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URINE FORMATION BY THE MALPIGHIAN TUBULES OF *CALLIPHORA*

I. CATIONS

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INTRODUCTION

The current concept of fluid transport in vertebrates is that water moves passively down the osmotic gradient created by an active transport of solute. Earlier suggestions that these tissues could transport water in the absence of a concomitant movement of solute have been rejected (Curran, 1965; Diamond, 1965; Robinson, 1965, 1966). Interest is now focused on the mechanism by which water movement is coupled to active solute transport. A simple explanation would be that active solute transport across an epithelium might increase the total solute concentration on one side and thus provide the osmotic gradient for a passive flow of water. This simple hypothesis, however, cannot account for water transport against an osmotic gradient. Curran (1960) extended the hypothesis by postulating that active solute transport takes place into an enclosed compartment within which a large osmotic pressure gradient could be maintained. Long narrow channels (e.g. lateral intercellular spaces and basal infoldings), which are a constant feature of secretory or reabsorptive epithelia, appear to be the structural analogues of the enclosed compartment in Curran's model (Diamond & Tormey, 1966*a*, *b*; Kaye, Wheeler, Whitlock & Lane, 1966; Berridge & Gupta, 1967; Davis & Schmidt-Nielsen, 1967). A mathematical treatment of solute-linked water transport indicates that standing osmotic gradients within long narrow channels can adequately account for the coupling of water to solute transport (Diamond & Bossert, 1967).

Urine formation by the Malpighian tubules of insects resembles fluid transport in vertebrate epithelia in that water flow depends on an active secretion of potassium (Ramsay, 1953, 1955*b*, 1956). However, details of the exact linkage between solute and water transport have not been determined. This paper attempts to extend Ramsay's observations on the nature of cation transport and to consider the structural basis for the mechanism of solute-linked water transport in Malpighian tubules.

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MATERIAL AND METHODS

Adult female *Calliphora erythrocephala* were reared as described previously (Berridge, 1966*b*). The Malpighian tubules of 3-day-old females were set up in artificial media kept under liquid paraffin (Berridge, 1966*b*). Rate of urine production was always measured 2 hr. after setting up the tubules.

Table 1. *Composition of artificial media: the final concentration of phosphate in medium C was* 15 *mM/l.*

	A (g./l.)	B (g./l.)	C (g./l.)
$CaCl_2$	0·2	Variable	0·2
$MgCl_26H_3O$	2·0	Variable	2·0
Trehalose	1·8	1·8	1·8
Glucose	1·8	1·8	1·8
Proline	0·6	0·6	0·6
Glutamine	0·8	0·8	0·8
α-Alanine	0·4	0·4	0·4
Glycine	0·5	0·5	0·5
Penicillin	0·03	0·03	0·03
Streptomycin	0·1	0·1	0·1
Fumaric acid	—	1·0	1·0
Citric acid	—	1·0	1·0
Malic acid	—	1·0	1·0
KOH	—	2·25	2·25
NaCl	Variable	Variable	4·8
KCl	Variable	—	—
Sucrose	Variable	—	—
$NaH_2PO_4H_2O$	—	—	Variable
Na_2HPO_4	—	—	Variable

The composition of the artificial media used is shown in Table 1; the pH of all media (except C) was 7·2 and the osmotic pressure was adjusted to give a depression of the freezing point of approximately 0·7° C. To test the effect of ionic strength and osmotic pressure on the process of urine formation, different media were prepared by adding various amounts of sodium, potassium or sucrose to medium A. Medium B was used to test the ability of divalent cations to support urine formation. This medium contained a number of organic acids which helped to keep these divalent cations in solution, especially when they were present in high concentrations. Solutions of similar osmotic pressure were obtained by balancing the concentration of the divalent ions with sodium. The effect of pH on the activity of tubule cells was tested with medium C; different hydrogen ion concentrations were obtained by means of a phosphate buffer.

The sodium and potassium concentrations of urine and artificial media were measured by means of a Beckman flame-photometer. Micropipettes were used to obtain fluid samples from under liquid paraffin.

Osmotic pressure determinations were made by the cryoscopic method of Ramsay & Brown (1955).

RESULTS

Effect of potassium concentration on urine formation at constant osmotic pressure

(*a*) *Potassium transport in the presence of sodium*

Rate of urine formation by the Malpighian tubules of *Calliphora* is markedly affected by the external potassium concentration (Fig. 1). In the absence of potassium (i.e. when sodium was the major cation), urine formation occurred at a very slow rate (0·9 $mm.^3 \times 10^{-3}$/min.), but was considerably accelerated as potassium was progressively replaced by sodium. The increase occurred as a two-stage process; replacement of only 8 mM/l. sodium with an equivalent amount of potassium caused a large increase of urine flow, but thereafter the increase became asymptotic. The maximum rate of urine formation was recorded when potassium completely replaced sodium in the bathing medium.

