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# THE INFLUENCE OF AGE, DIET AND LIPID CONTENT ON SURVIVAL, WATER BALANCE AND $Na^+$ AND $K^+$ REGULATION IN DEHYDRATING COCKROACHES

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SUMMARY

In dehydrating conditions late instar nymphs lose weight more slowly and survive longer than adult *Periplaneta americana*. This appears to be due, at least in part, to the larger lipid stores which are found in the fat bodies of the nymphs. When the water loss from an animal is greater than the amount of metabolic water obtained from the catabolism of stored foods, water is removed from the haemolymph in order to maintain water balance in the tissues. Dehydration for 6 days causes the haemolymph volume to decrease markedly in most adults, but the haemolymph $Na^+$ and $K^+$ concentrations increase only slightly. During dehydration the mean $Na^+/K^+$ ratio of the fat body tissue increased in adults, except in those which had been fed on pure carbohydrate prior to dehydration. Although not always statistically significant on account of the large variances, the changes in mean $Na^+$, $K^+$ and in the $Na^+/K^+$ ratio suggest there is an increase in $Na^+$ and decrease in $K^+$ in the fat body of animals where the haemolymph volume is markedly reduced by dehydration.

## INTRODUCTION

Edney (1968) and Wall (1970) found that when the cockroach *Periplaneta americana* was dehydrated the haemolymph volume decreased by about 50% within a week, but there was only a slight increase in its osmotic pressure. It was suggested (Pichon, 1963, 1970; Wall, 1970) that during dehydration ions, especially $Na^+$ ions, might be stored somewhere in the tissues and become quickly available when rehydration caused a large increase in haemolymph volume. Tucker (1977*a*) found that dehydration caused an increase and rehydration a decrease in the mean $Na^+/K^+$ ratio of the fat body tissue of adult male *P. americana*. This finding was consistent with the hypothesis that the fat body helped to regulate the haemolymph $Na^+$ concentration by taking up $Na^+$ when an animal was dehydrated and then releasing it to help to maintain the normal ion concentration in the haemolymph when an animal was rehydrated. However, it was also found that the $Na^+$ and $K^+$ concentrations in the fat body tissue of normally fed and hydrated adult male cockroaches showed great variability, ranging from 38 to 252 $\mu$-mol/g wet tissue weight for $Na^+$ and from 216 to 1046 $\mu$-mol/g for $K^+$. Because of the variability in the fat body $Na^+$ and $K^+$ values, and because of unquantified changes in the amount of food reserves in the tissue, the real changes in the $Na^+$ and $K^+$ content in the fat body were still not clear.

With the aim of reducing both the variance and the absolute concentrations of the $Na^+$ and $K^+$ in the fat body, groups of adult male *P. americana* have been kept on restricted diets before being used in dehydration experiments. A reduction in the absolute amount of $Na^+$ and $K^+$ in the tissue was thought to be desirable because with low initial concentrations of ions any changes in the concentrations brought about by dehydration would be more pronounced than in tissues with higher ion content.

It had previously been noticed (personal observations) that last instar nymphs and very young adults usually had less $Na^+$ and $K^+$ in their fat bodies than did adults and Pichon (1963) noted that nymphs withstood dehydration better than adults. Therefore, nymphs and young adults, as well as older adults, were used in some experiments. From the experiments described in this paper it was hoped, firstly, to test the validity of the hypothesis that during dehydration the fat body of cockroaches takes up excess $Na^+$ ions from the haemolymph and, secondly, to see whether animals of different age and lipid content showed similar changes in their water and ion balance during desiccation.

## MATERIALS AND METHODS

Male cockroaches (*P. americana*) were used in all experiments. Several days before the beginning of any experiment animals were separated from the stock culture and placed in individual clear polystyrene containers, which were then kept in thermostatically controlled cabinets set at 27 °C.

Before being placed on experimental diets, animals were kept with water but no food for 5 days and then during the time they were on restricted diets they were allowed food and water *ad libitum*. The diets consisted of (*a*) rat pellets (also the food for the stock culture); (*b*) dextrin (carbohydrate) for 2 weeks; (*c*) casein (protein) for 2 weeks; (*d*) dextrin/casein/casein hydrolysate in the proportions 2:1:1 for 7 weeks (abbreviated to dextrin/casein in the text). Dehydration was achieved by withholding both food and water from the animals and during rehydration they were given ashless floc soaked with distilled water.

For $Na^+$ and $K^+$ analyses, haemolymph samples were collected in microcaps (Drummond Scientific Company) and other tissues were weighed immediately after dissection, dried for 24 h at 105 °C and then weighed again before being dry-ashed at 450 °C. $Na^+$ and $K^+$ concentrations were measured by flame photometry.

