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SALT AND WATER BALANCE IN THE SPIDER, *PORRHOTHELE ANTIPODIANA* (MYGALOMORPHA: DIPLURIDAE): EFFECTS OF FEEDING UPON HYDRATED ANIMALS

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SUMMARY

The spider, *Porrhothele antipodiana*, starved and provided with water, produced urine *via* the anal excretory system (Malpighian tubules, midgut diverticula and stercoral pocket) at a mean rate of about 2·5–5 $\mu l\,g^{-1}\,day^{-1}$ and with a mean Na^+/K^+ ratio of about 1·0. Salts ingested from the prey were eliminated by two mechanisms. A K^+-rich (Na^+/K^+ about 0·2) anal diuresis lasted about 3 days following a single meal and was maintained at more than 30 $\mu l\,g^{-1}\,day^{-1}$ during feeding *ad libitum*. The second mechanism, interpreted as coxal secretion, functioned only during feeding itself and delivered Na^+ into the prey at a constant rate of about 3 % h^{-1} of total body Na^+. This progressively raised the Na^+/K^+ ratio of the prey debris from 0·47 to 0·96 and, because of re-ingestion, recycled more Na^+ than was originally present in the prey.

Feeding was associated with large net increases in dry weight and ions, particularly K^+, which were mainly stored in the diverticular tissue (midgut diverticula and Malpighian tubules embedded in adipose tissue). The stercoral fluid (final urine) was slightly hyposmotic to the haemolymph in starved and fed spiders. Only about half of its osmolarity was accounted for by Na^+, K^+ and Cl^-. The volume of water gained from the meal was about equal to that lost in diuresis, and *P. antipodiana* drinks to maintain water balance because of relatively high transpirational and other losses. The primary function of the diuresis is probably elimination of ions from the meal, and not volume regulation.

INTRODUCTION

Spiders, are familiar, numerous and ecologically important inhabitants of a wide range of terrestrial habitats and yet remarkably little is known of their osmoregulatory physiology.

The rates of evaporative water loss by spiders are comparable with those of similar-sized insects (Davies & Edney, 1952; Cloudsley-Thompson, 1957; Stewart &

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Martin, 1970; Seymour & Vinegar, 1973; Humphreys, 1975) and, like insects, the relative impermeability of their cuticle is thought to be conferred by a thin epicuticular wax layer (Hadley, 1978, 1981). Reductions in respiratory water loss due to the action of spiracular valves present at the openings of the booklungs have also been demonstrated in spiders (Davies & Edney, 1952; Cloudsley-Thompson, 1957; Stewart & Martin, 1970).

Our understanding of the excretory physiology of spiders is limited to considerations of nitrogenous excretion. They are generally considered to be guanotelic (Vajropala, 1935; Schmidt, Liss & Thannhauser, 1955; Atkinson & Chorlton, 1956; Anderson, 1966), an adaptation which confers advantages in terms of water conservation, but it is unknown whether they are also capable of producing a hyperosmotic excretory fluid as do many terrestrial insects.

In addition to mechanisms of water conservation, terrestrial animals require mechanisms of compensation for short-term perturbations of salt and water balance associated with environmental change or with feeding. Spiders are carnivorous (mainly insectivorous) intermittent feeders and it is thought that some spiders obtain their water requirements from their prey (Comstock, 1940). However, Stewart & Martin (1970) reported that the tarantula, *Dugesiella hentzi*, drank water to replace blood volume lost during desiccation or bleeding.

Information on the overall salt and water balance and the routes for salt excretion is entirely lacking for any spider. Indeed, our knowledge of the excretory physiology of spiders is so limited that there is room for dispute as to which are in fact the principal excretory organs of spiders.

Spiders possess a hindgut-associated system composed of Malpighian tubules and a stercoral pocket which, at least superficially, resembles the Malpighian tubule/rectal system of insects whose excretory role is well documented (see Maddrell, 1971; Wall & Oschman, 1975 for reviews). In addition, spiders possess coxal glands which have close morphological similarities with the coelomoduct-derived organs of other arthropods including ticks, mites, scorpions, crustaceans, onychophorans and some insects (Goodrich, 1945; Clarke, 1979) and for which in some groups there is evidence for an excretory role (Lees, 1946; Woodring, 1973; Frayha, Dajani, Almaz & Sweatman, 1974; Riegel & Cook, 1975; Kaufman, Kaufman & Phillips, 1981).

Porrhothele antipodiana (Walckenaer) was selected for this first detailed study of the osmoregulatory physiology of a spider partly because of its abundance in New Zealand and its relatively large size. Another consideration was that it is a representative of the more primitive Mygalomorpha, a group which includes the tarantulas and trapdoor spiders and which perhaps exhibits more generalized features than the varied Araneomorpha or 'true' spiders. It is a hygric to mesic species, constructing funnel-like webs under stones and logs and also between rocks and shingle in the supra-littoral zone, often to a depth of 40 cm.

In this paper the possible components of the excretory system of *P. antipodiana* and other spiders are briefly described and the salt and water balance of starved and feeding animals is quantified. The relative contributions of the prey, drinking and excretion are estimated. The possible routes of salt excretion are also investigated

and a complete salt budget for feeding animals is presented. Interestingly, an important avenue for the elimination of salts in the diet involves excretion into the prey during feeding.

MATERIALS AND METHODS

Adult female *P. antipodiana* were collected from Third Bay and Whaler's Bay on the Kaikoura Peninsula, New Zealand. Before use in experiments at 20 ± 2°C, they were held for at least 1 week at this temperature and provided with water *ad libitum* and 4–5 large cockroach nymphs per week. These cockroaches were obtained from a culture which was maintained on a standard diet. Cockroaches from the same culture were used in the feeding experiments reported below. In all experiments spiders were placed in individual borosilicate glass dishes lined with Whatman 542 filter paper, changed daily. Moistened cotton wool rolls provided a supply of drinking water.

Droplets of excreta (urine plus faeces) which had dried on the filter paper lining were visualized under ultraviolet light. Daily urine production was thus estimated from the mean diameter of these spots calibrated by dropping onto filter paper known volumes of fluid sampled from the stercoral pocket. Diameter squared varied linearly with volume and was not affected by small differences in temperature, humidity, air movement or rate of delivery. In the range 5–25 μl the coefficient of variation of the diameters of replicates was about 2 % of the mean (corresponding to a volume error of less than 5 %). Spots were cut from the paper and eluted with nitric acid for estimation of daily rates of Na^+ and K^+ excretion. Coxal fluid and regurgitated fluid, which also contain Na^+ and K^+ (Butt, 1983), do not fluoresce and were therefore unlikely to be mistaken for urine. Chance contamination was also unlikely since, in the ^{22}Na experiments reported below, radioactivity was never located outside the excreta, drinking water or prey debris.

