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NITROGEN METABOLISM IN THE AMERICAN COCKROACH: AN EXAMINATION OF WHOLE BODY AND FAT BODY REGULATION OF CATIONS IN RESPONSE TO NITROGEN BALANCE

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SUMMARY

The effects of nitrogen balance on uric acid/urate, K^+ and Na^+ storage or mobilization were examined in the American cockroach *Periplaneta americana* (L.). Cockroaches on a high nitrogen diet increased in whole body uric acid/urates, K^+ and Na^+. Those on a semi-starvation diet maintained fairly constant levels of uric acid/urates, K^+ and Na^+. However, those on a low nitrogen diet mobilized stored uric acid/urates and K^+, but not Na^+. Analyses for uric/urates K^+, Na^+, Ca^{2+} and Mg^{2+} in fat body tissue from insects maintained on 12 diets containing different concentrations and sources of dietary nitrogen showed that only K^+ concentration could be correlated with fat body uric acid/urate storage. Whole body storage and mobilization of uric acid/urates, K^+ and Na^+ were reflected in the faecal/dietary ratios of K^+ and Na^+. A model for a uric acid/urate ion sink which might be associated with ionic and osmotic balances is proposed, and some evidence for its existence is discussed.

INTRODUCTION

Organisms from several taxonomic groups are known to store uric acid or urates internally. These include pulmonate snails (Duerr, 1967; Badman, 1971), tunicates (Nolfi, 1970), land crabs (Horne, 1968), terrestrial isopods (Hartenstein, 1968), and insects (Cochran, 1974). With the latter group, storage usually occurs in the fat body (Spiegler, 1962; Corbet & Rotheram, 1965; Clark & Smith, 1967; Salkeld, 1967; Tojo & Hirano, 1968), or associated with the cuticle (Friauf & Edney, 1969; Caveney, 1971), but a rather general internal distribution may also exist (Haydak, 1953; Zielinska, 1957; Kermack & Stein, 1959; Friauf & Edney, 1969).

The storage phenomenon has been especially well studied in cockroaches where it is known that urate stores are typically contained intracellularly in fat body urate cells (Gier, 1947; Bodenstein, 1953). The level of storage is related to the amount of nitrogen in the diet (Gier, 1947; Haydak, 1953; McEnroe, 1956; Mullins & Cochran 1974*a*), and under conditions of extreme storage large urate deposits may be precipitated extracellularly in the body cavity (Haydak, 1953). Because nitrogen balance studies have shown that uric acid/urate storage in the whole body can be quite significant (Mullins & Cochran, 1974*a*, *b*), the nitrogen balance being also related to ammonia excretion and water requirements (Mullins, 1974), it is of interest to obtain information regarding the storage form of uric acid/urates in various parts of the body.

Specific information pertaining to the storage form of uric acid/urates in insects is quite meagre. Jungreis & Tojo (1973) have shown that in *Hyalophora* fat body uric acid and potassium simultaneously increase at the larval–pupal molt. The uric acid apparently arises by *de novo* synthesis, but K^+ increases correspond with K^+ losses from the integument. These workers concluded that the fat body is a major site for uric acid and K^+ storage during the pupal stage. Presumably, potassium urate is involved. In addition, Hopkins & Lofgren (1968) have shown that labelled uric acid obtained from *Leucophaea* fat body is strongly bound to proteins and/or polypeptides, again indicating a possible storage form.

In this paper evidence is presented on the storage form of uric acid/urates in relation to nitrogen balance based on experiments with adult *Periplaneta americana* (L.). As a result of this study, we wish to draw attention to the possible importance of internally stored purines on ionic and osmotic balances in this insect, and perhaps in other organisms which store uric acid/urates internally.

MATERIAL AND METHODS

Examination of uric acid and metallic cation content of the American cockroach, *Periplaneta americana* (L.) was conducted in two steps: (1) whole body analysis of insects which had been maintained on three diets containing different concentrations of nitrogen and carbohydrate and (2) fat body analysis of insects maintained on one of 12 diets containing different concentrations and sources of nitrogen.

Whole body studies

Females were reared on commercial dog food containing 24% crude protein and were maintained under uncontrolled conditions of temperature, relative humidity and photoperiod. Special diets used in the whole body studies were modified from those described by Haydak (1953) and are as follows: (1) 50% casein protein diet according to Haydak: 10 g yeast extract, 4 g Hawk Oser salt mixture No. 3, 45 g casein protein and 41 g dextrin. (2) Cellulose + 5% protein diet: 10 g yeast extract, 4 g Hawk Oser salt mixture No. 3 and 106 g of chromatographic grade cellulose/120 g diet. (3) Dextrin diet: 4 g Hawk Oser salt mixture No. 3, 96 g dextrin and 20 g of chromatographic grade cellulose/120 g diet. (4) 42% casein protein diet: 100 g 50% casein protein diet diluted to 120 g with cellulose powder.

