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REGULATION OF IONS IN THE HAEMOLYMPH OF THE COCKROACH *PERIPLANETA AMERICANA* DURING DEHYDRATION AND REHYDRATION

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SUMMARY

Experiments using ^{22}Na-uptake and serial sampling techniques showed that during dehydration Na was taken up by the fat body in adult *Periplaneta americana*, but not in nymphs. Generally, accumulation of Na in the fat body was highest in animals which had little reserve lipid and in those which excreted the smallest amounts of Na during the dehydration period.

Dehydration caused little change in the Cl^- concentration in the haemolymph. From results of ^{36}Cl-uptake experiments, it appears that the fat body is not involved in the regulation of haemolymph Cl^- during dehydration. In old adults, the hindgut was the tissue in which dehydration caused the greatest increase in ^{36}Cl uptake.

The mechanisms which enable cockroaches to withstand dehydrating conditions are discussed.

INTRODUCTION

When analyses were made of the fat body tissue of *Periplaneta americana* (the American cockroach), it was found that, except for animals which had been on a pure carbohydrate diet, dehydration caused an increase in the mean fat body Na (Tucker, 1977*c*). Rehydration caused a decrease in the fat body Na (Tucker, 1977*a*). It appeared that the fat body helped to regulate the concentration of Na^+ in the haemolymph by taking up Na^+ ions when the haemolymph volume of an individual was reduced by dehydration and then liberated Na^+ when the haemolymph volume rapidly returned to normal during rehydration. However, even in animals which had been maintained on restricted diets, there was a wide range in the Na^+ concentrations in the fat bodies. Therefore ^{22}Na has been used and serial samples of the fat body tissue taken to verify whether dehydration causes a significant increase in Na^+ in the fat body.

Late-instar nymphs withstand desiccation better than adults and so both nymphs and adults have been used in the present study, to determine if animals of different ages show a difference in the pattern of movement of Na^+ ions between the haemolymph and the fat body.

In earlier work (Tucker, 1977*a*, *b*, *c*), the effects of dehydration and rehydration on the regulation of only Na^+ and K^+ were investigated. In order to gain a fuller understanding of the regulation of the osmotic pressure of the haemolymph when the volume

changes, this current study has also included an investigation of changes in Cl^- (the major anion) in the haemolymph of animals in different states of hydration, and of possible ways in which the Cl^- content of the haemolymph is regulated.

MATERIALS AND METHODS

Methods used for dehydration and rehydration of the cockroaches have been described elsewhere (Tucker, 1977*a*). In this study only male *P. americana* were used. Young adults refer to those less than 2 weeks old and old adults are those which are more than 2 weeks past their final moult.

Chloride determinations were made with a Chloride Titrator (American Instrument Company). Haemolymph samples were collected directly into microcaps (Drummond Scientific Company) which were then rinsed thoroughly with distilled water to ensure expulsion of all the haemolymph.

^{22}Na and ^{36}Cl were both obtained as NaCl solution from the Radiochemical Centre, Amersham. For injection of ^{22}Na, the high specific activity $^{22}NaCl$ was diluted with saline (with 10 mM-K^+, but concentrations of other ions as given by Treherne, Buchan & Bennett, 1975). With an Agla micrometer syringe, 25 μl of the radioactive saline was injected into each animal between the second and third abdominal tergites. If there was any sign of leaking of haemolymph or saline from the point of injection, it was sealed with New-Skin (Harwoods Laboratories), but usually this was not necessary. Animals were not allowed water for 2 days before injection of saline, because it was found that if animals were too well hydrated a considerable amount of haemolymph and radioactive solution was lost at the time of injection. Because of the lower specific activity of $Na^{36}Cl$, it was necessary to use a greater proportion of stock radioactive solution to saline than was the case with $^{22}NaCl$. Therefore, a small volume of modified saline was made up to dilute the $Na^{36}Cl$. The final solution of $Na^{36}Cl$ + modified saline, which was injected, had an ion composition similar to normal saline. At the end of the experimental period, tissues were dissected out, placed on weighed planchettes and flattened as evenly as possible around the centre of the planchette. They were then dried, weighed and the radioactivity of each sample measured with a GM tube and automatic sample changer.

In some experiments fat body samples were taken from adult males before and after a period of hydration or dehydration. In order to decrease the haemolymph volume slightly and so prevent haemolymph being lost, animals were not allowed water for 2 days prior to the operation. During the operation each animal was kept anaesthetized with carbon dioxide. A small flap was cut in the third abdominal sternite and after removal of a fat body sample the flap was sealed with New-Skin or a beeswax-resin mixture. The animal was then returned to its container, where it was kept without food and with or without water for 2–6 days. At the end of the experiment each animal was anaesthetized, dissected and a second sample of fat body tissue was removed from the ventral part of the third abdominal segment, on the opposite side of the body to that from which the first sample had been taken. Two further samples of fat body were removed for lipid and carbohydrate analyses.