Haemolymph was collected from the tarsal–metatarsal joint of the third or fourth walking leg of spiders lightly anaesthetized with CO_2 using paraffin-filled, silanized Pyrex micropipettes. Subsequent bleeding was prevented by ligature of the metatarsal segment and by covering the wound with wax. Ion analyses refer to whole haemolymph, no attempt being made to isolate the cellular component. The low values for K^+ concentration suggest their contribution is minor. For collection of fluid from the stercoral pocket, the pipette tip was flamed smooth to avoid damaging the delicate cuticle lining the rectal tube between the stercoral pocket and the anal tubercle.

Tissue samples were dried at 100°C for 48 h for estimation of water content and then ashed at 600°C and dissolved in 50 % nitric acid for cation determinations.

Osmotic pressure measurements were made by the method of Ramsay & Brown (1955) and chloride was determined potentiometrically by the second method of Ramsay, Brown & Croghan (1955). Na^+ and K^+ were estimated by atomic absorption spectroscopy (Varian Techtron AA1200) using an air–acetylene flame. Mutual interference of Na^+ and K^+ (ionization interactions in the flame) were

minimized by matching the Na^+/K^+ ratio of standards as closely as possible to that expected in the samples.

^{22}Na-labelled saline [15–25 μl containing approximately 0·07 MBq ^{22}Na; and (in mmol l^{-1}) NaCl 200, KCl 4·8, $CaCl_2$ 4·5, $MgCl_2$ 2·5, $NaHCO_3$ 3·0; pH 7·3] was injected into spiders anaesthetized with CO_2 using the following procedure to prevent bleeding. A ligature was tied around the tibial segment of the third walking leg and the syringe needle inserted into the tibio–metatarsal joint and tied into the tibial segment with a second ligature. The first ligature was then released and the saline injected over a period of 3–5 min. After a further 5 min, the first ligature was retightened, the second one removed and the needle withdrawn, and the wound covered with wax.

A well counting system (Ortec) was used to measure the ^{22}Na activities of the filter paper, which was divided into small pieces, the dried cotton wool used to supply drinking water and the food debris.

Except where noted otherwise, tests of significance of differences between means were performed using Student's unpaired *t*-test and means in the text and on figures are given ± one standard error. Where error bars are not shown they are smaller than the plotting symbols.

RESULTS

Morphology of the alimentary canal and associated structures

The arrangement of the mouth, gut and excretory structures of *P. antipodiana* is shown schematically in Fig. 1. In general, the descriptions of Millot (1931), Comstock (1940) and Legendre (1978) for other spiders have been confirmed and these should be consulted for more detail.

The mouth opens at the junction of the rostrum, labium and maxillary blades and is surrounded by hairs which function as a sieve. The foregut, derived from ectoderm and lined with cuticle, consists of the pharynx, oesophagus and sucking stomach. The pharynx passes vertically from the mouth to the oesophagus which then leads horizontally between the dorsal and ventral nerve masses to the sucking stomach. Muscles attached to the pharynx and the sucking stomach expand and constrict these structures generating suction during ingestion of the liquefied food.

The midgut arises from the sucking stomach and passes into the abdomen. Two systems of diverticula extend from the midgut. In the cephalothorax, a main branch on each side extends anteriorly and sends a branch into the coxal segments of each of the four walking legs, and a fifth branch anterodorsally towards the chelicera. The abdominal midgut diverticula are not simple tubes but branch extensively within a large mass of fatty storage tissue occupying much of the abdominal volume between the heart dorsally and the reproductive organs and spinnerets ventrally. The abdominal midgut diverticula arise as six main trunks (two pairs dorsolaterally and a pair mid-ventrally) and branch immediately.

After giving rise to the diverticula the central midgut tube is constricted and turns ventrally before entering the stercoral pocket. At the point of constriction, pellets of

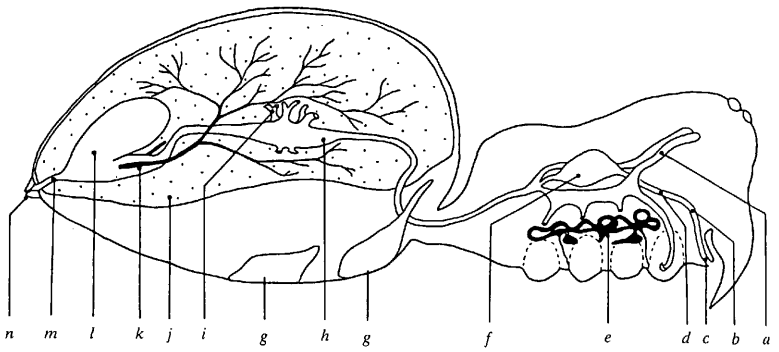

Fig. 1. Schematic diagram of the alimentary canal and excretory system of *Porrhothele antipodiana*. *a*, foregut diverticula; *b*, pharynx; *c*, mouth; *d*, oesophagus; *e*, coxal gland; *f*, sucking stomach; *g*, booklungs; *h*, midgut; *i*, abdominal midgut diverticula (proximal regions only shown); *j*, diverticular tissue (branches of Malpighian tubules and abdominal diverticula embedded in adipose tissue); *k*, Malpighian tubules (finest branches not shown); *l*, stercoral pocket; *m*, rectal tube; *n*, anal tubercle. Body length of 0·8 g spider, approx. 20 mm.

faecal material are invested with a membrane. Van der Borght (1966) described similar membranes in other spiders as 'peritrophic membranes'. However, in insects these membranes are produced at the anterior end of the midgut (Wigglesworth, 1972) and are therefore probably not homologous with the 'faecal membranes' of spiders.

The hindgut consists of the stercoral pocket and the rectal tube. The stercoral pocket is a simple, extensible sac in which faecal material and excretory fluid are stored before excretion. Comstock (1940) described the stercoral pocket as lined with cuticle and derived from the proctodeum but Millot (1926, 1949) did not mention a cuticle. In an ultrastructural study of the stercoral epithelium of *P. antipodiana*, Butt (1983) found no evidence of a cuticle, the luminal surface being covered with loosely packed microvilli.

A pair of Malpighian tubules branch from the midgut just anterior to the stercoral pocket. These extend forward parallel to the midgut for a short distance before branching dichotomously within the abdominal mass of storage tissue and midgut diverticula. The Malpighian tubules are not closely associated with the midgut diverticula but are surrounded by the fat cells. Ultrastructurally, the luminal membrane of the Malpighian epithelium is extended into thin lamellar processes (Butt, 1983).

The coxal glands, which also must be considered as potential excretory organs, are located in the cephalothorax (Fig. 1). In *P. antipodiana* they open at the base of walking legs 1 and 3. Further details of their structure and physiology will be given in a subsequent paper (A. G. Butt & H. H. Taylor, in preparation).

