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THE INFLUENCE OF DIET, AGE AND STATE OF HYDRATION ON Na^+, K^+ AND URATE BALANCE IN THE FAT BODY OF THE COCKROACH *PERIPLANETA AMERICANA*

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SUMMARY

The effect of diet, age, sex and state of hydration on the relationship between Na^+, K^+ and urate in the fat body tissue of the cockroach *Periplaneta americana* has been investigated. There is a significant correlation between K^+ and urate concentrations in the fat bodies of both males and females in late nymphal and adult stages. Animals fed on a dextrin/casein diet, which had a K^+ content of about one-third that of the rat pellets normally used for food, had a lower than normal K^+/urate ratio. In both the normally fed and dextrin/casein-fed groups of animals, dehydration caused a decrease in the K^+/urate ratio. Dehydration (and starvation) caused an increase in the amount of urate per unit weight of fat body. Some of the urate in the fat body is present in small spherical cellular inclusions. There is also a correlation between the K^+ concentration and the number of spherules. The ratio of spherules/urate is significantly higher in hydrated animals than in dehydrated ones.

INTRODUCTION

For many years terrestrial insects have been considered to be uricotelic and only in aquatic animals has ammonia been recognized as the major nitrogenous excretory product. The American cockroach *Periplaneta americana* was thought to excrete most of its waste nitrogen as uric acid (Nation & Patton, 1961; Bursell, 1967), but Srivastava & Gupta (1961) were unable to detect uric acid in its Malpighian tubules and Wharton & Wharton (1961) found that only about 3·5% of the nitrogen excreted could be attributed to uric acid. Recently, Mullins & Cochran (1973) reported that no uric acid could be detected in the excreta of *P. americana* maintained on a variety of diets. They found that faecal pellets of this species contained ammonia, amino nitrogen, three metabolites of tryptophan and some unidentified nitrogenous materials. The amount of ammonia excreted was found to increase with increased protein levels in the diet and they concluded that *P. americana* was ammonotelic (Mullins, 1974). As well as nitrogenous waste being eliminated from the body, some nitrogenous products may be stored in various tissues, and storage of uric acid in the fat body of cockroaches has long been recognized (see e.g. Cuénot, 1895; Philiptschenko, 1907; Faussek, 1911; Srivastava & Gupta, 1961).

Excretion of ammonia necessitates having plenty of water available, so that one would expect that dehydrating cockroaches might excrete less nitrogen in the form of ammonia than do normally hydrated animals. One way of achieving this would be to deposit more of the waste nitrogen as uric acid in the fat body. Dehydrating adult cockroaches will seldom eat dry food (Tucker, 1977*a*) and starvation increases the amount of urates in the fat body (Philiptschenko, 1907; Bodenstein, 1953; Anderson & Patton, 1955). Therefore, urate levels in the fat bodies of dehydrated animals would probably be higher than in normally hydrated ones.

Mullins & Cochran (1974) presented evidence that urate ions in *P. americana* may be stored as salts of K^+, Na^+ and possibly NH_4^+. They put forward a model of an ion sink for regulation of ion levels in *Periplaneta*, which proposed that Na^+, K^+ and NH_4^+ might be sequestered as urates in situations where solute concentrations of these ions were in excess of desirable osmolarity levels and then released from the precipitated urates when the body fluids approached lower than favourable osmotic levels.

Previous experiments (Tucker, 1977*a*) have shown that when *P. americana* is dehydrated the Na^+/K^+ ratio of the fat body tissue is increased and then it decreases again when animals are rehydrated. Because of the relationship between urate and potassium, which was reported by Mullins & Cochran, the relationship between Na^+, K^+ and urate in animals in different states of hydration was investigated.

Many factors other than the state of hydration of an individual are likely to affect the cation–urate balance in the fat body. Diets with high protein content increase urate deposition. Loss of uric acid from the fat body occurs when animals are fed on low-protein diets (Gier, 1947; Haydak, 1953; McEnroe, 1957; Mullins & Cochran, 1975*a*, *b*). Also the cation balance of the diet might affect the deposits of minerals accumulating in the cells. Uric acid levels in the haemolymph of females are higher than those in males (Hilliard & Butz, 1969). The rate of urate storage in animals on high-protein diets is higher in females than in males (Mullins & Cochran, 1975*a*). Although dehydration experiments were confined to males more than 2 weeks old, some analyses of urate and Na^+ and K^+ concentrations in the fat body were made on males on diets of different Na^+ and K^+ content, and on males and females of different ages, to see if diet, age or sex had a significant effect on the cation–urate balance in the fat body.

METHODS

Several days prior to an experiment individuals were separated from the stock culture and placed with food and water in polystyrene containers in a temperature-controlled cabinet set at 27 °C. The normal diet for cockroaches was rat pellets and water. Dehydrated animals are those deprived of water and food. Dehydrated animals were rehydrated by giving them water but no food. In one experiment, instead of rat pellets for food, animals were given a mixture of dextrin, casein and casein hydrolysate in the proportion 2:1:1 for 7 weeks prior to dehydration. Hydrated animals are those supplied with water and no food for 5 days after being on a normal rat pellet and water diet. Further details of maintenance have been described elsewhere (Tucker, 1977*a*). To obtain adults of known age, last instar nymphs were separated from the stock culture and then used at a known time after the final moult. The fat body of each individual was then dissected out and analysed.

