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DIURESIS IN THE TSETSE FLY *GLOSSINA AUSTENI*

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SUMMARY

After taking a blood meal, the tsetse fly *Glossina austeni* excretes the excess water and salts of the meal in approximately 30 min. During this period a volume of fluid equivalent to 80% of the unfed weight of the fly passes through the haemolymph, whose composition nevertheless remains almost constant. The fluid excreted has a higher sodium and lower potassium concentration than the haemolymph, indicating that sodium may be the prime mover in urine formation in *Glossina*.

INTRODUCTION

The rapid ingestion of a blood meal, which may be twice the tsetse fly's own weight (Lester & Lloyd, 1928; Moloo & Kutuza, 1970), creates certain problems for the fly. It is so heavy that, for example, the speed of flight of *Glossina swynnertoni* is reduced from about 15 to only 3 or 4 miles/h (Glasgow, 1961). Until it loses weight, *Glossina* is extremely susceptible to predation. Rapid drainage of the unwanted water and salts from the plasma of the blood meal is therefore of great advantage to the fly.

By a process of rapid diuresis, substantial amounts of water are excreted by tsetse flies immediately after the ingestion of a blood meal (Lester & Lloyd, 1928); in *Glossina brevipalpis*, for example, a loss of 38% of the weight of the meal within 30 min has been observed (Moloo & Kutuza, 1970). It is apparent that *Glossina* must possess an efficient excretory system, for it is able to discharge a volume of water approximately equivalent to its own unfed weight within $\frac{1}{2}$ h of feeding.

There are two routes by which excess water in the blood meal may be eliminated from the fly. Newstead, Dutton & Todd (Newstead, 1924) and Tobe (1974) have postulated that water passes directly down the alimentary canal; alternatively, Lester & Lloyd (1928) have suggested that water crosses the gut wall into the haemolymph, to be excreted by the Malpighian tubules, as occurs during diuresis in *Rhodnius* (Maddrell, 1971). It is unlikely that the former route accounts for rapid diuresis in *Glossina* , since the simple separation of the plasma of the meal from the corpuscles would produce a urine with a composition identical to that of the plasma,

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and it will be seen that this is not the case. Water excreted by the latter route mus pass through the haemolymph, whose volume is sufficient to cover the organs of the abdomen with no more than a thin film of fluid (Tobe & Davey, 1972). In this case the excretory system must be carefully controlled, for any imbalance between the absorption and excretion of water and ions would soon cause drastic changes in the volume and composition of the haemolymph.

The experiments described in this paper were the first step in an investigation of the mechanism of rapid diuresis in the tsetse fly. The rate of excretion, the composition of the urine and the effect of the rapid production of urine on the composition of the haemolymph were followed during the first hour of diuresis.

MATERIALS AND METHODS

Tsetse flies *Glossina austeni* Newstead were obtained as pupae from the Tsetse Research Laboratory, Langford, Bristol. The conditions in which the pupae were incubated and the flies emerged were based on those outlined by Nash, Jordan & Boyle (1968) and Jordan, Nash & Boyle (1968). To reduce variation within the results, only previously unfed male flies between 48 and 72 h after emergence were used in these studies.

The flies were fed singly on a rabbit. Feeding took between ½ and 2 min and was taken as ended when the proboscis was withdrawn. Urine was collected by suspending the flies over a dish of liquid paraffin (sp.gr. 0·87–0·89 at 20 °C). Drops of urine fell on to the surface of the paraffin and were immediately sunk using a fine, siliconed, glass rod. Haemolymph was collected from fed and unfed flies by inserting a finely drawn Pasteur pipette into the thorax at the junction of the pleural sclerites. The volumes of small samples ($\leqslant$ 1 μl) of urine and haemolymph under liquid paraffin were estimated by measuring their diameters and calculating their volumes by assuming the drops to be spherical.

Osmotic pressures were measured using either the cryoscopic method of Ramsay & Brown (1955) or a nanolitre osmometer (Clifton Technical Physics, New York). Concentrations of chloride ions were measured by potentiometric titration against silver nitrate solution (Ramsay, Brown & Croghan, 1955). Concentrations of sodium were estimated using the emission mode of a Unicam SP 90 Series 2 Atomic Absorption Spectrophotometer. Potassium concentrations were estimated using an integrating flame photometer similar to that described by Ramsay, Falloon & Machin (1951). Details of the use of this apparatus and its calibration are given by Ramsay (1950, 1952).