For Na^+ determinations, fat body samples were dry-ashed and Na^+ estimated by flame photometry. The lipid content of the fat body tissue was determined colori

Table 1. *Cl^- in the haemolymph of adult male* P. americana *in different states of hydration*

State of hydration	Cl^- (μ-mol/ml) Mean ± S.E.	*n*	% of normal
Hydrated (H_2O only 7 days)	128·7 ± 1·8	8	98
Normal (rat pellet + H_2O)	131·3 ± 6·4	5	—
Dehydrated 7 days	135·2 ± 2·5	9	103
Dehydrated 7 days, rehydrated 1 day (with food)	125·4 ± 2·7	5	96
Dehydrated 7 days, rehydrated 7 days (with food)	130·2 ± 3·1	5	99
Dehydrated 7 days, rehydrated 1 day (without food)	114·2 ± 5·1	8	87
Dehydrated 7 days, rehydrated 7 days (without food)	123·1 ± 3·2	5	94
(Dehydrated 7 days, rehydrated 1 day (without food)) 2 ×	112·1 ± 3·0	5	85

metrically with a Merckotest reagent kit for total lipids. The method is based on the sulphophosphovanillin reaction described by Zöllner & Kirsch (1962). Carbohydrate in the fat body was measured using the colorimetric method of Marshall & Orr (1962). The phenol-sulphuric reagent used in this method is suitable for determining the presence of sugars and polysaccharides such as glycogen (Dubois *et al.* 1956).

RESULTS

Haemolymph Cl^- concentration. The effects of starvation, dehydration and rehydration on the haemolymph Cl^- concentration of adult male *P. americana* are shown in Table 1. When the animals were dehydrated for a week the haemolymph Cl^- concentration did not change significantly from its normal value. When they were rehydrated and given food, there was an initial slight drop in the haemolymph Cl^-, but it returned to its normal concentration within a week. However, when animals were rehydrated without food, the haemolymph Cl^- showed a larger initial decrease than that in the animals which were allowed food, and the concentration did not return to normal after a week of rehydration. The lowest haemolymph Cl^- concentrations were found in animals which had been subjected to two consecutive dehydration–rehydration cycles without food.

^{22}Na- and ^{36}Cl-uptake. After ^{22}Na in saline had been injected into the haemocoel, the uptake of ^{22}Na by tissues in normally hydrated animals was not proportional to the absolute amount of Na^+ in each tissue. It can be seen (Table 2) that in adults the fat body tissue had the highest Na^+ concentration, but the lowest ^{22}Na-uptake of any tissue. Tissue rankings for ^{22}Na-uptake in hydrated and dehydrated animals were compared to determine whether dehydration changed the uptake by any tissue. On the right-hand side of the table changes in rank caused by dehydration are listed. In all age groups, dehydration causes a marked reduction (i.e. decrease in rank) in the amount of excreted ^{22}Na. However, it can be seen that changes in the ^{22}Na-uptake by the body tissues differ in the three age groups. In old adults, dehydration causes a noticeable increase in ^{22}Na-uptake by the fat body, but this is not true for the young adults or last instar nymphs. Nymphs showed an increase in ^{22}Na in the hindgut, while old adults showed a decrease.

Table 2. *Comparison of ^{22}Na-uptake in tissues of* P. americana *of different ages and different states of hydration*

(All animals were injected with the same amount of ^{22}Na and 4 days after injection tissues were removed and the uptake of ^{22}Na measured. In each group tissues were then ranked according to the mean cpm/mg tissue dry weight and changes in rank caused by dehydration are shown on the right-hand side of the table.)

Tissue	Na^+ (μ-mol/g) in hydrated adults*	Cpm/mg in hydrated animals			Cpm/mg in dehydrated animals			Change in rank (hydrated–dehydrated)		
		Old adults	Young adults	Last-instar nymphs	Old adults	Young adults	Last-instar nymphs	Old adults	Young adults	Last-instar nymphs
Foregut	42	108	167	95	192	203	171	+2	+2	+1
Midgut	50	96	180	160	165	166	234	+2	−1	+2
Midgut caeca	59	63	119	61	120	111	71	+1	0	0
Malpighian tubules	74	143	261	208	201	259	212	+2	0	−1
Hind gut	49	169	201	129	144	187	253	−2	−1	+4
Fat body	133	42	74	44	125	102	35	+4	0	0
Nerve cord	80	231	349	183	348	397	205	+1	0	−1
Integument	72	78	120	70	115	130	98	−1	0	+1
Muscle	25	49	60	86	51	63	94	−1	0	−1
Faeces	Very variable	350	976	532	70	604	103	−8	0	−5

* Data from Tucker (1977*a*).

Table 3. *Uptake of ^{36}Cl in the tissues of* P. americana *in different states of hydration*

(Animals were dissected 6 days after injection of ^{36}Cl. The solution used for injection of the older adults had a higher specific activity than that which was injected into the young adults.)

Tissue	Cpm/mg tissue dry weight				Change in rank	
	Hydrated		Dehydrated			
	Old adults	Young adults	Old adults	Young adults	Old adults	Young adults
Foregut	139	104	215	115	+1	0
Midgut	119	86	142	90	0	−1
Midgut caeca	83	64	127	79	+1	0
Malpighian tubules	134	77	209	111	−1	+1
Hind gut	200	170	410	144	+1	0
Fat body	17	13	19	13	0	0
Nerve cord	154	110	239	126	0	0
Integument	82	50	99	69	−1	0
Muscle	33	20	33	27	0	0
Faeces	239	810	406	802	−1	0

In Table 3 ^{36}Cl-uptake values are shown. A comparison of tissue rankings was made in the same way as was done for ^{22}Na and this shows that the relative uptake of ^{36}Cl by the various tissues is not affected by dehydration in the same way as ^{22}Na-uptake. The tissue rankings in dehydrated animals are little different from those in hydrated animals.

