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MECHANISM AND CHARACTERISTICS OF COXAL FLUID EXCRETION IN THE ARGASID TICK *ORNITHODORUS MOUBATA*

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SUMMARY

1. Adult ticks (*Ornithodorus moubata* Murray, Acari, Argasidae) were fed on human blood at 37 °C in a beaker covered with chicken skin.

2. ^{14}C-labelled inulin and 3-o-[$^{14}CH_3$]glucose were rapidly cleared from haemolymph to coxal fluid; the coxal tubule appeared not to reabsorb either substance. Glucose was reabsorbed from tubular fluid via a phlorrhizin-sensitive mechanism. Reabsorption of amino acids varied between 0 and 90% but was greatest for those amino acids which were scarce in the meal.

3. During normal feeding conditions, there was positive correlation between haemocoelic hydrostatic pressure and rate of coxal fluid excretion.

4. Unilateral cannulation of a coxal organ so as to eliminate back pressure of a valve at the coxal orifice led to a twofold increase in rate of coxal fluid production compared to the contralateral side.

5. The above data confirm that coxal fluid excretion occurs by a filtration–resorption mechanism.

INTRODUCTION

During the feeding cycle, ticks (Acari: Ixodoidea) of the family Argasidae excrete excess fluid of the blood meal via a pair of coxal organs (Fig. 1) to accomplish haemolymph volume and ion regulation (Boné, 1943; Lees, 1946; Kaufman, Kaufman & Phillips, 1981). Whereas Boné (1943) believed the coxal organ to consist of a coiled tubule which elaborated coxal fluid by a secretory mechanism, Lees (1946) demonstrated that the tubular lumen is in direct communication with a tortuous sinus bounded by a fine membrane to which are attached numerous small muscle fibres. Lees proposed that this structure functions like a filtration membrane analogous to that found in the vertebrate glomerular nephron.

Although his proposals for the creation of the driving force for filtration were speculative, Lees presented good circumstantial evidence that filtration occurred: the

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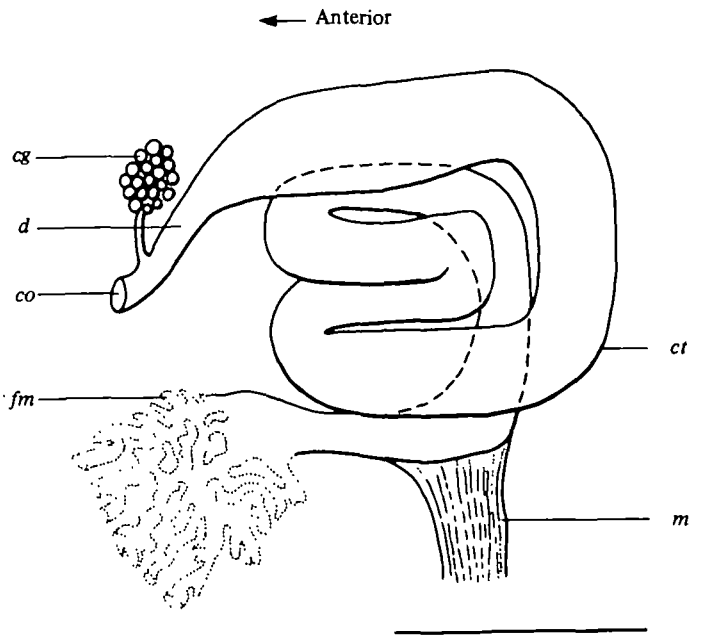

Fig. 1. Diagram of coxal organ from *O. moubata*. (*co*) Coxal orifice; (*d*) duct; (*cg*) coxal gland; (*ct*) coxal tubule; (*m*) large medial muscle; (*fm*) filtration membrane. Horizontal bar represents 0·5 mm. The coxal orifice exits between the first and second coxae. The coxal gland may play an important role in cuticular tanning (Binnington, 1975).

dyes bromophenol blue (MW = 670) and Congo red (MW = 697) passed into the coxal fluid quite readily, whereas they could not be detected in the Malpighian tubules up to 24 h later. Even haemoglobin (MW $\simeq$ 68000) passed freely into the coxal fluid. Serum albumin (MW $\simeq$ 69000 was retained by some ticks, but was filtered by others, and casein (MW $\simeq$ 200000) never appeared in the coxal fluid. In this paper we confirm Lees' filtration hypothesis and provide new observations on clearance and reabsorption of amino acids.