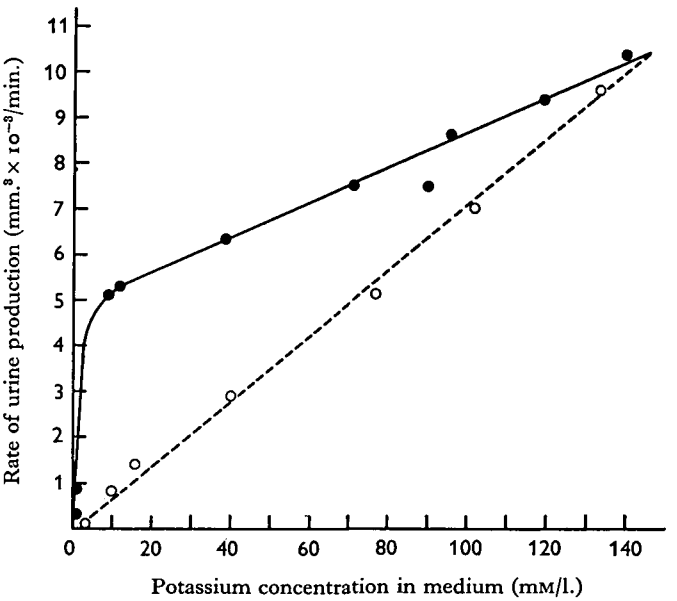

Fig. 1. Effect of potassium concentration on rate of urine formation when osmotic pressure was kept constant with (*a*) sodium (●), and (*b*) sucrose (○).

Transport of sodium and potassium independently of each other is shown more clearly when the concentration of these two ions in the urine is compared with their concentration in the bathing medium (Fig. 2). The slow rate of urine secretion in a potassium-free medium (Fig. 1) resulted from a secretion of sodium which appeared in the urine at a concentration similar to that in the bathing medium (Fig. 2). Replacement of a small quantity of sodium with an equivalent amount of potassium in the bathing medium produced a sudden shift in the potassium and sodium concentration of the urine; potassium almost completely replaced sodium. Appearance of potassium in the urine coincided with the large increase in rate of urine production (Fig. 1) and emphasizes further the extraordinary sensitivity of the tubule cells to potassium.

Potassium concentration in the urine (100–140 mM/l.) was always much higher than its concentration in the bathing medium, especially at low concentrations of the latter (i.e. 8–60 mM/l.). Sodium apparently does not interfere with the carrier mechanism for potassium, since potassium was transported against a large gradient even with a high concentration of sodium in the medium (Fig. 2).

Since tubules were capable of producing urine in a potassium-free medium by secreting sodium, this might indicate that at least one component of the cation transport mechanism is relatively unspecific. Therefore the specificity of cation transport was explored further by using different cations (Table 2). Potassium and rubidium maintained a high rate of urine production, whereas caesium and sodium were less

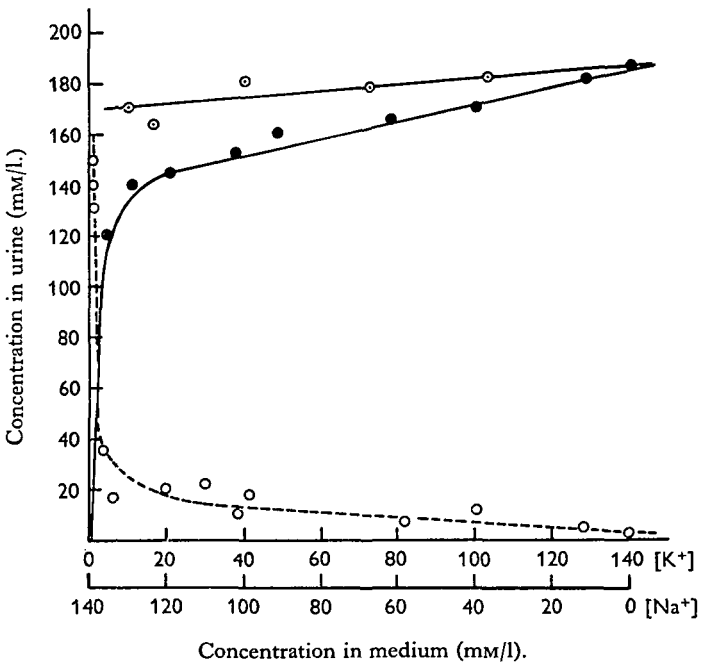

Fig. 2. Concentration of sodium and potassium in the urine collected during the experiments illustrated in Fig. 1*a* (●—●, potassium; ○---○, sodium) and 1*b* (⊙—⊙, potassium concentration when sucrose replaced sodium in the bathing medium).

Table 2. *The ability of individual cations to support a flow of urine: each cation was dissolved in medium A to give a final concentration of* 140 *mM/l.*

Cations	Rate of urine production ($mm.^3 \times 10^{-3}$/min.)
Potassium	13·8
Rubidium	10·9
Caesium	2·5
Sodium	0·4
Ammonium	0
Lithium	0
Choline	0

effective. Ammonium, lithium and choline were not able to maintain a flow of urine. These results demonstrate that tubule cells can secrete urine in the presence of a single univalent cation.