Table 4. *Groups of male* P. americana *used in radio-isotope experiments*

Group no.	*n*	Age	Injection	Conditions during experiment	Time (days)	Cpm/mg muscle (mean ± S.E.)
1	7	Old adult	^{22}Na in saline	H_2O only	4	49 ± 10
2	7		^{22}Na in saline	No food or H_2O	3–4	51 ± 7
3	3		^{22}Na in saline	No food or H_2O	7	51 ± 6
4*	4		^{22}Na in H_2O	H_2O only	4	54 ± 4
5	3	Young	^{22}Na in saline	H_2O only	4	60 ± 3
6	4	adult	^{22}Na in saline	No food or H_2O	4	63 ± 3
7	3	Last-instar	^{22}Na in saline	H_2O only	4	75 ± 13
8	3	nymph	^{22}Na in saline	No food or H_2O	4	94 ± 11
9	2		^{22}Na in saline	No food or H_2O	7	65
10	5	Old adult	^{36}Cl in saline	H_2O only	6	33 ± 2
11	5		^{36}Cl in saline	No food or H_2O	6	33 ± 3
12	5	Young	^{36}Cl in saline	H_2O only	6	20 ± 3
13	5	adult	^{36}Cl in saline	No food or H_2O	6	27 ± 8

* Animals in this group were dehydrated for 4 days prior to injection of the isotope, in contrast to other groups which were dehydrated for only 2 days – see text.

A more detailed picture of how dehydration affects the uptake of ^{22}Na and ^{36}Cl by the tissues can be obtained by comparing the change in uptake by each tissue in individuals in different states of hydration. To do this, one should ideally inject exactly the same amount of isotope into animals of the same size. Even though animals of approximately the same size were chosen for the experiments, and the same amount of radioactive solution was injected into each individual, there was some variation in body size and the relative sizes of the tissues and also, in some cases, a small amount of radioactive solution was lost due to bleeding at the time of injection. Therefore, to compare the amount of isotope taken up under different conditions, one must correct for individual differences in dilution of the isotope within the animals and also for any losses of isotope during injection. This correction is usually made by measuring the amount of isotope in the blood at the time of sampling, but this could not be done in these experiments because it was impossible to obtain haemolymph samples from some of the dehydrated individuals. As an alternative to using the specific activity of the haemolymph in each individual to correct values obtained for isotope uptake by other tissues, a similar kind of correction was made using uptake by another tissue – the muscle – which was reasonably homogeneous and in which dehydration did not cause changes in the concentrations of Na^+ and Cl^-.

In an earlier study (Tucker, 1977*a*), it was found that during the first week of dehydration there was no noticeable change in the Na^+ content of the muscle in adult male cockroaches. Also, the present investigation showed that the mean values for the uptake of both ^{22}Na and ^{36}Cl by the muscle were very similar for most animals of the same age, no matter what their state of hydration. For example, four groups of adult males in different states of hydration had mean ^{22}Na-uptake values of 50·0, 50·9, 50·6 and 54·2 cpm/mg tissue (see also Table 4). In spite of the very similar mean radio-isotope uptake values for the muscle in animals of the same age, there was sometimes considerable range in the values for the uptake by the muscle tissue between

Table 5. *Table to show how corrected uptake values* (C/M) *were calculated for* ^{22}Na*-uptake in the tissues of two individuals, and also how the ratios, which showed the differences in uptake by the tissues in dehydrated and hydrated animals, were obtained*

Tissue	Hydrated old adult		Dehydrated old adult		Ratio
	Cpm/mg ($=C_{hyd}$)	$C_{hyd}/56{\cdot}6$ ($=C/M_{hyd}$)	Cpm/mg ($=C_{dehy}$)	$C_{dehy}/57{\cdot}3$ ($=C/M_{dehy}$)	$\frac{C/M_{dehy}}{C/M_{hyd}}$
Foregut	173·4	3·06	224·5	3·92	1·28
Midgut	148·8	2·63	221·4	3·86	1·47
Midgut caeca	117·6	2·08	145·7	2·54	1·22
Malpighian tubules	186·0	3·29	250·8	4·37	1·33
Hind gut	227·5	4·02	143·0	2·49	0·62
Fat body	47·5	0·84	159·0	2·77	3·30
Nerve	361·0	6·38	417·5	7·28	1·14
Testis (+fat)	61·4	1·08	143·0	2·49	2·31
Utriculi	146·5	2·59	152·6	2·66	1·03
Conglobate	100·0	1·76	102·4	1·90	1·08
Integument	113·2	2·00	111·7	2·04	1·02
Muscle	56·6	1·00	57·3	1·00	1·00
Faeces	415·5	7·34	7·2	0·12	0·02

individuals within each group (see S.E.s listed in the table). This indicates that the specific activity of the isotope within animals did vary from individual to individual, or that the total Na^+ in the muscle showed a considerable range. A large proportion of the Na^+ in muscle tissue is likely to be from haemolymph trapped between the cells and the amount of haemolymph around the muscle cells might be expected to vary from individual to individual, especially when animals are in different states of hydration. However, the variability in muscle ^{22}Na-uptake within groups was greater than that for total Na^+ (see Tucker, 1977*a*) and this supports the view that at least some of the variability in muscle ^{22}Na-uptake is due to differences in the specific activity of the isotope in different individuals.

A 'corrected' uptake value (C/M) for each tissue was obtained by dividing the absolute cpm/mg tissue for that tissue by the cpm/mg muscle for the same individual. Such calculations for two individuals are shown in Table 5. On the right-hand side of the table a comparison of the ^{22}Na-uptake by the various tissues within the two individuals has been made by dividing the C/M value for each tissue of the dehydrated individual by the C/M value for the same tissue in the hydrated individual.