Estimates of total neutral lipid were made from weight differences before and after extraction with three changes of acetone at room temperature.

## RESULTS

Data obtained from some animals in the experimental groups are not included in the results presented here, because when they were dissected they were found to be infected with the sporozoan *Diplocystis schneideri* (see Jameson, 1920). In some of these animals the abdominal cavity was found to be packed with flaccid, ovoid sporocysts. Even though heavily infected animals did not exhibit any abnormal behaviour and showed weight decreases with dehydration similar to those of non-infected individuals, the parasite may have caused changes in the ion balance and haemolymph volume. Therefore, results from animals found to be infected were discarded, and

Table 1. *Mean values for the $Na^+$ and $K^+$ content of various foods used in the diets for* P. americana

| Food | $Na^+$ ($\mu$-mol/g) | $K^+$ ($\mu$-mol/g) | $Na^+/K^+$ |
|---|---|---|---|
| Rat pellet | 101·8 | 180·4 | 0·6 |
| Dextrin | 4·4 | 2·1 | 2·1 |
| Casein | 384·6 | 12·4 | 1·0 |
| Casein hydrolysate | 648·0 | 234·0 | 2·8 |

this means that data quoted for some of the experimental groups are for only a few animals.

*Foods.* The $Na^+$ and $K^+$ content of the foods used for the four diets are shown in Table 1. It can be seen that the four foods vary greatly in ion concentration, as well as in organic content. Because of the obvious dietary deficiencies of (*b*), (*c*) and (*d*), animals would not be expected to maintain their normal metabolism after extended periods on these foods but, during the short-term experiments used here, although a few animals died, this was also true for groups of animals on the rat pellet diet and the animals from which the data were obtained all showed normal activity throughout the course of the experiments. When given food after the 5-day period with only water, animals ate immediately and during the 2 weeks on the dextrin diet individuals gained weight by an average 5% of the initial body weight. Casein-fed animals showed a gain of about 3% over 2 weeks and animals fed with the dextrin/casein diet for 7 weeks increased by a mean 12·4%. Two animals died while they were on the dextrin/casein diet and they were two which had shown much greater than average weight gains – one had increased its body weight by 30% after 4 weeks on the diet and the other had shown an increase of 47% after 7 weeks.

*Weight changes during dehydration and rehydration.* Curves showing the weight loss caused by dehydration of previously normally fed nymphs, young adults and adults greater than 2 weeks old are shown in Fig. 1. During the first day or two of dehydration the nymphs lost weight more quickly than adults (probably due to their greater activity), but thereafter they showed a significantly lower rate of weight loss than the adults. Also, nymphs survived desiccation for much longer periods than adults, one last instar nymph surviving for 58 days without food or water. Under the same conditions, old adults died within 2–3 weeks. Generally, young adults showed weight losses and survival times intermediate between the nymphs and older adults. The nymphs, as well as showing less than the normal adult weight loss during dehydration, did not drink water as readily as the adults when they were provided with water after a period of dehydration. Weight gains of both nymphs and adults given food and water after a period of dehydration are also shown in Fig. 1.

Table 2 shows weight losses brought about by 6 days' dehydration of animals which had been fed on different diets prior to dehydration. Weight losses of adults which had been on either a dextrin or a casein diet for 2 weeks prior to dehydration were not significantly different from those of animals which had been kept on the normal rat pellet diet, but animals which had been on a dextrin/casein diet for 7 weeks lost significantly more weight. It is quite likely that this increased weight loss is due to abnormal metabolism caused by deficiencies in the diet.