MATERIALS AND METHODS

Experimental animals

Ornithodorus moubata were reared on chickens as described in a previous paper (Kaufman *et al.* 1981). Experimental ticks were fed on human blood maintained at 37 °C in a beaker covered by chicken skin through which ticks were allowed to feed (Kaufman *et al.* 1981).

Clearance of inulin, glucose and methylglucose

One hour prior to feeding, ticks were injected via an 'Agla' micrometer syringe (Patchin & Davey, 1968) with 2 μl ($\simeq$ 280 nCi) of aqueous inulin [carboxyl-^{14}C] (New England Nuclear). As soon as coxal fluid production started, serial samples of coxal fluid and haemolymph were taken as described by Kaufman *et al.* (198[illegible]). Haemolymph and gut contents were taken 20 h after feeding to detect any residual

[^{14}C]inulin. These fluids were dissolved in 10 ml Bray's solution and ^{14}C activity determined with a Nuclear Chicago Mark I Scintillation Counter. Clearance of 3-o-[$^{14}CH_3$]glucose (New England Nuclear) was determined in like manner 1 h after ticks were injected with 2 μl containing 45 nCi.

We determined glucose concentration in haemolymph and coxal fluid semi-quantitatively using strips of glucose oxidase-impregnated paper ('Tes Tape'; Eli Lilly & Co.) and calibration with the sugar standards o = < 2·8 mM, + = 5·6 mM, ++ = 14 mM, +++ = 28 mM, ++++ = 110 mM and above. Some ticks were pretreated with the glucose transport blocker, phlorrhizin (100 mg kg^{-1} delivered in 2 μl distilled water) 1 h before feeding.

Reabsorption of inulin

We tested for possible reabsorption of inulin in the coxal tubule in the following way: a glass micropipette, connected to an 'Agla' micrometer syringe, was introduced through the coxal orifice so that the tip lay just within the tubule. This micropipette was sealed in place with beeswax/resin, and about 0·25 μl of [^{14}C]inulin (2·5 g l^{-1}) was injected into the tubule lumen. A few minutes later, a haemolymph sample was taken from a leg segment adjacent to the injected coxal organ. If there was any radioactivity in this sample, the tick was discarded on the assumption that the coxal tubule had been punctured during injection. The successfully injected specimens were fed 2 h later and coxal fluid was collected from the collateral (i.e. uninjected) coxal gland over 10 min; as will be seen later (Fig. 2), this 10 min sample would have contained 90% of any inulin reabsorbed from the injected tubule into the haemolymph. The wax was then pulled away from the sealed coxal tubule, the micropipette removed and the first 4 μl of coxal fluid emerging was collected. Since the volume of the gland is only about 250 nl, the 4 μl of coxal fluid was considered adequate to contain the total amount of residual inulin.

Influence of hydrostatic pressure changes on coxal fluid excretion

Pressure measurements of the haemolymph were made using a Statham low-volume-displacement transducer (model no. P23 Gb) linked to a Gilson polygraph. The ticks were cannulated directly into the body cavity with a length of perforated PE 10 tubing filled with saline. The posterior rim of the tick's body was clamped so that the gut lobes were displaced towards the anterior. An incision was made posterior to the clamp and the PE tubing pushed gently into the haemocoel so that no air bubbles were trapped in the pressure line. The area was carefully dried and sealed with melted beeswax/resin. Although several traces were recorded up to and including the start of coxal fluid production, after many attempts we achieved only one complete record right through the feeding cycle to detachment.

