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Excretion of uric acid and amino acids during diuresis in the adult female *Glossina morsitans*

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Summary

Radiometric analysis was carried out on the urine collected for one hour following feeding of the adult female *Glossina morsitans* on day 1 of a pregnancy cycle, which had previously received haemocoelic injections of U-^{14}C labelled arginine, histidine, leucine, lysine, phenylalanine, threonine, tyrosine or valine. Mean radioactivity in the urine was quite high after labelled arginine (17.4% of injected activity) and histidine (21.8%) administration, most of the activity being in the amino acid fractions. With the remaining six labelled amino acids, mean radioactivity in the urine varied between 1.6 and 7.2% of injected activity, most of this activity occurred in a non-amino acid fraction (probably uric acid), though low radioactivity was also detected in a range of essential as well as non-essential amino acids.

Key words: G. morsitans; diuresis; excretion of uric acid and amino acids.

Introduction

Although uric acid is the main nitrogenous waste product in *Glossina*, the amino acids arginine and histidine also make up a substantial proportion of the dry weight of its excreta (Bursell, 1965). This observation has since been confirmed in other tsetse species, and it has further been demonstrated that amino acids other than arginine and histidine are also present in the excreta of this insect though in very small amounts (Moloo et al., 1974; Balogun, 1974a; 1974b). After the ingestion of a blood-meal a substantial amount of water is excreted very rapidly (Lester and Lloyd, 1928; Bursell, 1960; Moloo and Kutuza, 1970), and the present study examines the elimination of uric acid and

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amino acids during this diuretic process which continues for about one hour after feeding.

Materials and methods

G. morsitans were obtained from the self-supporting goat-fed colony maintained in this laboratory (Nash et al., 1971). The experiments were undertaken with female flies on day 1 of their second pregnancy cycle, which were collected as previously described by Langley and Pimley (1974). All experimental flies were fed on goats and kept at 25° C.

Adult female flies, five in each case, were individually injected through the thoracic cuticle with 2 μl of U-^{14}C labelled arginine (specific activity = 318 mCi/mmole), histidine (324), leucine (311), lysine HCl (318), phenylalanine (477), threonine (232), tyrosine (522) or valine (265) (Radiochemical Centre, Amersham). The radioactive concentration of the above labelled amino acids was the same at 0.05 μCi/μl. Following injections, the flies were fed and kept singly in scintillation vials each containing a filter paper disc for the collection of urine. One hour after feeding, the urine was individually eluted from the filter paper discs with 2 ml of distilled water. Total ^{14}C-activity was determined in each sample, after which the amino acid fraction was separated using a column of Zerolit 225 and radioactivity determined. As this insect is largely uricotelic (Bursell, 1965), the difference between the total radioactivity and that in the amino acid fraction of the urine was taken to be the activity in the uric acid. Details of the general procedure have been described elsewhere (Moloo, 1976a).

In another series of experiments, using three flies for each labelled amino acid, urine was eluted from the filter paper discs with 50% methanol, filtered (Millipore, 0.45 μm), and the pooled sample was dried in vacuo. The dry sample was redissolved in 100 μl of 80% methanol; a 50 μl sample was subjected to chromatographic separation of amino acids. Another 50 μl sample was co-chromatographed with a standard mixture of amino acids and radioactivity in various amino acids was determined using liquid scientillation techniques as previously described (Moloo, 1976a).

Results

Table 1 shows radioactivity in the urines, expressed as mean counts/min/fly, collected for one hour after feeding which followed the haemocoelic administration of different labelled amino acids. This table also shows the distribution of radioactivity between uric acid and amino acid fractions of the urines. It is apparent that ^{14}C-activity in the urine was considerably higher after the administration of arginine (17.4% of the injected activity) or histidine (21.8%) than that recorded for the remaining six labelled amino acids. Of the latter, the elimination of threonine-derived radioactivity in the urine was relatively quite high while ^{14}C-activity recorded after injections of the other five labelled amino acids was low at between 1.6 and 3.1% of the injected activity. Almost all the radioactivity was in the amino acid fraction after labelled arginine or histidine administration. Proportionate radioactivity in the amino acid fraction was markedly high after labelled valine administration (14.6% of the total recovery in the urine), but in the case of the remaining five amino acids more than 90% of the radioactivity was in the uric acid fraction of the urine. Amino acids identified in the pooled urine were alanine, arginine, glycine, histidine, leucine/isoleucine, lysine, methionine, proline and valine.

