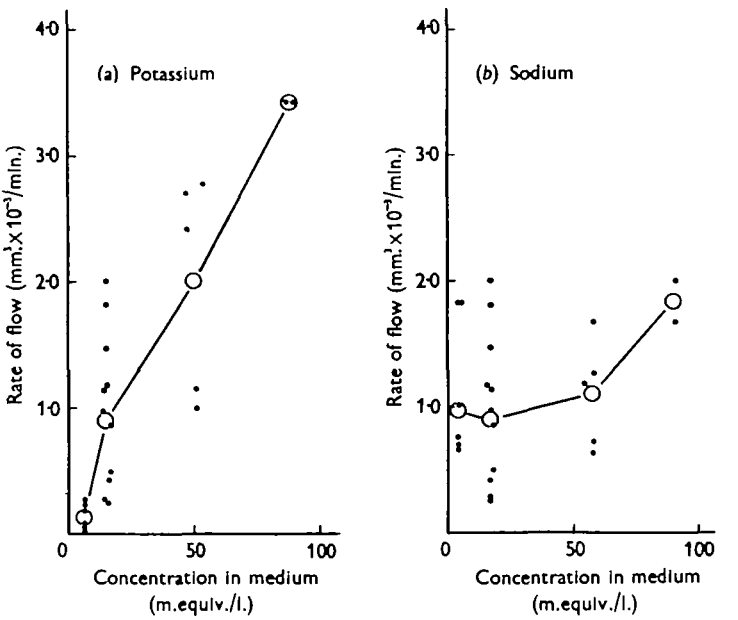

Fig. 2. (*a*) Rate of urine flow as a function of potassium concentration in the medium, the sodium concentration in the medium being constant at 16–17 m.equiv./l. (*b*) Rate of urine flow as a function of sodium concentration in the medium, the potassium concentration in the medium being constant at 15–16 m.equiv./l. The circles represent average values.

The rate of urine flow varies greatly from one insect to another, and the scatter of the observations makes it difficult to establish quantitative relations between the rate of urine flow and other factors. Nevertheless, it is very clear from Fig. 2*a* that with increase of the potassium concentration in the medium there is a very marked increase in the rate of flow, whereas with increase of the sodium concentration the increase in rate of flow is very much less and is barely significant in relation to the scatter of the observations (Fig. 2*b*).

The relation between potassium concentration in the medium and potassium concentration in the urine is shown in Fig. 3*a*, and the corresponding relation

from sodium concentrations in Fig. 3*b*. As expected from earlier work, the potassium concentration in the urine is always much greater than that in the medium, whereas the sodium concentration is generally less. Both curves show a tendency to rise steeply and then flatten off.

Further insight into these relations is gained by considering the rate of secretion of potassium (and of sodium) as a function of its concentration in the medium.

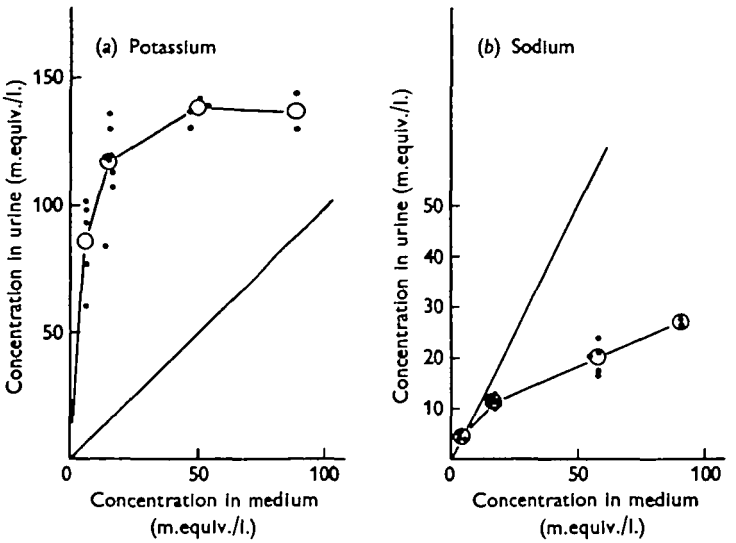

Fig. 3. (*a*) Potassium concentration in the urine as a function of potassium concentration in the medium, the sodium concentration in the medium being constant at 16–17 m.equiv./l. (*b*) Sodium concentration in the urine as a function of sodium concentration in the medium, the potassium concentration in the medium being constant at 15–16 m.equiv./l. The circles represent average values. The straight lines from the origin indicate equal concentration in urine and medium.