RESULTS

The extent of feeding and excretion

Unfed flies were weighed and allowed to feed, and urine was collected for 1 h after feeding. The flies were then reweighed and the volume of urine produced was measured. The results of these measurements are shown in Table 1 where they are compared with those obtained by Lester & Lloyd (1928) on *G. morsitans*. When the volume of urine produced during successive 10 min intervals was measured it was found that the majority was expelled during the first 30 min after feeding (Fig. 1)

Table 1. *The extent of feeding and excretion*

	Glossina austeni	*Glossina morsitans* (Lester & Lloyd, 1928)
Weight of unfed fly (mg)	14·2 (1·96)	23·6
Weight of meal (mg)	25·8 (1·64)	39·5
Weight excreted during first hour (mg)	9·9 (1·82)	16·9
Meal (% weight of fly)	184% (23·1%)	167%
Loss during first hour (% weight of meal)	38% (4·7%)	43%

Means from five male flies, with the value of twice the standard error of the mean in parentheses.

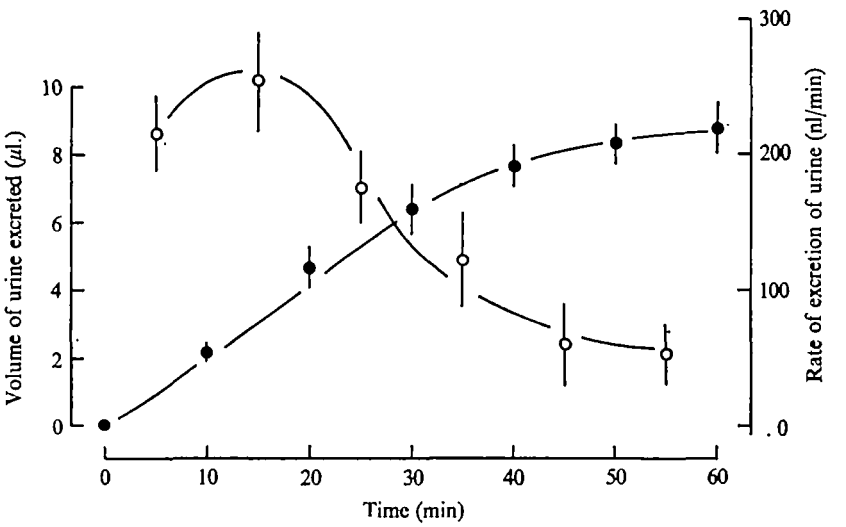

Fig. 1. The rate of excretion (○) and the volume of urine produced (●) during the first hour of diuresis in *G. austeni*. Each point is the mean of ten determinations and the vertical lines represent the extent of ± twice the standard error of the mean (± 2 × S.E.). The experiments were performed at room temperature (21–24 °C).

The rate of excretion reaches a peak during this period and then declines (Fig. 1). Over the range of temperatures at which these experiments were performed the flies had maximum rates of excretion of 200–300 nl/min. Excretion at such rates during the first 20 min after feeding enabled the flies to complete rapid diuresis in approximately 30 min. At laboratory temperatures above 24 °C maximum rates of excretion in excess of 300 nl/min were observed, indicating that the rate of excretion, as in the blood-sucking bug *Rhodnius* (Maddrell, 1964), may be temperature-dependent.

The osmotic pressure and ionic concentrations of urine and haemolymph

Estimations of the osmotic pressure of samples of urine obtained at intervals during the first hour after feeding are shown in Fig. 2(*a*). All show a slight decline in osmotic pressure followed by the maintenance of an almost constant value during the most rapid phase of excretion. In two examples where diuresis was completed