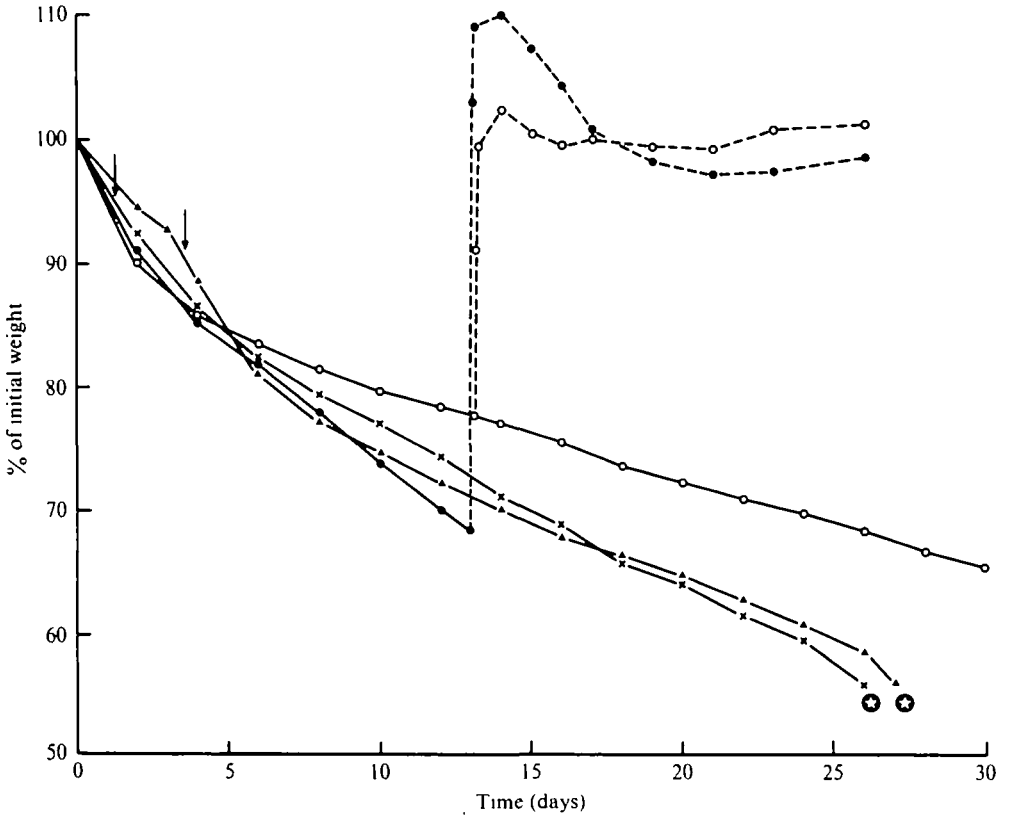


Fig. 1. The effect of dehydration and rehydration on the body weight of male *Periplaneta americana* of different ages. Solid lines represent dehydration, dashed lines rehydration. Closed circles = adults ($n$ = 10 for dehydration, $n$ = 4 for rehydration). Open circles = last instar nymphs ($n$ = 8 for first 13 days of dehydration and $n$ = 4 for continued dehydration and for rehydration). Triangles and crosses = individuals in which the final moult took place within the first few days of dehydration. Arrows mark the time of the moult and stars where the individuals died.

*Total lipid and water content.* Juvenile cockroaches accumulate lipid reserves in their fat bodies and by the time an animal has reached the final nymphal instar the abdomen is usually packed with fat body tissue which is cream, translucent and high in lipid. Even though the fat body may still be large in adults, they usually have considerably less lipid than the late instar nymphs. As the animals age, and particularly if they are on a high-protein diet, uric acid and/or urate salts are deposited in the fat body and the tissue becomes white and dense.

The total neutral lipid content in normally hydrated adults was highest in those which had been on a carbohydrate diet, but it was still not as high as in the normally fed, last instar nymphs. It was lowest in adults which had been fed with protein (Table 3). During dehydration, or when animals were kept with water but no food, lipid reserves were used. The lipid content of dehydrated, starved animals increased quickly when they were allowed food and water again.

Generally, normally hydrated animals with large lipid reserves had a lower percentage of total body water than did animals with small lipid reserves (Fig. 2). This

Table 2. *Effect of age and diet on the body weight changes of* P. americana *caused by* 6 *days' dehydration*

| Life stage | Diet before dehydration | *n* | Weight after dehydration as percentage of initial weight: Mean | S.E. |
|---|---|---|---|---|
| Last instar nymphs | Rat pellet | 10 | 83·96 | 1·09 |
| Adult | Rat pellet | 10 | 81·68 | 0·51 |
| | Dextrin | 6 | 82·56 | 0·92 |
| | Casein | 5 | 77·66 | 1·86 |
| | Dextrin/casein | 6 | 74·02 | 0·93 |

Table 3. *Lipid content of male* P. americana *after different diets and in various states of hydration. Values are expressed as a percentage of the total dry weight*

| | | Diet: Rat pellet | Dextrin | Casein |
|---|---|---|---|---|
| Life stage | State of hydration | Mean ± S.E. (*n*) | Mean ± S.E. (*n*) | Mean ± S.E. (*n*) |
| Last instar nymph | Normal | 24·4 ± 2·7 (4) | — | — |
| Adult | Normal | 13·4 ± 1·3 (4) | 17·8 ± 3·3 (6) | 5·4 ± 1·3 (6) |
| | Dehydrated 6 days | 6·4 ± 1·7 (10) | 13·7 ± 2·0 (6) | 3·0 ± 0·3 (6) |
| | Dehydrated 13 days | 4·8 ± 1·3 (6) | — | — |
| | Rehydrated (with food) 13 days | 15·0 ± 1·3 (4) | — | — |
| | Water only 5 days | 7·5 ± 2·1 (4) | — | — |