Na^+, K^+ and uric acid analyses

Tissue samples for Na^+ and K^+ determinations were dry-ashed after weighing and then analysed by flame photometry. Uric acid content of homogenates of fat body tissue was determined by the method of Appelt & Cvancara (1968). In this method comparisons are made of the absorbance at 293 nm of replicate samples of homogenate, one of which has been incubated with uricase, converting uric acid to allantoin, which does not absorb at 293 nm. Hog liver Type V uricase from the Sigma Chemical Company was used.

Microscopic examination of fat body tissue

Some fat body tissue samples were gently homogenized in saline in a ground glass homogenizer and then examined under a microscope (up to 1000×). Counts of some of the tissue constituents were then made with a haematocytometer. Smears of the tissue were examined. Other pieces of tissue were fixed for sectioning and staining. Many fixatives (see Results for list) were unsuitable for preserving urate inclusions. Methylene blue (Gabe, 1968) and Schultz's carmine-methylene blue (Lillie, 1965) were stains used to distinguish between uric acid and urate salts. For Schultz's method the time in the methylene blue solution was 3–5 min instead of the recommended 30 s.

RESULTS

Uric acid/urate analyses

In this paper, unless specifically mentioned, urate concentration refers to uric acid/urate as determined by the uricase assay. Concentrations of urate in the fat bodies of animals which had been on either a rat pellet + water diet, or a dextrin–casein + water diet for 7 weeks, are shown in Table 1. The effects on the urate content of dehydration, dehydration followed by a short period of rehydration and hydration are also shown. Deprivation of food and water and deprivation of food alone both significantly increase urate per unit weight of fat body. The absolute change in urate is not known because when animals are starved they metabolize food reserves in the fat body which causes an increase in the proportion of urate in the tissue, even if the total amount of urate is unchanged.

Relationship between Na^+, K^+ and urate

Although the dextrin/casein diet has a higher Na^+ content than the rat pellet one, fat body tissue from animals on a dextrin/casein diet had a lower Na^+ concentration (mean = 112·2 μ-mol/g tissue dry weight) than that from those fed with rat pellets (mean = 212·1 μ-mol/g). The differences in Na^+ concentration may be due to differences in the availability of water for the animals on the two diets. In the stock culture water was provided in a large flat container which was filled with cotton wool. The cotton wool usually dried out completely between the times when fresh water was added to the container (about once a week) and animals would thus have short periods (up to about 4 days) without water. Animals on the dextrin/casein diet were maintained in individual containers throughout the 7-week period on the experimental diet and were never without water during this time. Dehydration for 4–6 days

Table 1. *Uric acid content of the fat body tissue of adult male* P. americana *on two diets and in different states of hydration*

	Diet			
	Rat pellet		Dextrin/casein	
State of hydration	μ-mol/mg dry wt. Mean S.E.	n	μ-mol/mg dry wt. Mean S.E.	n
Normal	2·68 ± 0·45	6	2·81 ± 0·50	5
Dehydrated 6 days	3·50 ± 0·29	6	4·07 ± 1·14	5
Rehydrated 1 day (without food) after 6 days' dehydration	2·85 ± 0·37	6	4·12 ± 0·85	4
Hydrated	4·31 ± 0·57	5	—	

Table 2. *The ratio of K^+/urate in the fat body tissue of adult male* P. americana *in different states of hydration*

	Diet	
State of hydration	Rat pellet	Dextrin/casein
Normal	0·493 (6)	0·250 (5)
Dehydrated 6 days	0·297 (6)	0·156 (5)
Rehydrated 1 day (without food) after 6 days' dehydration	0·460 (6)	0·203 (4)
Hydrated	0·451 (5)	—

Numbers in parenthesis represent the numbers of individuals from which the mean value for the ratio was obtained. The K^+ content of the rat pellets was about 180 μ-mol/g while that of the dextrin/casein mixture was about 60 μ-mol/g.

increases the Na^+ in the fat body of *P. americana* (Tucker, 1977*c*). Many short periods without water, such as experienced by animals in the stock culture, may also cause a noticeable increase in the Na^+ in the fat body.

No correlation was found between the concentration of Na^+ and the amount of urate in the fat bodies of adult males. However, there was a positive and significant correlation between K^+ and urate in animals on both diets and in all states of hydration ($P \leqslant 0{\cdot}05$; Spearman rank correlation coefficient). The K^+/urate ratio was lower in dextrin/casein-fed animals than in the rat pellet-fed group which may be because the K^+ content of the dextrin-casein mixture was only one-third that of the rat pellets. The ratio of K^+/urate was lower in dehydrated animals than in normal, rehydrated or hydrated animals (Table 2).

