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EFFECT OF DEHYDRATION AND REHYDRATION ON THE WATER CONTENT AND Na^+ AND K^+ BALANCE IN ADULT MALE *PERIPLANETA AMERICANA*

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SUMMARY

Changes in live weight and in the water, Na^+ and K^+ content of the tissues and faeces have been examined in adult male *Periplaneta americana* in various states of hydration. During dehydration the haemolymph volume decreases markedly, but the Na^+ and K^+ concentrations rise only slightly. Less than 25% of the Na^+ removed from the haemolymph during dehydration was found to be excreted, but the K^+ excreted during dehydration was in excess of that removed from the haemolymph alone. It seems likely that the major tissue for regulating the haemolymph Na^+ during dehydration and rehydration is the fat body, in which dehydration causes an increase, and rehydration a decrease, in the Na:K ratio. The Na^+ and K^+ content of the fat body was found to be variable in both hydrated and dehydrated animals and absolute changes in the ion content of the tissue could not be estimated because of unknown changes in the amount of food reserves and excretory products.

INTRODUCTION

When the cockroach *Periplaneta americana* is dehydrated the haemolymph volume shows a marked decrease, but its osmotic pressure increases only slightly (Edney, 1968; Wall, 1970), indicating that during dehydration solutes are removed from the haemolymph. Heit, Sauer & Mills (1973) found that when *P. americana* drank hyperosmotic saline it was able to keep the haemolymph hypo-osmotic to the drinking medium. Pichon (1963) found that in juvenile cockroaches a short period of dehydration caused an increase in haemolymph Na^+, K^+ and Ca^{2+}, but extended dehydration caused a decrease in the concentrations of ions in the haemolymph. Regulation of the osmotic pressure of the haemolymph has also been found in other fairly closely related genera (e.g. *Arenivaga* – Edney, 1966; *Leucophaea* – Laird, 1972; *Chortoicetes* – Djajakusumah & Miles, 1966; *Schistocerca* – Shaw & Stobbart, 1972). Djajakusumah & Miles found that decreases in blood volume were associated with a loss of free amino acids and a gain of soluble protein in the haemolymph, but the amino acids contributed only 15% of the osmotically active constituents and therefore movement of other molecules or ions must be involved in the regulation of the osmotic pressure of the body fluid.

During dehydration, sodium has been shown to be removed from the haemolymph

of adult male *Periplaneta* (Wall, 1970), but, from faecal pellet analyses, she estimated that only 3·3% of the total sodium chloride removed from the haemolymph was excreted and she suggested that excess sodium ions were sequestered by some of the body tissues and then mobilized when a dehydrated animal had water to drink. The gut was found to contain only about 8·5% of the total sodium chloride needed for the new haemolymph formed when animals drank water after a period of dehydration. Pichon (1963, 1970) has also suggested that sodium may be stored in tissues and become quickly available when there is a large increase in haemolymph volume. Mullins & Cochran (1974) have proposed a uric acid/urate associated ion sink in *Periplaneta*, which would sequester Na^+, K^+ and NH_4^+ as urates in cases where solute concentrations of these ions are in excess of desired osmolarity levels.

The aim of the present study was to investigate the water content, sodium and potassium balance in *Periplaneta americana* during dehydration and rehydration and to try to determine whether or not sodium and/or potassium are sequestered within the animal as the haemolymph volume decreases.

MATERIALS AND METHODS

Adult male cockroaches were used in all experiments. The stage in the sexual cycle is known to affect the haemolymph volume in the female (Verrett & Mills, 1973), so that interpretation of the effects of changes in the haemolymph brought about by dehydration in females would be more difficult than in males. To avoid changes in haemolymph volume associated with ecdysis (Wheeler, 1963; Wharton, Wharton & Lola, 1965; Mills & Whitehead, 1970), adults were not used in any experiments until they were at least 1 week old. Animals were removed from the stock culture at least 4 days before the beginning of an experiment and placed in individual polystyrene containers with rat pellets (the same food as was used for the stock culture) and a small tube of ashless floc saturated with water. The containers were kept in a temperature-controlled cabinet set at 27 °C. The heat source for the cabinet was a bulb with a heating filament, so that animals received short intermittent periods of light throughout the day and night. Animals showed initial exploratory activity when placed in the individual containers, but within a few hours this decreased and thereafter they spent most of the time inactive on the floors of the containers.

Wall (1967) found that lowering the relative humidity of the atmosphere, supplying animals with hyperosmotic saline to drink, or withholding drinking water, all brought about similar dehydration effects. In the present series of experiments dehydration was always brought about by withholding drinking water from animals. Humidity was not controlled, but the relative humidity in the containers of animals without water was found to be as low as 25%.

In initial experiments animals were provided with food during periods of dehydration, but it was found that animals without water would seldom eat the dry food with which they were provided (the rat pellets were found to contain less than 10% water) and in further experiments dehydrating animals were not given food.