is because of the extremely low water content of high-lipid fat body tissue, compared with other tissues, including low-lipid fat body tissue. Thus, total body water by itself is not a good indicator of the state of hydration of an animal. A hydrated animal with a large, high-lipid fat body (such as a last-instar nymph) could well have a lower water content, expressed as a percentage of the live weight, than a partially dehydrated animal with a small, low-lipid fat body. The mean total water content for normally hydrated adults which had been on a diet of rat pellets was 68·6%, while values for those on dextrin and casein diets were 68·3 and 69·8%, respectively.

*Haemolymph $Na^+$ and $K^+$.* The effects of artificial diets and dehydration on the concentrations of $Na^+$ and $K^+$ in the haemolymph of adult males are shown in Table 4. When animals were placed on a dextrin diet, which had very low $Na^+$ and $K^+$, there were decreases in the concentrations of these ions in the haemolymph. However, when animals were dehydrated, the $Na^+$ and $K^+$ in the haemolymph of dextrin-fed animals increased proportionately more than in the normally fed ones, resulting in similar concentrations of $Na^+$ and $K^+$ in the haemolymph of both groups of dehydrated animals. Animals fed on casein and dextrin/casein had a haemolymph $Na^+$ concentration which was slightly lower and $K^+$ which was slightly higher than normal. As with normally fed animals, the small increase in the concentrations of these ions and the noticeable decrease in haemolymph volume which occurred during dehydration, indicated that both $Na^+$ and $K^+$, particularly $Na^+$, must have been removed from the haemolymph during dehydration. For all diets, the range in concentrations of ions in the haemolymph of dehydrated animals was greater than in the normally

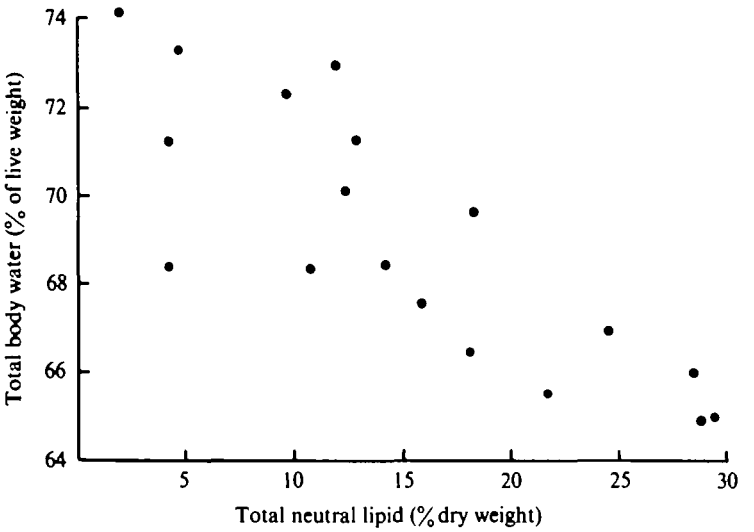


Fig. 2. The relationship between total body water and total body neutral lipid in fed, hydrated male *P. americana*.

Table 4. *The effect of dehydration on the $Na^+$ and $K^+$ concentrations in the haemolymph of adult male* P. americana *on different diets*

| | | State of hydration | |
|---|---|---|---|
| | | Normal | Dehydration 6 days |
| Ion | Diet | Mean S.E. | Mean S.E. |
| $Na^+$ ($\mu$-mol/ml) | Rat pellet | 127·7 ± 4·0 | 137·7 ± 2·9 |
| | Dextrin | 108·8 ± 0·5 | 136·4 ± 14·4 |
| | Casein | 120·2 ± 3·6 | 127·7 ± 7·4 |
| | Dextrin/casein | 115·0 ± 0·9 | 125·0 ± 3·0 |
| $K^+$ ($\mu$-mol/ml) | Rat pellet | 8·3 ± 0·5 | 11·7 ± 3·2 |
| | Dextrin | 6·4 ± 0·2 | 14·6 ± 3·0 |
| | Casein | 11·2 ± 1·0 | 18·1 ± 3·7 |
| | Dextrin/casein | 10·4 ± 2·6 | 14·2 ± 1·6 |

hydrated groups, but the variability in $Na^+$ concentration was particularly noticeable in animals which had been on a dextrin diet. The two of these dehydrated animals which showed the highest $Na^+$ in the haemolymph (178 $\mu$-mol/ml and 131 $\mu$-mol/ml) had the lowest concentrations of $Na^+$ in the fat body.