A sphincter at the coxal orifice appears to open and close regularly during the period of coxal fluid excretion. To learn whether this structure might also influence the filtration rate by exerting back-pressure through the tubule, in some ticks we introduced a fine glass cannula through the orifice of one coxal organ; this cannula lay just within the distal tubular segment. Rates of coxal fluid production were measured simultaneously from both the normal and cannulated sides.

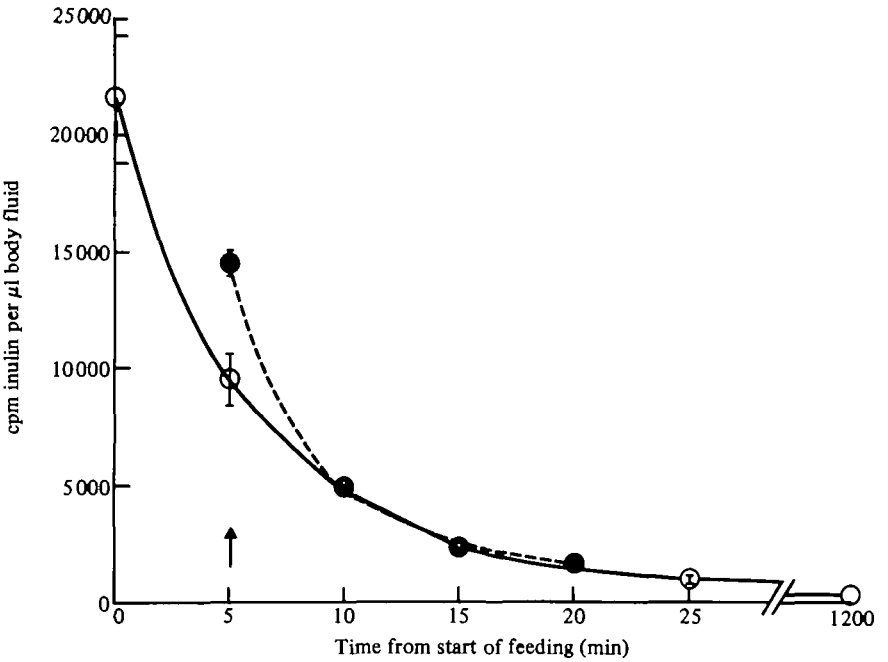

Fig. 2. Inulin clearance by coxal organs during normal feeding on blood. Ticks were injected with [^{14}C]inulin prior to feeding; haemolymph (○) and coxal fluid (●) were collected at the indicated times. Means ± S.E. (N = 4) are indicated. The arrow denotes initiation of coxal fluid excretion. At no point was the difference in radioactivity between haemolymph and coxal fluid statistically significant (Student's t test).

Analysis of amino acids in tick tissue fluids

Groups of 15 ticks were fed and 100 μl samples of pooled haemolymph were collected 2 h after feeding. Coxal fluid was collected during feeding. These fluids were mixed with 0·5 ml methanol and centrifuged. The supernatant was retained, pooled with two further 0·5 ml methanol washes of the pellet, and evaporated to dryness at 35 °C in a rotary evaporator, and the residue resuspended in 0·5 ml glass-distilled water. After evaporation to dryness again, the residue was dissolved in a Biocal buffer and the amino acids determined on a Biocal BC20 Amino Acid Analyser.

pH of body fluids

The pH of haemolymph and coxal fluid was measured with Radiometer capillary-type pH electrodes designed to avoid CO_2 loss from 5–100 μl volumes (models K150 and G252C), using a Radiometer PHM25a-PHA925a meter. The electrodes were rinsed in a small volume of test fluid before recording steady pH values.