(*b*) *Potassium transport in the presence of sucrose*

The next series of experiments tested the ability of Malpighian tubules to secrete urine at variable potassium concentrations when sucrose replaced sodium (Fig. 1). If a non-transportable molecule is used to adjust the osmotic pressure, rate of urine formation is linearly related to the external potassium concentration (Fig. 1). Under such conditions, potassium concentration of the urine was again much higher than its concentration in the bathing medium and remained relatively constant throughout the range of external potassium concentration (Fig. 2). However, potassium concentration in the urine was slightly higher than that secreted by tubules in a sodium + potassium medium.

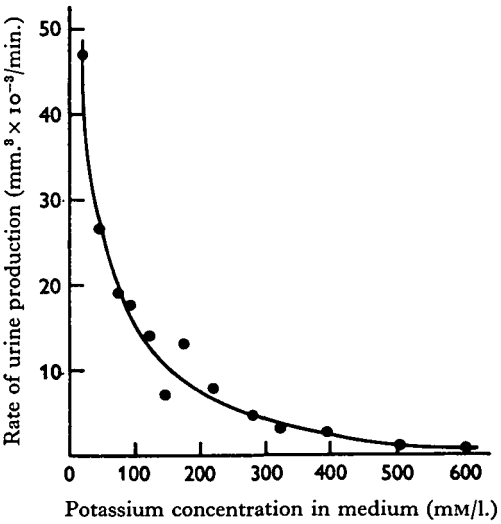

Fig. 3. Effect of potassium concentration on rate of urine formation when the osmotic pressure is not adjusted.

Effect of osmotic pressure on urine formation

(*a*) *Effect of osmotic pressure on urine formation at variable potassium concentration*

Rate of urine formation by the Malpighian tubules of insects is directly related to the external osmotic pressure (Ramsay, 1954; Berridge, 1966*a*); in the previous experiments by Ramsay and Berridge the osmotic pressure of serum was modified to give hypotonic or hypertonic solutions by adding distilled water or sucrose respectively. However, such a procedure introduces a complicating factor, because, although osmotic pressure is continuously variable, the potassium concentration is decreased by dilution but remains constant in the hypertonic media. Therefore, in the present study a range of osmotic pressure was obtained by adding different amounts of potassium chloride to medium A (Table 1). Consequently, both potassium concentration and osmotic pressure were continuously variable; Fig. 3 illustrates the change in the

rate of urine formation under such conditions. A maximum rate of urine formation was recorded at the lowest potassium concentration and hence the lowest osmotic pressure. An increase in both these parameters resulted in a consistent decrease in rate of urine formation. The tubules in hypotonic medium were considerably swollen but became progressively shrunken as the outside osmotic pressure increased. Clearly, the tubules of *Calliphora* can function over a wide range of potassium concentration and osmotic pressure.

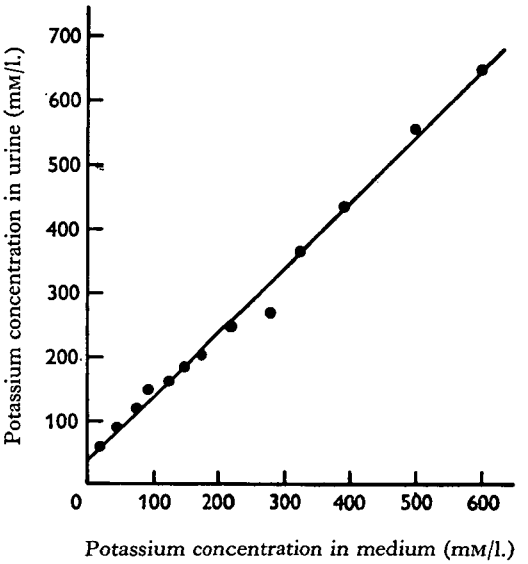

Fig. 4. Concentration of potassium in the urine secreted during the experiment illustrated in Fig. 3.

The potassium concentration of the urine remained consistently higher than that of the bathing medium throughout the range of external potassium concentration employed (Fig. 4). The average difference of $[K^+]_{urine} - [K^+]_{medium}$ for all the observations was 37 mM/l. Combination of the data in Fig. 3 and 4 showed that rate of potassium secretion (Fig. 5) is inversely related to the external potassium concentration. When the osmotic pressure was kept constant with sucrose, however, rate of potassium secretion (obtained from the data in Figs. 1 and 2) was directly related to the external potassium concentration (Fig. 5).

The osmotic pressure of urine was slightly, but consistently, hypertonic to the bathing medium throughout the range of osmotic pressures employed (Fig. 6). The average value of the $O.P._{urine} - O.P._{medium}$ was 0·066° C. At low osmotic pressures, rate of urine formation is increased (Fig. 3) because more water molecules must follow each ion in order to maintain this constant osmotic pressure relationship (i.e. urine osmotic pressure 0·066° C hypertonic to the medium).