Definitions

There is no established terminology to describe the excretory fluids of spiders. Labelling the fluid collected from the stercoral pocket as 'rectal fluid', by analogy with insects, would be misleading. The absence of a cuticle suggests that the stercoral pocket is not homologous with the insect rectum and there is, as yet, no evidence to suggest that they are physiologically similar. The following terms are used to refer to the various excretory fluids of spiders: *stercoral fluid*, fluid collected directly from the stercoral pocket; *urine*, fluid voided *via* the anus; *excreta*, urine plus solid material (faecal pellets mainly) voided *via* the anus; *coxal fluid*, fluid which emerges from the coxal glands.

The term *anal excretory system* refers to the organs which produce the excreta (some combination of the midgut, its diverticula, the Malpighian tubules and the stercoral pocket). *Diverticular tissue* refers to the tissue mass comprising the Malpighian tubules and abdominal midgut diverticula embedded in storage tissue.

Changes in the pools of water, ions and dry material in spiders fed ad libitum *for 5 days*

Sixteen spiders were starved of food, but allowed water for 3 weeks and divided into two groups of similar mean body weight. One group was then provided with a constant supply of water and live cockroach nymphs for 5 days while the other group was provided with water only. Live weights, rates of urine production and rates of cation excretion were determined daily and at the end of the 5-day period haemolymph samples and the contents of the stercoral pocket were collected from each spider. The diverticular tissue was dissected out and its water and cation content determined separately from the rest of the body.

Weight changes

Table 1 shows the mean live weight and the weights of dry material and water in the two groups. In the feeding spiders, more or less constant daily weight increments were observed, accumulating after 5 days to a total increase of 47 % in the fed spiders relative to the starved spiders. In absolute terms, the spiders gained more water than dry material during the feeding period, but the relative increase in total body water (39 %) was less than that of dry weight (75 %), resulting in a small decrease in fractional water content of the whole spiders. Most of the weight increases were associated with the diverticular tissue which accounted for 40 % of the total body weight in fed spiders but only 19 % in starved spiders. The size of the abdomen relative to the cephalothorax was visibly greater in the fed spiders. Thus, 84 % of the total weight increase, 78 % of the increase in water content and 93 % of the increase in dry material were within the diverticular tissue. Fractional water content of the diverticular tissue decreased to a greater extent than in the body as a whole (Table 1).

Sodium and potassium contents and concentrations

The mean Na^+ and K^+ contents of starved and fed groups of spiders are shown in Table 2. With feeding there were increases of about the same magnitude in the

amounts of Na^+ (37·6 μmol) and K^+ (43·4 μmol) present in the whole body of spiders. As whole body Na^+ increased by about 60 % while K^+ content increased by about 90 % the Na^+/K^+ ratio of the whole body decreased from 1·32 to 1·08. These trends were even more marked within the diverticular tissue. A large increase in K^+ content (from 12·8 to 40·3 μmol) and smaller increase in Na^+ content (from 8·6 to 15·2 μmol) produced a fall in Na^+/K^+ ratio from 0·69 to 0·38 in this tissue, whereas the Na^+/K^+ ratio of the 'rest of the body' (whole body minus diverticular tissue) actually increased slightly.

Table 2 also lists the Na^+ and K^+ concentrations in mmol kg^{-1} wet weight. In fed spiders the mean K^+ concentration of the whole body increased considerably from 55·0 to 72·6 mmol kg^{-1} and the Na^+ concentration increased slightly from 71·0 to 78·6 mmol kg^{-1}. Similar trends were seen in the 'rest of the body' (whole body minus diverticular tissue). However the large increases in cation content of the diverticular tissue were accompanied by increases in the water content and dry weight so that the K^+ concentration of this tissue remained about the same while the Na^+ concentration decreased by almost half.

Composition of the haemolymph and stercoral fluid

The major osmolar effectors in the haemolymph of *P. antipodiana* were Na^+ and Cl^- (Table 3), together accounting for about 83 % of the total osmotic pressure. Na^+ and Cl^- concentrations and osmotic pressure all increased with feeding but K^+

Table 1. *Comparison of live weight, dry weight and water content in spiders either starved or provided with food for 5 days*

	Starved 5 days ($N = 8$)	Fed 5 days ($N = 8$)	% Change associated with feeding
Live weight (g)			
Initial	0·861 ± 0·051	0·854 ± 0·04	
Final	0·856 ± 0·070	1·258 ± 0·059***	+47
Final weight of water (g)	0·631 ± 0·094	0·873 ± 0·123(NS)	+39
Final weight of dry material (g)	0·219 ± 0·02	0·381 ± 0·02***	+75
Fractional water content (kg kg^{-1})			
Whole spider	0·748 ± 0·007	0·695 ± 0·01***	−7
Diverticular tissue†	0·661 ± 0·021	0·584 ± 0·013**	−12
Rest of body‡	0·752 ± 0·005	0·770 ± 0·009(NS)	+2

Both groups provided with water.

Pretreatment, 3 weeks starvation with access to water.

Significance level of differences between means of starved and fed spiders (after corrections for slight difference in mean initial weight where appropriate), NS, not significant; *, $0·05 > P > 0·01$; **, $0·01 > P > 0·001$; ***, $0·001 > P$.

† Malpighian tubules, abdominal midgut diverticula and stercoral pocket epithelium.

‡ Principally the cephalothorax, abdominal cuticle, spinnerets, heart and ovaries.

Table 2. *Na^+ and K^+ contents and concentrations in spiders provided with water and starved or fed for 5 days*

	Starved spiders	Fed spiders	Diverticular tissue		Rest of body	
			Starved	Fed	Starved	Fed
Potassium						
content (μmol)	48·0 ± 5·0	91·4 ± 5·5***	12·8 ± 1·8	40·3 ± 2·0***	35·2 ± 3·3	51·1 ± 3·9**
concentration (mmol kg^{-1} wet tissue)	55·0 ± 3·0	72·6 ± 1·8***	79·6 ± 10·3	81·1 ± 2·5(NS)	50·5 ± 3·3	67·1 ± 2·8**
Sodium						
content (μmol)	60·8 ± 3·2	98·4 ± 6·7***	8·6 ± 0·91	15·2 ± 0·92***	52·2 ± 2·6	83·3 ± 6·8***
concentration (mmol kg^{-1} wet tissue)	71·0 ± 6·5	78·1 ± 3·3(NS)	54·8 ± 3·7	31·0 ± 2·2***	75·2 ± 2·7	109·7 ± 6·2***
Na^+/K^+	1·32 ± 0·098	1·08 ± 0·05*	0·69 ± 0·03	0·38 ± 0·02**	1·53 ± 0·13	1·64 ± 0·09(NS)

Mean weight of these groups and additional notes given in Table 1.