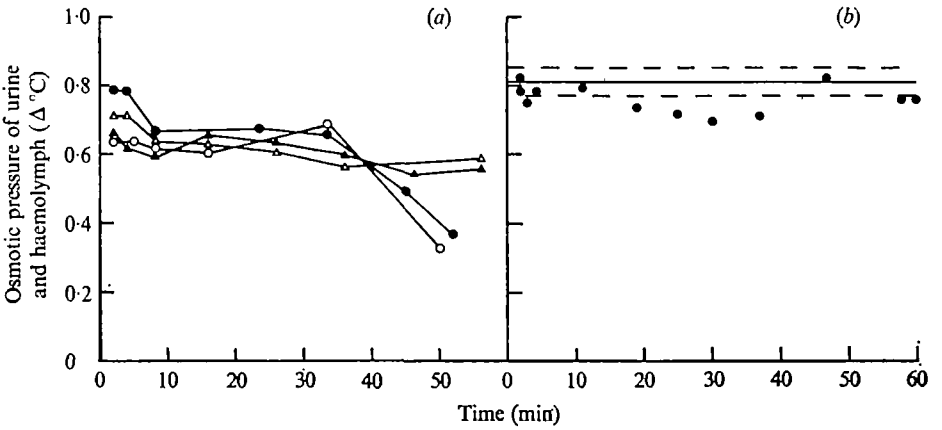

Fig. 2. (*a*) The osmotic pressure of the urine of four flies during the first hour of diuresis. (*b*) The osmotic pressure of samples of haemolymph from 12 flies during the first hour of diuresis (●). The continuous line shows the mean value of the osmotic pressure of the haemolymph of six unfed flies with the extent of ±2 × S.E. indicated by the broken lines.

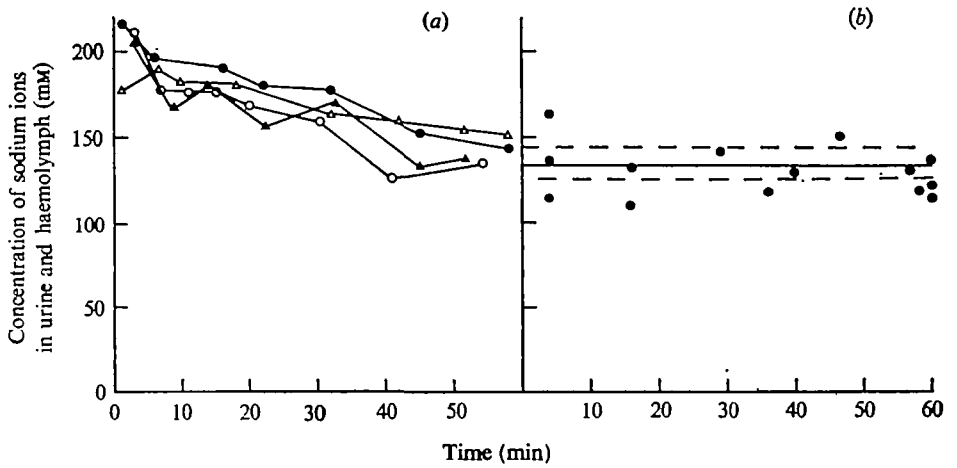

Fig. 3. (*a*) The concentration of sodium ions in the urine of four flies during the first hour of diuresis. (*b*) The concentration of sodium ions in samples of haemolymph taken from six unfed flies and from fourteen flies during the first hour of diuresis. (Symbols as in Fig. 2*b*.)

within the hour, the osmotic pressure of the urine can be seen to fall to a lower level. During the period of almost constant osmotic pressure, 80% of the urine produced during the first hour is excreted. This urine (Δ = 0·60–0·65 °C) is approximately isosmotic with the ingested blood (Δ, rabbit plasma = 0·59 °C). By producing a urine with an osmotic pressure close to that of the ingested meal, the fly should minimize the disturbance of its internal osmotic environment. This is found to be true when the osmotic pressures of the haemolymph before feeding and during diuresis are compared (Fig. 2*b*). Though fluid equivalent to 80% of the fly's unfed weight passes through the haemolymph, there is little disturbance of its osmotic pressure.

An almost constant concentration of sodium in the urine is maintained from 5 to 35 min after feeding (Fig. 3*a*). The sodium concentration of the urine (160–180 mM)