(b) Effect of osmotic pressure on urine formation at constant potassium concentration

Progressive addition of sucrose to medium A containing 140 mM/l. of potassium chloride caused a regular decrease in rate of fluid formation (Fig. 7). The presence of sucrose in the bathing medium also had a pronounced effect on the potassium

concentration of urine (Fig. 8). The curve in Fig. 8 represents the calculated concentration of potassium in the urine required to exactly counteract the increase in osmotic pressure of the bathing medium due to the presence of sucrose. Most of the observed values (open circles) lie very close to this curve, therefore the concentration of potassium in the urine increased by 1 mM/l. for each 2 mM/l. of sucrose added to the bathing medium. In rabbit gall bladder, a similar increase in concentration of the transported cation (in this case sodium) was observed when impermeant non-electrolytes such as raffinose and sucrose were added to the bathing solution (Diamond, 1964).

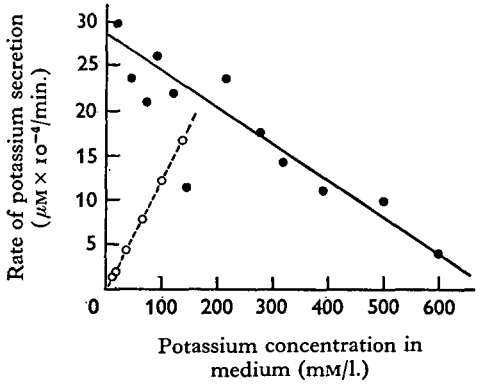

Fig 5.

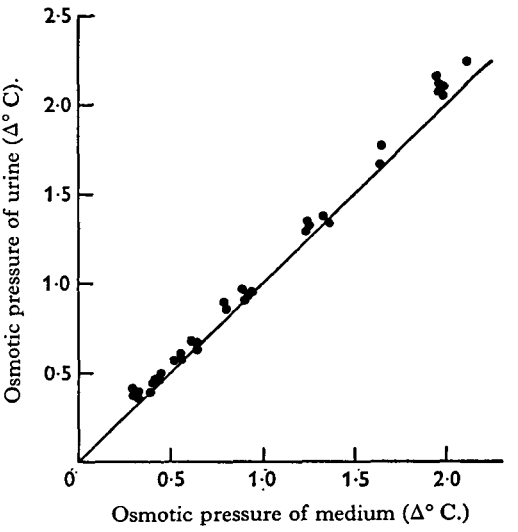

Fig 6.

Fig. 5. Rate of potassium secretion when (*a*) osmotic pressure is unbalanced (●; data taken from Figs. 3 and 4), and (*b*) osmotic pressure is balanced with sucrose (○; data taken from Figs. 1 and 2).

Fig. 6. The relationship between osmotic pressure of the medium and that of the urine secreted in the experiment illustrated in Fig. 3. The point where the two solutions are isosmolar is indicated by the straight line. Most of the points lie above the line, indicating that the urine is slightly hypertonic.

Effect of divalent cations on urine formation

The divalent cations, calcium and magnesium, cannot replace the univalent cations in the process of urine formation. Nevertheless, these divalent ions are known to play an important role in active transport mechanisms. These ions were tested on tubules provided with potassium so that they could secrete urine. In the complete absence of these divalent cations, urine production occurred at a slow rate but was accelerated after addition of either magnesium or calcium (Fig. 9). The optimal concentration of these cations was about 10 mM/l. Higher concentrations tended to depress urine production with calcium showing a greater inhibitory effect than magnesium. The isolated Malpighian tubules of *Calliphora* reacted to high concentrations of magnesium and calcium in much the same way as do the tubules of *Dixippus* (Ramsay, 1956).

Effect on pH and inhibitors on urine production

The ability of Malpighian tubules to secrete urine in the presence of a single monovalent cation is unusual and might suggest that the transport mechanism is electrogenic. Such a suggestion cannot be taken seriously until it is possible to exclude hydrogen as an exchange partner for potassium in the form of a coupled potassium-hydrogen pump.

