J. exp. Biol. (1981), 93, 225–242
With 10 figures
Printed in Great Britain

FLUID BALANCE IN THE ARGASID TICK, *ORNITHODORUS MOUBATA*, FED ON MODIFIED BLOOD MEALS

BY SUSAN E. KAUFMAN,[1] WILLIAM R. KAUFMAN[2] AND JOHN E. PHILLIPS

Department of Zoology, University of British Columbia, Vancouver, Canada V6T 1W5

(*Received* 13 *November* 1980)

SUMMARY

1. The argasid tick *Ornithodorus moubata* Murray was fed on modified blood meals, the ionic strength and/or osmotic pressure of which was varied by additions of NaCl or glucose, and by dilution with distilled water or isosmotic glucose. The following parameters were monitored: total volume increase of tick, total volume of meal ingested, net increase in haemolymph volume, volume of coxal fluid excreted and ion concentrations and osmotic pressures of the gut contents, haemolymph and coxal fluid, as well as potential differences across the gut lumen.

2. Absorption of fluid from the gut into the haemolymph appeared to be linked to the active transport of Na^+ and Cl^-, although the rate of absorption was an inverse function of the prevailing osmotic gradient across the gut epithelium.

3. The tick can maintain a reasonably constant [Na^+] and [Cl^-] in the haemolymph when fed meals in which the concentration of these ions varies up to 6-fold, but only if the meal remains isosmotic with blood.

4. The rate and volume of coxal fluid production are direct functions of the rate and volume of fluid absorption by the gut epithelium. Thus, during the feeding cycle, the coxal organ appears to function chiefly in an osmo- and volume-regulatory capacity in this species of argasid tick.

INTRODUCTION

At least three morphologically distinct systems to excrete excess fluid have evolved among blood-sucking arthropods. The haematophagous insects secrete a copious, dilute urine from the Malpighian tubules when a specific diuretic hormone is released into the haemolymph at the appropriate time (see reviews by Maddrell, 1971, 1976). The ixodid ticks have adapted their salivary glands to accomplish this 'diuresis' (Kaufman, 1979). Finally, it appears that the coxal organs (bilateral structures whose

[1] Permanent address: Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

[2] Permanent address (to which reprint requests should be directed): Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

orifices exit between the first and second pair of coxae) perform the same function in argasid ticks. It is the latter system which is the subject of the present communication.

Remy (1922) and Siderov (1960) believed that the fluid which emerged from the coxal organs of unfed ticks was simply a result of reflex bleeding following disturbance. Boné (1943) and Lees (1946), on the other hand, proposed that the coxal organ functions in ion and water regulation. Boné (1943) believed that the coiled tubules produced coxal fluid by a secretory process, whereas Lees (1946) demonstrated that the tubular structure communicated directly with a space bounded by a fine membrane. He ascribed to the latter the function of a filtration membrane but his evidence, though certainly suggestive, was mostly qualitative. So the objectives of the present study were (1) to determine the changes in volume and composition of the haemolymph which occur during a normal feeding cycle, and to examine the role of the gut and coxal organ in the regulation of these parameters, and (2) to determine the ionic regulatory capacity of these organs in the face of experimentally induced osmotic and ionic perturbations to the ingested fluid.

MATERIALS AND METHODS

Rearing and feeding procedure

The tick colony was reared from some specimens kindly provided by Dr G. Kohls of the Rocky Mountain Laboratory, Montana, U.S.A. They were maintained at 28 °C, 50% relative humidity, and were fed, confined in capsules, on constrained chickens of about 5 weeks of age.

Approximately 10 days after feeding, a female tick would lay about 60 eggs which would hatch after a further 16 days. After a larval stage, which did not feed, there were five (in the female) or four (in the male) moults before the adult emerged. Males and females remained together in the holding vials for several days, during which time they mated. The females were then separated from the males and used only after a period of 3 weeks had elapsed. This time interval was important to allow the excretion of guanine (end-product of nitrogen metabolism associated with moulting) to wane.

For all experimental work, adult ticks were fed on outdated, citrated whole human blood through a washed chicken skin. The blood was maintained at 38 °C in a water bath; both the blood and bath were constantly stirred. For ease of observation and collection of coxal fluid, the tick was tilted away from the chicken skin with thin adhesive strips (Fig. 1) and its ventral surface viewed from the side through a dissecting microscope.

Although our ticks would feed readily on human blood and initially produced viable eggs, progressively this laboratory strain developed a sensitivity to such a meal; the ticks turned purple and died about a week after feeding. Such a phenomenon has been recorded in *Argas persicus* (Nuttall, 1908) and in *Ornithodorus hermsi* (Gregson, 1956). Since pathological symptoms did not appear until several days after feeding, and since the behaviour of the ticks during the experimental period was very similar to that of ticks fed on live chickens, it was decided to contin

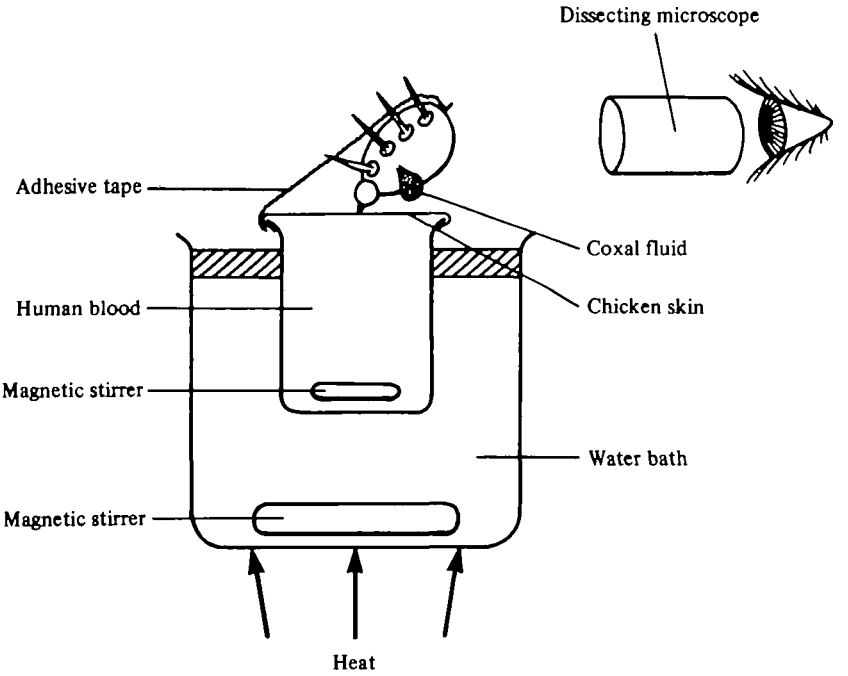

Fig. 1. Arrangement for feeding tick on human blood.

research using human blood, since this feeding system was far more suitable for the experimental manipulations required during this study.