*Fat body $Na^+$ and $K^+$.* The results of ion analyses of the fat body tissue of adults on different diets, and the effects of dehydration, are summarized in Table 5. Animals which had been fed on either the dextrin diet or the dextrin/casein diet had lower than normal $Na^+$ and $K^+$ in their fat bodies, while the casein-fed group showed a reduction in $K^+$, but not in $Na^+$. Animals fed with dextrin with added NaCl and KCl, to give the same concentration of $Na^+$ and $K^+$ as the rat pellets, still showed a reduction in fat body $Na^+$ and $K^+$, although the reduction was not as great as in those which had been on a salt-free dextrin diet. With dehydration, the mean $Na^+$ (expressed on a tissue dry weight basis) showed an increase in the normal, casein and dextrin/casein

Table 5. *Effect of diet and of 6 days' dehydration on the $Na^+$ and $K^+$ concentrations in the fat body of adult male* P. americana

| | $Na^+$ | | $K^+$ | | |
|---|---|---|---|---|---|
| | $\mu$-mol/g wet wt. | $\mu$-mol/g dry wt. | $\mu$-mol/g wet wt. | $\mu$-mol/g dry wt. | $Na^+/K^+$ |
| Rat pellet | | | | | |
| Normal ($n$ = 12) | 133·2 ± 17·6 | 210·4 ± 29·3 | 852·2 ± 43·7 | 1309·4 ± 62·0 | 0·16 ± 0·02 |
| Dehydrated ($n$ = 9) | 153·5 ± 26·7 | 233·1 ± 51·7 | 609·5 ± 103·4 | 831·1 ± 124·4 | 0·33 ± 0·08 |
| Dextrin | | | | | |
| Normal ($n$ = 4) | 68·9 ± 21·5 | 128·3 ± 45·9 | 250·6 ± 58·4 | 459·4 ± 143·2 | 0·36 ± 0·12 |
| Dehydrated ($n$ = 5) | 106·8 ± 17·3 | 134·3 ± 18·8 | 347·7 ± 93·9 | 432·8 ± 99·5 | 0·36 ± 0·05 |
| Casein | | | | | |
| Normal ($n$ = 6) | 196·9 ± 51·5 | 306·4 ± 82·6 | 590·3 ± 43·3 | 919·4 ± 59·0 | 0·33 ± 0·08 |
| Dehydrated ($n$ = 5) | 250·2 ± 57·3 | 417·8 ± 84·9 | 544·2 ± 56·1 | 941·1 ± 90·0 | 0·44 ± 0·06 |
| Dextrin/casein | | | | | |
| Normal ($n$ = 5) | 68·0 ± 9·2 | 112·3 ± 18·5 | 444·4 ± 41·4 | 702·3 ± 63·7 | 0·16 ± 0·03 |
| Dehydrated ($n$ = 5) | 111·1 ± 23·8 | 185·8 ± 35·9 | 346·5 ± 151·9 | 579·8 ± 265·4 | 0·47 ± 0·11 |

All values are mean ± S.E.

fed animals, but little change in those which had fed on dextrin. $K^+$ values in all groups showed great variability and although dehydration caused a significant decrease in $K^+$ in normally fed animals, in the groups which had been on dextrin or casein diets dehydration did not cause any significant change in the mean $K^+$ content. In the animals which had been on a dextrin/casein diet, dehydration caused a decrease in mean $K^+$, but there was a very large variance in the values for the dehydrated animals and the difference between the mean $K^+$ for normally hydrated and dehydrated animals was not statistically significant. However, the large variance in the dehydrated group was caused mainly by a single animal which had an abnormally high $K^+$ concentration (mean ± S.E. for a triplicate sample = 1628·7 ± 6·0 $\mu$-mol/g tissue dry weight) compared with a mean value of 317·6 ± 50·6 $\mu$-mol/g for the other four animals in the group. There is a correlation between the $K^+$ concentration in the fat body and the urate content (Mullins & Cochran, 1974; Tucker, 1977*b*) and the above-mentioned animal with the very high $K^+$ had an exceptionally high concentration of urate in the fat body. Even though the $K^+$ concentration in the fat body of this abnormal, dehydrated individual was greater than the mean value obtained for the normally hydrated animals which had been on a dextrin/casein diet, it was considerably lower than would be expected in a normally hydrated animal with the same urate concentration.