After termination of a feeding series, the insects were frozen, lyophilized and ground in a Wiley Intermediate Mill (No. 20 Mesh). Two hundred mg of ground carcasses were extracted with a 0·6% $LiCO_3$ solution at 80 °C for 30 min with continuous stirring. The uric acid content was determined using a uricase assay method modified from Dubbs *et al.* (1956). Two hundred mg samples of the diets, whole body tissues and faeces were wet-ashed and the Na^+ and K^+ concentrations determined by Atomic Absorption Spectrophotometry (Perkin Elmer 303 Atomic Absorption Spectrophotometer; analyses performed by the Biochemistry Analytical Testing Laboratory, Department of Biochemistry, VPI and SU using the absorption mode).

Fat body studies

The 12 diets containing different concentrations or sources of dietary nitrogen used in the fat body and faecal/diet studies were formulated as described in the preceding paper (Mullins, 1974) and outlined in Tables 4 and 5.

Two-month-old males reared on dog food were placed on one of 12 diets for 8 weeks after which their fat bodies were removed. Fat bodies from three insects were pooled (replicated three times), frozen, and lyophilized for two days prior to dry weight determinations. The dried samples were homogenized in 2·0 ml 0·6% $LiCO_3$ in a glass homogenizer at 80 °C for 10 min, centrifuged at 1000 **g** for 10 min and the supernatant analysed using the uricase assay method described above.

Analyses for K^+, Na^+, Ca^{2+} and Mg^{2+} were also carried out on the supernatant. Lanthanum chloride and cesium chloride solutions were added to aliquots of the samples during determination of Ca^{2+}, due to ionization interference (Sanui & Pace, 1972). Samples of diets and faecal material were wet-ashed and analysed as previously described (Mullins, 1974).

RESULTS

Because body weight fluctuates in response to the diet on which cockroaches are maintained, the data concerning whole body responses with respect to uric acid, K^+ and Na^+ are presented in two forms for comparative purposes. The values plotted in Figs. 1, 2, and 3 are expressed on the basis of μM/mg tissue and do not take into account changes in body weight which tend to conceal what is actually happening in the whole organism. Information in Table 1 presents the whole body changes in weight, total body uric acid, K^+ and Na^+ occurring over the specific feeding intervals.

Fig. 1 shows that when female *Periplaneta* were maintained on a high protein diet, the whole body uric acid/urate and K^+ undergo obvious increases, while Na^+ show only a very slight increase. Data presented in Table 1, however, make it clear that the body weight increased 166% along with large increases in total body uric acid, K^+, and Na^+ (723, 363 and 158%, respectively). The importance of comparing the results on a μM/mg basis and the whole body-weight change basis should be evident here. Fig. 1 indicates only a slight increase in whole body build up of Na^+ when expressed on a μM/mg basis, but a significant increase (158%) if whole body weight changes (166%) are taken into account. Although cockroaches maintained on diets containing elevated nitrogen levels excrete some of the nitrogen externally (Mullins & Cochran, 1973, 1974*a*; Mullins, 1974) a large amount of it appears to be stored internally as uric acid nitrogen along with K^+ or Na^+.

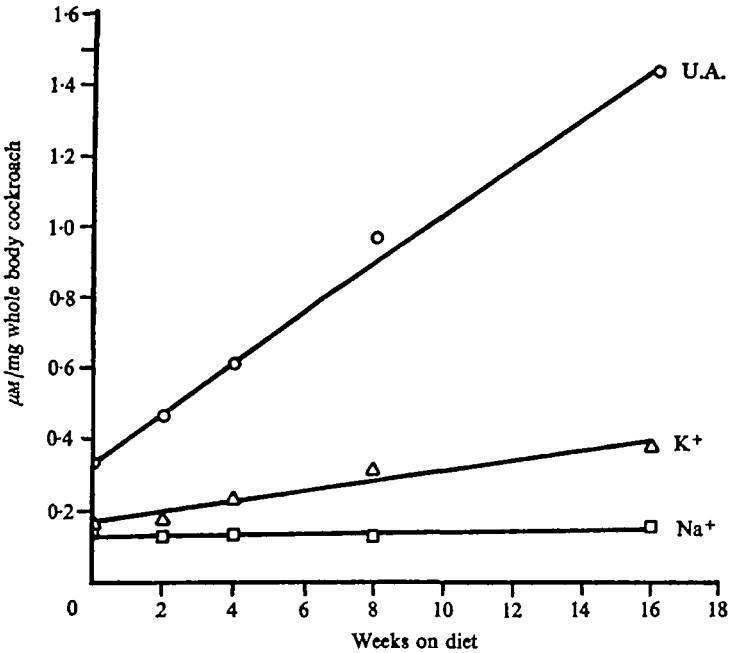

Fig. 1. The changes observed in uric acid (U.A.), K^+ and Na^+ levels in newly emerged female cockroaches maintained on a 50% casein protein diet for 16 weeks. The values are expressed as μM/mg whole body cockroach.