Preparation of blood meal

In some experiments, the ionic concentration of the blood meal was reduced to one-half or one-quarter of the value for whole blood by dilution with isosmotic glucose (53·5 g l^{-1}) so as to maintain a constant osmotic pressure. A meal of lower osmotic pressure was produced by diluting the blood with distilled water. Meals of increased osmotic pressure were prepared by the addition of either NaCl or glucose in osmotically equivalent amounts. The abbreviations used in the text to designate the composition of these blood meals are as follows:

Blood	Outdated human blood as received from the Red Cross. ACD solution added as anticlotting agent (295 m-osmole kg^{-1} water).
Blood + NaCl	Human blood plus 2·96 g l^{-1} NaCl (470 m-osmole kg^{-1} water).
Blood + glucose	Human blood plus 31 g l^{-1} glucose (isosmotic with blood + NaCl).
1 blood : 1 DW	Human blood diluted 50/50 with distilled water (148 m-osmole kg^{-1} water).
1 blood : 1 glucose	Human blood diluted 50/50 with an isosmotic solution of glucose (53·5 g l^{-1}).
1 blood : 3 glucose	Human blood diluted 25/75 with isosmotic glucose.

Collection of body fluids and rate measurements

Coxal fluid was collected, as it exuded from the coxal orifice (Fig. 1), into a micropipette attached to a Gilmont micrometer syringe; micrometer readings were taken at 5 sec intervals to measure the rate of coxal fluid excretion. For monitoring changing composition with time, the samples were collected in micropipettes and were stored under liquid paraffin.

Haemolymph was sampled by amputating a leg and collecting two aliquots in 1 μl pipettes. Between sampling, the flow of haemolymph was halted with a fine haemostat. Samples were stored for up to several hours under liquid paraffin until preparing them for ion analysis. Whole haemolymph was used for analyses.

Before gut samples were taken, much of the haemolymph was first drained by amputating the legs. When a fine incision was then made into the dorsal integument, a lobe of gut protruded which was then punctured and sampled. Gut contents were collected in haematocrit tubes and, except where indicated to the contrary, the tubes were centrifuged for 5 min in a clinical microhaematocrit centrifuge. The supernatant (essentially host plasma) was stored frozen in the sealed tubes.

Analysis of ion concentration

Chloride determinations were carried out according to the method of Ramsay, Brown & Croghan (1955), whereby 1 μl samples were diluted in 100 μl of 50% acetic acid and titrated potentiometrically with $AgNO_3$. Na^+ and K^+ concentrations were measured by standard techniques of flame emission spectrophotometry (Unicam SP900 or a Techtron AA1120 Flame Spectrophotometer).

Total body chloride was measured by homogenizing the whole tick in 4 ml of distilled water. A 50 μl sample added to 50 μl of glacial acetic acid was then titrated against $AgNO_3$ (Ramsay *et al.* 1955).

Volume measurements

To estimate increase in body volume, tick weights were determined before feeding and after cessation of coxal fluid excretion. We were satisfied that weight and volume were interconvertible since 10 μl of gut fluid weighed the same as 10 μl of water to two significant figures.

The total blood ingested was estimated by a direct spectrophotometric assay of haemoglobin in homogenates of whole ticks; standards were prepared from human blood. These values were corrected for haemoglobin present in a group of unfed ticks. The haemoglobin spectrum from tick homogenates was the same as that for human blood, indicating that no significant breakdown of haemoglobin had occurred during feeding.

The total blood ingested was also estimated by comparing the haematocrit of the gut contents of the fed tick with that of the blood meal on which it had been feeding. The gut fluid and blood meal were collected in microhaematocrit tubes after coxal fluid production had ceased, the samples were centrifuged for 5 min and haematocrits measured directly. This method could be used only for those feeding conditions in which haemolysis of the erythrocytes did not occur.

Haemolymph volumes were estimated by standard tracer-dilution methods. [^{14}C] Inulin-carboxyl solution, 1 μl (New England Nuclear), was injected into the haemocoel via a leg by means of a syringe needle of suitable gauge fixed to an 'Agla' micrometer syringe unit (Burroughs Wellcome). The injection procedure used was essentially that of Patchin & Davey (1968). We determined from preliminary experiments that tracer equilibration in the haemocoel had occurred within 1 h. Samples of haemolymph of 2 μl were added to 10 ml of Bray's solution (Bray, 1960) and the samples were counted on a Nuclear Chicago Mark I liquid scintillation counter. When measuring post-prandial haemolymph volumes, care was taken to ensure that coxal fluid production had indeed ceased before injection of the tracer; otherwise, an unknown proportion of the tracer was immediately washed out in any coxal fluid produced.

Method for calculating volume and composition of gut absorbate

The ion concentrations of the haemolymph before feeding are presented below as the mean ± S.E.

$$[Cl^-] = 200 \pm 2{\cdot}0 \text{ m-equiv } l^{-1} \quad (N = 9),$$
$$[Na^+] = 231 \pm 5{\cdot}8 \text{ m-equiv } l^{-1} \quad (N = 4),$$
$$[K^+] = 11{\cdot}6 \pm 0{\cdot}7 \text{ m-equiv } l^{-1} \quad (N = 4).$$

The amount of any ion transported across the gut was taken either as the difference between the total ion ingested and the amount remaining in the gut, or alternatively as the sum of the total ion in the coxal fluid and the net increase in haemolymph ion content. These values were calculated from measured concentrations and volumes of body fluids.

These two independent methods of calculation agreed to within a difference of 10% and thus provided a check on experimental error. With these estimates for amount of ion crossing the gut epithelium, the concentration of the absorbate could be calculated from estimates of the volume of fluid crossing the gut. That volume, as will be shown in the Results section, is equal to the total volume of coxal fluid excreted plus the net increase in haemolymph volume.

Measurement of osmotic pressure

Osmotic pressure was measured cryoscopically by the method of Ramsay (1949), with the exception that the drops of sample were interspersed with liquid paraffin in 1 μl pipettes instead of the type of capillary tube described by Ramsay. Gut samples were centrifuged and a drop of the supernatant was placed under liquid paraffin. Tiny droplets could then be taken up from this pool into a microlitre pipette. Coxal fluid samples were treated similarly but not centrifuged. Unfortunately, haemolymph clotted when immersed directly in oil and thus it had to be collected into the pipette directly from the site of haemorrhage.