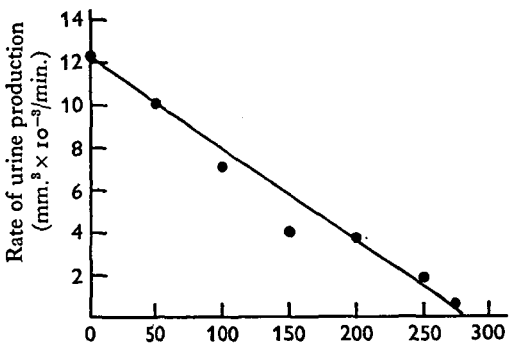

Fig. 7

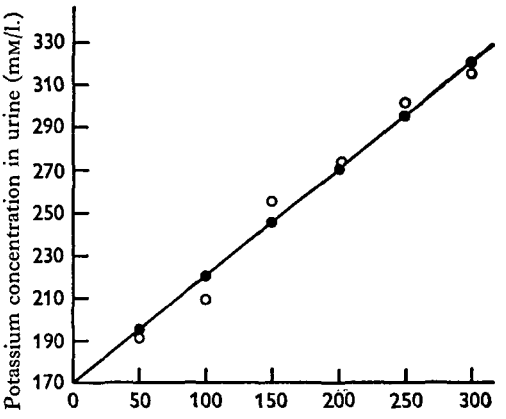

Fig. 8

Fig. 7. The effect of adding sucrose to the bathing medium (medium A + 140 mM/l. KCl)on rate of urine formation.

Fig. 8. Increase in the potassium concentration of the urine caused by the progressive addition of sucrose to the bathing medium. ●, Calculated values; ○, observed values. See text for further details.

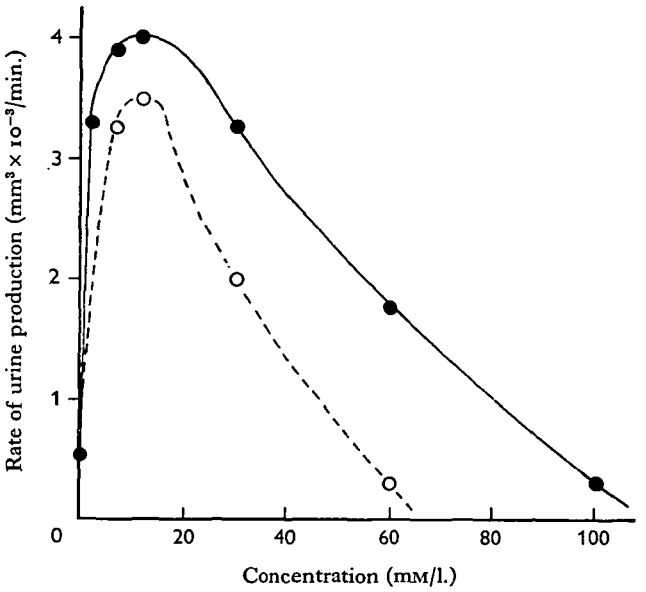

Fig. 9. Effect of magnesium (●) and calcium (○) on rate of urine production.

The inhibitors acetazolamide (Diamox) and sulphanilamide were tested on isolated Malpighian tubules but were found to have no effect. In certain vertebrate epithelia which secrete hydrogen these agents inhibit the enzyme carbonic anhydrase which makes hydrogen ions available to the transport mechanism (Berliner, 1963; Potts, 1965). Further studies determined the effect of pH on rate of urine production. If potassium secretion is linked to a movement of hydrogen in the opposite direction, an increase in hydrogen-ion concentration in the external medium would be expected to drive the pump in the opposite direction and result in a slowing down of urine flow. This did not occur, however, since the results (Fig. 10) showed that rate of urine production is little affected by hydrogen ion concentration despite the wide range of pH employed (5·5–9·2).

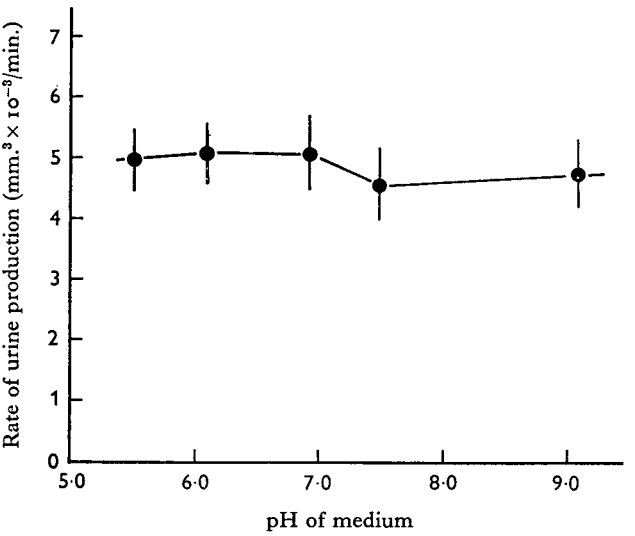

Fig. 10. Effect of pH on rate of urine production. (Vertical lines represent ± twice the standard error of each mean.)

The ion-transport mechanism of the Malpighian tubules of *Calliphora* was completely insensitive to the cardiac glycoside ouabain, which is a specific inhibitor of active ion transport in a large number of tissues (Glynn, 1964). Ouabain appears to act directly on the sodium–potassium activated adenosine triphosphatase which may be intimately involved with the carrier mechanism itself. Evidence for this has come from studies which showed that the isolated enzyme is affected by ions and cardiac glycosides in much the same way as the intact cell. The intimate relationship between drug action and ionic environment is emphasized by the observation that an increase in potassium concentration can antagonize the inhibitory effect of ouabain on sodium transport. Therefore, ouabain was tested on Malpighian tubules maintained in three media which differed markedly with respect to their potassium and sodium content. The first medium was sodium-free, the second contained both ions with a K/Na ratio of 0·4 and the third was a potassium-free medium. When ouabain (1×10^{-3} M) was tested on Malpighian tubules maintained in any one of these three media, no inhibitory effect was observed (Fig. 11).