The average values for rate of flow (Fig. 2) are multiplied by the corresponding average values for concentration (Fig. 3) and the products are plotted in Fig. 4. From this it is seen that the rate of secretion of each ion can be regarded as roughly proportional to concentration of the ion in the medium, and that the rate of secretion of potassium is some 11 times greater than that of sodium.

Consideration has so far been restricted to the relation between the concentration of an ion in the urine and the concentration of the same ion in the medium. The possibility that the sodium concentration in the urine is affected by the potassium concentration in the medium (and vice versa) has still to be examined. As can be seen from Fig. 5*b* there is undoubtedly an inverse relation between the sodium concentration in the urine and the potassium concentration in the medium, and there is a suggestion of the same thing in the converse case (Fig. 5*a*). This at first sight suggests the possibility of mutual interference between sodium and potassium in the secretory mechanism. Allowance has to be made, however, for the increased flow of urine which accompanies an increase in the potassium concentration in the

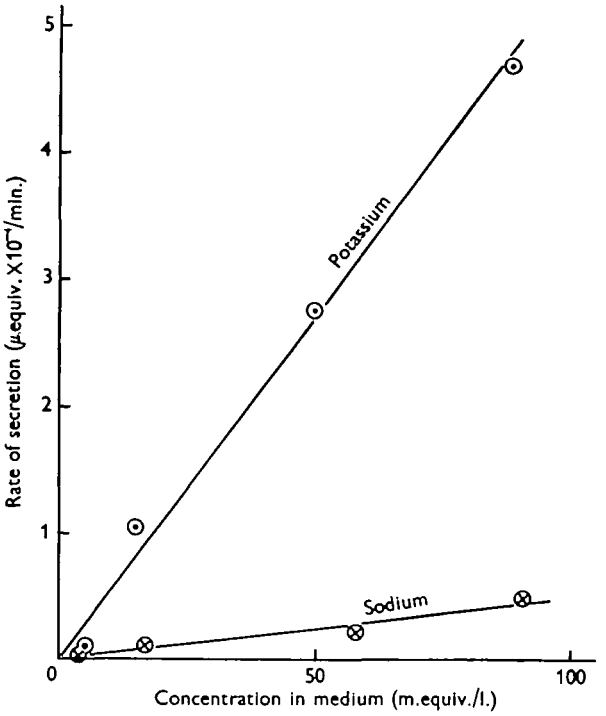

Fig. 4. The rate of secretion of potassium as a function of the potassium concentration in the medium (from data of Figs. 2*a* and 3*a*) and the rate of secretion of sodium as a function of the sodium concentration in the medium (from data of Figs. 2*b* and 3*b*).

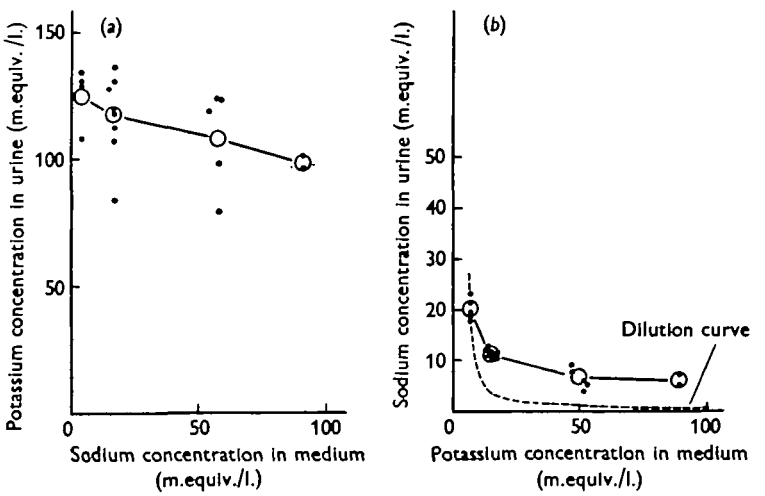

Fig. 5. (*a*) Potassium concentration in the urine as a function of sodium concentration in the medium, the potassium concentration in the medium being constant at 15–16 m.equiv./l. (*b*) Sodium concentration in the urine as a function of potassium concentration in the medium, the sodium concentration in the medium being constant at 16–17 m.equiv./l. The circles represent average values. For explanation of 'dilution curve' see text.