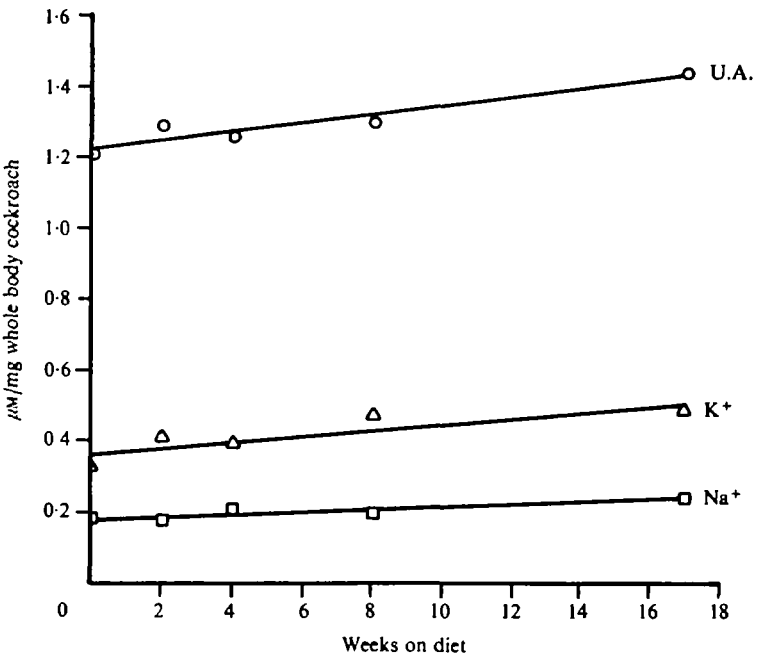

Fig. 2. The changes observed in uric acid (U.A.), K^+ and Na^+ levels in female cockroaches maintained on a cellulose + 5% protein diet (semi-starvation) for 17 weeks. The young females had been maintained on a 42% casein protein diet for 6 weeks prior to placement on the semi-starvation diet in order to build up their uric acid/urate reserves. The values are expressed as μM/mg whole body cockroach.

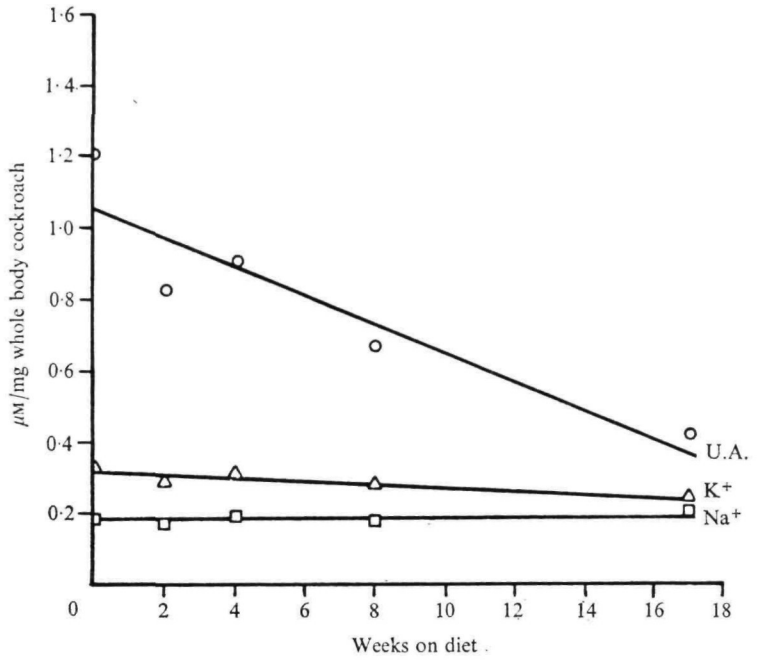

Fig. 3. The changes observed in uric acid (U.A.), K^+ and Na^+ levels in female cockroaches maintained on a dextrin diet for 17 weeks. The young females had been maintained on a 42 % casein protein diet for 6 weeks prior to placement on the dextrin diet in order to build up their uric acid/urate reserves. The values are expressed as μM/mg whole body cockroach.