C/M values were obtained for each tissue in all the individuals used in the study and then mean C/M values were calculated for each tissue in each of the 13 groups of animals listed in Table 4. Comparisons between the mean C/M uptake values for the various groups are shown in Table 6. These ratios (mean C/M_{dehy} : mean C/M_{hyd}) show that most of the tissues took up more ^{22}Na in dehydrated animals than in hydrated ones. However, even the ratios obtained from the C/M values cannot be interpreted quantitatively as a measure of real changes in Na^+ and Cl^- in the various tissues. Hydrated animals excrete more Na^+ and Cl^- than dehydrating ones and this will mean that changes in the specific activity of the isotopes in the haemolymph and tissues during the course of the experiments will be different in hydrated and dehydrated individuals. Also, the exchange of isotope in the muscle in dehydrated animals

Table 6. *Comparison of* ^{22}Na- *and* ^{36}Cl-*uptake in tissues of* P. americana *of different ages and different states of hydration*

(The values listed for each tissue represent the ratio obtained by dividing the corrected uptake value for one group of animals by the corrected uptake value for the second group of animals whose group numbers are shown at the top of each column. For fuller explanation of method for obtaining ratios, see text.)

Ion ...	^{22}Na							^{36}Cl	
Age ...	Nymphs		Young adults	Old adults				Young adults	Old adults
	Dehy. 4 days	Dehy. 7 days	Dehy. 4 days	Dehy. 3–4 days	Dehy. 7 days	Dehy. 3–4 days	Dehy. 7 days	Dehy. 4 days	Dehy. 4 days
	Hyd.	Hyd.	Hyd.	Hyd.	Hyd.	H_2O inj.	H_2O inj.	Hyd.	Hyd.
Group nos. ...	8/7	9/7	6/5	2/1	3/1	2/4	3/4	13/12	11/10
Foregut	1·63	1·74	1·14	1·70	1·74	1·16	1·20	1·00	1·60
Midgut	1·36	1·21	0·89	1·85	2·06	1·08	1·20	0·97	1·22
Midgut caeca	1·78	1·77	0·91	1·73	2·24	1·23	1·60	1·14	1·56
Malpighian tubules	0·93	1·19	0·94	1·41	1·68	1·40	1·66	1·15	1·63
Hind gut	1·79	2·34	0·87	0·78	1·07	0·88	1·20	0·81	2·13
Nerve cord	1·01	1·14	1·08	1·45	1·36	1·56	1·46	1·06	1·51
Testis (+fat)	0·97	1·35	1·22	1·84	2·73	1·35	2·00	0·77	1·16
Utriculi	—	—	0·97	1·47	1·55	1·68	1·77	1·14	1·29
Conglobate gland	—	—	1·09	1·12	1·46	1·37	1·77	1·00	1·03
Fat body	0·74	0·89	1·40	2·69	2·72	3·72	3·42	0·75	1·16
Muscle	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
Integument	1·02	1·35	1·03	1·38	1·34	1·63	1·58	1·15	1·20

* Group numbers refer to those listed in Table 4.

could well differ from normal because of alterations in haemolymph circulation and this would bias the figures listed in the table.

Differences in ^{22}Na-uptake due to age are noticeable, particularly for the Malpighian tubules and the fat body, where older animals showed much greater uptake than young ones. The hindgut showed an accumulation of Na in nymphs, but not in adults. Dehydration caused the biggest increase in ^{22}Na-uptake in the fat body tissue of old adults. Dehydration does not bring about any marked changes in the ^{36}Cl-uptake in young adults, but in old adults there is a greater than normal uptake by most tissues, particularly the hindgut.

In summary, the radioisotope experiments indicated that dehydration has different effects on the patterns of ^{22}Na- and ^{36}Cl-uptake in animals of different ages. In nymphs dehydration causes some accumulation of ^{22}Na in the hindgut, but in old adults both the ^{22}Na-uptake by the hindgut and excreted ^{22}Na are reduced and there is a large increase in ^{22}Na-uptake by the fat body. Other tissues show some increase in ^{22}Na, but not nearly as much as the fat body. However, in old adults there is no increase in ^{36}Cl-uptake by the fat body during dehydration. Instead, there is an increase in the amount of ^{36}Cl in the hindgut and much more Cl is excreted than Na.

Fat body Na^+. During the course of the radio-isotope experiments it was observed that although there was a positive correlation between the ^{22}Na-uptake and the total