RESULTS

Inulin clearance

To confirm that inulin was not rapidly reabsorbed by the coxal tubule, [^{14}C]inulin was introduced by retrograde injection into the coxal tubule as described in Method

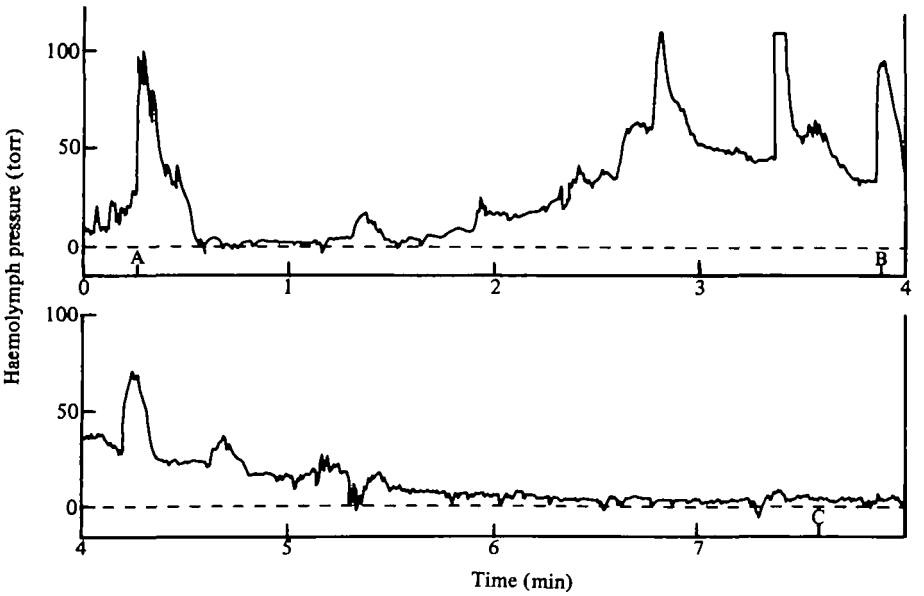

Fig. 3. Hydrostatic pressure of haemolymph in a tick feeding on blood. At A, the tick attached to the chicken skin covering the beaker of blood. Coxal fluid made its first appearance at B, and excretion ceased at C. Note the gradual rise in baseline pressure leading to coxal fluid excretion and a return to near atmospheric thereafter.

Two hours later, the total haemolymph activity was only $4 \pm 2\%$ (mean ± S.E.; $N = 4$) of injected ^{14}C. Injected [^{14}C]inulin was recovered in the first 4 μl of coxal fluid issuing from the cannulated organ after removing the wax seal. Thus, only about 4% of the injected ^{14}C activity crossed the tubule wall within 2 h. Since a volume of coxal fluid equivalent to the total organ volume is cleared every 10 s, reabsorption of 'filtered' inulin by the coxal tubule is not significant.

Fig. 2 shows that the coxal organs eliminated 50% of inulin injected into the haemolymph within 5 min and 90% within 10 min. No radioactivity was found in the haemolymph or gut 20 h later. The coxal fluid to haemolymph ratio of inulin (CF/H) was not significantly different from unity over the entire course of the experiment. The apparent departure of CF/H ratio from unity at 5 min after feeding (Fig. 2) was not statistically significant by Student's *t* test. Small differences would be expected because of the unavoidable delay (30 s) between the taking of coxal fluid and its paired haemolymph sample.