Table 1. *Whole body changes in body weight, uric acid, potassium and sodium in female cockroaches maintained on three diets*

Diet	Weeks on diet	Body weight (mg)	Uric acid (μM)	K^+ (μM)	Na^+ (μM)
50 % Casein protein	0[1]	238	80	41	36
	16	394	579	149	57
		166 %[3]	723 %	363 %	158 %
Cellulose + 5 % protein	0[2]	383	460	126	70
	17	301	437	146	72
		78 %	95 %	116 %	103 %
Dextrin	0[2]	383	460	126	70
	17	327	139	83	69
		85 %	30 %	66 %	99 %

[1] Newly moulted adults were reared and maintained on dog food (24 % crude protein) prior to placement on the 50 % casein protein diet.

[2] Young adults were maintained on 42 % casein protein for 6 weeks prior to placement on either the cellulose + 5 % protein or dextrin diet.

[3] Percent of original values.

Fig. 2 illustrates that when females were placed on a cellulose + 5 % protein diet (essentially a starvation diet, low in carbohydrate and protein) for 17 weeks, the uric acid, K^+ and Na^+ levels all increase slightly. However, these changes (expressed in μM/mg tissue) apparently reflect to some extent the decreases in body weight as shown in Table 1. After 17 weeks body weight decreased to 78 % of the initial weight,

Table 2. *Total potassium and sodium consumption and excretion by female cockroaches maintained on three diets*

Diet	Weeks on diet	K^+ Consumed (μM)	K^+ Excreted (μM)	Na^+ Consumed (μM)	Na^+ Excreted (μM)
50% Casein protein	16[1]	243	98 (40%)[3]	161	63 (39%)
Cellulose + 5% protein	17[2]	268	231 (86%)	110	104 (94%)
Dextrin	17[2]	112	176 (157%)	68	72 (105%)

[1] Newly moulted adults were reared and maintained on dog food (24% crude protein) prior to placement on the 50% casein protein diet. The total K^+ and Na^+ consumption was determined by weight measurements of the total amounts of diet the insects consumed during the experimental feeding intervals multiplied by their respective ionic contents (determined by atomic absorption analysis on wet-ashed samples).

[2] Young adults maintained on 42% casein protein for 6 weeks prior to placement on either the cellulose + 5% protein or dextrin diet.

[3] ( ) percent excreted compared with consumption.

but the total body uric acid, K^+, and Na^+ content remained relatively constant (95, 116 and 103%, respectively). These data indicate that under semi-starvation conditions uric acid, K^+, or Na^+ were neither stored nor mobilized to any large extent, the utilization of other reserves probably accounting for the decreases in body weight.

When females were maintained on a dextrin diet for 17 weeks uric acid and K^+ levels decreased, the Na^+ levels showed a slight increase when compared on a μM/mg basis (Fig. 3). The body weights decreased to 85% of the initial weight (Table 1). The total body uric acid and K^+ decreased to 30 and 66% of the original values, respectively, but Na^+ remained about the same (99%). This information strongly suggests that when female *Periplaneta* are placed on a carbohydrate diet for an extended period of time the uric acid and potassium reserves are mobilized. Presumably the uric acid nitrogen is utilized as a source for nitrogen requirements (Mullins & Cochran, 1974*b*).

A comparison of total K^+ and Na^+ consumption and excretion for the time intervals studied is presented in Table 2. These data corroborate the changes observed in the analysis of whole bodies. Those females maintained on the 50% casein protein diet excreted only 40% of the K^+ and 39% of the Na^+ which they consumed in their diet during the 16 week feeding interval. This reflects the whole body storage of these metals under conditions of positive nitrogen balance (Table 1). Females maintained on the cellulose + 5% protein diet excreted 86% of the K^+ and 94% of the Na^+ they consumed in 17 weeks, indicative of a slight amount of whole body storage. However, this information generally reflects a more or less static uric acid/urate, K^+, and Na^+ situation which may occur under conditions of semi-starvation. Contrarily, dextrin-fed females excreted more K^+ (157%) than, and about the same amount of Na^+ (99%) as they consumed in 17 weeks. Thus, under conditions of negative nitrogen balance where uric acid is mobilized, K^+ are mobilized and excreted. Sodium levels appear to remain constant in the whole body (Table 1) and the consumption/excretion levels are almost equivalent.