Table 3. *The composition of the haemolymph and stercoral fluid collected from spiders allowed water and starved or fed 5 days*

	Starved		Fed	
	Haemolymph	Stercoral fluid	Haemolymph	Stercoral fluid
Osmotic pressure (mosmol l^{-1})	436 ± 5·2	427 ± 5·5	515 ± 9·9***	504 ± 9·6***
Na^+ (mmol l^{-1})	196·3 ± 3·9	50·5 ± 11·5	226 ± 5·3***	34·6 ± 8·1(NS)
K^+ (mmol l^{-1})	5·08 ± 0·2	64·3 ± 12·1	4·5 ± 0·26(NS)	125·4 ± 6·1***
Cl^- (mmol l^{-1})	155·7 ± 4·8	56·5 ± 12·9	225 ± 2·7***	78·3 ± 8·0(NS)
Na^+/K^+	38·9 ± 2·2	1·04 ± 0·28	51·7 ± 2·9**	0·28 ± 0·07***

Additional details and notes as for Table 1.

Note: tests of significance refer to differences between means of haemolymph and stercoral fluid in fed spiders with the same fluid in starved spiders. See text for haemolymph/stercoral fluid comparison.

concentration showed a slight decrease from 5·08 to 4·5 mmol l^{-1}. A few measurements of Ca^{2+} and Mg^{2+} concentrations in the haemolymph of fed spiders indicated that these were also in low concentrations (mean, 5·1 and 3·6 mmol l^{-1}, respectively, $N = 3$). The osmotic pressure of the stercoral fluid also increased on feeding. However, the stercoral fluid of both starved and fed spiders was slightly, but significantly, hyposmotic to the haemolymph (paired Student's t-test, $0{\cdot}001 > P$ for both groups). Interestingly, less than half of the osmotic pressure of the stercoral fluid is accounted for by the inorganic ions Na^+, K^+ and Cl^- in both starved and fed spiders. On feeding, the K^+ concentration of the stercoral fluid increased greatly (64·3 to 125·4 mmol l^{-1}) but Na^+ concentration decreased. Thus the Na^+/K^+ ratio of the fluid decreased dramatically from 1·04 ± 0·28 to 0·28 ± 0·07.

Rates of urine production

Discrete spots of dried urine, each containing a few faecal pellets, were observed on the filter paper lining the experimental chambers of all spiders. Blockage of the anal tubercle of several spiders with wax confirmed that this material was voided from the anus. Starved spiders provided with water excreted about 2·5–5·0 µl of urine per day (0·4–0·8 % body water per day) (Fig. 2A). On provision of food the rate increased dramatically, reaching about 30 µl day^{-1} within 48 h and being maintained at about this level throughout the feeding period. These increases resulted from increases in both the mean volume of urine produced per defaecation (from 5 to 17 µl) and the frequency of excretion.

Rates of Na^+ and K^+ excretion

Urine production by the starved, drinking spiders resulted in a small loss of Na^+ (0·2 µmol day^{-1}, 0·3 % day^{-1} of total pool) and K^+ (0·3 µmol day^{-1}, 0·6 % day^{-1}) which represented a steady depletion of body reserves. Associated with the feeding diuresis there were large increases in the urinary Na^+ and K^+ losses (Fig. 2B,C) and a very marked change in the Na^+/K^+ ratio of the excreta (Fig. 2D). The Na^+/K^+

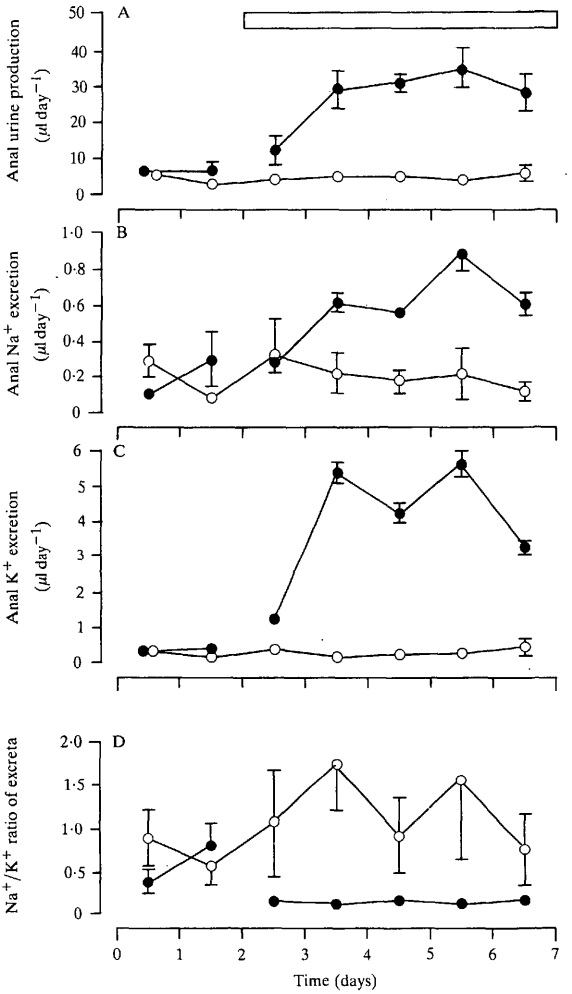

Fig. 2. The effect of prolonged feeding [5 days (horizontal bar) provided with live cockroach nymphs plus water *ad libitum*] of *Porrhothele antipodiana* on daily anal excretion of (A) urine, (B) Na^+ and (C) K^+. (D) Na^+/K^+ ratio of excreta. Closed symbols fed spiders, open symbols control spiders provided with water only. $N = 8$ for both groups.

ratio of the control group was highly variable (range 0·05 to 2·4), but during the 5-day period roughly equal quantities of Na^+ and K^+ were excreted (mean of 5-day Na^+/K^+ ratio for each animal $1·04 \pm 0·4$). Similar variability was seen in the experimental animals prior to feeding. With the onset of feeding, however, the mean ratio fell to 0·16 and remained at about this level with little variation throughout the feeding period (mean of 5-day ratio for each animal $0·154 \pm 0·038$).

The reduced Na^+/K^+ ratio of the excreta of feeding spiders resulted from a large increase in K^+ excretion (maximum rate, 5·7 μmol day^{-1}; total 19·9 μmol during 5 days feeding) and a smaller increase in Na^+ excretion (maximum, 0·9 μmol day^{-1}; total 3·0 μmol).

These results are consistent with the above observation that the Na^+/K^+ ratio of the stercoral fluid was also low (Table 3). As the Na^+/K^+ ratio of the food provided was much higher (0·47), the feeding spiders ought to have been either accumulating Na^+ relative to K^+, or depleting themselves of K^+. In contrast, measurements of the cation content of feeding spiders (Table 2) indicated that the increase in K^+ content was slightly greater than that of Na^+.