As with the older males, young adult males (up to 4 days old) showed a positive correlation between K^+ and urate in the fat body, the Pearson correlation coefficient being 0·978. The mean urate and K^+ concentrations in the fat bodies of adults more than 2 weeks old were greater than the values for nymphs and adults up to 4 days old (see Fig. 1*a*), but there was no obvious change in the fat body Na^+, K^+ or urate concentrations at about the time of moulting. Clark & Smith (1967) found that a population of *Habrobracon* was heterogeneous with respect to the amount of urate material found in the abdomens of newly emerged adults. In the nymphs and young adults there was a slight but significant increase in Na^+ with increasing urate.

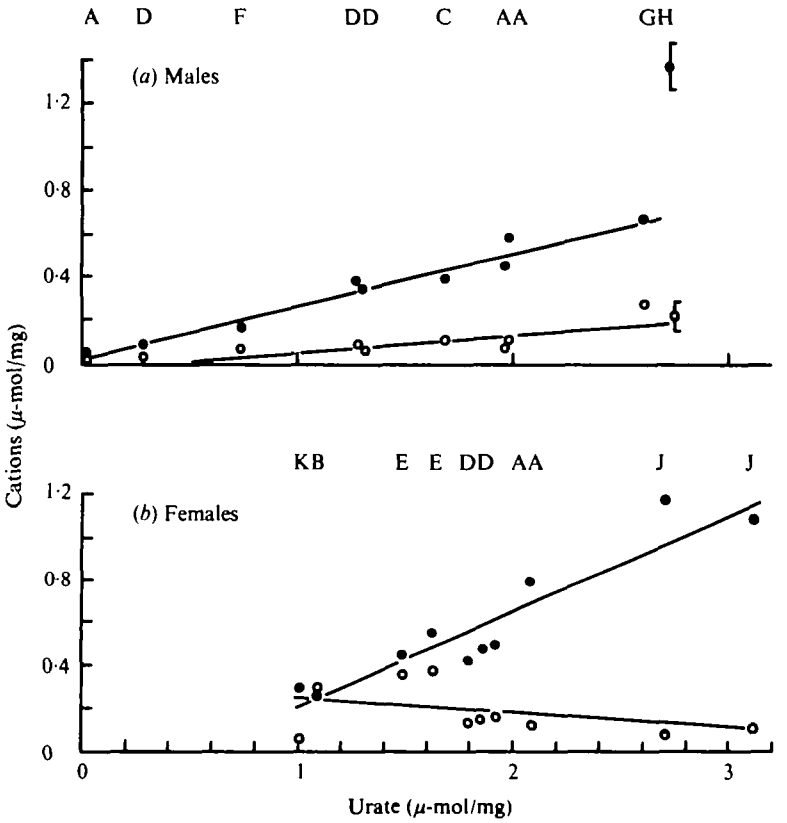

Fig. 1. Relationships between cations and urate in the fat body tissue of *P. americana* of different ages. Closed circles = K^+, Open circles = Na^+. Ages are: A = last instar nymph; B = last instar nymph just before final moult; C = 2 h-old adult; D = 1-day-old adult; E = 2-day-old adult; F = 3-day-old adult; G = 4-day-old adult; H = mean ± S.E. for adults > 2 weeks old; I = females carrying oothecae; J = 3-week-old female just after depositing ootheca.

The regression lines are: For males (omitting group of old adults), K^+: $y = 0{\cdot}245x - 0{\cdot}16$ (Pearson's $r = 0{\cdot}978$) and Na^+: $y = 0{\cdot}079x - 0{\cdot}020$ ($r = 0{\cdot}700$). For females, K^+: $y = 0{\cdot}450x - 0{\cdot}244$ ($r = 0{\cdot}931$) and Na^+: $y = -0{\cdot}071x + 0{\cdot}327$ ($r = -0{\cdot}429$).

In young females the ion content, particularly Na^+, in the fat body was more variable than in young males (Fig. 1*b*). Three of the females (one nymph just before the final moult and two adults which were 2 days old) had higher Na^+ levels and higher Na/K ratios than were ever found in normally hydrated males. Two adults which were carrying oothecae were found to have higher K^+ and urate than other females, and one which had just deposited an ootheca had a very low K^+ and urate. After a study on nitrogen balance in female *Periplaneta*, Mullins & Cochran (1975*b*) suggested that a portion of the uric acid nitrogen is utilized during egg production and may be incorporated into the ootheca. However, more detailed analyses of females with and without oothecae would have to be made to see to what extent the sexual cycle affects the level of cations and urate in the fat body. As with the males, there was a significant correlation between K^+ and urate (Pearson's $r = 0{\cdot}931$) but there was none between Na^+ and urate.