The fat body is known to be a major site for uric acid storage (Bodenstein, 1953)

Table 3. *Distribution of uric acid, K^+ and Na^+ in the head plus thorax and the abdomen of old females maintained on a 79% casein protein diet for three months*

	Uric acid (μM/mg)	K^+ (μM/mg)	Na^+ (μM/mg)
Head + thorax	0·86	0·33	0·18
Abdomen	2·91	0·52	0·24

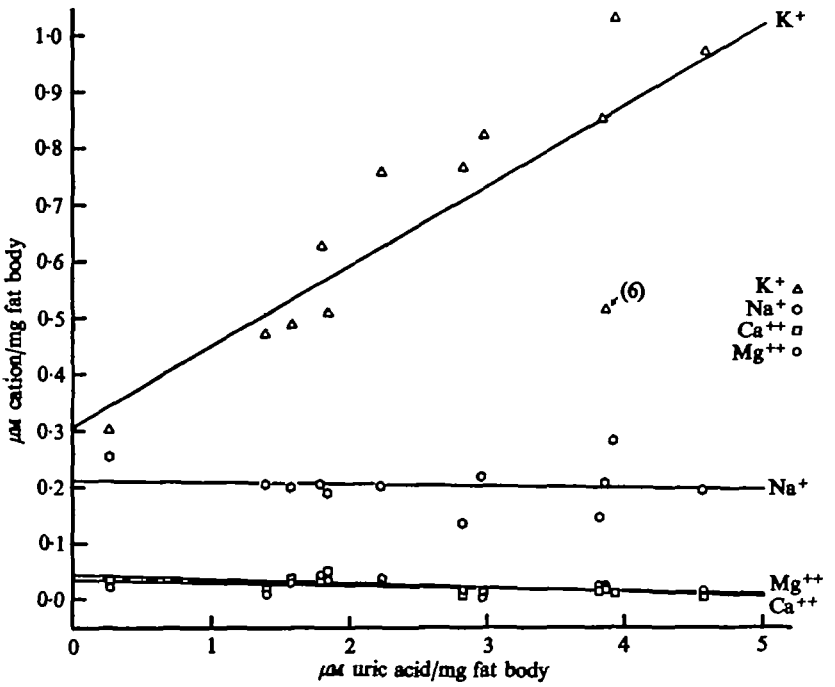

Fig. 4. The relationships of fat body uric acid/urates and K^+, Na^+, Ca^{2+}, and Mg^{2+} in response to feeding groups of male cockroaches on 12 different diets for 8 weeks. The diets contained different sources and various concentrations of dietary nitrogen (see Table 4). The slopes of the regression lines are: (K^+) $y = 0{\cdot}145x + 0{\cdot}304$, ($Na^+$) $y = -0{\cdot}004x + 0{\cdot}214$, ($Ca^{2+}$) $y = -0{\cdot}008x + 0{\cdot}044$ and (Mg^{2+}) $y = -0{\cdot}005x + 0{\cdot}035$. The point designated as △ (6) represents the K^+: uric acid value obtained from males maintained on diet number 6 (25% casein protein diet + 1% uric acid nitrogen).

Donnellan & Kilby, 1967), but when cockroaches are maintained on high protein diets, uric acid deposits have been noted to accumulate in other body parts (Gier, 1947; Haydak, 1953). Old females maintained on a 79% casein protein diet for 3 months were dissected into head + thorax and abdomens, and analysed for uric acid, K^+ and Na^+ content. Table 3 shows that although the abdomen contained higher concentrations of uric acid, K^+ and Na^+ the head and thorax contained relatively high levels of uric acid/urate salts presumably K^+ and Na^+. Precise composition analysis of various body parts was beyond the scope of this study, but it appears that under conditions of high dietary nitrogen consumption for prolonged periods, uric acid storage may occur in body parts/tissues other than the fat body.

Abdominal fat body tissue was examined from male *Periplaneta* which had been

Table 4. *Comparison of dietary nitrogen levels, fat body uric acid and potassium levels and the ratio of faecal/dietary potassium from adult males maintained for eight weeks on twelve diets*

Diet	Dietary nitrogen content (%)	Fat body: Uric acid (μM/mg)	Fat body: K^+ (μM/mg)	Fat body: Uric acid/K^+	K^+ (faecal/diet)
1. Dog food (25 % crude protein)	4	1·40	0·47	3·0	2·7[1]
2. 25 % Casein protein	4	2·96	0·83	3·6	3·4
3. 25 % Casein hydrolysate	4	3·93	1·04	3·8	3·4
4. 42 % Casein protein	7	3·82	0·86	4·4	1·6
5. 76 % Casein protein	12	4·58	0·98	4·7	1·4
6. 25 % Casein protein + 1 % UA-N[2]	5	3·86	0·52	7·4	2·7
7. Dextrin	0	0·27	0·30	0·9	3·6
8. Dextrin + 1 % UA-N	1	2·81	0·77	3·6	3·0
9. Dextrin + 1 % NH_3-N[3]	1	1·58	0·49	3·2	2·4
10. Dextrin + 2 % NH_3-N	2	1·80	0·63	2·9	2·5
11. Dextrin + 4 % NH_3-N	4	2·23	0·76	2·9	2·0
12. Dextrin + 6 % NH_3-N	6	1·84	0·51	3·6	1·9