DISCUSSION

The experiments described here confirm the observations of Ramsay (1955*b*) on the Malpighian tubules of *Dixippus*, that rate of urine production is critically dependent on the concentration of potassium ions. The tubules of *Calliphora*, like those of *Dixippus*, are capable of concentrating potassium in the urine, thus confirming Ramsay's theory 'that the secretion of potassium is the prime mover in generating the flow of urine' (Ramsay, 1956). In *Calliphora*, potassium secretion is relatively non-specific because urine formation is possible with related cations such as rubidium, caesium and sodium. Moreover, these experiments also indicate that tubule cells can secrete urine in the presence of a single univalent cation. The implications of this observation are worth considering further.

Active transport of a cation across a membrane is often accompanied by an obligate transfer of a different cation species in the opposite direction, and such a coupled pump is thought to be responsible for maintaining the high intracellular potassium concentration found in most cells (Ussing, 1960). It is postulated that sodium, which diffuses passively into the cell, is pumped out in exchange for potassium. In the case of reabsorptive epithelia, deployment of such coupled sodium-potassium pumps on

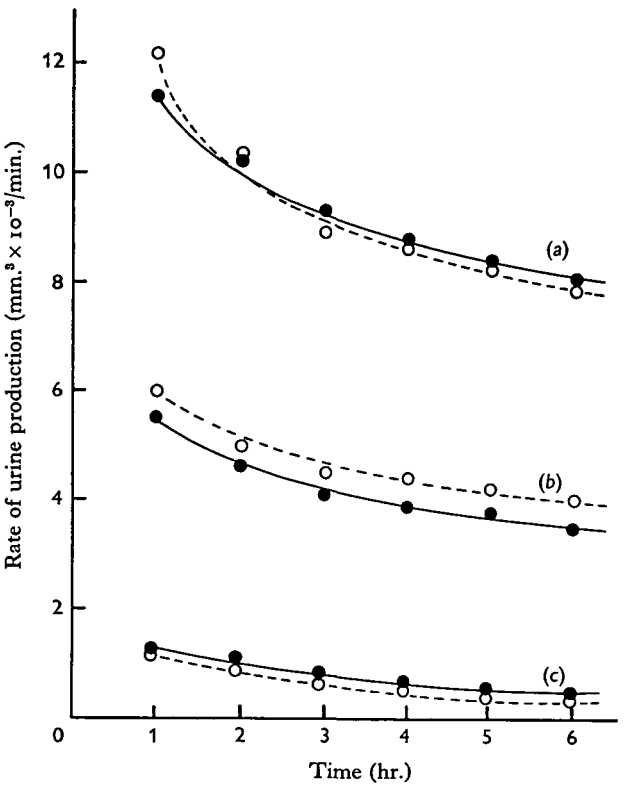

Fig. 11. Effect of ouabain on rate of urine production at different concentrations of sodium and potassium in the bathing medium. (*a*) Sodium-free medium (medium A + 140 mM/l. potassium); (*b*) medium A + sodium (84 mM/l.) + potassium (56 mM/l.); (*c*) potassium-free medium (medium A + 140 mM/l. sodium). ●, Control; ○, artificial medium + ouabain (1×10^{-3} M).

one side of the cell, such as on the basal plasma membrane of kidney cells (Schmidt-Nielsen, 1965) or on the lateral membrane of frog skin (Farquhar & Palade, 1966), can result in a directional movement of sodium. An important characteristic of these pumps is their dependence on the simultaneous presence of both cations for maximal activity. Removal of one ion from the system usually results in a decreased flux of its partner. For example, efflux of sodium from the nerve cord of *Periplaneta* is considerably suppressed in a potassium-free solution, suggesting a coupling of sodium and potassium transport (Treherne, 1961). Consequently, the ability of the Malpighian tubules of *Calliphora* to function in the presence of single univalent cations suggests that at least one component of potassium transport is not a coupled pump. Insensitivity of tubule cells to a wide range of pH seems to exclude effectively the possibility of a coupling between hydrogen and potassium ions as is thought to occur in the midgut of *Hyalophora cecropia* (Harvey & Nedergaard, 1964; Haskell, Clemons & Harvey, 1965). This component of potassium transport might be electrogenic. On the other hand, sodium can markedly stimulate the secretion of potassium, especially at low external concentration of the latter (Fig. 1). Choline (Berridge, 1967*a*) or sucrose, however, have no such effect, and, in their presence, potassium secretion is linearly related to the external potassium concentration. The following hypothesis has been devised in an effort to explain these observations.