Fixation and appearance of fat body urate deposits

Uric acid is found in the fat body cells of *P. americana* in birefringent spherical inclusions as illustrated in Fig. 2 in Srivastava & Gupta's paper (1961). A variety of media dissolve or partially dissolve these spherules which makes many histological and histochemical methods unsatisfactory. (As the structure, chemical nature and function of these inclusions is still not known in detail, the word *spherule* (meaning simply 'little sphere') has been used to refer to all the cellular spherical inclusions discussed in this paper.) Dilute acids dissolve the spherules, and crystals, presumably mainly uric acid, separate out. If the fat body tissue is placed in mildly alkaline solutions, such as dilute ammonia or carbonate solutions, the spherules partially dissolve leaving a stroma, in which concentric rings are clearly visible. From this behaviour in acids and alkalis it appears that the spherules in the fat body of *Periplaneta* are very similar to the urate spheres in the urine of *Rhodnius* which were described by Wigglesworth (1931). The spherules are not fixed by formaldehyde or glutaraldehyde fixatives even when buffered. However, low temperature, making up the aldehyde in saline, and adding cobalt chloride to the fixative all delay solution of the spherules. Thus the structure of the spherules and other similar cellular inclusions are likely to be altered during some standard fixation procedures used for both light microscope and E.M. studies. Skaer & Lane (1974) commented on 'the highly structured appearance of fat droplets' in freeze-etched preparations of the fat body cells around the ventral nerve cord of *Periplaneta*, in comparison with fat droplets in the same tissue as seen in conventionally prepared ultra-thin sections. Inclusions with the highly structured appearance may be urate spherules, which are present in the fatty tissue on the nerve cord as well as in the free fat body (personal observation). Absolute alcohol and Carnoy's fluid are both suitable fixatives.

The main purpose of the histological study was to see if any changes in urate distribution could be seen in the fat body tissue of animals in different states of hydration, and to differentiate between the amount of free uric acid and urate salts present. Schultz's and Gabe's methods were employed to differentiate between free uric acid (blue-green with these staining procedures) and the urate salts (yellow-green). Sections and tissue smears from normal, dehydrated and rehydrated animals were stained in parallel. The blue-green 'uric acid-positive' material could readily be distinguished in fat body tissue from animals in all states of hydration. However, some highly refractile cellular inclusions appeared greenish even in unstained preparations. Thus it was impossible in such cases to say whether a yellow-green colour signified 'urate-positive' material, or merely the refractive nature of the material. This was particularly true for small amorphous granules, which were present in varying numbers in tissue from different individuals. In some of the larger spherules, the blue-green material appeared as a ring around the 'nucleus' of the spherules but in others, particularly in small ones, blue-green material was restricted to the central portion of the spherule. In most animals the blue-green (uric acid) material was restricted to the spherules, but in a few cases in rehydrated and hydrated animals some of the amorphous granular material also appeared blue-green. Tissues from animals in different states of hydration showed a wide range in the amounts of positively stained material. Thus, it was impossible with these histological methods to

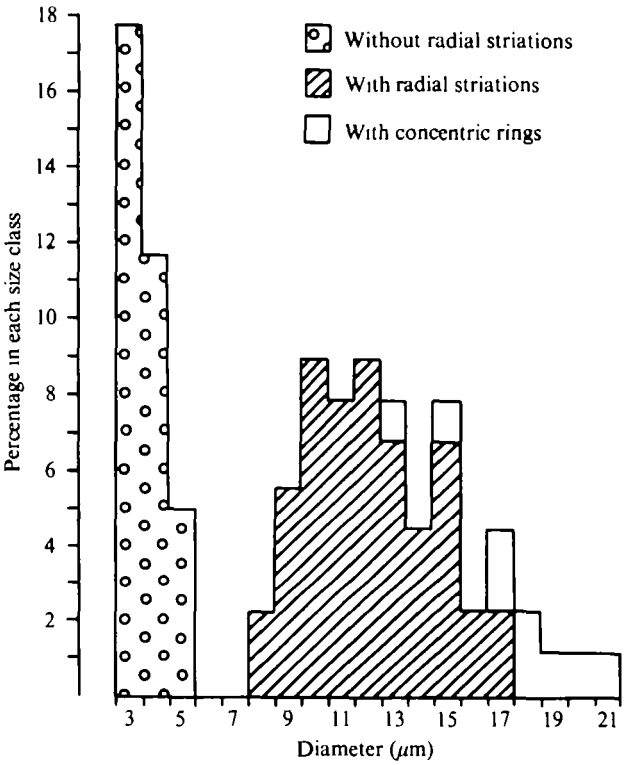

Fig. 2. Size of spherules in the fat body tissue of *P. americana*. The distribution shown is for an individual adult male which was 4 days old. $n = 60$.

detect any consistent differences in the distribution of uric acid and urate in normal, dehydrated and rehydrated animals.

Counts of the numbers of spherules were made on homogenates containing known weights of fat body tissue. The distribution of the sizes of three types of spherules in one individual are shown in Fig. 2. Spherule sizes in other animals showed similar distributions. Some amorphous granules greater than about 3 μm in diameter could clearly be distinguished from spherules of a similar size, but with the light microscope it was not possible to distinguish the spherules (which have a 'nucleus' of granular material) less than about 3 μm from the small amorphous highly refractile granules. Therefore, the lower limit for the size of the spherules is not known. Spherules with a diameter greater than about 6 μm differed from smaller spherules in having radial striations which may represent lines of fusion of small spherules. The bimodal distribution of the spherule size supports this hypothesis. The size of the spherules with the radial striations ranged from about 6–16 μm in diameter, with most of them being about 9–11 μm, although the mode varied slightly in different animals. A few even larger spherules (up to about 20 μm) were present in the tissues of most animals. Several concentric rings were visible in each large spherule.