Possible explanations for these apparently contradictory results are that the excretion of Na^+ *via* a route other than the anus was overlooked or that the material ingested by the spiders was relatively richer in K^+ than the prey as a whole.

Water and ion balance associated with a single meal

In order to examine these possibilities further, a series of observations was made in which the excretion and accumulation of ions by the spiders could be related more precisely to the amounts of ions ingested.

Sixteen spiders were starved of food, but allowed water for 3 weeks then divided into two groups of similar mean weight ($0·7703 \pm 0·09$ and $0·7814 \pm 0·07$ g). The first group was starved for a further day and each spider then provided with three cockroach nymphs of known weight for 24 h only. The daily production of urine and rates of Na^+ and K^+ excretion were measured before, during and for 5 days after, completion of the meal. Water was available to spiders at all times. The second (control) group of spiders was treated identically but was not provided with a meal.

Fig. 3 shows the time course of changes in body weight, urine production and cation excretion. Changes in the cation concentrations of serial samples of haemolymph for similar fed and control groups (mean weights $0·8216 \pm 0·08$ and $0·8023 \pm 0·10$ g, respectively, $N = 8$) in a parallel experiment are also shown.

The mean increase in body weight associated with the single meal was about 11 % (Fig. 3A). About two-thirds of this weight gain was lost during the first 3 days after the meal, and this was associated with a pronounced diuresis commencing during the feeding period itself (Fig. 3B). Five days after feeding, the rates of urine production and body weight were still slightly elevated compared with controls but this was not statistically significant. The total urine production in excess of control values over the 5-day post-prandial period (about 50 μl) actually corresponded quite closely to

the weight lost in this time. However, as discussed below, the spiders would also have been losing water by transcription and gaining weight by drinking. The lack of exact temporal agreement between weight changes and urine production in both

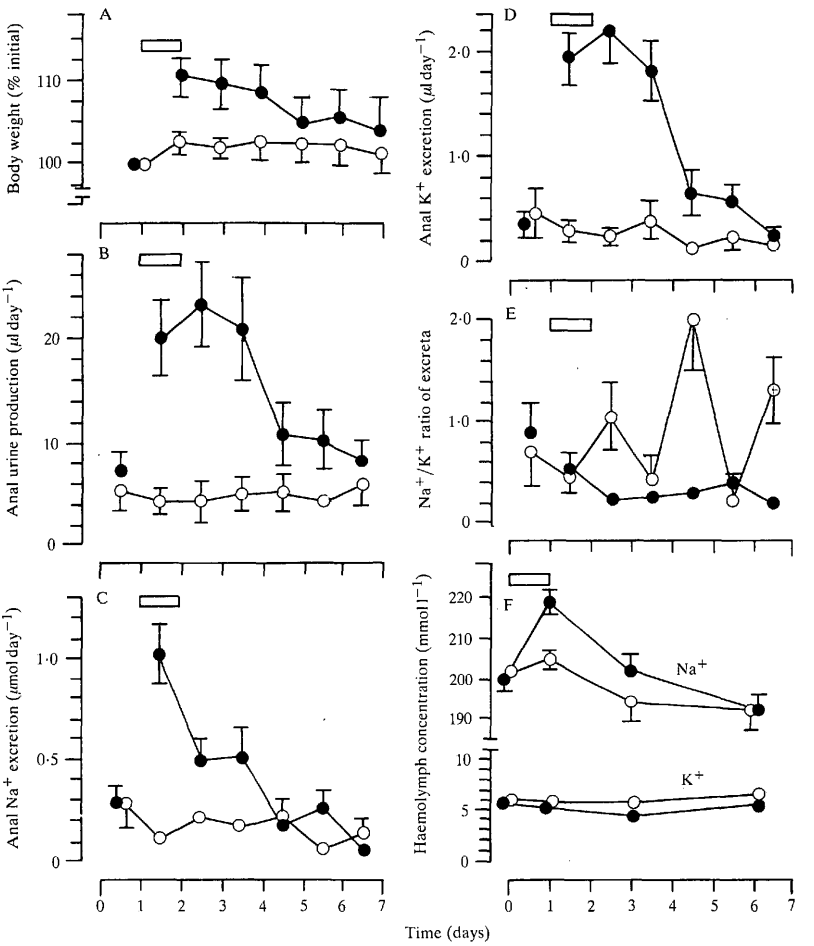

Fig. 3. Effects of a single meal on salt and water of *Porrhothele antipodiana*. Spiders were provided with live cockroach nymphs as prey for 1 day only (horizontal bar). (A) body weight; (B) anal excretion of urine, (C) Na^+ and (D) K^+; (E) Na^+/K^+ ratio of excreta; (F) Na^+ and K^+ concentration of haemolymph. Closed symbols fed spiders, open symbols control spiders provided with water only. $N = 8$ in all cases.

groups (Fig. 3A,B) (the controls actually gained weight despite continual urine production) is presumably related to these components of the water balance.

The post-prandial diuresis was associated with a large increase in K^+ excretion, the rate rising to 2·22 μmol day^{-1} on the day after the meal (Fig. 3D). Thereafter, K^+ excretion decreased in parallel with urine excretion, returning to control levels 5 days after the meal. The effect of feeding on anal Na^+ excretion was not as great, the peak of 1·01 μmol day^{-1} occurring on the day of feeding (Fig. 3C). On the day after feeding, when K^+ excretion was greatest, Na^+ excretion dropped considerably and it had fallen to control levels 3 days after the meal.

In the control group, the Na^+/K^+ ratio of the excreta was high and very variable (Fig. 3E) as noted in the previous experiment (overall mean over 7 days, 0·90; range of individual daily excretion, 0·06 to 2·30). The mean Na^+/K^+ ratio of the daily excretion of the fed group dropped to 0·22 in the day after the meal and remained low over the whole 5-day period of observation (mean; 0·28 ± 0·03 for 5-day period).

From Table 4 it is possible to construct a complete budget for the 6-day feeding plus post-prandial period, relating the intake, accumulation and excretion of Na^+ and K^+ associated with a single meal, and thus determine whether any major route for ion loss (or uptake) has been omitted. Thus for each ion:

$$P_i - P_f = S_f - S_i + E_s,$$

where P_i and P_f are the quantities of this ion in the prey and prey debris respectively, S_i and S_f are the ion contents of the fed spiders before feeding and at the end of the observation period respectively, and E_s is the total quantity of the ion excreted by

Table 4. *A summary of the ion budget for spiders during 1 day plus 5 days post-feeding and control spiders unfed for 6 days* (N = 8)

	Na^+ content (μmol)	K^+ content (μmol)	Dry material (mg)
Prey			
Initially (P_i)*	10·7 (10·1–11·3)	22·7 (21·6–23·7)	39·5
Debris (P_f)	8·2	8·5	14·0
Net ingested ($P_f - P_i$)	2·5	14·2	25·5
Fed spiders			
Initially ($S_i = aC_i$)	78·6	56·5	
Finally (S_f)	79·6	61·3	
Total anal excretion (E_s)	2·8	7·8	
Control spiders			
Initially ($C_i = C_f + E_c$)	79·7	57·3	
Finally (C_f)	78·5	55·5	
Total anal excretion (E_c)	1·2	1·8	

All spiders provided with water.