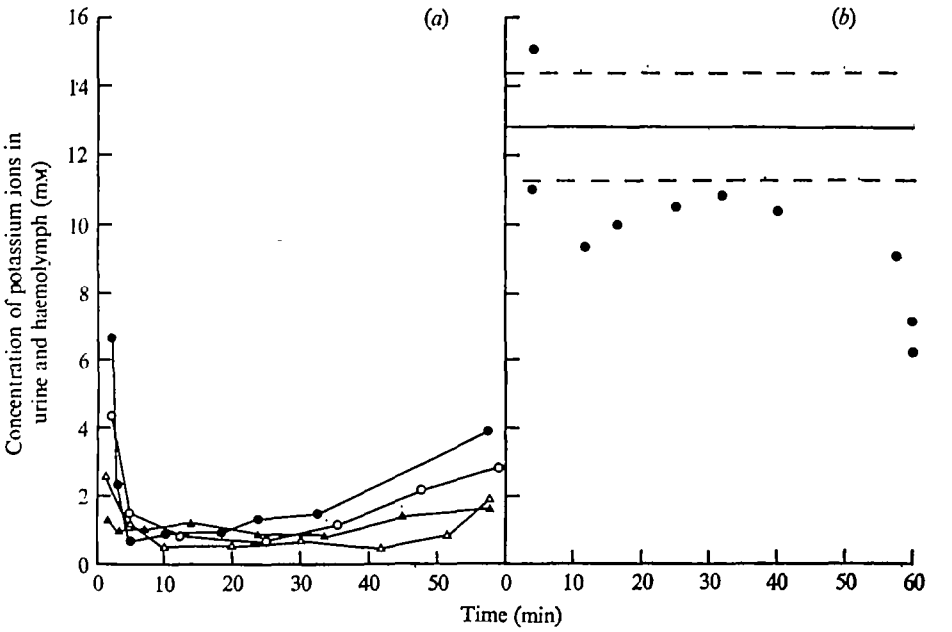

Fig. 4. (*a*) The concentration of potassium ions in the urine of four flies during the first hour of diuresis. (*b*) The concentration of potassium ions in samples of haemolymph taken from six unfed flies and from ten flies during the first hour of diuresis. (Symbols as in Fig. 2*b*.)

is higher than that of the ingested blood (rabbit plasma 140–150 mM). It is also higher than that of the haemolymph (120–140 mM), the concentration of which remains almost constant during diuresis, at a level similar to that found in unfed flies (Fig. 3*b*). The haemolymph sodium concentration remains constant even though the concentration in the urine is higher than in the ingested meal; the gut contents must therefore become depleted of sodium. The sodium concentration of the gut contents will fall below that of the haemolymph, and sodium must then be transported against its concentration gradient across the midgut wall.

Estimations of the concentration of potassium in the urine are shown in Fig. 4(*a*). The concentration falls rapidly after the first one or two drops of urine, during rapid diuresis it is very low, but as the rate of excretion decreases the potassium concentration of the urine begins to rise. The elevated concentration of potassium in the initial drops of urine is probably due to contamination of the urine by the contents of the rectum. The rise in concentration towards the end of the hour may be due to the release of potassium from the corpuscles (Wigglesworth, 1931), though it is not certain that the red cell walls will retain their impermeability to potassium ions for so long under the conditions of 'pseudoclotting' that occur in the midgut of *Glossina* (Lester & Lloyd, 1928). The haemolymph potassium concentration during diuresis is maintained at a level slightly lower than that found in the unfed fly (Fig. 4*b*). This indicates that the solution crossing the gut wall must have a very low potassium concentration, for very little potassium is lost in the urine. The concentration of potassium in the haemolymph falls as rapid diuresis is completed,

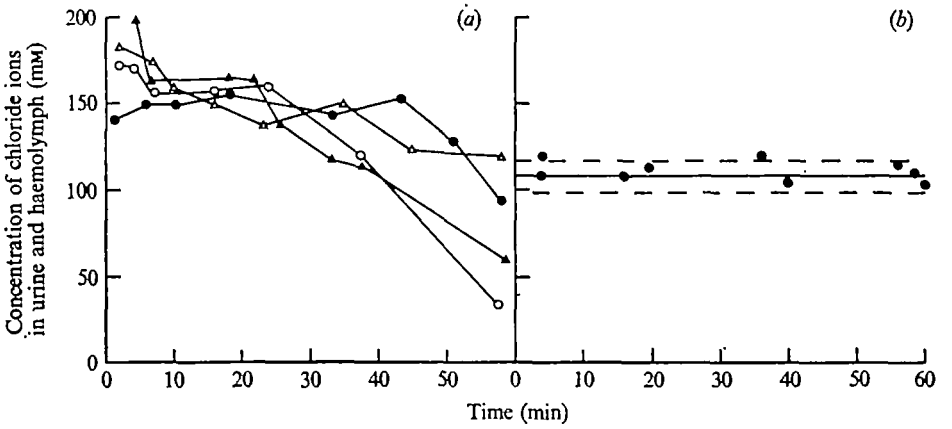

Fig. 5. (*a*) The concentration of chloride ions in the urine of four flies during the first hour of diuresis. (*b*) The concentration of chloride ions in samples of haemolymph taken from six unfed flies and from nine flies during the first hour of diuresis. (Symbols as in Fig. 2*b*.)