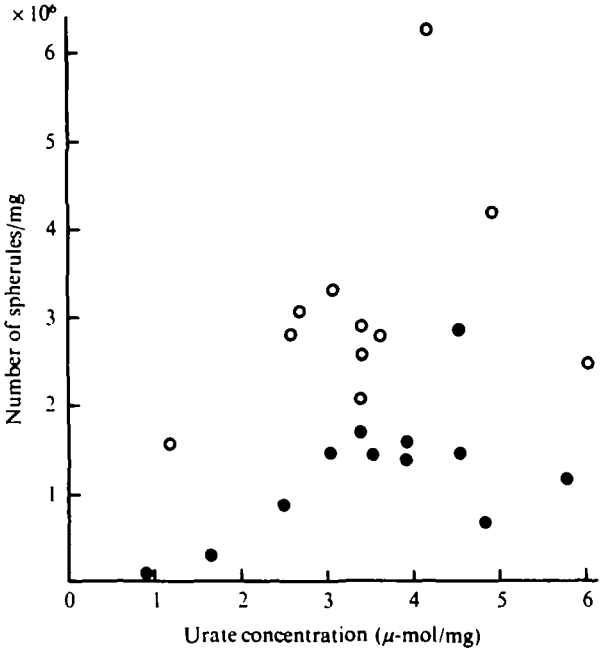

Fig. 3. The relationship between numbers of spherules and urate concentration in the fat body tissue of adult males. Open circles = hydrated animals; closed circles = dehydrated animals.

Relationship between spherules, cations and urate in the fat body

Because of the difficulty in distinguishing the smallest spherules from the amorphous granules, and because the largest spherules with the concentric rings were present in only small numbers, counts of only the medium-sized spherules with the radial striations (about 6–16 μm) were made in the investigation of the relationship between spherules and the urate and cation content of the fat body.

In the normal and hydrated groups of animals there was a correlation between the numbers of spherules and the urate content of the fat body (Spearman rank correlation test, $P = 0{\cdot}05$), but in dehydrated animals the correlation was not statistically significant at the 0·05 level. The ratio of spherules/urate was significantly higher in hydrated animals than in dehydrated ones ($P < 0{\cdot}001$). Fig. 3 shows the relationship between numbers of spherules and the urate concentration in the fat bodies of 11 dehydrated animals and 11 animals which had been kept with water but no food for 5 days.

There was no correlation between the number of spherules and the Na^+ concentration, but the positive correlation between the number of spherules and the K^+ concentration was highly significant ($P < 0{\cdot}001$, Spearman rank test). The relationship between the number of spherules and the K^+ concentration is shown in Fig. 4.

DISCUSSION

As Bursell (1970) pointed out, the widely used term 'storage excretion' may not be appropriate for the accumulation of uric acid in the fat bodies of insects. It is incorrect to think of the urate in the fat body as a deposit of nitrogenous waste which

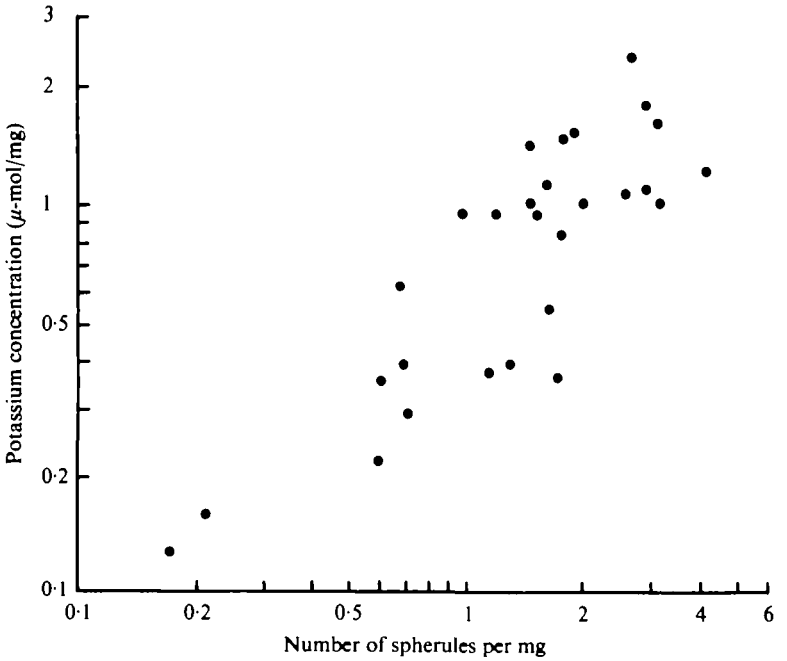

Fig. 4. The relationship between K^+ and number of spherules in the fat body tissue of adult males.

has been excreted and is no longer of use to the animal, because the urate can readily be mobilized when animals are on high-carbohydrate, low-protein diets (Gier, 1947; Haydak, 1953; McEnroe, 1957; Mullins & Cochran, 1975*b*). The extent of the importance of the bacteroids in utilization of the urate in the fat body is not known, but the bacteroids, when cultured on urate, have been shown to degrade it, eventually through to glycerate and the tricarboxylic acid cycle (Donellan & Kilby, 1967). Also bacteroids have the ability to synthesize amino acids from glucose and for this synthesis uric acid would be a possible source of nitrogen (Henry & Block, 1962; Lipke, Leto & Graves, 1965). Aposymbiotic cockroaches accumulate urate earlier than do normal animals (Brooks & Richards, 1956) and clearly urate utilization by the bacteroids is important and must be considered in a study of the overall urate metabolism in the cockroach.