[1] Insects were maintained on the diets for 6 weeks prior to a 2-week collection period for faecal analysis. See Mullins (1974) for values of K^+ content in the faeces.
[2] UA-N. Uric acid nitrogen incorporated into the diet as free uric acid.
[3] NH_3-N. Ammonia nitrogen incorporated into the diet as NH_4Cl.

maintained on one of twelve diets containing different levels and sources of dietary nitrogen. Fig. 4 compares the fat body uric acid, K^+, Na^+, Ca^{2+} and Mg^{2+} content after 8 weeks on the various diets (Tables 4 and 5). It can be seen that K^+ is the only one of the four cations studied which increases in response to fat body urate storage ($r = 0{\cdot}818$, $P < 0{\cdot}01$; $y = 0{\cdot}145x + 0{\cdot}304$). The test for the significance of Pearson's r used here was described by Downie & Heath (1970). The regression lines for Na^+, Ca^{2+} and Mg^{2+} have slightly negative slopes. However, these decreases may merely reflect the increases of fat body uric acid and K^+. The fat body K^+ in males fed diet No. 6 (25 % casein protein + 1 % uric acid nitrogen) appears to be much lower than expected for the corresponding uric acid concentration. This may reflect a difference in the manner in which metabolically derived and absorbed uric acid is deposited under conditions of an elevated nitrogen balance.

It has already been noted that whole body excretion of K^+ and Na^+ show a direct relationship with whole body urate storage/mobilization. Closer examination of fat body urate, K^+, and Na^+ levels with respect to the faecal/dietary ratios of K^+ and Na^+ is necessary to gain a better understanding of ion and nitrogen balance in the cockroach. Tables 4 and 5 compare the effects of dietary nitrogen levels on fat body uric acid, K^+, or Na^+ and the faecal/diet ratio of K^+ or Na^+, obtained when males were maintained on one of the 12 diets for 8 weeks.

Information presented in Table 4 shows that males maintained on high nitrogen-containing diets (positive nitrogen balance diets) not only contained higher uric acid and K^+ levels in the fat body, but tended to show a lowering of the K^+ faecal/diet ratio. Those cockroaches maintained on diets containing low levels of nitrogen (negative nitrogen balance diets), tended to contain lower levels of urate and K^+ in the fat body and tend to have higher K^+ faecal/diet ratios. The evidence presented here

Table 5. *Comparison of dietary nitrogen levels, fat body uric acid and sodium levels and the ratio of faecal/dietary sodium from adult males maintained for eight weeks on twelve diets*

Diet	Dietary nitrogen content (%)	Fat body: Uric acid (μM/mg)	Fat body: Na^+ (μM/mg)	Fat body: Uric acid/Na^+	Na^+ faecal/diet
1. Dog food (25% crude protein)	4	1·40	0·20	7·0	4·1[1]
2. 25% Casein protein	4	2·96	0·22	13·5	4·8
3. 25% Casein hydrolysate	4	3·93	0·29	13·6	4·9
4. 42% Casein protein	7	3·82	0·15	25·5	3·5
5. 76% Casein protein	12	4·58	0·20	22·9	2·4
6. 25% Casein protein + 1% UA-N[2]	5	3·86	0·21	18·4	4·7
7. Dextrin	0	0·27	0·25	1·1	5·6
8. Dextrin + 1% UA-N	1	2·81	0·14	20·1	5·0
9. Dextrin + 1% NH_3-N[3]	1	1·58	0·20	7·9	4·6
10. Dextrin + 2% NH_3-N	2	1·80	0·20	9·0	4·6
11. Dextrin + 4% NH_3-N	4	2·23	0·20	11·2	3·7
12. Dextrin + 6% NH_3-N	6	1·84	0·19	9·7	3·2

[1] Insects were maintained on the diets for 6 weeks prior to a two-week collection period for faecal analysis. See Mullins (1974) for values of Na^+ content in the faeces.

[2] UA-N. Uric acid nitrogen incorporated into the diets as free uric acid.

[3] NH_3-N. Ammonia nitrogen incorporated into the diet as NH_4Cl.

indicates that the levels of K^+ consumption and excretion generally may reflect an inverse relationship with the insects' nitrogen balance and urate storage in the fat body.