medium; even if sodium continues to be secreted at the same rate its concentration in the urine will fall as the rate of urine flow increases. From Fig. 5*b* the sodium concentration in the urine is 21 m.equiv./l. when the potassium concentration in the medium is 6 m.equiv./l. In the same medium, from Fig. 2*a*, the rate of urine flow is $0{\cdot}13 \times 10^{-3}$ mm.^{3}/min. The rate of secretion of sodium is therefore $2{\cdot}7 \times 10^{-6}$ μequiv./min. Assuming that the rate of secretion of sodium remains constant at this value and that the rate of urine flow varies with the potassium concentration in the medium as indicated in Fig. 2*a*, it is possible to calculate the expected sodium concentration in the urine for the other potassium concentrations in the medium. In this way the 'dilution curve' of Fig. 5*b* has been calculated and is seen to lie well below the observed values for the sodium concentration in the urine. This means that as the potassium concentration in the medium is increased and the rate of secretion of potassium increases the rate of secretion of sodium also increases, which does not suggest that sodium and potassium are in competition for the same secretory channel—at least, not under the conditions of these experiments.

The ability of the tubule to concentrate potassium is well marked. Fig. 3*a* shows that the ratio K_{urine}/K_{medium} increases as the potassium concentration in the medium is decreased, reaching a value of about 14 when the potassium concentration in the medium is 6 m.equiv./l. In view of this progressive increase and of the higher concentration ratios found for the Malpighian tubules of other insects, particularly those living in fresh water (Ramsay, 1953)* it seems possible that in the stick insect further decrease in the potassium concentration in the medium might reveal powers of concentration hitherto undisclosed. Unfortunately, by reason of the fact that it is necessary to add 1 part of serum to 3 parts of Ringer, artificial media having very low potassium concentrations cannot be prepared. There is, however, another means whereby the effect of very low potassium concentrations can be studied and that is by allowing the potassium concentration in the medium to be lowered by the activity of the tubule itself. The tubule can be immersed in a very small droplet of medium, and samples of urine and of medium can be taken at intervals. Since the potassium concentration in the medium is falling all the time it is more difficult to interpret the results of experiments carried out in this way, but at least they give some idea of the power of the tubule to remove potassium from low concentrations.

In one such experiment two tubules were set up in the same small droplet of serum. The results of the experiment are given in full in Table 8 and are plotted for one tubule only in Fig. 6. The concentration of potassium in the medium has been reduced to 0·4 m.equiv./l. and the corresponding concentrations in the urine are of the order of 50 m.equiv./l. When the concentration of potassium falls to 1 m.equiv./l. or less there is no doubt that the percentage error of analysis increases;

* In the paper to which reference is made there are two errors in Table 1. $\frac{C_1}{C_2}$ (Na) for *Dytiscus* should be 0·31 and $\frac{C_1}{C_2}$ (K) for the Tabanid should be 32·0.

Table 8

Time of collection (min.)	Medium					Urine, tubule (1)						Urine, tubule (2)					
	Sodium (m.equiv./l.)	Potassium (m.equiv./l.)	Osmotic pressure (mM./l. NaCl)	Volume of sample ($mm.^3 \times 10^{-3}$)	Potassium ($\mu equiv. \times 10^{-3}$)	Sodium (m.equiv./l.)	Potassium (m.equiv./l.)	Osmotic pressure (mM./l. NaCl.)	Volume ($mm.^3 \times 10^{-3}$)	Rate of flow ($mm. \times 10^{-3}$/min.)	Potassium ($\mu equiv. \times 10^{-3}$)	Sodium (m.equiv./l.)	Potassium (m.equiv./l.)	Osmotic pressure (mM./l. NaCl)	Volume ($mm.^3 \times 10^{-3}$)	Rate of flow ($mm.^3 \times 10^{-3}$/min.)	Potassium ($\mu equiv. \times 10^{-3}$)
0	11·5	17·3	177	—	—	—	—	—	—	—	—	—	—	—	—	—	—
145	12·0	10·0	178	84	0·84	6·5	125	165	210	1·45	26·2	—	101	—	235	1·62	23·7
270	11·2	5·9	171	100	0·59	7·3	113	164	190	1·52	21·6	7·8	85	153	165	1·32	14·0
505	12·3	3·6	171	155	0·56	8·6	93	165	280	1·19	26·1	8·5	79	157	260	1·11	20·5
695	11·7	0·5	165	103	0·05	13·5	76	165	155	0·81	11·8	11·7	71	155	180	0·95	12·8
1330	12·5	0·4	150	116	0·05	16·2	48	160	142	0·22	6·8	14·7	51	147	148	0·23	7·5
Totals	—	—	—	558	2·09	—	—	—	977	—	92·5	—	—	—	988	—	78·5
Medium remaining at end of experiment																	
		0·4		6200	2·60												