Results obtained in the present study on urate concentrations in the fat bodies of animals with and without food and in different states of hydration, confirm the reports by Philiptschenko (1907), Bodenstein (1953) and Anderson & Patton (1955) that starvation of *P. americana* brings about an increase in the amount of urate per unit weight of fat body. However, in this study no total body urate estimations were made and one can only say that there is an increase in urate ***relative*** to the other constituents of the fat body. During starvation reserve foodstuffs are metabolized, and one would expect a decrease in the total mass of the fat body. Therefore, the present results do not necessarily conflict with those of Mullins & Cochran (1974), which showed that the total urate in semi-starved cockroaches remained relatively constant.

Uric acid selective staining methods (this work and Srivastava & Gupta, 1961) and the positive correlation found between the urate concentration and the numbers of spherules, indicate that much of the uric acid in the fat body cells of *P. americana* is present in the spherules. The accumulation of mineralized spherical inclusions in cells in a variety of tissues of many species has been reported (see e.g. Berkaloff, 1960; Wigglesworth & Salpeter, 1962; Gouranton, 1968; Krzysztofowicz, Jura & Bilinski, 1973; Turbeck, 1974; Ballan-Dufrançais, 1975). Walker (1965) suggested that inclusions with concentric lamellae in the fat body of starved *Blaberus* were derived from degenerating mitochondria. However, Gouranton (1968) and Ballan-Dufrançais (1975) considered that spherules arose from cisternae of the endoplasmic reticulum. Locke & Collins (1965) described the separation of isolation bodies within paired membranes in the fat body cells of *Calpodes* larvae and found that several of these could coalesce with fusion of their outer membranes. They considered that isolation within paired membranes derived from Golgi vesicles might be of general importance in separating regions of either massive lysis or massive sequestration. Even though the appearance of spherules found in a variety of tissues is very similar, their composition varies widely depending on the tissue and the physiological state of the animal at the time at which the spherules are formed.

Much work is still required in order to determine what controls the formation and composition of the spherules but, from the present study and from the results obtained by Ballan-Dufrançais (1975) and Mullins & Cochran (1974), it appears that when *P. americana* has an adequate supply of water, urate accumulates in the fat body cells in spherules which have a high K^+ content. The K^+/urate ratio of 0·33 found by Mullins & Cochran for adult males on a dog food diet was intermediate between the values of 0·49 and 0·25 found in this work for adult males on rat pellet and dextrin/casein diets, respectively.

In the present investigation an attempt was made to separate the spherules from the rest of the fat body tissue by differential centrifugation, so that isolated spherules could be analysed. However, the method used by Jungreis & Tojo (1973) for the separation of urate spheres in tissue homogenates from cecropia larvae was unsatisfactory because it caused the separation of some crystalline uric acid. Isosmotic lithium carbonate (a solvent for uric acid) caused a marked decrease in the K^+, but not the Na^+, content of the pellet of cellular constituents spun down at 20000 **g**, compared with a pellet from a replicate tissue sample homogenized in normal saline. However, incubation of intact fat body tissue in lithium carbonate solution caused no such large decrease in the K^+ content.

Because of the presence of both K^+ and urate in the spherules, one would expect that when urates are mobilized in animals fed on a pure carbohydrate diet some K^+ might also be released. Mullins & Cochran (1974) did find a higher than normal faecal/diet K^+ ratio in animals on a pure dextrin diet. The decrease in the proportion of urate present in spherules in dehydrated animals, as compared with hydrated ones, and the decrease in K^+ in the fat body which is found in dehydrating animals (Tucker, 1977*a*), suggest that urate and K^+ are mobilized from the spherules when animals are dehydrated. However, Mullins & Cochran (1974) found that there was little change in the total urate in animals when they were semi-starved. Urate per unit weight of fat body is higher in unfed, dehydrated animals than in normally fed, hydrated indi-

viduals. Therefore, although dehydration and/or starvation may bring about mobilization of urate in the spherules, to maintain the total urate content in dehydrated and starved animals, urate must be deposited somewhere other than in spherules. During dehydration the urate may be deposited in the small amorphous granules which gave a positive reaction with the Schultz and Gabe staining methods for uric acid. In colloidal solutions urate can be in excess of the solubility level without being precipitated. Porter (1963) found that if the concentration of Mg^{2+}, Na^{+} or K^{+} was increased in such a solution it caused flocculation, always in the form of sodium urate. Deposition of urate as sodium urate during dehydration would be consistent with the finding that the fat body Na^{+} increases when an animal is dehydrated (Tucker, 1977*b*, *c*). However, the urate need not be in the form of a normal monobasic urate salt. Lonsdale & Sutor (1971), from X-ray diffraction studies, came to the conclusion that the excrement from a budgerigar consisted of mainly uric acid dihydrate, present along with some soluble material including mineral ash. They found that initially the uric acid was in a smectic form and only when it aged or when soluble components were removed with water or dilute acids did it transform into uric acid dihydrate crystals. So also, in the fat body of *P. americana*, urate may be present as uric acid in a non-crystalline form in close association with cations and some organic substances.