Pressure dependence of coxal fluid formation

A record for haemolymph pressure during feeding and coxal fluid production is shown in Fig. 3. This was the only complete trace obtained after many attempts. However, five additional pressure records were obtained up to the initiation of coxal fluid production. The haemolymph pressures consistently rose from near 0 to an average of 39 ± 4 torr (mean ± S.E.) prior to fluid production (Fig. 3, point B). The sudden transient pressure increase recorded when the tick attached (Fig. 3, point A)

Table 1. *Amino acid composition of body fluids in* Argas arboreus (*A.a.; from Boctor* 1972) *and* Ornithodorus moubata (*O.m.; this study*)

	Concentration (μM l^{-1})					
	Haemolymph		Coxal fluid		CF/H	
Amino acid	*A.a.**	*O.m.*†	*A.a.*	*O.m.*	*A.a.*	*O.m.*
Ala	449	256 ± 91	—	173 ± 52	—	0·68
Arg	—	158 ± 47	—	30 ± 10	—	0·19
Asp	474	20 ± 13	68	24 ± 11	0·14	1·20
Cys	364	48 ± 36	—	7 ± 5	—	0·15
Glu	5456	100 ± 47	82	156 ± 55	0·02	1·56
Gly	933	223 ± 107	13	125 ± 45	0·01	0·56
His	458	30 ± 10	58	32 ± 13	0·13	1·07
Ile	565	89 ± 4	15	29 ± 14	0·03	0·33
Leu	412	180 ± 11	23	75 ± 23	0·06	0·42
Lys	575	—	82	—	0·14	—
Met	trace	25 ± 6	29	8 ± 1	—	0·32
Phe	trace	206 ± 174	—	55 ± 25	—	0·27
Pro	174	46 ± 13	—	46 ± 5	—	1·00
Ser	771	178 ± 84	—	75 ± 20	—	0·42
Thr	630	94 ± 45	17	67 ± 15	0·03	0·71
Tyr	—	172 ± 118	77	68 ± 32	—	0·40
Val	2128	177 ± 62	—	94 ± 28	—	0·53
Gln + Asn‡	—	—	—	—	—	0·10

* *N* and ± S.E. not given.
† For *O.m.*, mean ± S.E., $N = 2$–4.
‡ Absolute concentrations are not known for these amino acids since standards were not run, but the CF/H ratio could be estimated. Ratio shown is for combined peak, Gln + Asn.

was consistently observed and was associated with the ejaculation of a pool of saliva. Invariably, pressure returned to near atmospheric after attachment. During the appearance of pressure peaks immediately preceding and following the onset of coxal fluid production, the tick seemed to be flexing the large intercoxal muscles. Note the increase in baseline haemolymph pressure leading up to coxal fluid production and the gradual return to atmospheric pressure by the time coxal fluid production had ceased.

Since the valve at the coxal orifice opens and closes rhythmically during excretion, we monitored the valve's influence on rate of coxal fluid production by cannulating one coxal orifice. The rate of coxal fluid production from the uncannulated organ was 0·72 ± 0·07 μl min^{-1} (mean ± S.E., $N = 6$); that for the cannulated collateral organ of the same ticks was 1·38 ± 0·18 μl min^{-1}, $N = 6$, a difference which was significant at the 5% level (pairs *t* test).

Reabsorption

Because filtration does not discriminate between small solutes, most renal organs possess efficient mechanisms for recapturing useful organic substrates before excreting the waste fluid. Table 1 displays amino acid concentrations of haemolymph and coxal fluid. Variability was high, but about 0–90% of filtered amino acid was reabsorbed, a comparatively low percentage. Glutamic and aspartic acids were unique in having CF/H ratios slightly exceeding unity. We noticed a greater tendency to reasorb a given amino acid if its concentration in human blood is low (Fig. 4).