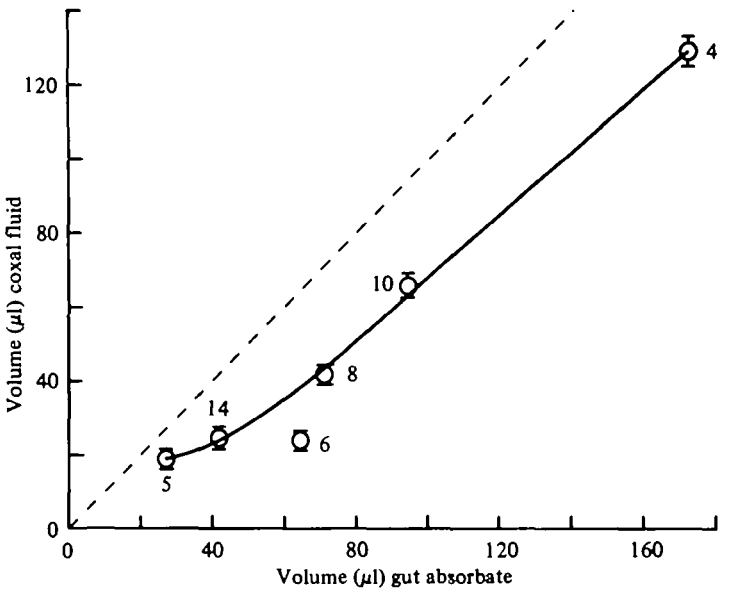

Fig. 2. Correlation between total volume of gut absorbate and total volume of coxal fluid excreted over 2 h for ticks fed on blood. The S.E. and *n* is indicated for each point.

Measurement of electropotential differences across the gut

A V-shaped incision was made in a coxa, allowing a lobe of gut and a pool of haemolymph to exude through the incision. The indifferent electrode consisted of an Ag/AgCl wire embedded in a Pasteur pipette filled with 1·5 M-KCl in 1% agar gel. The tip of this pipette (drawn out to about 1 mm in diameter) was touched to the drop of haemolymph. The recording electrode (glass micropipette filled with 1·5 M-KCl, resistance ≏ 10 MΩ) was attached to a Medistor cathode follower. The electrode was gently lowered into the drop of haemolymph, the asymmetry potential was backed-off and the potential then measured on a Tektronix cathode-ray oscilloscope as the electrode passed across the gut wall into the lumen.

RESULTS

Time sequence of feeding and excretion

The ticks became very active when placed on the chicken skin (Fig. 1). After choosing a site, they released a droplet of saliva on to the skin and probed with the hypostome until, with a prolonged thrust, they punctured the skin and began sucking blood. After feeding for 2–10 min, the ticks started to produce coxal fluid; droplets of about 1/4 μl appeared simultaneously from each organ. After about 30 min of feeding the tick detached, although coxal fluid continued to be produced for a further 30 min during which time body size diminished.

Table 1. *Volumes (μl) of body fluids during and after feeding*

Meal	Total vol. increase	Total vol. ingested	Final vol. of gut	Vol. of gut absorbate	Final haemolymph vol.	Total vol. of coxal fluid	*N*
Blood	235 ± 7	364	192	172	58 ± 4	129 ± 5	4
Blood + NaCl	225 ± 22	291	197	94	43 ± 4	66 ± 3	10
Blood + glucose	237 ± 12	261	197	64	55 ± 6	24 ± 2	6
Blood + DW	181 ± 10	223	152	71	44 ± 6	42 ± 9	8
1 blood : 1 glucose	177 ± 8	202	160	42	32 ± 3	25 ± 2	14
1 blood : 3 glucose	248 ± 20	267	240	27	23 ± 4	19 ± 2	5

'Total' and 'final' values refer to those existing 2 h from start of feeding. Parameters that were measured directly are presented as mean ± S.E. The other values were calculated (see Methods).

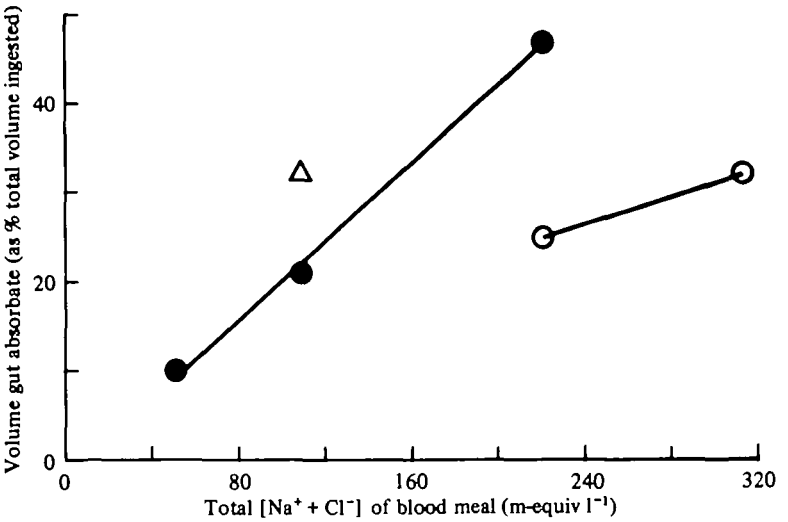

Fig. 3. Correlation between volume of gut absorbate (as a percentage of ingested fluid) and total $[Na^+ + Cl^-]$ of blood meal. ●, Blood meals 100% osmotic pressure of blood (i.e. meals:blood, 1 blood:1 glucose, and 1 blood:3 glucose). ○, Blood meals 158% osmotic pressure of blood (i.e. meals:blood + NaCl and blood + glucose). △, Blood meal 50% osmotic pressure of blood (i.e. blood + DW).

Volume changes during feeding and coxal fluid excretion

Table 1 shows how the volumes of several fluid compartments were affected when the ticks fed on human blood, the ionic strength and/or osmotic pressure of which was varied independently as described in Methods. Although modification of blood composition caused some diminution of meal size (the maximum reduction (44%) being for 1 blood:1 glucose), such modifications reduced gut absorbate volume (by 60%) and coxal fluid volume (by 85%) to a far greater extent. Fig. 2 illustrates a correlation that one would have predicted, namely that the volume of coxal fluid excreted is dependent upon the volume of gut absorbate; the displacement between the experimental points and the isometric line is accounted for largely by the increase in haemolymph volume.

In Fig. 3 we have plotted the volume of the gut absorbate as a function of the total $[Na^+ + Cl^-]$ of the meal. There is a linear relationship between these parameters when the osmotic pressure is maintained equal to that of blood. There is additional limited data in Fig. 3 to suggest that for a given ionic concentration of the meal, the volume of gut absorbate is related inversely to the osmotic pressure of the meal.