Hopkins & Lofgren (1968) found that, in the cockroach *Leucophaea*, bound urates in the fat body always migrated with a ninhydrin-positive substance, possibly a peptide or amino acid. Ballan-Dufrançais (1975) thought that the organic stroma of the mineralized spheres in *P. americana* was a glycoprotein. With the use of electron microscope, X-ray microprobe, X-ray diffraction and cytochemical techniques, she made a detailed study of spherical inclusions in many tissues of several insect species. In discussing her results, she stressed the importance of bioaccumulation of minerals and purines in maintaining constancy of the *milieu intérieur*. Hydromineral regulation by Malpighian tubules and rectum is well known to be under hormonal control (see e.g. Wall, 1967; Cazal & Girardie, 1968; Maddrell, 1971) but, although it is known that urates disappear from the cells of the fat body after removal of the corpora allata and corpora cardiaca and that reimplantation of the corpora cardiaca restores urate deposition (Bodenstein, 1953), the extent to which the cation–urate balance in the fat body is under hormonal control remains to be elucidated.

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REFERENCES

ANDERSON, A. D. & PATTON, R. L. (1955). *In vitro* studies of uric acid synthesis in insects. *J. exp. Zool.* **128**, 443–451.

APPELT, A. W. & CVANCARA, V. A. (1968). An improved procedure for the determination of uric acid in biological homogenates. *Analyt. Biochem.* **25**, 588–590.

BALLAN-DUFRANÇAIS, C. (1975). Bioaccumulation minérale, purique et flavinique chez les insectes. Thèse, Université Pierre et Marie Curie.

BERKALOFF, A. (1960). Contribution a l'étude des tubes de Malpighi et de l'excrétion chez les insectes. *Annls Sci. nat. (Zool.) sér. 12* **2**, 869–947.

BODENSTEIN, D. (1953). Studies on the humoral mechanisms in growth and metamorphosis of the cockroach *Periplaneta americana*. III. Humoral effects on metabolism. *J. exp. Zool.* **124**, 105–115.

BROOKS, M. A. & RICHARDS, A. G. (1956). Intracellular symbiosis in cockroaches. III. Reinfection of aposymbiotic cockroaches with symbiotes. *J. exp. Zool.* **132**, 447–465.