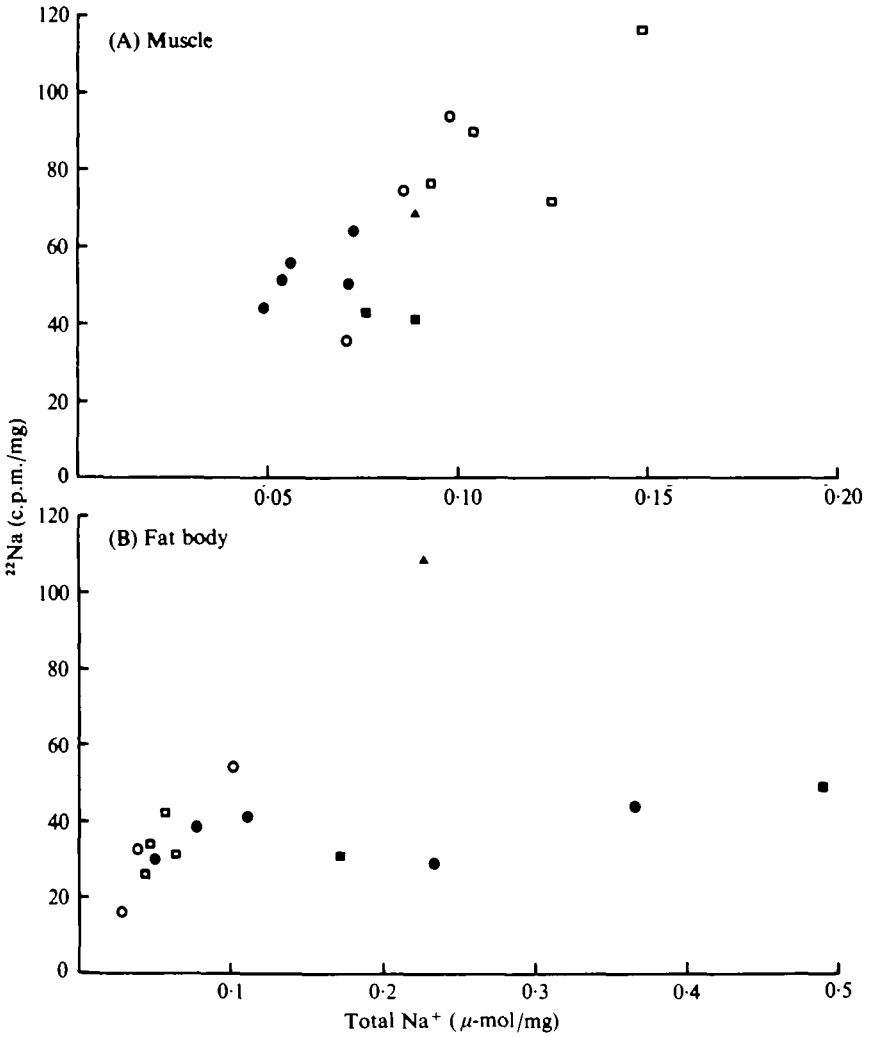

Fig. 1. Relationship between ^{22}Na-uptake and total Na$^+$ in replicate samples of tissues from 15 individuals. Open symbols = last instar nymphs; closed symbols = adults; circles = hydrated; squares = dehydrated 6 days during which time a large amount of Na$^+$ was excreted; triangle = individual which excreted very little Na$^+$ during a 6-day dehydration period.

Na$^+$ in the muscle tissue in animals of different ages and in different states of hydration (Fig. 1*a*), in the fat body the relationship between ^{22}Na-uptake and total Na$^+$ was more complicated. In nymphs, both hydrated and dehydrated, there was a positive correlation between ^{22}Na-uptake and total Na$^+$, but in adults there was no such correlation (Fig. 1*b*). This indicates that some of the Na$^+$ in the fat body tissue of adults is not freely exchangeable. The highest ^{22}Na-uptake by the fat body tissue was recorded for an individual which excreted very little Na$^+$ while it was being dehydrated.

The effects of dehydration on the movements of Na$^+$ into the fat body are clearly seen in results from a series of experiments in which samples of fatty tissue were taken from individuals before and after a period of hydration or dehydration (Table 7).

Table 7. *Results of experiments in which serial samples of fat body were taken from adult male* P. americana

(All groups initially contained 6 animals but one animal in Group C and 3 in Group D died during the dehydration period. In all cases the animals which died excreted few or no faecal pellets. For ease of comparison, animals in each group have been ranked according to the ratio of final/initial fat body sodium.)

Group	Conditions after initial fat body sample taken	Animal	Final fat body Na^+ / Initial fat body Na^+	Final fat body Na^+ (μ-mol/g)	Lipid in fat body at end of expt. mg lipid / mg tissue	μ-mol Na^+ excreted
A	Water only	1	0·628	82·7	*	Faeces
	2 days	2	0·702	181·8	0·110	semi-fluid
		3	0·713	66·0	0·214	and *entire*
		4	0·770	125·4	0·457	sample was
		5	0·976	306·5	0·088	not
		6	1·476	403·5	0·131	collected
	Mean		0·878	194·3	0·200	
B	Dehydrated	1	0·588	78·9	0·382	0·132
	(without food)	2	0·617	72·2	0·146	0·204
	2 days	3	0·784	227·4	*	*
		4	1·367	198·1	0·099	None
		5	1·410	359·3	*	*
		6	1·420	130·9	0·121	0·109
	Mean		1·031	177·8	0·187	0·110
C	Dehydrated	1	1·423	186·1	0·181	0·207
	(without food)	2	1·589	77·2	0·495	0·135
	4 days	3	1·623	194·5	0·231	0·129
		4	2·067	357·6	0·101	1·710
		5	2·346	465·8	0·051	0·520
	Mean		1·810	256·2	0·212	0·540
D	Dehydrated	1	1·128	466·1	0·106	4·890
	(without food)	2	1·753	214·2	0·063	8·460
	6 days	3	1·869	255·3	0·051	1·050
	Mean		1·583	311·9	0·073	4·800

* Analyses not made.

Rehydration caused a mean decrease and continued dehydration an increase in the amount of Na^+ per unit weight of fat body. There was considerable variation within each group. Some of this variation is likely to be caused by differing degrees of desiccation of the individuals in each group.