It is postulated that potassium secretion is achieved by ion pumps situated on both the basal and apical surface. The pump on the basal plasma membrane is thought to resemble a linked sodium–potassium pump, whereas that on the apical surface may be the electrogenic pump mentioned earlier. Since urine is continuously secreted, the fluid in the lumen cannot function as a reservoir of ions to participate in an exchange pump on the apical surface. Therefore, the apical membrane must achieve a net secretion of potassium from the cell into the lumen without the concomitant movement of another cation in the opposite direction. Conceivably, the pump could be an electrically neutral cation–anion pump similar to that found in the gall bladder (Diamond, 1962); however, evidence will be presented elsewhere suggesting that such a close coupling is absent in Malpighian tubules (Berridge, 1967*b*). The existence of an electrogenic potassium pump on the apical plasma membrane is therefore worth serious consideration. Electrogenic pumps have been reported in muscle (Kernan, 1962, 1967), nerves (Kerkut & Thomas, 1965), frog skin (Bricker, Biber & Ussing, 1963), toad bladder (Frazier & Leaf, 1963) and the gastric intima (Rehm, 1964).

Effectiveness of the electrogenic pump will probably depend on the rate at which potassium can enter the cell across the opposite surface. When the external potassium concentration is low, the sodium–potassium exchange pump on the basal surface probably facilitates potassium entry into the cell so that a high intracellular concentration of potassium can be maintained. If sodium is removed from the outside medium and replaced with choline (Berridge, 1967*a*) or sucrose, this coupled pump becomes ineffective and potassium entry into the cell will then depend upon diffusion. Such an explanation is consistent with the observation that, under these conditions, rate of potassium secretion is linearly related to the external potassium concentration. Saturation of the potassium transport mechanism in the presence of sodium probably represents saturation of the exchange pump on the basal surface. However, it must be stressed that the nature of potassium transport across the two surfaces of the

cell is not certain, and the model outlined above will provide a basis for future study.

There is considerable cytological and histochemical evidence suggesting that both basal and apical surfaces of tubule cells are involved in the process of urine formation. The surface area of the tubule cells of *Calliphora* is greatly enlarged. The basal plasma membrane is extensively infolded to form a complex system of tubular channels; numerous large mitochondria lie within the cytoplasmic compartments between these infolded membranes. The surface area of the apical membrane is increased by the formation of regular microvilli, many of which contain long thin mitochondria. Furthermore, a Mg^{2+}-activated adenosine triphosphatase has been localized on the cytoplasmic surface of both these membranes (unpublished observation).

Fluid transport by Malpighian tubules depends not only on potassium secretion into the lumen but also on the simultaneous transfer of an anion to neutralize the positive charge. If chloride, for example, is replaced in the bathing medium by a non-transportable anion such as sulphate, or if the permeability of the cell membrane to anions is reduced by the presence of trace amounts of copper, net solute transport stops and rate of urine flow falls to zero (Berridge, 1967*b*). A wide variety of anions can accompany the secretion of potassium, apparently ruling out the possibility of a close coupling of cation and anion transport similar to that found in the gall bladder (Diamond, 1962; Dietschy, 1966). It is more likely that the active secretion of potassium generates an electrical gradient for the movement of anions so that the over-all process would be a net transport of neutral salt. A lag between the primary transfer of potassium and the resulting movement of anions, however, could result in a measurable potential gradient similar to that already recorded across the tubule cells of *Pieris*, *Tenebrio*, *Dytiscus* and *Dixippus* (Ramsay, 1953).

In order to understand fully how water movement is coupled to active solute transport, it is necessary to locate the sites of ion transport and the route of water flow through the cell. Considerable information is now available concerning fluid transport by reabsorptive epithelia such as gall bladder (Diamond, 1964; Diamond & Tormey, 1966*a*, *b*; Dietschy, 1966; Kaye *et al.* 1966), vertebrate ileum (Curran, 1965) and the rectum of insects (Phillips, 1965; Berridge & Gupta, 1967). A consistent feature of all these epithelia is the presence of complex intercellular spaces, and Diamond & Tormey (1966*a*, *b*) have suggested that active solute transport into these spaces establishes a standing osmotic gradient which promotes a passive flow of water. Furthermore, these structural devices also 'prevent actively transported solute from diffusing away before water can follow osmotically' (Diamond & Tormey, 1966*b*). Reabsorptive epithelia, therefore, appear to function by maintaining a standing osmotic gradient within an intercellular compartment which communicates with that surface of the cell towards which fluid transport is directed. The model proposed by Diamond & Tormey (1966*a*,*b*) is applicable only to water movement across one surface of the cell (i.e the lateral plasma membrane) and does not attempt to explain how water movement occurs across the apical surface; however, water transport across the luminal surface is probably also an active process and Diamond & Bossert (1967) have suggested that microvilli may function in isotonic water-to-solute coupling across the apical membrane.