Table 5 shows that fat body Na^+ levels are not directly related to the fat body uric acid/urate levels. However, the faecal/dietary levels of Na^+ are similar to those trends established for K^+. Positive nitrogen balance diets, particularly diet No. 5 (76% casein protein) reflect a decreased Na^+ faecal/diet ratio. Negative nitrogen balance diets correspondingly show a higher Na^+ faecal/diet ratio. It should be noted that the accumulation of Na^+ by the whole body (Table 1) cannot be attributed to storage in the fat body as sodium urate. It is quite possible that the uric acid/urate deposits occurring extracellularly, in the haemocoel (Gier, 1947; Haydak, 1953) may be precipitates of sodium urate. Although critical analysis of the precise composition of these extracellular urates has not been performed, they may be composed predominantly of sodium urate because the haemolymph contains a higher level of Na^+ than K^+ (Tobias, 1948; van Asperen & van Esch, 1956; Pichon, 1970; Brady, 1967). In support of this possibility, Seegmiller (1969), has shown that sodium urate is precipitated extracellularly when human serum is supersaturated with uric acid. It is the accumulation of extracellular deposits of sodium urates that in some manner cause the pathological condition of gouty arthritis in man (Seegmiller, 1969).

DISCUSSION

Nolfi (1970) has suggested that there are at least four possible biological functions for permanently stored or translocated purines in various organisms: (1) storage excretion, (2) pigmentation, (3) a source of reserve carbon or nitrogen and (4) a stable

purine source for nucleic acid synthesis. In addition, we would like to propose (5) a uric acid/urate-associated ion sink. This concept suggests that the stored urates deposited in body spaces or specific tissues, particularly in cockroaches, might contribute to ionic balance and osmoregulation by ion exchanges in tissue fluids or haemolymph. Some experimental evidence reported by other workers may be used in support of this concept. Edney (1968) has shown that *Periplaneta* and the desert cockroach *Arenivaga* both respond to dehydration by losses in haemolymph water, but are subject to very strong osmoregulation. Similar findings have been reported in *Leucophaea* (Laird, 1970). In an examination of the effects of dehydration and rehydration in *Periplaneta* Wall (1970), found that extreme dehydration and subsequent rehydration resulted in less than expected changes in haemolymph osmolality with excess solutes apparently being sequestered and later released by some tissue(s). The observed changes could not be explained by excretory functions. Pichon (1970) examined haemolymph ion levels in *Periplaneta* under various conditions of dietary status and hydration. The general findings were that some tissue(s) are involved in sequestering and releasing ions into the haemolymph, and that variations in K^+ and Na^+ levels appear to be independent of one another. This could hardly be explained by variations in blood volume alone. Steele (1969) has shown that pre-incubation of cockroach fat body in Na^+- or K^+-free media results in significant changes in these ion levels in the tissue. Such information suggests that this tissue may have the capability of sequestering/releasing ions in the maintenance of a stable ionic environment. In addition Pichon (1970) found that as haemolymph flowed away from the abdomen, its K^+ level decreased. This suggests that the fat body might be involved in maintaining an elevated K^+ level in the area surrounding it. The results of the present study indicate that the fat body is a site for K^+ storage.

Studies of the physico-chemical properties of uric acid indicate that it may exist in several states. Indeed, the crystalline forms of uric acid and urates are numerous. Free uric acid crystallizes in an orthorhombic crystal system forming rhombic plates from pure solutions, and very fine amorphous crystals are produced by rapid precipitation (Seegmiller, 1969). Other crystalline forms of urates may be present in biological fluids (Oser, 1965), and may be in a dynamic state. Recent studies have shown that well-formed crystals of urinary calculi excreted by some vertebrates may transform from an ordered to a disordered state once eliminated from the body (Lonsdale & Sutor, 1971; Minnich and Piehl 1972). The solubility of various urates is greatly influenced by pH, and NH_4^+ have been shown to take part in ion exchange reactions resulting in the precipitation of ammonium urate (Porter, 1963). In addition, flocculation of uric acid-containing colloids can be accomplished by altering the pH or by the presence of certain cations, again notably NH_4^+ (Porter, 1963).

Although the precise storage form of uric acid/urates in *Periplaneta* is not known, information presented here and elsewhere (Mullins & Cochran, 1974*a*) strongly suggests that it may be stored as K^+, Na^+ and possibly NH_4^+ salt(s). In addition, Hopkins and Lofgren (1968) have suggested that uric acid in the fat body of *Leucophaea* may be bound to polypeptides and/or proteins, which appear to dissociate upon separation in electrophoretic fields with buffers increasing in pH.