Table 1. Radioactivity in the urine of *G. morsitans* females and its distribution in the uric acid and amino acid fractions after haemocoelic injections individually of eight U-^{14}C amino acids followed by feeding

U-^{14}C amino acid injected	Counts/min in urine $\bar{x} \pm se$	Per cent of injected activity	Uric acid %	Amino acid %
Arginine	30,473 ± 5,542	17.4	0.4	99.6
Histidine	38,060 ± 4,625	21.8	0.1	99.9
Leucine	3,320 ± 226	1.6	97.0	3.0
Lysine	3,387 ± 357	1.9	93.5	6.5
Phenylalanine	3,069 ± 272	1.6	96.8	3.2
Threonine	12,938 ± 1,939	7.2	94.2	5.8
Tyrosine	4,396 ± 565	2.3	98.6	1.4
Valine	6,249 ± 646	3.1	85.4	14.6

Table 2. Distribution of ^{14}C-activity in various amino acid fractions from the pooled urine of *G. morsitans* females, co-chromatographed with a standard mixture of amino acids, after haemocoelic injections individually of eight U-^{14}C amino acids followed by feeding

Amino acid	Counts/min**	%
Alanine	180	0.5
Arginine*	16,760	48.9
Aspartic acid	80	0.2
Cystine/Cysteine	–	–
Glutamic acid	90	0.3
Glycine	100	0.3
Histidine*	13,900	40.6
Leucine*/Isoleucine	400	1.2
Lysine*	180	0.5
Methionine	–	–
Phenylalanine*	160	0.5
Proline	520	1.5
Serine	60	0.2
Threonine*	100	0.3
Tyrosine*	210	0.6
Valine*	1,500	4.4
Total	34,240	100.0

* U-^{14}C amino acids injected

** Radioactivity above background

Table 2 shows the distribution of radioactivity in various amino acid fractions from the 50 μl sample of the pooled urine (co-chromatographed with a standard mixture of amino acids) of 24 female flies. The highest ^{14}C-activity was recorded in arginine (48.9% of total recovery) followed by that in the histidine fraction (40.6%). Apart from methionine and cystine/cysteine, radioactivity was also detected in the other amino acids, of which that in valine (4.4%) and proline

(1.5%) was higher than in the remaining fractions. It is of interest that radioactivity was recorded in a range of non-essential amino acids in the urine within about one hour after administration of the labelled essential amino acids.

Discussion

The elimination of large quantities of arginine and histidine is a characteristic feature of the excretory physiology of *Glossina*. These two amino acids are eliminated quantitatively in relation to their concentration in blood-meals ingested by male tsetse, supposedly because the metabolic cost of their deamination is quite high (Bursell, 1965). In the female fly, however, these two amino acids are utilized in the synthetic processes associated with larval nutrition, there being a smaller surplus for elimination during late pregnancy when protein synthetic activity is high (Moloo, 1977a). In the present study the female flies used were on day 1 of a pregnancy cycle when there was little metabolic demand for these two amino acids (Moloo, 1976b; Moloo, 1977a), so that substantial amounts of the injected radioactive arginine and histidine were eliminated very rapidly in the urine during diuresis. This is suggestive of a rapid diffusion of arginine and histidine into the Malpighian tubules during diuresis. It is likely that other amino acids also diffuse into the urine, but that these would be resorbed by the rectal glands for return to the haemolymph, hence the very low radioactivity recorded in amino acids other than arginine and histidine in the urine.

The excretion of specific amino acids is probably an adaptation to specific diets. In the carpet beetle, *Attagenus piceus*, the sulphur-containing amino acid, cystine, accounts for a considerable proportion of excretory nitrogen (Powning, 1953), but since its keratin diet has a high cystine content its presence in the excreta probably represents a faecal material rather than excretory product (Bursell, 1967). Haematophagous insects, whose diet contains a high proportion of nitrogen-rich amino acids may eliminate these unchanged as is the case with tsetse (Bursell, 1965; Balogun, 1974; Moloo, 1977a) and to some extent with mosquito species (Irreverre and Terzian, 1959). However, in the case of the blood-sucking hemipteran, *Rhodnius prolixus*, a number of amino acids have been detected in the excreta but together making up only 0.2% of the total dry weight, with histamine and histidine predominant among them (Wigglesworth, 1931; Harrington, 1956; 1961).

The present study has also revealed that the metabolism of other amino acids, such as leucine, lysine, phenylalanine, threonine, tyrosine and valine, results in the elimination of uric acid as nitrogenous waste. After their injections into the haemolymph, radioactivity was detected mainly in the uric acid fraction of the urine within one hour of diuresis though low activity was also recovered in the amino acid fraction. In view of the fact that the food of this insect is vertebrate blood which contains about 95% proteins by dry weight, the

appearance of very small quantities of a variety of amino acids other than arginine and histidine in the urine probably represents a loss rather than an excretion of amino acids, particularly at diuresis when copious amounts of water from the blood-meal is eliminated. It has been demonstrated that this insect can synthesize a range of non-essential amino acids from leucine, lysine, phenylalanine, threonine, tyrosine or valine (Moloo, 1976c; 1977a; 1977b; 1977c), but not from arginine or histidine (1977a). It is of interest that a small proportion of most of the non-essential amino acids synthesized from at least a few of the injected former ones were also lost during diuresis. The present study has thus served to illustrate that during diuresis *G. morsitans* excretes nitrogen-rich arginine and histidine, loses very small quantities of a range of other amino acids, and excretes a non-amino acid material (probably uric acid) which has been formed from several of the amino acids studied.

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