In order not to disturb the animals after the removal of fat body samples, their weight changes were not monitored. However, the same operation was later carried out on another group of animals and their weight changes were then followed during 6 days' dehydration. The weight changes of these five animals are shown in Fig. 2, along with mean weight changes for unoperated adults, 2-day-old adults and late-instar nymphs. It can be seen that weight losses for operated animals were greater than for unoperated ones and also, for the first 1–2 days after the operation, individuals showed a very wide range in weight loss. Such a range in weight loss is probably true also for the groups of animals for which data are presented in Table 7.

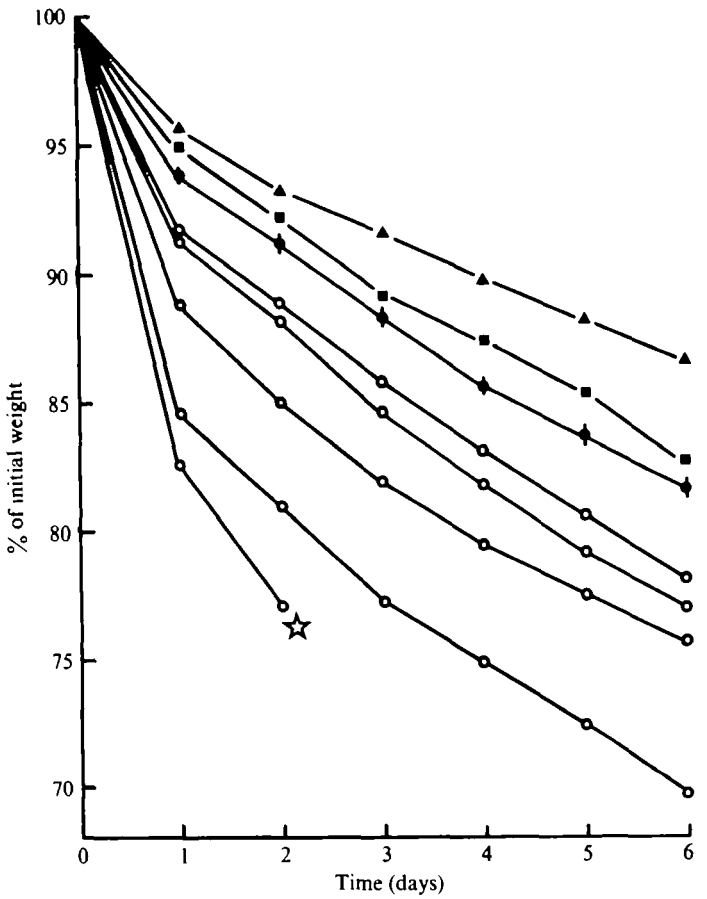

Fig. 2. Weight loss in dehydrating male *P. americana*. Triangles = last instar nymphs (mean 2 animals); crosses = 2-day-old adults (mean 3 animals); closed circles = adults greater than 2 weeks old (n = 10); open circles = adult individuals from which a fat body sample was taken at the beginning of the dehydration period; star = individual died.

Two other variables are likely to affect the Na^+ balance, the first being the amount of Na^+ excreted during the dehydration period. The amount of Na^+ excreted by individuals varied greatly and, if the fat body takes up excess Na^+ from the haemolymph during dehydration, one would expect the greatest increases in fat body Na^+ to be in individuals which excreted only small amounts of Na^+ during the dehydration period. Results obtained for Groups C and D give support to this hypothesis. Although Group D animals were dehydrated for 2 days longer than Group C, they did not show such a large increase in fat body Na^+, but they excreted Na^+ at a much higher rate.

The other variable which had previously been found to affect water balance (and thus ion balance) during dehydration was the lipid content of the animal (Tucker, 1977*c*). No analyses of the total lipid content of animals were made in this series of experiments, but analyses of lipid and carbohydrate concentrations were made at the end of each experiment on small fat body samples. There was no correlation between the carbohydrate concentration and changes in the Na^+ in the fat body but, in the

most dehydrated animals (i.e. Groups C and D), in general those whose fat bodies still had a high lipid content at the end of the dehydration period showed smaller increases in fat body Na^+ than those with low lipid values. However, it is the total amount of lipid available for catabolism which will most affect the animal's state of hydration when no water is available for drinking. The size of fat bodies in adults show a very wide range. For instance, Animal 1 in Group C had a very large fat body, while Animal 2 in the same group, although it had a much higher proportion of lipid in its fatty tissue, had only a small amount of fatty tissue. The total lipid in Animal 2 could actually have been less than that in Animal 1.

DISCUSSION

A study has been made of the ion regulation involved in preventing large changes in the osmotic pressure of the haemolymph of *Periplaneta americana* when the haemolymph volume changes during dehydration and rehydration. The results of this investigation have been presented in this and three earlier papers (Tucker, 1977*a*, *b*, *c*). From the evidence available, one may conclude that the ability of cockroaches to withstand dehydrating conditions and to be able to prevent large increases in the osmotic pressure and concentration of ions in the haemolymph during dehydration, is due to the factors which are discussed in the following paragraphs.