A model of the ion sink as it might function in the maintenance of ion levels in *Periplaneta* is illustrated in Fig. 5. This model calls for a dynamic equilibrium betwee

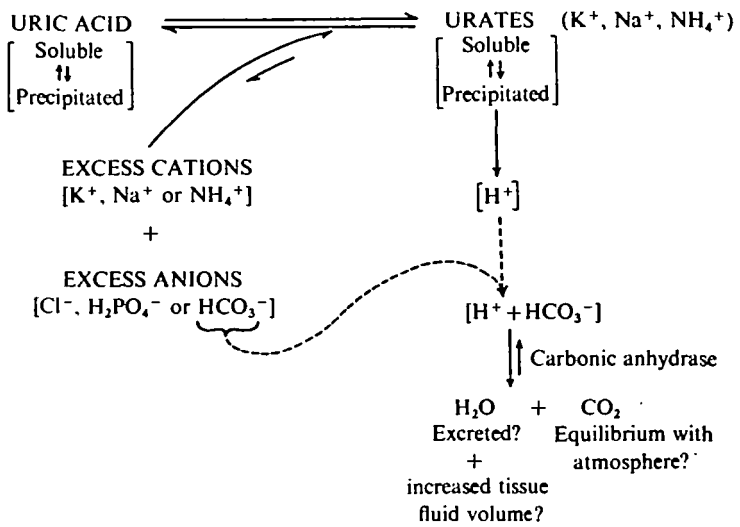

Fig. 5. A model of the ion sink operating under conditions where tissue fluids have increased in solute concentrations, raising the osmolarity. Sequestering of the monovalent cations K^+, Na^+, or NH_4^+ could occur by formation of their respective salts. The excess anions might be dealt with by the employment of carbonic anhydrase producing water and CO_2 which might then be eliminated by normal processes.

Under the opposite circumstances, where tissue fluid solutes might be lowered, the reversal of carbonic anhydrase activities and the formation of uric acid from the salt forms might allow for the elevation of the solute levels.

free uric acid and the urate salts of K^+, Na^+, and perhaps NH_4^+ in both the soluble and precipitated forms. The operation of the ion sink might involve a sequestering of Na^+, K^+ and NH_4^+ as urates in situations where solute concentrations of these ions are in excess of the desirable osmolarity levels. On the other hand, these ions may be released from the precipitated urates when the body fluids approach lower than favourable osmotic levels.

An anionic balance might be required, and the model proposes that bicarbonate may act in this capacity. In mammals, chloride, bicarbonate and phosphate are the major blood anions (Starling & Evans, 1962). Bicarbonate plays a particularly important role in acid-base balance in man (Guyton, 1971). Similar information reported for insects is much less satisfactory (Wigglesworth, 1972), but the literature indicates the same anions to be present with chloride being the most important (Sutcliffe, 1963; Bursell, 1970). Phosphate and bicarbonate are regarded as the principal buffers (Chapman, 1969). Furthermore, most of the CO_2 present in the haemolymph apparently occurs as bicarbonate while most carbonic anhydrase activity is associated with specific tissues (Bursell, 1970). Carbonic anhydrase has been found in various tissues of *Periplaneta* (Anderson & March, 1956) and might play an important role in controlling the levels of the bicarbonate anions present in tissue fluids. It is possible that other mechanisms might be involved in the establishment of cation–anion balances upon the removal of excess cations from tissue fluids.

Brady (1967) has provided an hypothesis which implicates haemocytes of *Periplaneta* as being capable of sequestering and releasing K^+ in the establishment of

osmotic balances. His observations were based on correlations between blood cell density and whole blood ion concentrations, but they did not include determinations of the K^+ content of the cells themselves. Laird, Winston & Braukman (1972) have found that the salivary glands (reservoirs) in *Leucophaea* appear to function as water-storage organs, possibly allowing for maintenance of nearly constant haemolymph osmolality. That haemocytes and other tissues are capable of sequestering and releasing solutes and water must not be discounted, for indeed, they may be quite important in maintaining ion balances, especially under certain conditions of physiological stress where excretion may not provide a satisfactory alternative. However, that uric acid/urates present in the tissues and haemocoel of *Periplaneta* might function as an ion sink, is also an attractive hypothesis, particularly in achieving ion balances under circumstances where external excretion is not desirable, and under conditions where ion storage would involve little expenditure of energy.

The removal/release of organic solutes in the haemolymph has not been considered here, but may be quite important. Djajakusumah & Miles (1966) showed that in the locust, *Chortoicetes terminifera*, interconversions between haemolymph amino acid pools and proteins may play a limited role in maintaining osmotic balances during rapid changes in the absence of dietary salts. Our understanding of ionic balances in relation to general metabolism of the cockroach is indeed quite limited.

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