Changes in the $Na^+/K^+$ ratio of the tissue are shown in the right hand column of Table 5. There is an increase in the mean $Na^+/K^+$ in all groups of dehydrated animals, except for those which had been fed with dextrin. However, the increase is statistically significant at the 0·05 level only for the animals which had been on the dextrin/casein diet prior to dehydration ($P < 0·001$). For the rat pellet-fed animals $0·06 > P > 0·05$ and for casein-fed ones $P = 0·3$.

It was noticed that animals which had high concentrations of $Na^+$ and $K^+$ in their fat bodies were often large individuals and data from the 12 normally hydrated animals on the rat pellet diet were tested to see if there was any correlation between body

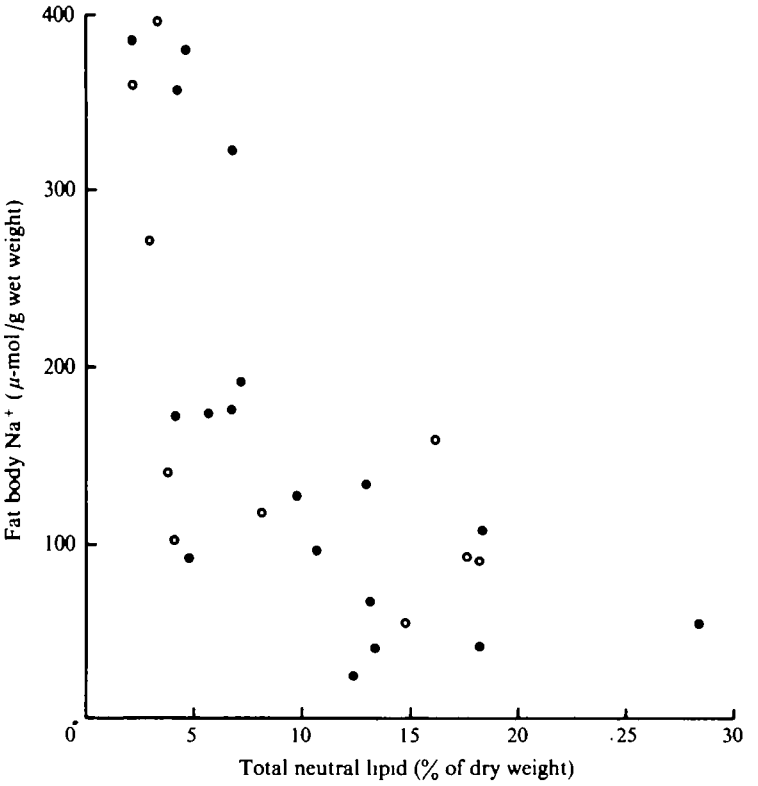


Fig. 3. Relationship between fat body $Na^+$ and whole body neutral lipid in male *P. americana*. Closed circles = normally hydrated animals. Open circles = dehydrated animals.

weight and the $Na^+$ and $K^+$ content of the fat body. For $Na^+$, the Spearman rank correlation cofficient ($r_s$) was 0·655, which indicates that there is a correlation between body weight and the fat body $Na^+$ concentration ($P < 0{\cdot}05$). The $r_s$ for $K^+$ and body weight was found to be 0·664, again significant at the 0·05 level. There was no correlation between the $Na^+/K^+$ ratio in the fat body and the weight of the animal. It appears, therefore, that both $Na^+$ and $K^+$ accumulate in the fat body as an animal ages.

During analyses of fat bodies it was observed that animals which had a high lipid content never had a high concentration of $Na^+$ in the fat body, even after 6 days' dehydration, while those which had the highest fat body $Na^+$ were those with very low total neutral lipid (see Fig. 3). Again, this indicates that $Na^+$ gradually accumulates in the fat body as an animal ages, because it is the young animals which have large lipid stores and the old ones which have large urate deposits and little reserve lipid.

## DISCUSSION

The influence of organic constituents of the diet on the growth and life span of *P. americana* has been studied by Zabinski (1929), Gier (1947) and Haydak (1953). Melampy & Maynard (1937) investigated the effects of a variety of diets on *Blattella*

*germanica* and Lafon (1951) studied the protein requirements of *Blatta orientalis*. Lafon said that casein was a complete and balanced dietary protein and it has been stated that both *B. germanica* (Melampy & Maynard, 1937) and *P. americana* (Schweet, 1941, cited by Scoggin & Tauber, 1950) are able to synthesize fat from protein. However, Melampy & Maynard concluded that milk products were unsatisfactory as the sole food for growing cockroaches. Also Gier found that cockroaches on a pure protein diet lived only until the supply of fat in their bodies was exhausted and Zabinski (1929) noted that neither *Periplaneta orientalis* nor *B. germanica* could survive on exclusively protein diets. The present experiments support the view that when *P. americana* is fed on a pure protein diet it mobilizes its lipid reserves, because the lipid content of animals which had been on a casein diet for 2 weeks after being on a rat-pellet diet was significantly lower than that of the rat-pellet fed animals.