Original volume of medium: $6200 + 558 + 977 + 988 = 8723$

Amount of potassium originally present in medium: $8723 \times 17{\cdot}3 \simeq 151\ \mu equiv. \times 10^{-3}$

Amount of potassium finally present in medium: 2·60

Amount of potassium in samples of medium: 2·09

$4{\cdot}69 \simeq 5{\cdot}0$

Amount of potassium lost from medium: $151 - 5 = 146\ \mu equiv. \times 10^{-3}$

Amount of potassium recovered in urine: $92{\cdot}5 + 78{\cdot}5 = 171\ \mu equiv. \times 10^{-3}$

Discrepancy: 14·6 %

but even making generous allowance for this it would seem that the ratio K_{urine}/K_{medium} can reach the order of 50.

The rise of the sodium concentration in the urine as the potassium concentration in the medium falls confirms the observations recorded in Fig. 5*b*.

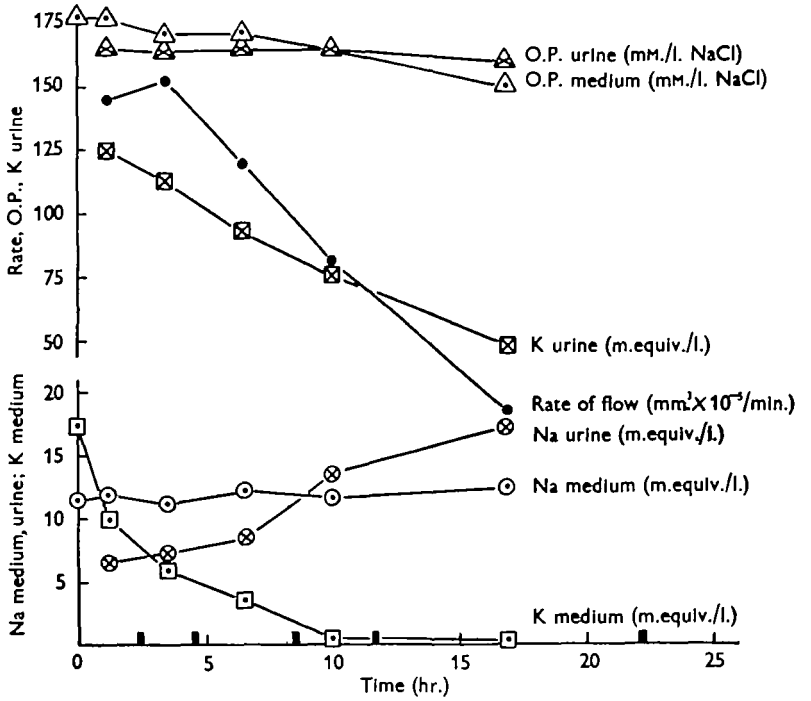

Fig. 6. Results of an experiment using a small volume of serum, showing the ability of the tubule to reduce the potassium concentration in the medium. The times of collection are indicated on the abscissa, and the points are plotted at the middle of the time intervals between collections.

Table 9

Tubule	Potassium lost from medium (μequiv. $\times 10^{-3}$)	Potassium recovered in urine (μequiv. $\times 10^{-3}$)	Discrepancy (%)
1	114	122	7
2	127	131	3

In experiments of this type it is possible to make out a balance sheet for potassium, comparing the amount of potassium which disappears from the medium with the amount which can be recovered from the urine. The object of preparing such a balance sheet is to provide a check upon the errors involved in measurements of concentration and of volume. In the case in question 146×10^{-3} μequiv. were lost from the medium and 171×10^{-3} μequiv. were recovered in the urine. This is a discrepancy of the order of 15%. In two other cases, in which inferior tubules were set up each in its own droplet of serum, the discrepancies were less (Table 9).