(1) *Water lost by transpiration is kept to a minimum* by the low permeability of the cuticle (Beament, 1958), but *P. americana* has been found to lose water at R.H.s of 82% or above (Edney, 1967). Berridge (1970) has suggested that, on theoretical grounds, the apical plasma membrane of the underlying epidermal cells could have an equally important role in restricting water loss through the integument of insects. Treherne & Willmer (1975) have proposed the existence of a hormone which controls the integumentary water loss. However, further experiments are required in order to see if the increases in weight loss during dehydration, which they observed in decapitated cockroaches, were due to increases in the amount of water lost through the integument, or to changes in the basal metabolism of the animals (see following paragraph). In some insects, for example the tsetse fly (Bursell, 1957), the degree of spiracular opening is influenced by the humidity of the atmosphere and the physiological state of the animal, but, to my knowledge, there is no experimental evidence that this is true for cockroaches. When in desiccating conditions, locusts have been shown to decrease the rate, amplitude and discontinuities in their respiratory movements, thereby decreasing the amount of water lost by transpiration (Loveridge, 1968) and this may be true for cockroaches also.

(2) *Production of metabolic water during dehydration and starvation.* The presence of food reserves (mainly lipid and glycogen) in the fat body appears to be an important factor in enabling cockroaches to withstand periods of desiccation. Cockroaches in dehydrating conditions will seldom eat dry food (Tucker, 1977*a*) and during desiccation they must therefore catabolize some of their stored food. If a cockroach is oxidizing carbohydrate or protein, its rate of weight loss in dehydrating conditions will be greater than if it were oxidizing lipid, because more than twice as much water is produced during the oxidation of lipid as is produced by the oxidation of the same weight of carbohydrate or protein. The retrocerebral system of cockroaches is well

known to affect the metabolism of both carbohydrate and lipids (see e.g. Steele, 1961, 1963; Ralph & McCarthy, 1964; Vroman, Kaplanis & Robbins, 1965; Wiens & Gilbert, 1967). Thus, individual differences in the rate of weight loss during dehydration and starvation could be brought about by differences in basal metabolism (i.e. whether predominantly lipids, carbohydrates or proteins are being catabolized) as well as by differences in the amount of water transpired and excreted. At 27 °C and ambient humidity, metabolic water is insufficient to maintain the normal percentage of water in adult cockroaches, when they are denied water intake with food (Tucker, 1977*a*). It was found (Tucker, 1977*c*) that late-instar nymphs, which have larger lipid stores than adults, remained better-hydrated than adults in dehydrating conditions. Last-instar nymphs were found to survive for up to about 2 months without water intake.

(3) *Movement of water to the tissues from the salivary gland reservoirs and the haemolymph.* When cockroaches are well hydrated the salivary gland reservoirs may contain up to about 0·1 ml fluid (Sutherland & Chillseyzn, 1968) and this fluid has an osmotic pressure which is about 300 mOsM lower than the haemolymph (Wall, 1970). During dehydration the fluid is reabsorbed from the reservoirs (Sutherland & Chillseyzn, 1968; Laird, Winston & Braukman, 1972) and this will help to maintain water balance in the tissues when there is no water intake with food. However, even after only a few days' dehydration these reservoirs are empty in adults (Tucker, 1977*a*) and, if the water lost by excretion and transpiration is greater than the amount of metabolic water produced, water moves from the haemolymph into the tissues. Edney (1968) found that during 4 days' dehydration in dry air *P. americana* nymphs showed a decrease in haemolymph water of about 34%, compared with a decrease in tissue water of only about 15%. The decrease in haemolymph volume caused by dehydration of insects has often been recorded (see e.g. Edney, 1968; Wall, 1970).

(4) *Uptake of Na^+ and liberation of K^+ from the urate stores in the fat body.* There is a positive correlation between the amount of K^+ and the urate in the fat body tissue of normally hydrated adult cockroaches (Mullins & Cochran, 1974; Tucker, 1977*b*). During dehydration the concentration of K^+ and the K^+/urate ratio of the fat body tissue of adults both decrease (Tucker, 1977*a*, *b*). At the same time there is an increase in the fat body Na^+ (see Results section of this paper), except in adults which have been on a pure carbohydrate diet prior to dehydration (Tucker, 1977*c*). As a pure carbohydrate diet causes the fat body tissue to be low in urate and high in lipid, it appears that during dehydration Na^+ is only taken up by the fat body in animals which show decreases in haemolymph volume and have urate deposits in their fat bodies. This uptake of Na^+ by the fat body enables the animal to maintain a fairly constant level of Na^+ in the haemolymph, without having to excrete large amounts of Na^+, when water moves from the haemolymph into the various tissues. The Na^+ which is sequestered in the fat body during dehydration can then be released during rehydration, to help to prevent large decreases in the Na^+ concentration when the haemolymph volume suddenly increases. In normally hydrated cockroaches urate and K^+ are known to be deposited in spherical cellular inclusions which, in cross section, characteristically have concentric rings visible (Ballan-Dufrançais, 1975; Tucker, 1977*c*). There is a correlation between the numbers of these spherules and the urate content of the tissue, but in dehydrated animals the ratio of number of

spherules/urate is lower than normal. It is probable that in dehydrated animals the urate is found in amorphous granules (? sodium urate), as well as in the larger spherical inclusions. It is known that if urate is in colloidal solution and the concentration of Mg^{2+}, Na^+ or K^+ is increased, flocculation of the sol always takes place in the form of sodium urate (Porter, 1963). It could be that slight increases in the osmotic pressure of the haemolymph or cellular fluids during dehydration are sufficient to cause precipitation of sodium urate. However, other controlling factors are necessary to explain how in normally hydrated animals urate is deposited in association with K^+ in the spherical inclusions and how K^+ is liberated from the fat body during dehydration and Na^+ during rehydration. Bodenstein (1953) found that removal of the corpora allata and cardiaca caused the disappearance of urates from the cells of the fat body and retransplantation of the corpora cardiaca restored urate deposition, but there is as yet no experimental evidence that deposition and liberation of Na^+ and K^+ in the fat body are under hormonal control.