Both Gier and Haydak found that animals on low-protein diets lived longer than those on high-protein ones. Gier found that animals fed on purified carbohydrates (including dextrin) lived very well and increased in weight, 68% of them surviving for at least 300 days. Therefore, maintaining animals on pure carbohydrate for 2 weeks, as was done in the present study, would not be expected to cause great physiological stress to the animals. Gier noted, as was found in the present experiments also, that animals fed on carbohydrate had rich lipid stores and no, or very few, urate deposits in their fat bodies. (The so-called 'protein crystals' of Gier were presumably urate.) Mullins & Cochran (1975) showed that when animals were fed on a low-protein diet the urate stores were mobilized. In the present work it was found that the fat bodies of dextrin-fed adults usually had a similar appearance to those of nymphs, which also have large lipid stores and little or no urate deposits.

Estimates of lipid content in insects have been made most frequently by extraction with ether, and values for *Periplaneta* in the literature show a very wide range, from 6·2% of the total body dry weight (Buxton, 1932) to 25·5% for males and 28·6% for females (Schweet, 1941, cited by Scoggin & Tauber, 1950). The acetone extractions used in the experiments reported here will have removed only the neutral lipids (Gurr & James, 1971) and so would be expected to give slightly lower values than ether extractions, which would have removed some of the complex lipids as well. Nelson, Terranova & Sukkestad (1967) showed that 97% of the lipid in the fat body of *P. americana* was in the form of triglyceride, so acetone-extractable lipid values should give a useful estimate of the lipid available as reserve food in this species.

Tucker (1977*a*) showed that dehydrating animals would seldom eat dry food and Willis & Lewis (1957) found that cockroaches provided with dry food but no water survived no longer than individuals with neither food nor water. Feeding inhibition with dry food has been observed in locusts also (Loveridge, 1975). Thus, in order to withstand periods of desiccation, *Periplaneta* must have reserve food supplies upon which it can draw as an energy source. Also, the metabolic water gained from the catabolism of this reserve food will be important to the animal in the absence of the normal water intake with food. It would seem that in this respect animals with large food reserves in their fat bodies would be better able to withstand long periods without water than those with little reserve food. In spite of the considerable attention which has been given to studying the mechanisms by which cockroaches might decrease water loss in dehydrating conditions, little attention has been directed to studying the

significance of metabolic water in these conditions. Metabolic water is known to be an important source of water in insects such as the mealworm (Buxton 1930) whose food is very dry. For *Locusta*, Weis-Fogh (1967) calculated that under certain conditions of temperature and humidity (e.g. 25 °C and 35% R.H.) it would be possible for an animal to maintain a positive water balance during flight if fat was metabolized, while Loveridge (1975) found that in non-flying locusts the metabolic water produced was insufficient to balance transpiratory losses in dry air.

At 27 °C and humidities of around 35% or less, if adult male cockroaches are deprived of food and water the total body water, expressed as a percentage of the live weight, becomes slightly reduced (Tucker, 1977*a*). Thus, under these conditions metabolic water is insufficient for the animals to maintain their normal water balance. However, the reduction in the amount of water in the body may not affect most of the body tissues. It is mainly a reflexion of the reduction in haemolymph volume and reabsorption of fluid from the salivary reservoirs.

Frequently, in studies of effects of dehydration, weight loss has been used as an indicator of water loss (e.g. Penzlin, 1971; Treherne & Willmer, 1975). However, unless one knows what kind of foodstuffs are being metabolized in a dehydrating animal, and whether different groups are metabolizing similar proportions of fat, carbohydrate and protein, a comparison of weight losses in dehydrating animals is difficult to interpret in terms of water loss and/or water balance. For every gram of fat oxidized 1·07 g of water is produced, whereas complete oxidation of 1 g of carbohydrate yields only 0·6 g water (Prosser & Brown, 1961). Unfortunately, it is impossible to check whether a dehydrating cockroach is oxidizing predominantly fats, carbohydrates or proteins simply by measuring the R.Q., because if protein is catabolized to uric acid it gives an R.Q. very close to that for lipid oxidation (Wigglesworth, 1972). Also, for flying adult *Periplaneta*, Polacek & Kubišta (1960) obtained R.Q. values averaging 0·64, along with other evidence which indicated that the animals were using carbohydrate for fuel. They attributed the very low R.Q. to incomplete oxidation of some products. Gourevitch (1928) obtained R.Q. values for fasting cockroaches which ranged from 0·65 to 0·85. In the present study R.Q.s were obtained for a few dehydrating animals and a mean value of 0·76 was obtained for adults and 0·67 for nymphs.