*P_i estimated from regression analysis, 95% confidence limits in brackets: all other values measured or evaluated from expression given; a = mean weight fed spider/mean weight controls = 0·986.

these spiders in the same period. S_i was not measured directly but may be estimated from the control group:

$$S_i = aC_i = a(C_f + E_c),$$

where C_i and C_f are initial and final ion contents, and E_c is the ion excretion of the control group over the same period. a is a scaling factor which corrects for the small difference in mean weights. Therefore,

$$P_i - P_f + aC_f - S_f + aE_c - E_s = 0.$$

Using the values for these parameters listed in Table 4, this expression is evaluated as $-1{\cdot}3$ and $+1{\cdot}6\,\mu$mol for Na^+ and K^+ respectively. The values are not significantly different from zero, being somewhat less than the compounded uncertainties of the six parameters (the greatest uncertainties are in the prediction of S_i and P_i, the analytical errors being relatively small). Thus within the limits of the experiment, Na^+ and K^+ intake are totally accounted for by accumulation within the spider and anal excretion during and after the meal. Indeed the small discrepancies are in the wrong direction to imply an extra-anal elimination of a Na^+-rich fluid.

It is also clear from Table 4 that the 'explanation' of the low Na^+/K^+ ratio of the excreta of fed spiders is that the net extraction of these ions from the prey is in approximately these proportions. Although the spiders ingested 65% of the dry material from the three cockroaches provided, surprisingly, this was accompanied by only 62% of the K^+ and 23% of the Na^+. Thus the Na^+/K^+ ratio of the prey debris increased from 0·47 at the start of feeding to 0·96 at the end.

The secretion of Na^+ into the prey

Two main mechanisms could account for the discrepancy between the proportions of Na^+ and K^+ in the prey and in the net ingestate. Either the spiders fed selectively on K^+-rich regions of the prey, or Na^+ was returned to the prey during or after feeding.

The first proposal is not substantiated by observations of feeding behaviour in *P. antipodiana*. Ingestion of each prey item required up to 3 h, during which it was thoroughly macerated with the chelicerae and maxillary blades. A fine grey paste, in which no specific parts of the prey were identifiable, remained. Sieving devices are present in the pre-oral region of spiders (Legendre, 1978) and these could prevent the ingestion of large pieces of tissue. However, spiders generally secrete powerful proteolytic enzymes into the prey which solubilize the tissues (Snow, 1970; Mommsen, 1978). Moreover, as the tissues of the cockroach are much richer in K^+ than is the more easily ingested haemolymph (Tucker, 1977), a relative depletion of K^+ in the ingestate would be more probable. Secretion (or excretion) of Na^+ into the prey therefore seemed the more likely explanation. Another arachnid, the tick *Dermacentor andersoni*, secretes a Na^+-rich fluid into the host during feeding (Kaufman & Phillips, 1973) and spiders are known to secrete an extra-oral digestive fluid into their prey (Snow, 1970; Legendre, 1978).

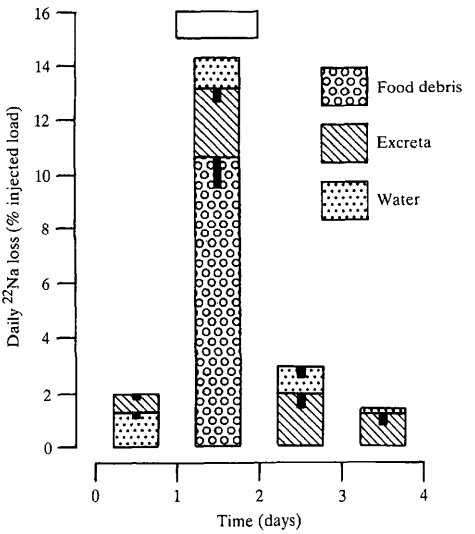

Fig. 4. Partition of the loss of ^{22}Na from spiders' body Na^+ pool before, during (horizontal bar) and after a meal. Height of each bar represents total loss and is divided into that appearing in the water, food debris and anal excreta. S.E. of mean ($N = 10$) of each component is shown as small bar, negative direction only for clarity.

In order to partition the routes for Na^+ loss, spiders were injected with ^{22}Na and allowed 48 h for recovery and equilibration. They were then placed into chambers lined with filter paper and provided with water for 4 days. Chambers were changed daily and, on day 2 only, each spider was provided with several cockroaches as food. For each 24-h period the excreta, visualized under ultraviolet light, were cut from the paper and their ^{22}Na activity measured. The remaining paper was then divided into small squares and checked for ^{22}Na activity. The radioactivity of the water supply and food debris was also measured.

Fig. 4 shows the total daily ^{22}Na loss and its compartmentalization for each of the 4 days. In the day prior to feeding, 2 % of the injected ^{22}Na was lost from the spiders, all of this located in the water and the excreta. On the day of feeding 14·2 % of the injected ^{22}Na was lost but only 2·6 % was present in the excreta and 1·1 % in the water. A large component, equivalent to 10·5 % of that injected, was located in the food debris. Following feeding, the total rates of ^{22}Na loss dropped almost to pre-feeding levels, again all being located in the food and water supply. No ^{22}Na was present in the chambers which was not associated with either the excreta, the water or the prey debris. While it is probable that the appearance of ^{22}Na in the prey debris is a result of fluid secretion directly into the prey, its presence in the water supply could be due to washing off of salts which may be observed dried on the labium and maxillae, particularly in recently fed spiders.

The time course of Na^+ loss into the prey during feeding

If the Na^+ is secreted into the prey after feeding ceases, then this might be considered as a truly regulatory (i.e. excretory) response to excess Na^+ taken in during feeding. However, if it is lost during the initial stages of, or continuously during, feeding, then the process could be either an 'anticipatory regulation' or an integral component of the feeding mechanism itself.

To determine the time course of Na^+ secretion into the prey, ^{22}Na-injected spiders were fed a single cockroach and feeding was terminated at various times to measure the ^{22}Na activity of the debris. ^{22}Na was lost steadily into the prey at about 3 % h^{-1} of the injected load with no obvious delay or initial pulse of secretion (Fig. 5).