(5) *Reduction in rate of fluid secretion by the Malpighian tubules.* Wall (1970) found that in dehydrated cockroaches there was a reduction in the rate of production of the primary fluid by the Malpighian tubules and there is evidence that the rate of fluid secretion by the tubules is under hormonal control (Wall & Ralph, 1964; Wall, 1965; Mills, 1967). The primary urine is virtually isosmotic or slightly hyperosmotic to the haemolymph and therefore production of this fluid is not directly important in the control of the osmotic pressure of the haemolymph. However, by continuing to secrete urine even under dehydrating conditions, the Malpighian tubules provide a flow of fluid which enables the rectal epithelium to regulate ion balance in the haemolymph. Shaw & Stobbart (1972) pointed out that in order for the excretory system to be adequate to explain the osmotic changes in a dehydrating insect, the solute output through the Malpighian tubule-rectal system would have to be maintained at a constant level – that is, if the tubules' fluid production fell, the solute reabsorption by the rectum would have to decrease. They drew attention to the fact that if reabsorption of K^+ was reduced during dehydration and K^+ was necessary to maintain production of fluid by the Malpighian tubules the limited reservoir of K^+ in the haemolymph would soon be depleted. They suggested that a possible explanation would be that the K^+ output was sustained by the release of K^+ from the tissues and that if the released K^+ was exchanged for Na^+ (or some other cation) the conditions for osmotic control would be maintained. Results from the present study (Tucker, 1977*a*, *b*, *c*, and present paper) provide evidence that in dehydrating *Periplaneta* this does in fact happen, the K^+ being liberated from the fat body. The function of the ampullae at the bases of the Malpighian tubules is still not known, but they may play a part in ion regulation of the haemolymph. Wall, Oschman & Schmidt (1975) consider that ultrastructural evidence supports an absorption role for the ampullae and Wall (1970) found that the colon fluid was usually hypo-osmotic to the primary urine.

(6) *Increased reabsorption of water and decreased reabsorption of solutes by the rectum.* In dehydrating cockroaches there is increased reabsorption of water by the rectum (Wall, 1970; Wall & Oschman, 1970) and this is under hormonal control (see e.g. Wall, 1967; Goldbard, Sauer & Mills, 1970). Wall & Oschman found that during dehydration K^+, but not Na^+, became concentrated in the rectal lumen. They suggested that the rectum absorbed Na^+ in proportion to water during dehydration.

However, in hydrated animals they found that the rectal lumen consistently had lower Na^+ and K^+ than the colon fluid, indicating that both Na^+ and K^+ were reabsorbed by the rectum in hydrated animals. Phillips (1964) found that the rate of Cl^- absorption by the recta of the locust *Schistocerca gregaria* was governed by the rate of absorption of the accompanying cation, the rate of Cl^- absorption being greater in the presence of K^+ than from a NaCl solution of equal concentration. If a similar relationship exists in the cockroach rectum, and absorption of K^+ in dehydrated animals is lower than in hydrated ones, the reabsorption of Cl^- would be lower than normal in dehydrating animals. Some preliminary experiments, in which ileum, colon and rectal total Cl^- were measured, have shown that dehydration does cause some increase in the amount of Cl^- in the rectum. This would be expected to occur if the Malpighian tubules continued to secrete Cl^- while reabsorption of the ion by the rectal epithelium decreased. Further experiments are being carried out in this laboratory to determine the extent to which the hind gut regulates haemolymph Cl^- in dehydrating and rehydrating cockroaches. (The possibility that other tissues might also be involved in Cl^- regulation is also being investigated.) Wall (1970) observed that when an animal was rehydrated the dry material in the rectum was hydrated and then dehydrated again before being excreted. This would allow salts present in the rectum to be reabsorbed.

(7) *Decreased movement of material through the gut.* During dehydration there is little movement of material through the gut (Wall, 1970; Tucker, 1977*a*). If material is retained in the gut during periods of dehydration, necessary solutes can be absorbed from it when water again becomes available.

(8) *Increase in K^+ in the midgut caeca.* It is not known if the increase which dehydration causes in the K^+ content of the midgut caeca (Tucker, 1977*a*) is due to an active removal of K^+ from the haemolymph, or to a lack of absorption of K^+ from food which has moved into the caeca during the period of dehydration. Also, there is the possibility that some reverse peristalsis could occur. Active transport of K^+ from the haemolymph to the lumen has been observed in the midgut of *Hyalophora* larvae (Harvey & Nedergaard, 1964) and Sauer & Mills (1969) found that in a midgut ventriculus *in vitro* preparation from *P. americana* there was generally a small but variable net flow of K^+ from the haemolymph side to the lumen. The K^+ which accumulates in the midgut caeca during dehydration can be absorbed during rehydration, so helping to prevent a decrease in the concentration of K^+ in the haemolymph when its volume increases.

In addition to the eight factors discussed above, it is possible that changes in the amounts of soluble proteins and amino acids are also important in helping to prevent changes in the osmotic pressure of the haemolymph when the state of hydration of a cockroach changes. Djajakusumah & Miles (1966) found that during dehydration of the desert locust *Chortoicetes*, there was a loss of free amino acids and gain of soluble protein in the haemolymph and when the haemolymph volume returned to normal during rehydration there was a loss of soluble protein and gain of amino acids.

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