These figures give some assurance that the accuracy claimed for the methods used in this investigation has not been grossly overestimated.

DISCUSSION

The decision to treat the whole of the superior tubule as a single physiological unit may appear to take insufficient account of the very definite evidence for gradation in physiological properties.

In the Malpighian tubules of *Rhodnius* there is a striking discontinuity between the proximal and distal regions: urine is rapidly secreted in the distal region and appears to be partly reabsorbed in the proximal region (Wigglesworth, 1931*b*). Potassium is actively secreted in the distal region and is partly reabsorbed in the proximal region (Ramsay, 1952). In *Rhodnius* the composition of the urine as it leaves the tubule is therefore determined by the net effect of processes acting in opposition. If it were found that the potassium concentration in the urine increased with increasing potassium concentration in the medium this might be the result either of more rapid secretion in the distal region or less rapid reabsorption in the proximal region. It would therefore be necessary to study the two regions separately. But in the stick insect it has been found that sodium, potassium and water are secreted into the tubule at all levels. There is no indication that the opposing process of reabsorption is anywhere at work. The relative rates of secretion of sodium and potassium certainly do vary from one region to another; it may well be that some regions of the tubule respond more readily to changes in the medium than do others, and this possibility might be worth following up. It seems unlikely, however, that the general conclusions which have been reached from the investigation of the whole tubule will be contradicted by more detailed studies of its different regions.

While this position is maintained with respect to sodium, potassium and water, complete reservation must be made with respect to other constituents of the urine, which may be secreted in some regions and not secreted or even reabsorbed in others. In this connexion may be mentioned the observation that the urine produced by the proximal and middle regions is brown in colour, whereas that produced by the distal region of the superior tubule is colourless.

There is no evidence to suggest that the proximal and middle regions of the inferior tubule are in any way different from the corresponding regions of the superior tubule. The distal dilatation of the inferior tubule has quite different properties. It does not secrete urine and it is packed with granules in which calcium carbonate predominates (de Sinéty, 1901; Ramsay, 1955). Such evidence as there is suggests that it acts as a storage organ for calcium which is required in considerable quantity for hardening the shell of the egg (Moscona, 1950).

The main purpose of this investigation may be said to be achieved in the results presented in Figs. 2 and 3. Increase of the potassium concentration in the medium results in a spectacular increase in the rate of urine flow and also in an increase of the potassium concentration in the urine. On the other hand, increase of the sodium concentration in the medium results in a smaller increase of the sodium concentration in the urine and in a barely significant increase in the rate of urine flow. For both sodium and potassium the rate of secretion appears to bear a linear relationship to the concentration in the medium (Fig. 4); the simplest interpretation is that under

the conditions of these experiments the rate of secretion is limited by the availability of the ion and not by the failure of the secretory mechanism to transport all the ion that can be fed into it. But although the points in Fig. 4 lie reasonably near the lines it must not be forgotten that they represent average values to which considerable errors attach, so that the linearity of the relationship may be more apparent than real. Fig. 4 also serves to show the very great difference in the rates of secretion of sodium and potassium, and about this there is no doubt at all.

In the earlier investigation (Ramsay, 1953), which included the Malpighian tubules of the stick insect, it was stated that 'there are insufficient grounds for assuming the active transport of sodium, in fact, it is admissible as a working hypothesis that the differences in concentration of sodium are brought about by passive diffusion'. It is now possible to be more precise. In 'high Na' media the electrochemical gradient is such as to promote the diffusion of sodium into the tubule, but in 'normal' media and *a fortiori* in 'low K' media (where the sodium concentration in the urine can exceed that in the medium) active transport against the electrochemical gradient must take place. It is perhaps worth while to make clear the very limited implications of this statement. There is no suggestion that a different mechanism is involved according to whether the medium is of the 'low K' type (active transport) or the 'high Na' type (passive diffusion) any more than that a different mechanism is involved when a motor car is driven uphill under its own power or is allowed to run downhill with the engine being turned passively; in the one case energy must be supplied, in the other case energy need not be supplied, and that is all. It has now been shown that there must be a mechanism capable of transporting sodium against an electrochemical gradient, just as it has previously been shown that such a mechanism must exist for potassium. Although no evidence has been put forward to show that sodium and potassium are in competition for the same mechanism, the possibility that the same mechanism serves both ions is not excluded.