DISCUSSION

A steady, low rate of anal urine production of 2·5–5 $\mu l\, g^{-1}\, animal^{-1}\, day^{-1}$ was observed in starved, fully hydrated spiders, draining the body reserves of Na^+ and K^+ at about 0·3 % day^{-1} and 0·6 % day^{-1} respectively. The Na^+/K^+ ratio of this maintenance urine production, which is derived from the combined activities of the Malpighian tubules, stercoral pocket, gut and diverticula, was about unity. This is reasonable, since it was observed that when starved spiders were fed *ad libitum* for 5 days, Na^+ and K^+ were accumulated in the body in approximately equimolar proportions.

In the spiders fed for a single day, the quantities of Na^+ and K^+ which were retained from each meal were quite small. Most was rapidly excreted. However, the spiders which were fed for 5 days showed very large increases in the total Na^+ and

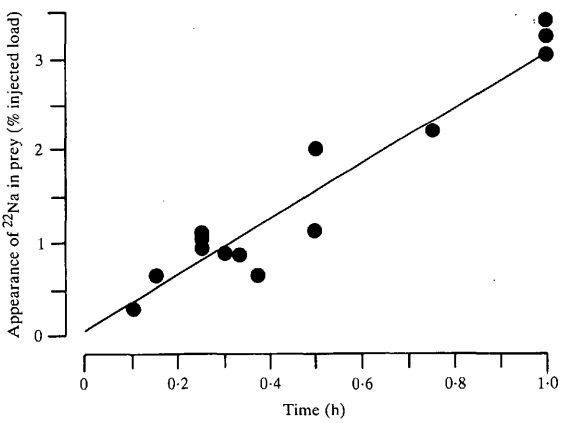

Fig. 5. Time course of appearance in prey debris of ^{22}Na initially injected into spiders' haemolymph. Each point represents an individual spider in which feeding was interrupted after the indicated time. Regression line fitted is: $y = 3{\cdot}0x + 0{\cdot}112$.

K^+ storage (60 % and 90 % respectively). Thus it appears that the spiders did not take the opportunity to regulate the Na^+ and K^+ content of the body to a preferred level. Rather, storage of Na^+ and K^+ was matched to nutrient storage. These stored ions could be viewed as providing a vehicle for the elimination of excretory products released during subsequent catabolism of the meal. As these spiders are opportunistic, intermittent feeders, large changes in total ion content would probably be a normal occurrence.

In contrast to maintenance excretion, the anal diuresis associated with feeding produced a K^+-rich fluid. This diuresis lasted about 3 days after a single meal and was prolonged in animals feeding *ad libitum*. The rate of urine production increased to about 7 times the maintenance rate and the Na^+/K^+ ratio dropped to <0·2. During feeding Na^+ was secreted (or excreted) into the prey so that although the Na^+/K^+ ratio of the prey was initially about 0·47 (rising to about 1·0 in the debris during feeding), the *net* ingestion was of a K^+-rich fluid with a Na^+/K^+ ratio similar to that of the diuresis fluid.

The ^{22}Na experiments demonstrated that the increase in the relative proportion of Na^+ in the prey debris resulted from secretion into the prey of Na^+ from the spider's body pool and not simply from selective concentration of prey Na^+ by, for example, the selective ingestion of the K^+-rich regions of the prey. As argued above, effective maceration and enzymatic solubilization of the prey should result in the ingestion of equal fractions of the Na^+, K^+ and dry weight originally present in the prey. Indeed, the proportions of ions ingested might be expected to exceed that of total dry weight because relatively indigestible, sclerotized cuticle of low ion content would presumably predominate in the debris. In fact, the ingestion of 65 % of the prey dry weight was associated with a net extraction of 62 % of K^+ and only 23 % of Na^+, suggesting that a small quantity of K^+ and much Na^+ was secreted into the prey. In a subsequent paper (A. G. Butt & H. H. Taylor, in preparation) it will be demonstrated that ions are delivered into the prey in the coxal fluid which has a high Na^+ and low K^+ concentration. From the value of 65 % of prey dry weight ingested, it is calculated that the spiders which fed for 1 day actually ingested 7·0 μmol of prey Na^+ and 14·7 μmol of prey K^+ (compared with a *net* ingestion of 2·5 and 14·2 μmol, respectively, Table 4). The fate of these prey ions may be deduced from the data in Table 4. Over the 6-day feeding and post-prandial period, 2·7 μmol (38 %) of the Na^+ were excreted anally, 1·1 μmol (16 %) were stored and, by difference, 3·2 μmol (46 %) were excreted back into the prey in the coxal fluid. The corresponding values for K^+ are 7·8 μmol (55 %) anal excretion, 4·9 μmol (34 %) stored and 1·5 μmol (11 %) in the prey remains. It should be emphasized that these values for prey Na^+ and K^+ ingestion and their subsequent partition are notional quantities. The net amounts ingested were, as indicated, somewhat lower than this, whereas the total quantities of ions entering *via* the mouth would have been rather higher due to recycling of the body ions secreted into the prey.

During feeding, ^{22}Na was lost from the body pool at about 3 % h^{-1}, equivalent to 1·6 μmol h^{-1} of body Na^+ (Fig. 5). Even allowing for the fact that feeding was not

continuous during the 24-h period, it is clear that the quantity of body Na^+ recycled must have been much greater than that in the prey itself.

Does the Na^+ lost into the prey during feeding have primarily a regulatory (i.e. excretory) role or should it be regarded as essentially incidental to the feeding process? The excretion of ions and water by systems other than the Malpighian tubules and rectum is observed in several other arachnids. The blood-sucking gamasid mite, *Ornithonyssus bacoti*, excretes water from the salivary glands after feeding (Belozerov, 1958) and salivary glands are responsible for the excretion of water and ions by the ixodid ticks *Boophilus microplus* (Tatchell, 1967, 1969) and *Dermacentor andersoni* (Kaufman & Phillips, 1973). The coxal glands of the argasid tick, *Ornithodorus moubata*, function in volume and osmotic regulation, excreting water and ions during and after feeding (Lees, 1946; Kaufman *et al.* 1981). In the tick *D. andersoni*, Na^+ and K^+ excretion are partitioned in a similar way as in *P. antipodiana*. Na^+ is excreted into the prey during feeding by the salivary glands, while the bulk of the K^+ is excreted *via* the anus (Kaufman & Phillips, 1973). The relative sizes of the tick and vertebrate host ensure that little of the Na^+ excreted into the host would be re-ingested by the tick, so the salivary glands function as an efficient route for the excretion of Na^+ and water. However, *P. antipodiana* consumes prey which are usually much smaller than itself, so that recycling of Na^+ and K^+ is inevitable and the effectiveness of this system as a means of excretion of ions is reduced. This question of an excretory *versus* feeding role for the fluid secreted into the prey will be returned to in a subsequent paper where the actual source of the fluid is demonstrated and the effects of salt and water stress upon this process are investigated (A. G. Butt & H. H. Taylor, in preparation).