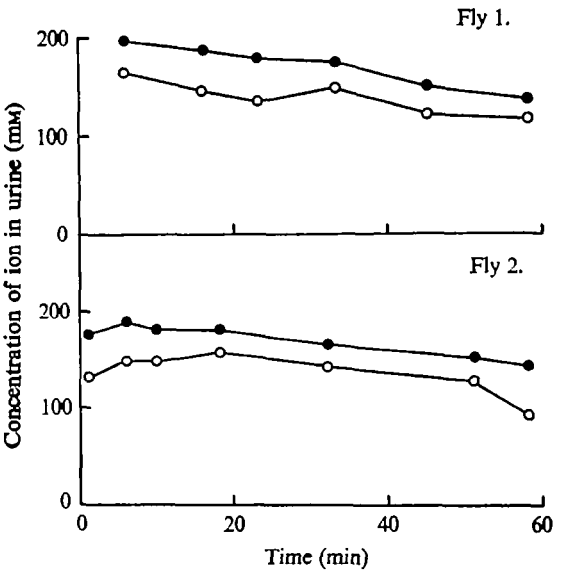

Fig. 6. The concentrations of sodium (●) and chloride (○) ions in the urine of two flies during the first hour of diuresis. The points represent paired estimations of samples obtained by pooling the urine produced over successive intervals.

though at this time the midgut contents probably have a higher potassium concentration than the haemolymph (rabbit blood plasma 5 mM, whole blood 40 mM). The midgut wall must have a low permeability to potassium, and, as in *Rhodnius* (Ramsay, 1952), it forms a barrier to the absorption of potassium during diuresis. The decrease in potassium concentration of the haemolymph at around 60 min may therefore be due to a decline in the rate of excretion in advance of the decline in the rate of absorption, a situation necessitated by the low haemolymph volume. Potassium in the haemolymph would then be diluted by the solution of low potassium concentration crossing the midgut wall.

The chloride concentration of the urine remains almost constant during rapid diuresis but it declines as the rate of excretion falls after about 60 min (Fig. 5*a*). The urine concentration (140–160 mM) is higher than that of the haemolymph (100–120 mM); however, the haemolymph concentration remains constant throughout the first hour of diuresis at a level similar to that found in unfed flies (Fig. 5*b*). The urine chloride concentration is also higher than that of rabbit blood plasma (100–110 mM). Chloride, like sodium, must therefore be moved across the gut wall against its concentration gradient, leaving the fluid in the midgut depleted of chloride ions. The close connexion between the excretion of sodium and chloride is apparent if urine samples are analysed for both ions (Fig. 6).

DISCUSSION

The primary function of the excretory system of the tsetse fly immediately after feeding is to remove the excess water from the meal as rapidly as possible. The results reported here show that as in other species of tsetse, for example *G. morsitans* (Lester & Lloyd, 1928) and *G. brevipalpis* (Moloo & Kutuza, 1970), *G. austeni* is able to excrete this excess water in about 30 min. It appears from preliminary observations that the rate of excretion of *G. austeni* like that of *Rhodnius* (Maddrell, 1964) is temperature-dependent. It is therefore possible that at the higher temperatures that occur in the natural environment, the problems created by the size and nature of the blood meal may be alleviated even more rapidly.

In unfed insects the haemolymph has a higher concentration of sodium than potassium – a relationship characteristic of carnivorous and omnivorous insects (Boné, 1944). During diuresis, although a large volume of fluid in relation to the blood volume passes through the haemolymph, the composition of the latter varies little, a situation which also occurs during diuresis in *Rhodnius* (Ramsay, 1952). This indicates that the actions of the absorptive and excretory systems are well balanced, for any imbalance would rapidly be reflected in a change in the composition or volume of the haemolymph.