A great many measurements of p.d. were made upon tubules in various artificial media, but it does not seem worth while to report these in detail. It can be stated briefly that the p.d. is notably unresponsive to changes in the sodium and potassium concentrations in the medium. An average increase of 16 mV. was noted in 'high K' as compared with 'low K', that is, for an eightfold increase in potassium concentration; corresponding changes in sodium concentration were without effect. This speaks against the idea that passive diffusion plays an important part in the movements of sodium ion.

The results here presented reinforce the suggestion made in earlier papers that there is some connexion between the secretion of potassium and the processes of formation of urine. It has now been shown that the greater the concentration of potassium in the medium the greater is the flow of urine. At the same time, in so far as it has been demonstrated that sodium can be actively transported and seems unlikely to enter the tubule by passive diffusion as at one time seemed possible, potassium is no longer to be regarded as having a unique role. Some re-assessment of the position is called for.

There is no doubt that the Malpighian tubules of insects are generally very active in removing foreign materials (e.g. dyes) from the haemolymph. They are also active in removing the natural excretory products (e.g. uric acid). If the tubule merely removed these substances from the haemolymph to its lumen and allowed them to accumulate there, there would be a limit to its usefulness; it is obviously advantageous from the insect's point of view that the excretory substances should be rapidly flushed out of the tubule and so eliminated from the body via the hindgut. This might be brought about by the simple secretion of water into the tubule, but it appears that the tubule is not able to secrete water against anything more than a slight osmotic gradient. It follows that a brisk flow of urine can only be maintained by the secretion of other substances into the urine to make up the osmotic difference. Potassium ion appears to be one of the most important of these substances. Just as most of the water secreted by the tubules is reabsorbed in the hindgut, so also is most of the potassium reabsorbed (Ramsay, 1955). The circulation of potassium is thus bound up with the circulation of water and its significance is to be traced to the inability of the tubule to secrete water rapidly against a concentration gradient.

This interpretation, however, entirely begs the question of why it is mainly potassium rather than sodium which is circulated. The tubule is capable of actively secreting sodium, at least in the stick insect. In many insects sodium is available in the haemolymph in much higher concentration than potassium. The fact remains that in the stick insect, and possibly in many other insects, the mechanism of potassium secretion works very much faster than the mechanism of sodium secretion. Why this should be so is at present a matter of pure speculation.

Of the many problems associated with the study of urine formation in Malpighian tubules none is more challenging than the problem of why the secretory cells are unable to function in balanced salt solutions similar in composition to the haemolymph. It is striking to observe how the contractile elements of the tubule wall can maintain normal activity in Ringer solution long after the secretory cells have completely disintegrated. All the evidence suggests that it is not a question of high sensitivity to the precise composition of the Ringer, but that it is a question of the absence of some substance which is present in the natural medium. Attempts have been made, using the methods of partition chromatography, to separate this essential principle from serum, but so far it has not been possible to recover from the paper any substance which when added to Ringer will significantly prolong the effective life of the secretory cells. Further investigation of this problem will be undertaken when adequate supplies of serum have been accumulated.

SUMMARY

1. The excretion of sodium, potassium and water by the Malpighian tubules of the stick insect has been further studied in preparations of single tubules isolated in droplets of medium under liquid paraffin.

2. There is some gradation of physiological activity along the length of the superior tubule. Sodium, potassium and water are secreted into the tubule at all levels, but the sodium/potassium ratio is greater in the proximal region.

3. The proximal and middle regions of the inferior tubule have not been shown to differ in any way from the corresponding regions of the superior tubule. The distal dilatation has quite different properties and does not produce urine.

4. The rate of urine flow increases markedly as the potassium concentration in the medium is increased; the corresponding effect of sodium is barely detectable.

5. Sodium, like potassium, can be actively transported against an electrochemical gradient, and does not appear to compete with potassium in the secretory mechanism.

6. The rates of secretion of sodium and potassium vary in direct proportion to the respective concentrations of these ions in the medium. The rate of secretion of potassium is more than ten times greater than that of sodium.

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