The arrangement of the midgut, its diverticula and the Malpighian tubules embedded within the adipose tissue of the abdomen has so far prevented direct sampling of fluid from these organs. Thus the sources of ions and water in the stercoral fluid and the mechanism of primary urine formation have not been elucidated. Whether the stercoral epithelium, like the insect rectal epithelium, functions in secondary modification of the final urine is also unknown. The faecal pellets which form the main solid component of the excreta are formed from material which passes from the diverticula to the gut lumen. They are invested by the faecal membranes in the posterior midgut.

As the Malpighian tubules of insects are the most important source of primary urine in insects, it is often assumed that the structures of the same name in spiders and ticks play a similar role. However, they do not lie free in the haemolymph in spiders so some means of supplying haemolymph to the tubules at an adequate rate would be a requirement. Certainly, the high K^+ concentration in the stercoral fluid of diuretic spiders would support its generation by the Malpighian tubules. In most insects, fluid secretion by the Malpighian tubules is coupled to the active transport of K^+, which appears at high concentration in the primary urine (Ramsay, 1953; Berridge, 1968; Irvine, 1969; Maddrell, 1969; Pilcher, 1970). However, the active transport of K^+ is a characteristic of several other insect tissues including the salivary glands of *Calliphora* (Oschman & Berridge, 1970), goblet cells in the gut of

lepidopteran larvae (Anderson & Harvey, 1966), the labial glands of *Antheraea* (Kafatos, 1968), *Schistocerca* rectum (Williams, Phillips, Prince & Meredith, 1978) and the posterior rectum of *Aedes taeniorhynchus* (Bradley & Phillips, 1975). Kaufman & Phillips (1973) proposed that the Malpighian tubules were not involved in the production of the K^+-rich fluid excreted *via* the anus of the tick, *D. andersoni*. They suggested that the midgut transported Na^+, Cl^- and water into the haemolymph but was relatively impermeable to K^+ so that most of the K^+ of the ingested host blood passed directly from the midgut to the rectal sac. A similar mechanism could operate in *P. antipodiana*. Despite a favourable gradient for movement of K^+ from the food (217 mmol kg^{-1}) to the haemolymph (5 mmol l^{-1}) and an unfavourable gradient for Na^+ entry (prey, 105 mmol kg^{-1}; haemolymph, 196 mmol l^{-1}), haemolymph Na concentration increased while haemolymph K^+ concentration actually decreased during both short-term (Fig. 3) and long-term (Table 3) feeding. This decrease could be explained by the secretion of coxal fluid, which has a higher K^+ concentration than the haemolymph (A. G. Butt & H. H. Taylor, in preparation), or by uptake of prey water or of drinking water if the gut is indeed relatively impermeable to K^+. However, the alternative explanation that the K^+ was absorbed but was rapidly excreted by the Malpighian tubules or other organ, is consistent with the demonstration by Van Hook (1971) that ingested ^{22}Na, ^{42}K and ^{47}Ca were fully assimilated by the spider, *Lycosa punctata*.

Spiders are usually thought of as fluid feeders, the prey being partially digested in the pre-oral cavity by enzymes regurgitated *via* the mouth (Meglistch, 1972) and it has been considered that, for some spiders at least, the water available in the prey is sufficient to meet their needs fully (Comstock, 1940). Thus it is of interest to consider the importance of the prey water in the overall water balance of *P. antipodiana*. For the spiders fed for 1 day only and followed through 5 days post-prandially, the estimated mean total water content of the three cockroach nymphs provided as food was 142 mg. The mean proportion of dry weight ingested was 65 % so that, at most, a similar proportion, or 92 mg, of prey water would have been ingested by the spiders. In practice, evaporation from the prey and feeding secretions would probably have reduced this considerably.

Transpirational water loss from spiders kept under similar conditions, but without food or water, is 19 mg day^{-1} (Butt, 1983). Thus, over the 6-day period at least 114 mg would be lost by transpiration (perhaps more due to feeding activity). In the same period the anal urine production was 93 μl (Fig. 3D). Although much of the Na^+-rich (coxal) fluid secreted into the prey may be re-ingested, some is not because Na^+ is left behind in the prey debris (difference between net Na^+ ingestion and theoretical ingestion calculated from change in dry weight is 4·5 μmol). From the Na^+ concentration of the coxal fluid (125 mmol l^{-1}, A. G. Butt & H. H. Taylor, in preparation) it may be estimated that at least 36 μl of water is lost by this route.

In summary, less than 92 μl of water would have been obtained from the prey while more than 243 μl were lost in feeding, diuresis and transpiration. However, the spiders showed a small increase in water content of about 5 μl at the end of the period (total weight increase, about 4 % or 30 mg, Fig. 3A; dry weight ingestion was 25 mg,

Table 4). Obviously, the spiders must have drunk the free water provided and this formed the major component of the water intake associated with feeding. Dew or rain droplets may frequently be observed on the entrance of the web at dawn and this is probably the natural source of drinking water. During late spring and summer this water may not always be available. Under these conditions they are found deeper in the web so that it is uncertain whether they normally experience serious dehydration. The effect of water deprivation on the salt and water balance of feeding spiders will be the subject of a further communication.

A post-prandial diuresis occurs in many blood-sucking arthropods, e.g. *Aedes aegypti* (Stobbart, 1977), *Rhodnius prolixus* (Maddrell, 1964), *D. andersoni* (Kaufman & Phillips, 1973), *Glossina austeni* and *G. morsitans* (Gee, 1975*a*,*b*) and *Ornithodorus moubata* (Kaufman *et al.* 1981). Less dramatic diuretic responses follow feeding in the stick insect, *Carausius morosus* (Pilcher, 1970) and the cotton stainer, *Dysdercus fasciatus* (Berridge, 1965).

Probably the main function of the diuresis in the blood feeders is in the rapid reduction of body volume. In *P. antipodiana* the volume increase is not large (11 %) and, because of the high transpirational and other water losses, the spider must actually drink to support the diuresis. Thus its main function in this case is more likely to be in the elimination of excess ions ingested with the meal, as was also proposed for *D. fasciatus* (Berridge, 1965). In both of these animals the magnitude of the diuresis required is increased by their inability to produce urine hyperosmotic to the blood (Berridge, 1965; Butt, 1983). For the spider an even larger volume is required because the ions Na^+, K^+ and Cl^- contribute less than 50 % of the osmotic pressure of the urine. A role for the diuresis in salt excretion is also supported by an increase in its magnitude and duration in spiders fed salt-loaded prey (A. G. Butt & H. H. Taylor, in preparation).

The unidentified major fraction of the osmotic pressure of the urine deserves further study. Most likely it represents a nitrogenous excretory product. However, spiders are thought to be generally guanotelic (Anderson, 1966) and guanine has been identified chromatographically in high concentration in the stercoral fluid of *P. antipodiana* (Butt, 1983). However, its low solubility means that it could not be a major osmolar effector.

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