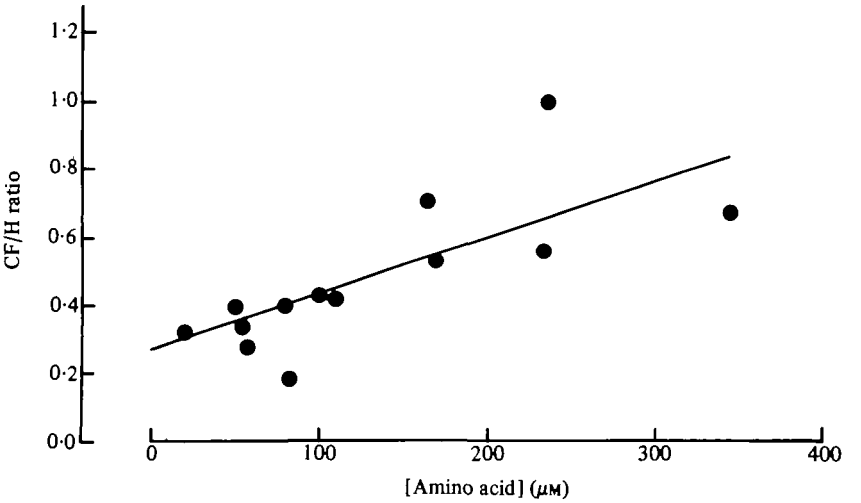

Fig. 4. CF/H ratios for amino acids as a function of human plasma concentration. CF/H ratios were taken from Table 1. Concentrations in human plasma were taken from the *Handbook of Biochemistry* (Chemical Rubber Co., 1968). The line was fitted by the method of least squares ($r = 0{\cdot}74$; $P < 0{\cdot}01$). The points for aspartic acid (CF/H = 1·2) and for glutamic acid (CF/H = 1·56) were not included for this analysis; since their ratios tended to exceed that for inulin (i.e. unity) they might be secreted into the coxal fluid.

Filtered glucose is mostly reabsorbed. In six ticks we observed a positive reaction (+→++) with 'Tes Tape' in every haemolymph sample, but no trace of glucose in coxal fluid (limit of detection ≃ 3 mM). Haemolymph and coxal fluid of ticks which were pretreated for 1 h with phlorrhizin, a blocker of glucose transport (Pitts, 1963), gave similar positive reactions for glucose (+ → ++). Thus haemolymph glucose concentration (≃ 6–14 mM) is slightly higher than the fasting blood levels for mammals (≃ 4–6 mM) and the coxal gland tubule must contain a phlorrhizin-sensitive reabsorptive transport mechanism for glucose. We attempted to use 3-O-methylglucose to probe this transport mechanism more thoroughly (Csáky & Wilson, 1956; Csáky & Thale, 1960), but as with some other renal tubules (Riegel, 1972), the coxal tubule did not reabsorb it (unpublished observations).

The pH of body fluids

The pH of haemolymph sampled 2 h after initiation of feeding was $6{\cdot}82 \pm 0{\cdot}05$ (mean ± S.E., $N = 4$), and that of pooled coxal fluid was $7{\cdot}40 \pm 0{\cdot}07$ ($N = 4$), the difference being statistically significant at the 95% confidence level.

DISCUSSION

Filtration

Our experiments are in accord with Lees' (1946) hypothesis that the mechanism of coxal fluid excretion in argasid ticks is filtration/resorption. Since we demonstrated that inulin is not reabsorbed from the tubular lumen (p. 347) and since CF/H ratios for inulin and 3-O-methylglucose remained at unity during coxal fluid production,