In one group of ticks, the net weight increase was measured at the time when the initial drops of coxal fluid first appeared in order to see whether the trigger for coxal fluid production might be a critical increase in body volume. No such evidence was found.

Distribution of ions in body fluids

To test the ionic regulatory powers of ticks, ionic concentrations of gut contents, haemolymph and pooled coxal fluid were measured 2 h after feeding on blood meals of varying ion concentration and osmotic pressure (Table 2). Fig. 4 demo

Table 2. *Ion concentrations (m-equiv l^{-1}) in body fluids during and after feeding*

Meal	Ingested fluid		Final gut contents		Mean gut absorbate	Final haemolymph	Mean coxal fluid	*N*
	Centrifuged	Whole	Centrifuged	Whole				
				Sodium				
Blood	177	124	197 ± 1	95 ± 2	115–156	154 ± 3	111 ± 3	4
Blood + NaCl	223	170	218 ± 5	119 ± 3	235–261	237 ± 14	217 ± 2	4
Blood + glucose	177	124	135 ± 4	89 ± 4	232–236	239 ± 8	225 ± 4	5
Blood + DW	62	62	73 ± 3	73 ± 3	38–48	108 ± 13	75 ± 8	3
1 blood : 1 glucose	62	62	51 ± 2	49 ± 2	109–109	169 ± 3	105 ± 7	4
1 blood : 3 glucose	31	31	33 ± 1	—	—	—	—	4
				Chloride				
Blood	116	96	49 ± 1	77 ± 2	117–127	170 ± 2	117 ± 5	6
Blood + NaCl	162	142	70 ± 3	88 ± 6	230–255	239 ± 9	217 ± 3	5
Blood and glucose	116	96	36 ± 3	58 ± 2	200–214	206 ± 4	187 ± 5	5
Blood + DW	45	45	51 ± 3	52 ± 4	30–46	95 ± 4	51 ± 3	4
1 blood : 1 glucose	45	45	26 ± 2	37 ± 1	75–103	173 ± 3	70 ± 2	5
1 blood : 3 glucose	21	21	20 ± 1	19 ± 1	39–59	172 ± 3	34 ± 1	4
				Potassium				
Blood	4	41	52 ± 2	62 ± 3	4	5 ± 0	5 ± 1	4

'Final' values refer to those existing 2 h from start of feeding. Parameters which were measured directly are presented as mean ± S.E. Values for gut absorbate were calculated (see Methods) and hence are not accompanied by S.E.'s. Values for ingested fluid are also unaccompanied by S.E.'s since any series of experiments under a given feeding condition was carried out using the same batch of blood.

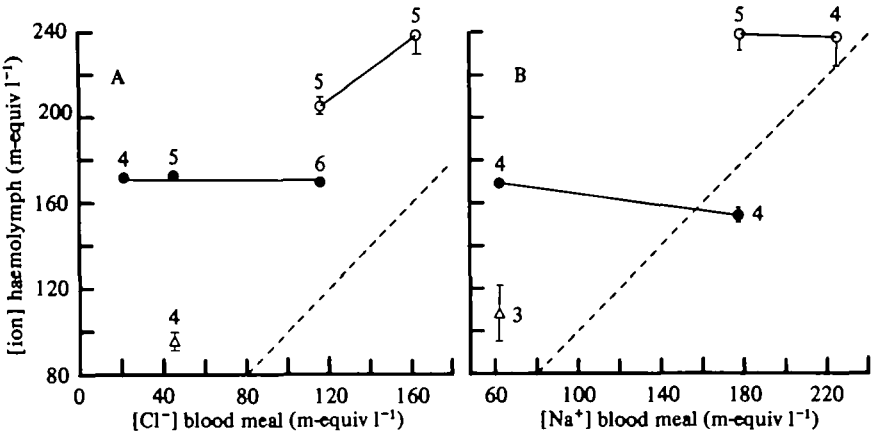

Fig. 4. Regulation of (A) [Cl^-] and (B) [Na^+] of haemolymph in ticks fed various meals. All samples were taken 2 h after feeding. Means, S.E.'s (where not included in the point) and n's are indicated as well as the isometric line (dashed). Symbols as in Fig. 3.

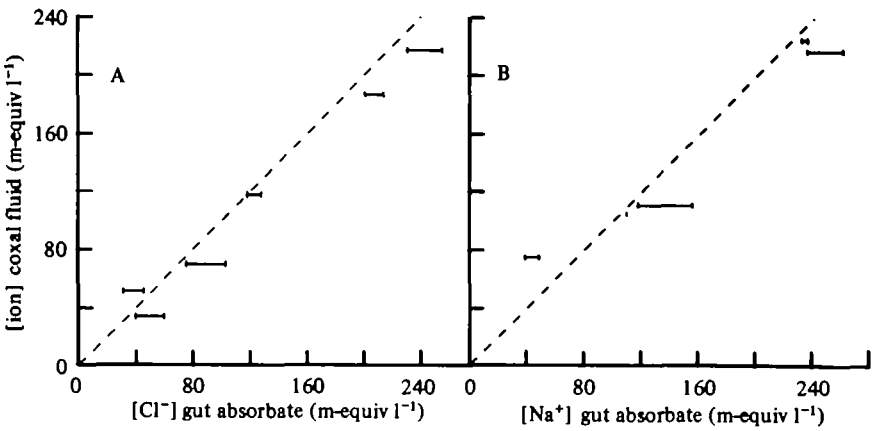

Fig. 5. Relation between mean ion concentration of gut absorbate and coxal fluid. (A) = [Cl^-], (B) = [Na^+]. Limits shown are from Table 2. Measurements were made over the 2 h experimental period. The isometric line (dashed) is shown.

strates that ionic regulation of the haemolymph occurred only for meals in which the osmotic pressure was maintained equal to that of normal blood by means of glucose. The [$Na^+ + Cl^-$] of the haemolymph was reduced when the meal was hyposmotic, and was increased when the meal was hyperosmotic. Changes of haemolymph composition should depend directly, of course, on the composition and quantity of gut absorbate. In these experiments, values for the latter could be estimated by calculation (see Methods). Fig. 5 shows that the concentrations of Na^+ and Cl^- were very nearly equal in the coxal fluid and gut absorbate over a sixfold range of concentration; this correlation between the two fluids indicates that the coxal organ is responsible for haemolymph ion regulation. Moreover, if one plots the total amount of Na^+ or Cl^- in the coxal fluid plus haemolymph as a function of the total amount of the same ion in the gut absorbate, the data fall very close to the