Live weights during dehydration and rehydration were determined by weighing animals at the same time each day in weighed plastic containers on a Sartorius electrobalance, correct to 0·1 mg. Animals were cold-immobilized before dissection

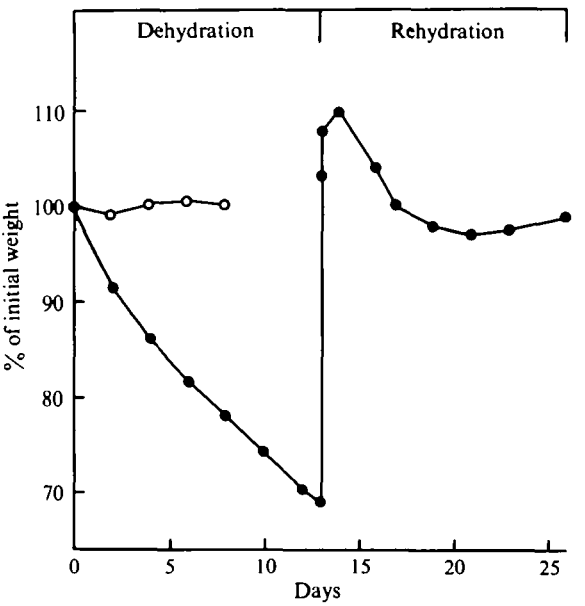

Fig. 1. Effect of dehydration and rehydration on the body weight. Open circles = mean for control group with food and water. Closed circles = mean for the experimental group.

and haemolymph samples collected from the dorsal vessel directly into 5 μl or 10 μl Microcaps (Drummond Scientific Company). Each microcap was rinsed several times with distilled water to ensure expulsion of any haemolymph adhering to the capillary and samples were then diluted in volumetric flasks for ion determinations.

Wet and dry tissue weights were measured to the nearest 0·5 μg on a Mettler electrobalance. Wet tissue weights were determined as soon as possible after dissection, each tissue being placed in a weighed crucible in a high-humidity container during the period between dissection and weighing. For dry weight estimations tissues were dried for 24 h at 105 °C. Tissues for Na^+ and K^+ analyses were dry-ashed at 450 °C and concentrations of ions determined by flame photometry.

RESULTS

Live weight changes

On a diet of rat pellets and water, adults showed little variation in body weight over a period of 1–2 weeks, but when drinking water was withheld they showed a marked loss in weight, and after about 12 days' dehydration they weighed only about 70% of their initial body weight. There was no correlation between initial body weight and the percentage of weight lost during dehydration. When given water again, dehydrated animals immediately began to drink and there was a rapid increase in weight. No attempt was made to expel water from the lumen of the gut before weighing an animal. Fig. 1 illustrates the mean body weight changes for 10 animals which were dehydrated for 13 days and four of these animals which were then allowed food and water for the following 13 days. One day after being allowed food and water the mean

Table 1. *Total water content of adult male* P. americana *in different states of hydration*

State of hydration	Water content (% of live weight) Mean	S.E.	*n*
Normal	68·60	1·69	4
Dehydrated 6 days	66·05	1·06	9
Dehydrated 13 days	64·04	0·58	4
Dehydrated 14 days	62·10	—	2
Rehydrated 1 day (no food) after 6 days' dehydration	74·31	0·56	6
Rehydrated 13 days (with food) after 13 days' dehydration	70·36	0·38	4
Hydrated	73·66	0·96	5

body weight was 10% higher than the initial weight, but within a few days this gain was lost and animals returned to close to their initial weight. If water alone was given after dehydration, animals did not show such a large initial weight gain and never regained their original weight.

Water content

The water content of animals in various states of hydration is summarized in Table 1. There is a 4·6% decrease in the total water content in a 13-day dehydrated animal compared to a normal animal. Upon rehydration the water content of an animal increases to a value greater than normal, but it returns to normal if the animal is allowed food. If an animal is kept with water and no food, its water content is higher than normal.

Defaecation

When animals are well hydrated the faecal material is semi-fluid with a water content of 80% or greater. When an animal does not have water to drink, the water content of the faeces is quickly reduced and discrete faecal pellets are produced. Thus, the water loss due to defaecation is much greater on the first day of dehydration than on subsequent days, when a greater amount of water is reabsorbed from the faeces by the rectum (see Fig. 2).

The dry weight of faeces defaecated per day falls rapidly after a day without water (Fig. 3*a*). This decrease is shown both in the decreased number of pellets (Fig. 3*b*) and in the size of each pellet (Fig. 3*c*). The average time from feeding to egestion in *P. americana* has been found to be 20·6 h (range 9·1–33·4) (Snipes & Tauber, 1937) but, as Wall (1970) noted, in dehydrated animals there is little movement of material through the gut.