It is not known whether the kind of food mobilized in starving cockroaches (i.e. lipid, carbohydrate or protein) is affected by the state of hydration of the animal. Melampy & Maynard (1937) found that there was little difference between the lipid content of fed and starved adult male *Blattella*. However, the initial lipid content measured by them was only 1·7% of the total weight of fresh tissue and this is low compared with the lipid content of most of the cockroaches used in the experiments reported in this paper. Pilewiczówna (1926) reported that during starvation for 33 days *P. orientalis* lost 12% of its protein, 18% of its lipid and 70% of other non-nitrogenous substances (presumably mainly glycogen). Červenková (1960) found that extended starvation of *P. americana* caused a large decrease in both lipid and glycogen and she calculated that during 35 days' starvation 2-month-old adults gained 66% of their energy from lipid catabolism, 22·2% from glycogen and 11·8% from protein. Results of the experiments reported here showed that the total body lipid decreased during starvation and dehydration, but no analyses were made of the total carbohydrate and protein in the animals.

Last-instar nymphs, which have considerably greater lipid reserves than adult males, show significantly slower rates of weight loss than the adults when water is withheld from them. Because prolonged dehydration causes a large decrease in haemolymph volume, it is impossible to collect a haemolymph sample and so one cannot accurately determine the haemolymph volume of severely dehydrated cockroaches. However, one can tell from the ease with which haemolymph can be collected from animals which have been dehydrated for short periods, and from visual inspection upon dissection, that the haemolymph volume in dehydrating nymphs shows much less reduction in a given dehydration period than does that of adults. Even though the haemolymph volume in dehydrated nymphs is greater than in adults dehydrated for the same time, there may be little difference in the total body water of animals in the two groups. This is because of the extremely low water content of high-lipid fat body tissue. This negative correlation between the percentage of body water and the neutral lipid content was also noted by Bongers & Eggermann (1975) in the bug *Oncopeltus*.

Not only the amount of reserve lipid, but also the lipid patterns of the haemolymph, vary with the age of cockroaches. Nelson *et al.* (1967) found that the haemolymph of eighth instar male cockroaches contained 83% triglyceride and 10% diglyceride, whereas young adults contained 36% triglyceride and 60% diglyceride. If the pattern of lipid metabolism is very different in nymphs and adults (as indicated by the work of Nelson *et al.*) and the catabolism of lipids is important during dehydration, it is not surprising that nymphs and adults show differing abilities to withstand desiccation.

Because adults show a greater reduction in haemolymph volume than nymphs, the problem of regulation of salt content of the haemolymph during dehydration is more acute in adults. Although not always statistically significant on account of the large variances within groups, the changes which dehydration causes in the concentrations of $Na^+$ and $K^+$ and in the $Na^+/K^+$ ratio in the fat bodies of adult males support the hypothesis that the fat body helps to regulate the osmotic pressure of the haemolymph in dehydrating cockroaches by taking up $Na^+$ ions as the haemolymph volume decreases. This $Na^+$ uptake possibly involves an exchange with the $K^+$ which is associated with the urate in the fat body (Mullins & Cochran, 1974; Tucker, 1977*b*), the excess $K^+$ ions then either being excreted or accumulated in some of the tissues of the gut (Tucker, 1977*a*). The only group of adult males in which dehydration (for 6 days) did not cause an increase in the mean $Na^+/K^+$ ratio of the fat body tissue, was that group fed on a low-ion, dextrin diet prior to dehydration. Most of these animals had high-lipid, low-urate fat bodies and after a period of dehydration it was easier to obtain free haemolymph from them than from dehydrated animals which had been on the other diets. Thus, lipid catabolism in the dextrin-fed animals may have provided enough metabolic water to prevent a marked decrease in haemolymph volume in the absence of drinking water. It appears that if the fat body is low in urate and $K^+$ and high in lipid (as in nymphs and carbohydrate-fed adults), $Na^+$ does not enter the fat body when the animal is in dehydrating conditions. This would explain why dehydration of dextrin-fed animals produced a greater increase in haemolymph $Na^+$ than was found in the other groups of adults, even though their haemolymph volume was less reduced.

Experiments using radioactive sodium and serial sampling techniques have been conducted on groups of male, juvenile and adult *Periplaneta* to examine further the

effects of lipid content and age on $Na^+$ movements during dehydration. The results of these experiments are presented in the following paper.

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