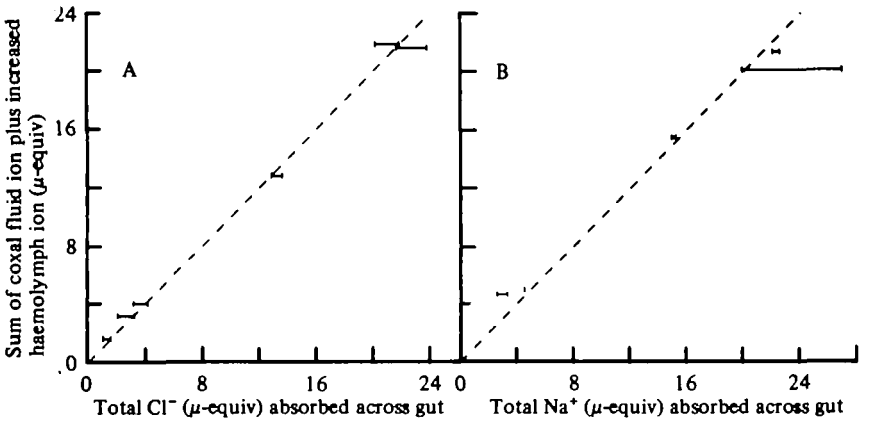

Fig. 6. (A) Relation between total Cl^- of gut absorbate and total Cl^- excreted in coxal fluid plus increase in haemolymph Cl^- during the 2 h experimental period. Limits shown are from Table 2. The isometric line (dashed) is shown. (B) As in (A) but for Na^+.

Table 3. *Ion concentrations (m-equiv l^{-1}) in initial droplets of coxal fluid and in samples pooled over the excretory period (mean ± S.E.; n)*

	Cl^--concentration		Na^+-concentration		K^+-concentration	
Meal	Initial drops	Pooled	Initial drops	Pooled	Initial drops	Pooled
Blood	68 ± 7 (8)	117 ± 5 (6)	112 ± 5 (4)	111 ± 3 (4)	20 ± 2 (4)	5 ± 1 (4)
Blood + NaCl	132 ± 19 (5)	217 ± 3 (5)	—	—	—	—
Blood + glucose	65 ± 2 (3)	187 ± 5 (5)	—	—	—	—
Blood + DW	16 ± 6 (5)	51 ± 3 (4)	—	—	—	—

isometric line (Fig. 6). This indicates that during the feeding period increase in tissue ion content must be negligible.

Changes in ion composition of body fluids over the time-course of coxal fluid production

Thus far we have only been considering volumes and compositions that exist 2 h post-engorgement, by which time equilibrium has been largely achieved. In the following experiments, we monitored in individual ticks the modulations which occur in the haemolymph and coxal fluid from the time of attachment to the end of coxal fluid excretion (Table 3). During the initial 10 min or so of feeding, $[Cl^-]$ of the haemolymph fell until such time as coxal fluid excretion commenced. The first droplets of coxal fluid were consistently lower in $[Cl^-]$ than the mean values, for a variety of feeding conditions. In contrast, $[K^+]$ of initial coxal fluid was significantly higher than the mean value and there was no significant difference in $[Na^+]$ (Table 3).

In a number of cases, ticks were killed at the first moment that coxal fluid appeared in order to sample the gut contents (Table 4); when fed isosmotic meals, the $[Cl^-]$ in the gut at this time was not significantly different from that existing 2 h after the meal, suggesting that the large chemical gradient against which Cl^- was being

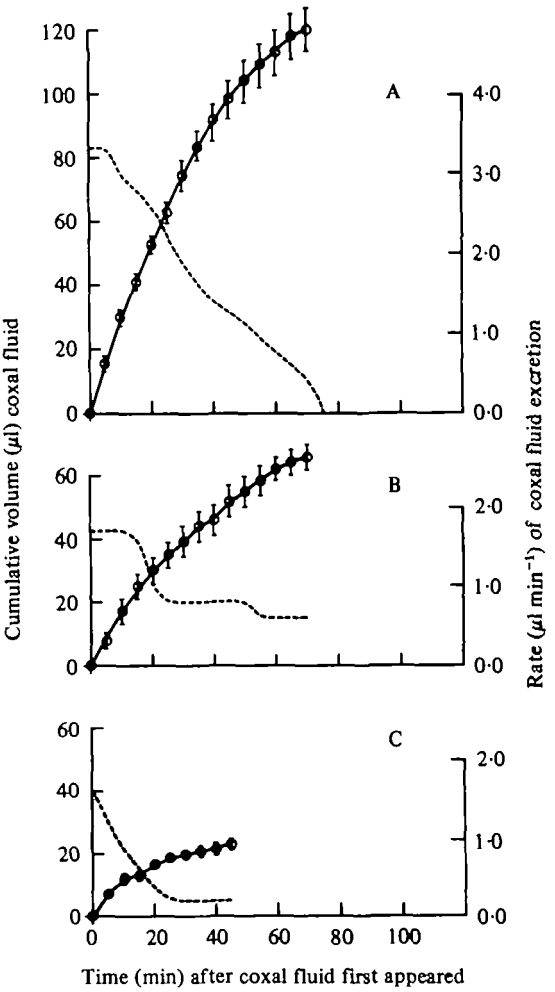

Fig. 7. Time course of coxal fluid production for the various meals. Solid line is cumulative volume, dashed line is rate of excretion. The S.E. is indicated for each point. (*a*) Ticks fed blood ($n = 6$); (*b*) ticks fed blood+NaCl ($n = 5$); (*c*) ticks fed blood+glucose ($n = 5$); (*d*) ticks fed blood+DW ($n = 4$); (*e*) ticks fed 1 blood:1 glucose ($n = 5$); (*f*) ticks fed 1 blood:3 glucose ($n = 4$).

Table 4. *Cl^--concentration (m-equiv l^{-1}) in haemolymph and whole gut fluid when coxal fluid first appeared and after coxal fluid excretion ceased (mean ± S.E.; n)*

	[Cl^-] in haemolymph		[Cl^-] in gut fluid	
Meal	Start*	Finish*	Start	Finish
Blood	155 ± 2 (9)	170 ± 2 (6)	75 ± 1 (9)	77 ± 2 (6)
Blood + NaCl	190 ± 1 (6)	239 ± 9 (5)	113 ± 1 (6)	88 ± 6 (5)
1 blood:1 glucose	153 ± 1 (9)	173 ± 3 (5)	37 ± 1 (9)	37 ± 1 (5)
1 blood:3 glucose	145 ± 2 (9)	172 ± 3 (4)	—	—

Initial [Cl^-] in haemolymph 200 ± 2 m-equiv l^{-1} (9).
* Start = at time when coxal fluid production began.
* Finish = at end of coxal fluid production (2 h after feeding commenced).