The amount of Na^+ and K^+ and the Na/K ratio in faecal pellets of both normally hydrated and dehydrating animals was very variable, but the mean values of both Na^+ and K^+ per unit weight of faecal material decreased when animals were dehydrated (Fig. 4).

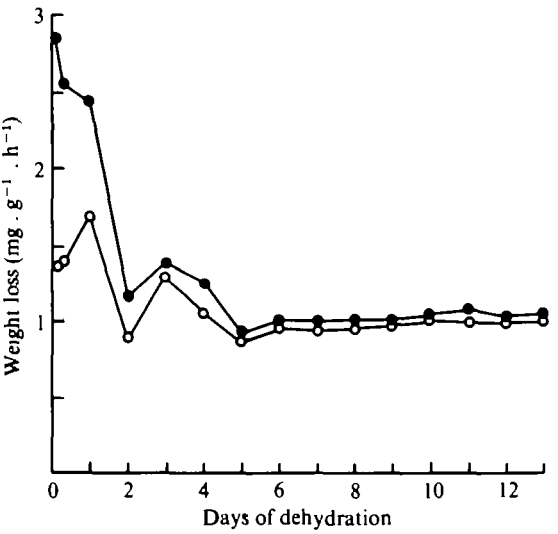

Fig. 2. Effect of dehydration on total water loss (closed circles) and water lost by transpiration, i.e. total loss minus water lost through defaecation (open circles). Curves represent mean values for 10 individuals.

Total body Na^+ and K^+

The concentration of Na^+ and K^+ in the head, thorax and abdomen of animals in different states of hydration, and the total body Na^+ and K^+, are shown in Table 2. The mean values for both total Na^+ and total K^+ per unit weight of dry tissue were slightly higher in animals which had been dehydrated for 6 days than in normally hydrated animals, and the greatest differences were found in the abdominal tissue. However, there was wide variability between animals in each group; this wide range of values for both Na^+ and K^+, particularly noticeable in the abdominal tissue, was later found to be a reflexion of the variability of the fat body tissue (see p. 58).

Haemolymph

Results of ion analyses of haemolymph samples collected from animals in different states of hydration are given in Table 3. It was impossible to collect samples from severely dehydrated animals. When dissected open, no free haemolymph could be seen in the body cavity of these animals. In moderately dehydrated animals it was sometimes possible to obtain a little haemolymph from around the muscles in the legs when none could be obtained from the dorsal vessel or abdominal cavity. As can be seen in the table, neither Na^+ nor K^+ show very large increases in animals which have been dehydrating for 6 days, although the proportional increase in K^+ is much greater than that of Na^+. Unless rehydrating animals were allowed food, the haemolymph Na^+ did not return to its normal value, but when animals were given only distilled water after dehydration, the K^+ did return to normal.

Other body tissues

Results for the analyses of Na^+ and K^+ in various body tissues are all presented as mean $\pm$ S.E. and are expressed on both a wet weight and on a dry weight basis. Wet

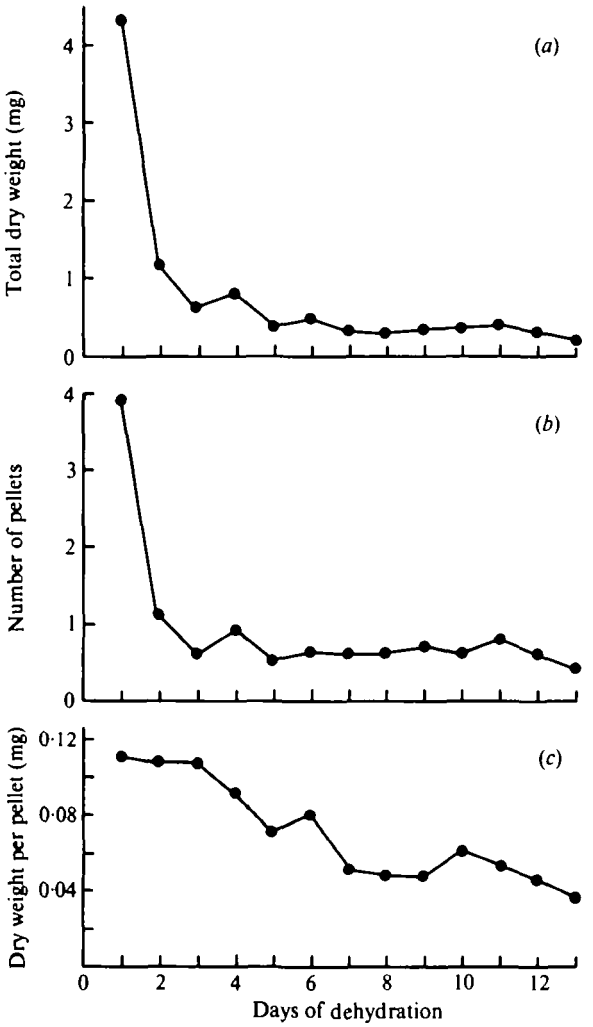

Fig. 3. Effect of dehydration on the amount of material defaecated. Curves represent mean values for 10 individuals. (*a*) Total dry weight of material defaecated. (*b*) Mean number of pellets defaecated per individual in 24 h. (*c*) Mean dry weight per pellet.