The mechanism of fluid transport by secretory epithelia is not as well understood as the reabsorptive mechanisms described above. For example, the exact location of

the ion pumps and the route of water flow in secretory epithelia such as salivary glands, sweat glands, parietal cells, the choroid plexus and the aglomerular kidney remain to be determined. In the case of Malpighian tubules, fluid secretion is dependent upon active solute transport to create the osmotic gradients necessary to promote a passive flow of water from the blood into the lumen. When sucrose was added to the bathing medium, the potassium concentration of urine had to be increased (Fig. 8) to counteract the increased osmotic pressure of the outside medium before urine formation was possible. Solute-linked water movement from the bathing medium into the lumen may be considered as a two-stage process: (*a*) water movement into the cell and (*b*) extrusion of water from the cell into the lumen across the apical plasma membrane. Of course, fluid transport across the basal plasma membrane may generate a sufficient hydrostatic pressure to drive fluid across the apical membrane. Although there is indirect evidence that the stria vascularis of the vertebrate cochlea may secrete endolymph by such a mechanism (Johnstone, 1964), it seems that the calculated hydrostatic pressure necessary for such a process is very much higher than that normally found in animal cells. Physiological and structural observations on Malpighian tubules, however, suggest that solute transport occurs across both surfaces of the cell. Perhaps the geometrical arrangement of the basal membrane in the form of long tubular channels and the apical membrane in the form of microvilli may have important implications with respect to the linkage between solute and water transport.

If solute is transported into the cell from the fluid within the narrow tubular channels, this fluid will become progressively hypotonic towards the closed end and provide the necessary osmotic gradient for a passive flow of water. In effect, a standing osmotic gradient similar to that found in the gall bladder will be established within a localized region of the epithelium. However, in Malpighian tubules the direction of water flow and hence the osmotic gradient is in the opposite direction to that found in the gall bladder (Diamond & Tormey, 1966*b*; Diamond & Bossert, 1967). Fluid will enter the extracellular channels from the bathing medium to replace fluid entering the cell.

A possible structural basis for water transport across the apical surface is less evident. The presence of long thin mitochondria within the microvilli of the tubule cells of *Calliphora* would seem to indicate that solute is transported across the entire microvillus surface. Furthermore, such microvilli have a geometrical arrangement which resembles the long narrow channels on the basal side, although they differ from the latter in being filled with cytoplasm. Nevertheless, if solute is transported out of the microvillus, and if osmotic equilibration takes place progressively down the length of the microvillus, a standing osmotic gradient could be established. The fluid in their extremities will be considerably hypotonic and provide the osmotic gradient for a passive flow of water into the lumen.

Therefore, the transport of water from the outside medium into the lumen probably involves a complex series of standing osmotic gradients situated within localized regions of the cell; the final osmotic pressure of urine will depend on a number of parameters such as the dimensions of the channels, transport rates and the water permeabilities of the basal and apical membranes. For example, differences in water permeability may explain why the fluid secreted by vertebrate salivary glands is isotonic (Burgen & Seeman, 1958), whereas that produced by the nasal salt glands of

marine birds is considerably hypertonic (Schmidt-Nielsen, 1960). Although the intracellular osmotic pressure of the tubule cells of *Calliphora* was not determined, the osmotic pressure of the urine was slightly hypertonic to the bathing medium throughout a wide range of osmotic pressures of the latter. Similarly, Ramsay (1952) found that the urine secreted by the distal tubules of *Rhodnius* was also hypertonic. In the case of *Dixippus*, however, Ramsay (1954) has pointed out that a slight hypotonicity of the urine apparently argues against the hypothesis outlined above. But the Malpighian tubules of *Dixippus* do show some degree of regional differentiation (Ramsay, 1955*a*, *b*), and hypotonicity of the urine may have resulted from solute reabsorption in the proximal part of the tubules.

SUMMARY

1. Rate of urine formation is very sensitive to potassium concentration.

2. Potassium is concentrated in the urine by a mechanism which is independent of other monovalent cations. Rubidium, caesium and sodium are also capable of maintaining a flow of urine. At low external potassium concentrations, sodium stimulates potassium secretion.

3. Rate of urine secretion is stimulated by low osmotic pressures; the osmotic pressure of urine was slightly hypertonic throughout the range of external osmotic pressure employed. Addition of sucrose depresses rate of urine secretion; the potassium concentration of the urine increased by 1 mM/l. for each 2 mM/l. of sucrose added to the bathing medium.

4. Urine formation is insensitive to sulphanilamide, acetazolamide, ouabain and a wide variation of pH.

5. These observations are discussed in relation to the hypothesis that potassium secretion takes place across both surfaces of the cell. The pump on the basal surface may be a coupled sodium–potassium pump, whereas that on the apical surface may be electrogenic.

6. Microvilli at the apical surface or channels formed by a complex infolding of the basal plasma membrane may represent structural devices by which standing osmotic gradients can be established during solute-linked water transport across the cells of Malpighian tubules.

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