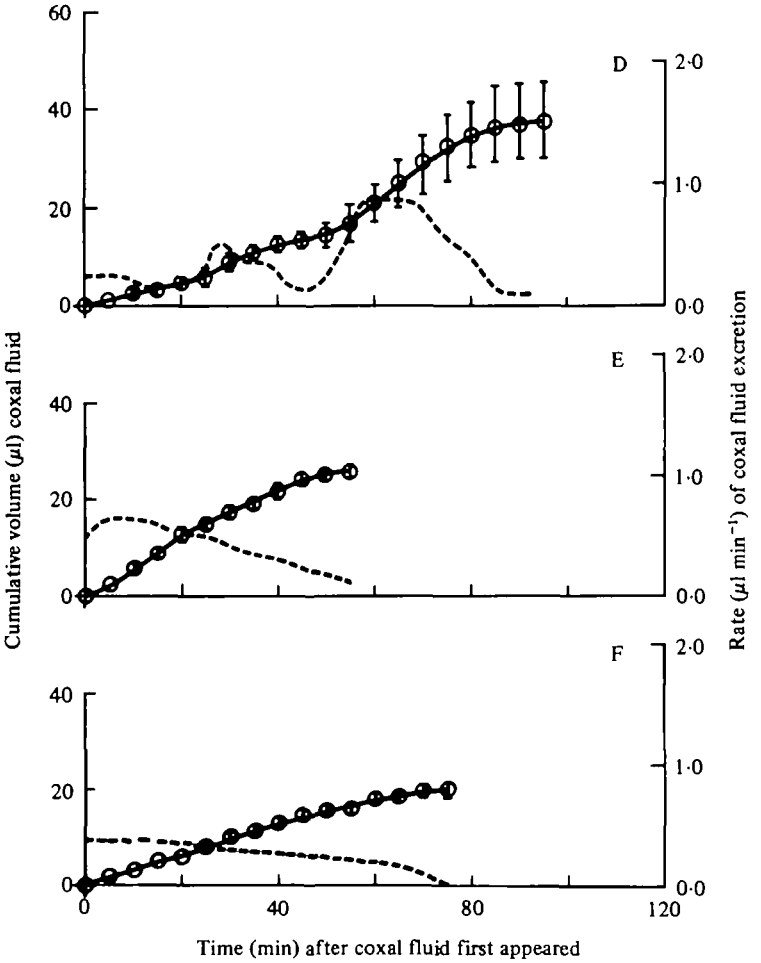

Fig. 7. (*cont.*)

transported from the gut was fairly constant throughout the main absorption period. On the other hand, the gut contents of those ticks fed on the hyperosmotic meal was significantly higher in [Cl^-] at the start of coxal fluid production compared to the equilibrium value 2 h post-feeding (Table 4).

Rate of coxal fluid excretion as a function of meal composition

Not surprisingly, the absolute rate of coxal fluid production was a function of initial tick weight. The following observations were made on ticks whose unfed weights ranged between 30 and 50 mg. Care was taken to ensure equal weight distributions in each experimental group.

The highest initial rate of coxal fluid excretion (3·3 μl min^{-1}) was associated with ticks fed blood, although the rate fell off steadily with time (Fig. 7*a*). Within any series of isosmotic meals, the initial and average rates of coxal fluid production fell

Table 5. *Osmotic pressures (m-osmol kg^{-1} water) of gut contents and haemolymph 2 h after feeding (mean ± S.E.; n)*

Meal	Ingested fluid	Gut fluid	Haemolymph
Blood	295*	478 ± 11 (5)	457 ± 5 (5)
Blood + NaCl	470	457 ± 11 (4)	527 ± 27 (5)
Blood + glucose	470	468 ± 11 (4)	473 ± 27 (3)
Blood + DW	148	215 ± 11 (4)	269 ± 11 (4)
1 blood : 1 glucose	295	360 ± 5 (4)	—
1 blood : 3 glucose	295	258 ± 16 (4)	290 ± 16 (3)

* Only the value for blood was measured cryoscopically; values for other ingested fluids were calculated from this.

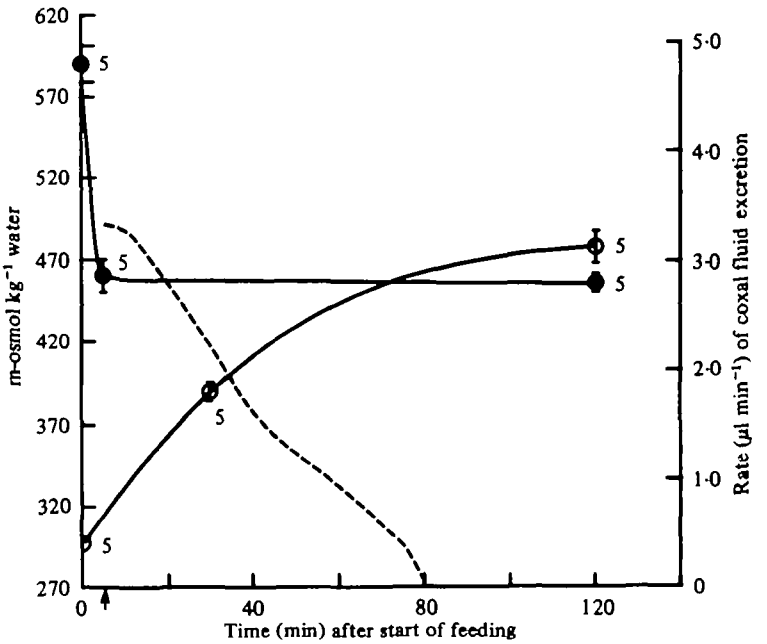

Fig. 8. Relation between osmotic pressures of gut fluid and haemolymph, and rate of coxal fluid excretion in ticks fed blood. S.E. and *n* are indicated for each point. Arrow denotes time that coxal fluid excretion began. ●, Haemolymph; ○, gut fluid; ---, rate of coxal fluid production.

with decreasing [Cl$^-$] in the blood meal. The duration of excretion was unchanged at 60–80 min for all meals isosmotic with blood (Fig. 7*a*, *e*, *f*). Initial and average rate of coxal fluid production was inversely related to osmotic concentration of the meal irrespective of which solute was added; however, at any given time during the excretory period, the rate was depressed more in ticks fed on blood + glucose (Fig. 7*c*) compared to ticks fed on blood + NaCl (Fig. 7*b*). When fed on meals of reduced ionic strength ticks excreted coxal fluid at a similar average rate irrespective of whether the diluent was distilled water or isosmotic glucose, but the initial rates differed and the course of excretion followed different patterns (Fig. 7*d*, *e*).