it appears that access of haemolymph solutes < 5000 MW to the tubular fluid is unrestricted. Moreover, little if any fluid was reabsorbed by the tubule. This is not surprising, since the main function of the coxal organ at this stage of the life cycle is to excrete excess fluid (Kaufman *et al.* 1981). We also demonstrated that hydrostatic pressure in the haemocoel rises from near atmospheric just after attachment to a plateau of 40–50 torr at the start of coxal fluid production, with peak pressures approaching or even exceeding 100 torr (Fig. 3). The steady decline in hydrostatic pressure thereafter (Fig. 3) correlated with decreasing production of coxal fluid (see Kaufman *et al.* 1981). Moreover, cannulated organs released coxal fluid at double the rate of their collateral (control) organs, presumably due to a significant reduction in tubular back-pressure opposing the filtration mechanism. We have no direct proof that this influence of the valve is exerted at the level of filtration, although Lees (1946) provided evidence that such is probably the case. Other evidence supports the filtration hypothesis. Kaufman (1971) injected fluorescein-labelled bovine-serum albumin into ticks during the first 10 min of coxal fluid production and prepared the coxal organs for visualisation by fluorescence microscopy. Fluorescence was clearly observed trapped within pockets of the filtration membrane, suggesting the latter as the site at which the primary ultrafiltrate of haemolymph is formed. Furthermore, ultrastructure of the putative filtration membrane of the coxal organ (Hecker, Diehl & Aeschlimann, 1969; Groepler, 1969; Kaufman, 1971) resembles that of other renal organs believed to initiate fluid excretion by a filtration process (Schmidt-Nielsen, Gertz & David, 1968; Altner, 1968; Tyson, 1968; Haupt, 1969; Menefee & Mueller, 1967; Berridge & Oschman, 1972). The spacing between the foot processes (200–500 nm) is remarkably similar in various filtration membranes. Indeed, it is only absence of a capillary endothelium and presence of tracheae in the arthropod systems that distinguish them easily from the vertebrate. Molecular size limitation of the tick coxal organ filter (Lees, 1946; this study) is similar to that of vertebrates (Wallenius, 1954) and somewhat lower than that of the crayfish (Kirschner & Wagner, 1965). But the permselectivity appears to vary depending on how coxal fluid production is elicited. Under normal feeding conditions, only a few haemolymph proteins appear in the coxal fluid, whereas all the haemolymph proteins are present in coxal fluid stimulated by mild trauma. In the studies of Remy (1922), Lees (1946) and Siderov (1960) coxal fluid excretion was usually induced by the latter method.

Reabsorption of metabolites

Fine structure of the coxal tubule (Hecker *et al.* 1969) is typical of reabsorptive epithelia (Berridge & Oschman, 1972). Lees (1946) described a shunt pathway in the tubular system, suggesting that it might be possible under certain physiological conditions for tubular fluid to bypass much of the 'proximal segment', thus allowing less opportunity for absorption to occur. Hecker *et al* (1969) found no structural evidence for this shunt pathway and neither did we.

Since inulin and 3-O-methylglucose are readily excreted and since glycosuria can be induced by phlorrhizin (p. 349), absence of detectable glucose in normal coxal fluid is likely due to the presence of an efficient glucose transport mechanism in the tubule rather than to exclusion of glucose from the primary filtrate. In contrast, only 0–90%

of filtered amino acids were reabsorbed by the coxal tubule (Table 1), there being a relatively greater degree of reabsorption of those amino acids which are low in the meal (Fig. 4). This loss of amino acids is probably of no great consequence because an enormous metabolic pool of amino acids is locked in the undigested protein stored in the tick's gut: there is no equivalent polymeric storage of glucose.

Boctor (1972) reported far lower CF/H ratios for amino acids in *Argas arboreus* than we found for *Ornithodorus* (Table 1). Also, CF/H ratios for amino acids are not correlated between these two species; other than a possible species-specific difference, we have no explantion for this discrepancy. The concentration of total amino acids in *O. moubata* haemolymph ($\simeq$ 260 mg l^{-1}) is similar to levels in crustaceans and vertebrates (200–800 mg l^{-1}), whereas the value for *A. arboreus* (1700 mg l^{-1}; Boctor, 1972) approaches those characteristic of exopterygote insects (2000–4000 mg l^{-1}; Jeuniaux, 1971). In vertebrate distal tubules, conversion of glutamine to glutamate and ammonia is the main source of ammonia in the kidney (Pitts, 1948). The high CF/H ratio for glutamic acid (1·6), the low ratio for glutamine ($<$ 0·1) and the relatively high pH of coxal fluid (7·4) compared to haemolymph (6·8) suggest that a similar system may be present in ticks.

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