Recently it has been proposed that compartmentalization of the blood space may prevent the free exchange of haemolymph between the abdomen and thorax during diuresis (Tobe, 1974). Samples from the thorax may therefore show constant composition even though the composition of the haemolymph in the abdomen varies during diuresis. The absence of a sufficient volume of free haemolymph in the abdomen and the fragile nature of the distended gut prevented samples of abdominal haemolymph from being obtained during diuresis. Until such samples can be obtained or until the rate of exchange of haemolymph between abdomen and thorax is investigated, the possibility that free circulation of water and ions between these two regions is restricted during diuresis cannot be ruled out.

Eighty per cent of the clear urine produced by *Glossina* is discharged during a 30 min period between 5 and 35 min after feeding. During this period the osmotic pressure and ionic concentrations of the urine and haemolymph remain almost constant (see Figs. 2–5). These approximately steady-state values are shown in Table 2, where they can be compared with the equivalent data for the blood meal.

The urine is hyposmotic to the haemolymph, a situation which occurs in several

Table 2. *The ranges of osmotic pressures and ionic concentrations of the haemolymph and urine during rapid diuresis*

	Haemolymph	Urine	Rabbit blood plasma*	Rabbit whole blood*
Osmotic pressure (Δ °C)	0·7–0·8	0·6–0·65	0·59	
Sodium (mM)	120–140	160–180	140–150	100
Potassium (mM)	8–12	0·5–1·5	5	40
Chloride (mM)	100–120	140–160	100–110	100

* Figures from Biological Handbooks (1961), *Blood and Other Body Fluids* (ed. P. L. Altman and D. S. Dittmer). Fed. Amer. Soc. Exp. Biol.

other insects, for example, in the stick insect *Carausius morosus* (Ramsay, 1954), in the cotton stainer, *Dysdercus fasciatus* (Berridge, 1965) and in *Rhodnius* (Ramsay, 1952). As in the majority of insect excretory systems (Maddrell, 1971), the primary urine produced by the Malpighian tubules of *G. austeni* is isosmotic with their bathing medium (unpublished observation). For the urine, which is eventually discharged from the insect, to be hyposmotic to the haemolymph, ions or molecules must be reabsorbed in the hind gut. Sodium and chloride, which are the major ionic constituents of the urine, may be reabsorbed. Useful molecules – for example, sugars and amino acids – may also be returned to the haemolymph by the hind gut in order to prevent their indiscriminate loss during diuresis, because the Malpighian tubules of insects are passively permeable to a wide variety of molecules (Ramsay, 1958; Maddrell & Gardiner, 1974).

The concentrations of sodium and chloride in the urine are high but the concentration of potassium is very low (Table 2), and it must be concluded that the excretory system of *Glossina* is either highly impermeable to potassium or possesses a highly efficient reabsorption mechanism for this ion. In all insects previously investigated (for examples, see Ramsay, 1953) potassium was found in the urine in a higher concentration than in the haemolymph. Sodium on the other hand was found to be more concentrated in the haemolymph than in the urine. Since this appeared to be true for all insects, Ramsay (1953) suggested that potassium must be the prime mover in the production of urine by insects. The excretory system of *Glossina*, however, appears to concentrate sodium in the urine and excludes potassium from it. These results suggest that *G. austeni* is an exception to the rule and may be the first insect shown to produce urine by the active movement of sodium rather than potassium.

The composition of the haemolymph during diuresis is almost constant and its volume does not appreciably increase (Tobe & Davey, 1972). We must therefore conclude that the loss of ions from the haemolymph in the urine is balanced by an equivalent gain by absorption from the meal. As the osmotic pressure of the urine and blood plasma are similar (Table 2), the osmotic pressure of the gut contents should not be affected by the rapid excretion of water. The total osmotic pressure will be determined by the rate of release and absorption of amino acids as the serum and corpuscular proteins are digested. Sodium and chloride must be absorbed from the midgut against their concentration gradients, as was suggested by Langley & Pimley (1973). Potassium must be retained in the midgut, a situation which

Ramsay (1952) suggested may occur in *Rhodnius*. Therefore during diuresis the midgut contents of *Glossina* must become depleted of sodium chloride and rich in potassium.

This investigation has shown that during the first hour of diuresis the tsetse fly excretes much of the excess water and salts of the blood meal and, though this must require a rapid flow of fluid through the haemolymph, there is little disturbance of the haemolymph composition. Of equal interest is the finding that in *Glossina* urine production appears to be generated by the transport of sodium – a possibility that requires further investigation.

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