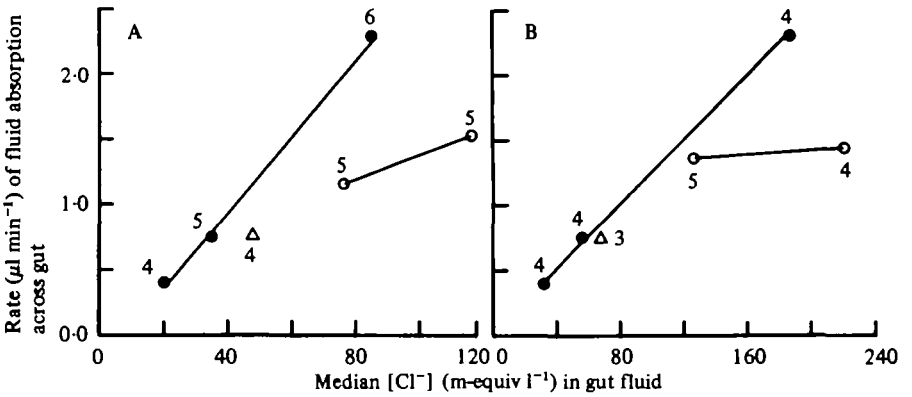

Fig. 9. Relation between median ion concentration in gut fluid and mean rate of fluid absorption by gut epithelium during the 2 h experimental period. Symbols as in Fig. 3. (A) Cl^-, (B) Na^+.

Osmotic pressure of body fluids

By 2 h after any meal, osmotic pressures in the gut and haemolymph had reached similar values (Table 5). But during the time of rapid feeding, there would undoubtedly exist a variable osmotic gradient across the gut epithelium. We thus monitored osmotic changes in these compartments during the feeding cycle so as to evaluate the importance of such passive gradients to ion and water flux across the gut epithelium.

Fig. 8 shows that an initial drop in haemolymph osmotic pressure precedes initiation of coxal fluid excretion when ticks were fed blood. This suggests first that the gut absorbate is hyposmotic with respect to the haemolymph and that the increase in haemolymph volume on feeding (Fig. 2) might occur at this time. If this were so, both the rate of coxal fluid excretion and osmotic concentration of the coxal fluid probably reflect the rate of absorption of fluid from the gut and osmotic concentration of the absorbate. In Fig. 8 we notice that as the osmotic pressures of gut contents and haemolymph approach each other, so does the rate of coxal fluid excretion fall. This pattern suggests that the rate of fluid absorption from the gut (as reflected by coxal fluid excretory rate) is influenced considerably by the prevailing osmotic pressure gradient.

Although there is little doubt that the prevailing osmotic gradient influences the rate of fluid absorption from the gut, other evidence indicates that this rate is also dependent upon the $[Na^+]$ and $[Cl^-]$ of the gut contents (Fig. 9). Indeed, it can be further shown that the volume of gut absorbate depends directly on the total Cl^- absorbed by the epithelium, though for any given amount of Cl^- absorbed the volume is an inverse function of osmotic pressure (Fig. 10).

Electropotential difference across the gut epithelium

Electropotential differences across the gut epithelium were recorded 2 h after feeding in ticks fed on blood and 1 blood:3 glucose as described in Methods. In the five ticks fed on blood, the potentials were 14–16 mV, lumen negative with respect

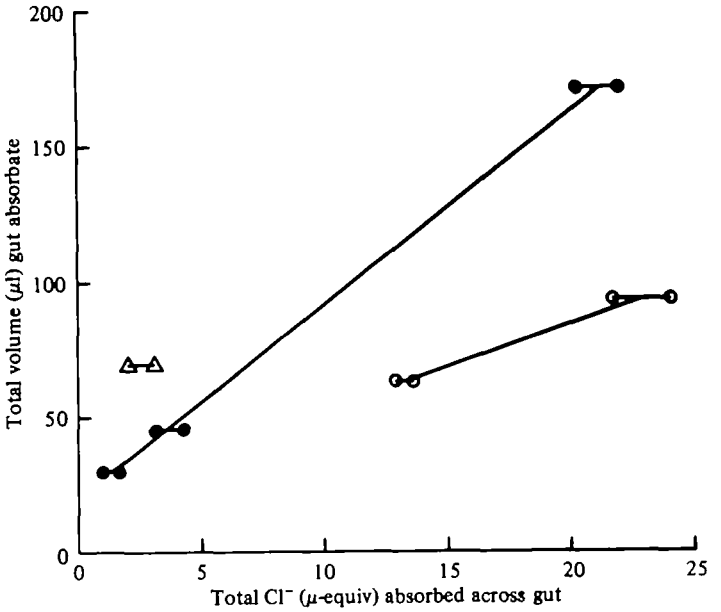

Fig. 10. Relation between total Cl^- and total fluid absorbed across gut walls during 2 h experimental period. Horizontal limits show calculated range from Table 2. Symbols as in Fig. 3.

to haemolymph. In those ticks fed on the isosmotic meal of low ionic strength, the magnitude of the potential was the same, but the polarity was reversed.

DISCUSSION

Ticks were fed blood meals with NaCl concentrations ranging from 31 to 223 m-equiv l^{-1} Na^+ and 21 to 162 m-equiv l^{-1} Cl^-. The osmotic pressures varied from 148 to 470 m-osmol kg^{-1} water. Despite these variations (7-fold for ion concentration and 3-fold for osmotic pressure), meal volume showed a less than 2-fold variation (Table 1). This suggests that in the osmotic and ionic ranges studied, the tick had an unremarkable ability to distinguish between the different conditions and showed no clear pattern of aversion to specific meals. However, no blood meals having ionic or osmotic concentrations higher than that of the haemolymph of unfed ticks could be tested because blood proteins precipitated out during preparation of such meals. Nevertheless, Fig. 4 indicates that the tick would have no regulatory capability for such a meal.

Table 2 shows that Na^+ and Cl^- move from gut to haemolymph against considerable concentration gradients under some feeding conditions. When ticks were fed 1 blood: 3 glucose, Cl^- moved into the haemolymph against an 8-fold concentration gradient and against an electropotential difference of 15 mV. Moreover, the large concentration gradient was maintained throughout the absorption period (Table 4). Thus we have evidence for active transport of Cl^- across the gut wall.