BURSELL, E. (1967). The excretion of nitrogen in insects. *Adv. Insect Physiol.* **4**, 33–67.
BURSELL, E. (1970). *An Introduction to Insect Physiology*. London and New York: Academic Press.
CAZAL, M. & GIRARDIE, A. (1968). Contrôle humoral de l'équilibre hydrique chez *Locusta migratoria migratoriodes*. *J. Insect Physiol.* **14**, 655–668.
CLARK, A. M. & SMITH, R. E. (1967). Urate accumulation and adult life span in two species of *Habrobracon*. *Exp. Gerontol.* **2**, 217–226.
CUÉNOT, L. (1895). Études physiologiques sur les Orthoptères. *Archs Biol. Paris* **14**, 293–341.
DONNELLAN, J. F. & KILBY, B. A. (1967). Uric acid metabolism by symbiotic bacteria from the fat body of *Periplaneta americana*. *Comp. Biochem. Physiol.* **22**, 235–252.
FAUSSEK, V. (1911). Vergleichend-embryologische Studien. *Z. wiss. Zool.* **98**, 529–625.
GABE, M. (1968). *Techniques Histologiques*. Paris: Masson.
GIER, H. T. (1947). Growth rate in the cockroach *Periplaneta americana* (L.). *Ann. ent. Soc. Am.* **40**, 303–317.
GOURANTON, J. (1968). Composition, structure, et mode de formation des concrétions minérales dans l'intestin moyen des homoptères cercopides. *J. Cell Biol.* **37**, 316–328.
HAYDAK, M. H. (1953). Influence of protein level of the diet on longevity of cockroaches. *Ann. ent. Soc. Am.* **46**, 547–560.
HENRY, S. M. & BLOCK, R. J. (1962). Amino acid synthesis, a rumen-like effect of the intracellular symbionts of the German cockroach. *Fedn Proc. Fedn. Am. Socs exp. Biol.* **21**, 9.
HILLIARD, S. D. & BUTZ, A. (1969). Daily fluctuations in the concentrations of total sugar and uric acid in the hemolymph of *Periplaneta americana*. *Ann. ent. Soc. Am.* **62**, 71–74.
HOPKINS, T. L. & LOFGREN, P. A. (1968). Adenine metabolism and urate storage in the cockroach, *Leucophaea maderae*. *J. Insect Physiol.* **14**, 1803–1814.
JUNGREIS, A. M. & TOJO, S. (1973). Potassium and uric acid content in tissues of the silkmoth *Hyalophora cecropia*. *Am. J. Physiol.* **224**, 21–26.
KRZYSZTOFOWICZ, A., JURA, C. & BILINSKI, S. (1973). Ultrastructure of midgut epithelial cells of *Tetrodontophora bielanensis* (Waga) (Collembola). *Acta biol. cracov. Ser. zool.* **16**, 257–265.
LILLIE, R. D. (1965). *Histopathologic Technic and Practical Histochemistry*, 3rd ed. New York: McGraw-Hill.
LIPKE, H., LETO, S. & GRAVES, B. (1965). Carbohydrate–amino acid conversions during cuticle synthesis in *Periplaneta americana*. *J. Insect Physiol.* **11**, 1225–1232.
LOCKE, M. & COLLINS, J. V. (1965). The structure and formation of protein granules in the fat body of an insect. *J. Cell Biol.* **26**, 857–884.
LONSDALE, K. & SUTOR, D. J. (1971). Uric acid dihydrate in bird urine. *Science, N.Y.* **172**, 958–959.
MCENROE, W. D. (1957). Uric acid metabolism in the American cockroach. *Diss. Abstr.* **17**, 2338.
MADDRELL, S. H. P. (1971). The mechanisms of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.
MULLINS, D. E. (1974). Nitrogen metabolism in the American cockroach: an examination of whole body ammonium and other cations excreted in relation to water requirements. *J. exp. Biol.* **61**, 541–556.
MULLINS, D. E. & COCHRAN, D. G. (1973). Nitrogenous excretory materials from the American cockroach. *J. Insect Physiol.* **19**, 1007–1018.
MULLINS, D. E. & COCHRAN, D. G. (1974). Nitrogen metabolism in the American cockroach: an examination of whole body and fat body regulation of cations in response to nitrogen balance. *J. exp. Biol.* **61**, 557–570.
MULLINS, D. E. & COCHRAN, D. G. (1975*a*). Nitrogen metabolism in the American cockroach. I. An examination of positive nitrogen balance with respect to uric acid stores. *Comp. Biochem. Physiol.* **50A**, 489–500.
MULLINS, D. E. & COCHRAN, D. G. (1975*b*). Nitrogen metabolism in the American cockroach. II. An examination of negative nitrogen balance with respect to mobilization of uric acid stores. *Comp. Biochem. Physiol.* **50A**, 501–510.
NATION, J. L. & PATTON, R. L. (1961). A study of nitrogen excretion in insects. *J. Insect Physiol.* **6**, 299–308.
PHILIPTSCHENKO, J. A. (1907). Ueber den Fettkörper der schwarzen Küchenschabe (*Stylopyga orientalis*). *Revue Russe Entom.* **7**, 188–189.
PORTER, P. (1963). Physico-chemical factors involved in urate calculus formation. II. Colloidal flocculation. *Res. vet. Sci.* **4**, 592–602.
SKAER, H. LE B. & LANE, N. J. (1974). Functional complexes, perineurial and glia-axonal relationships and the ensheathing structures of the insect nervous system; a comparative study using conventional and freeze-cleaving techniques. *Tissue & Cell* **6**, 695–718.
SRIVASTAVA, P. N. & GUPTA, P. D. (1961). Excretion of uric acid in *Periplaneta americana* L. *J. Insect Physiol.* **6**, 163–167.
TUCKER, L. E. (1977*a*). Effect of dehydration and rehydration on the water content and Na^+ and K^+ balance in adult male *Periplaneta americana*. *J. exp. Biol.* **71**, 49–66.

TUCKER, L. E. (1977*b*). The influence of age, diet and lipid content on survival, water balance and Na^+ and K^+ regulation in dehydrating cockroaches. *J. exp. Biol.* **71**, 67–79.

TUCKER, L. E. (1977*c*). Regulation of ions in the haemolymph of the cockroach *Periplaneta americana* during dehydration and rehydration. *J. exp. Biol.* **71**, 81–93.

TURBECK, B. O. (1974). A study of the concentrically laminated concretions, 'spherites', in the regenerative cells of the midgut of lepidopterous larvae. *Tissue & Cell* **6**, 627–640.

WALKER, P. A. (1965). The structure of the fat body in normal and starved cockroaches as seen with the electron microscope. *J. Insect Physiol.* **11**, 1625–1631.

WALL, B. J. (1967). Evidence for antidiuretic control of rectal water absorption in the cockroach *Periplaneta americana* L. *J. Insect Physiol.* **13**, 565–578.

WHARTON, D. R. & WHARTON, M. L. (1961). Effects of radiation on nitrogen and phosphorus excretion by the cockroach, *Periplaneta americana* (L.). *Rad. Res.* **14**, 432–443.

WIGGLESWORTH, V. B. (1931). The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). I. Composition of the urine. *J. exp. Biol.* **8**, 411–427.

WIGGLESWORTH, V. B. & SALPETER, M. M. (1962). Histology of the Malpighian tubules in *Rhodnius prolixus* Stål (Hemiptera). *J. Insect Physiol.* **8**, 299–307.