When ticks were fed blood, the electropotential difference across the gut w

was 15 mV (gut negative) and the $[Na^+]$ in the gut fluid (2 h after feeding) was 197 m-equiv l^{-1} (Table 2). According to the Nernst equation, the haemolymph $[Na^+]$ should be only 110 m-equiv l^{-1} if the ions were passively distributed, but the measured haemolymph concentration was 154 m-equiv l^{-1} (Table 2). This suggests that Na^+ is actively transported into the haemolymph. Stronger evidence for active transport of Na^+ was also obtained when ticks were fed 1 blood:3 glucose. In this case the $[Na^+]$ in the haemolymph was not measured (Table 2). However, it may be reasonably assumed from Fig. 4 that, since the meal was isosmotic with blood, the haemolymph $[Na^+]$ would be maintained at about 160 m-equiv l^{-1}. The observed electropotential difference (15 mV, lumen positive) could only have supported a haemolymph concentration of 59 m-equiv l^{-1} (gut concentration = 33 m-equiv l^{-1}; Table 2).

During the period of absorption across the gut, $[K^+]$ in the gut rose from 4 to 52 m-equiv l^{-1} and the concentration gradient increased 10-fold when ticks were fed blood (Table 2). The measured electropotential gradient of 15 mV (gut negative) could not account for this concentration gradient if rapid free diffusion is assumed. This can mean either that K^+ was being actively transported from the haemolymph to the gut as Harvey & Nedergaard (1964) found in the *Cecropia* moth, or that the gut epithelial cells had a very low permeability to K^+, but we do not have sufficient data to distinguish between these two mechanisms.

The volume of blood ingested varied less than 2-fold over the range of feeding conditions investigated, whereas the volume of fluid absorbed across the gut varied over 6-fold (Table 1) and was dependent on the composition of the blood meal (Fig. 3). What then controls rate of fluid absorption by the gut? Under all conditions studied, there was an initial osmotic gradient favourable for passive movement of water from gut to haemolymph. An initial high rate of water absorption across the gut was suggested by the initial rapid fall in haemolymph osmotic pressure (Fig. 8). A high rate of water absorption might have resulted simply from a reduction in osmotic pressure of gut fluid by the incoming blood meal. This suggestion is supported by Fig. 8, which shows that rate of coxal fluid excretion (shown in Fig. 2 to be an index of absorption rate across the gut) falls as the osmotic gradient between gut contents and haemolymph diminishes. But there are reasons to doubt that this is the complete story. (1) Amongst all the experimental conditions studied, the most favourable osmotic gradient for fluid absorption from the gut occurred when ticks were fed blood + DW. Although there was a 41% reduction in meal size under these conditions (compared to ticks fed on blood) there was a 61% reduction in the volume of gut absorbate (Table 1). (2) Furthermore, one can calculate from Table 1 that the ratio (volume of gut absorbate):(total volume ingested) is virtually identical for the meals blood + DW, and blood + NaCl (0·32), despite the fact that the initial osmotic gradient for water movement across the gut wall would have been far more favourable for the meal blood + DW. (3) There is a direct correlation between volume of gut absorbate and total Cl^- absorbed by the epithelium but for any given amount of Cl^- transported absorbate volume is an inverse function of osmotic pressure (Fig. 10). (4) Finally, rate of fluid absorption by the gut epithelium is dependent upon the $[Na^+]$ and $[Cl^-]$ of the gut contents (Fig. 9). The lower absorption ratio for ticks fed on blood + glucose might be explained if glucose were

absorbed more slowly than monovalent ions. A concomitant accumulation of glucose in the gut, and hence reduction in the osmotic gradient, would retard fluid absorption.

We propose the following scheme for regulation of haemolymph volume and ionic composition. NaCl is actively transported from the gut, water movement being coupled to this (Fig. 10). The general decrease in rate of coxal fluid production with meals of increased osmotic pressure possibly reflects a lower rate of osmotic flow across the gut wall due to a less favourable osmotic gradient. The signal that initiates coxal fluid production might be simply an increase in haemolymph hydrostatic pressure beyond a threshold value. In support of this, the rate of coxal fluid production is modified when the hydrostatic pressure of the haemolymph is altered (S. E. Kaufman, W. R. Kaufman & J. E. Phillips, in preparation). Thus a high rate of absorption across the gut would lead to a high rate of NaCl loss due to increased rate of filtration and decreased ion reabsorption. Conversely, a low rate of NaCl transport across the gut would lead to a low rate of water absorption. This in turn would lead to a low rate of coxal fluid production providing more time for NaCl to be reabsorbed and hence conserved.

This research was generously supported by NSERC Canada. S.E.K. gratefully acknowledges tenure of a NSERC postgraduate scholarship during the course of this study.

REFERENCES

BONÉ, G. (1943). Recherches sur les glandes coxales et la régulation du milieu interne chez l'*Ornithodorus moubata*. *Annls Soc. r. zool. Belg.* **74**, 16–31.

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* **1**, 279–285.

GREGSON, J. D. (1956). *The Ixodoidea of Canada*. Ottawa: Canadian Department of Agriculture.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the *Cecropia* silkworm. *Proc. natn. Acad. Sci. U.S.A.* **51**, 757–765.

KAUFMAN, W. R. (1979). Control of salivary fluid secretion in ixodid ticks. In *Recent Advances in Acarology*, vol. 1 (ed. J. G. Rodriguez), pp. 357–363. New York, San Francisco and London: Academic Press.

LEES, A. D. (1946). Chloride regulation and the function of the coxal gland in ticks. *Parasitology* **37**, 172–184.

MADDRELL, S. H. P. (1971). The mechanisms of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.

MADDRELL, S. H. P. (1976). Functional design of the neurosecretory system controlling diuresis in *Rhodnius prolixus*. *Am. Zool.* **16**, 131–139.

NUTTALL, G. M. F. (1908). The Ixodoidea or ticks. *Jl R. Inst. publ. Hth* **16**, 385–403.

PATCHIN, S. & DAVEY, K. G. (1968). The effects of injected aminopterin on egg production in *Rhodnius prolixus*. *J. Insect Physiol.* **14**, 1545–1551.

RAMSAY, J. A. (1949). A new method of freezing-point determination for small quantities. *J. exp. Biol.* **26**, 57–64.

RAMSAY, J. A., BROWN, R. H. J. & CROGHAN, P. C. (1955). Electrometric titration of chloride in small volumes. *J. exp. Biol.* **32**, 822–829.

REMY, P. (1922). Sur le rejet de sang par les Argasidae. *Archs Zool. exp. gén.* **61**, 1–16.

SIDOROV, V. E. (1960). The nature of the coxal fluid of the argasid ticks *Ornithodorus lahorensis*, *Ornithodorus papillus* and *Argas persicus*. *Dokl. Akad. Nauk SSSR* (Biol. Sci. Sect. Transl.) **130**, 176–178.