weight values have been included to give some indication of changes in the concentrations of ions in the tissues which might affect the normal functioning of the tissues, and dry weight values are included to enable comparison of possible changes in the total Na^+ and K^+. The difficulties in comparing Na^+ and K^+ content in tissues before and after periods of starvation and/or dehydration are most apparent for the fat body tissue, which is the major source of reserve food and also the main site for the storage of nitrogenous waste products within the animal. In the absence of information on changes in the organic matter in the tissues during the course of the experiments, it is impossible to calculate absolute changes in the Na^+ and K^+ content unless the entire tissue can be dissected out. Although this difficulty in trying to assess absolute changes in ions is most pronounced for the fat body, it is also true to a much lesser

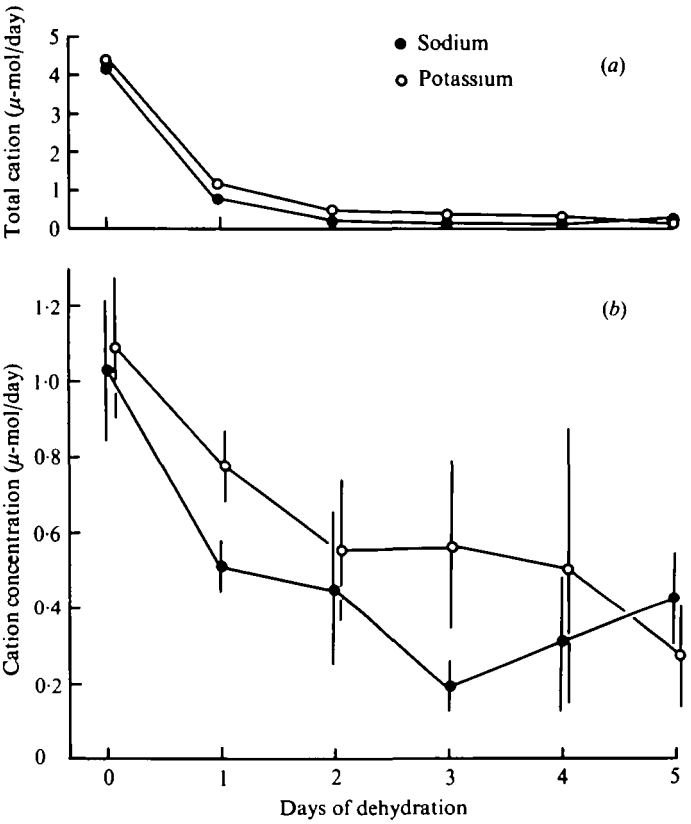

Fig. 4. Effect of dehydration on the amount of Na^+ and K^+ defaecated. Curves represent mean values for 11 individuals. (*a*) Total excreted per individual per day. (*b*) Changes in concentrations of ions in the faecal pellets. Vertical lines represent ± the standard error of the mean.

extent for some of the other body tissues which cannot be dissected out in their entirety.

Unless otherwise stated, hydrated animals are those which were kept with water, but no food for 5 days, normal animals are those which were allowed both food (rat pellets) and water, and dehydrated animals were kept without food and water for 6 days.

Water and ion analyses for various parts of the gut were made for each portion of the gut with its enclosed lumen contents and the results are listed in Table 4. In hydrated and dehydrated animals there was little solid material in the lumen and in only one of the normally fed animals did the crop contain a large amount of food. The water and ion content of the foregut of this animal did not differ significantly from the other normally fed animals. In all sections of the gut, and in the associated salivary glands and Malpighian tubules, the water content of the tissue is highest in hydrated and lowest in dehydrated animals; however, only in the salivary glands is there a very large difference. In this tissue the extremely high water content in hydrated animals is brought about by the fluid in the salivary reservoirs. The reservoirs in hydrated animals always contained fluid and sometimes appeared completely full, while in

Table 2. *Total Na^+ and K^+ in adult male* P. americana in *different states of hydration*

Ion	Part analysed	Hydrated (μ-mol/g dry wt.) Mean ± S.E.	Normal (μ-mol/g dry wt.) Mean ± S.E.	Dehydrated (μ-mol/g dry wt.) Mean ± S.E.	Change from normal (%)
Na^+	Head	187·7 ± 10·5	194·2 ± 7·9	217·4 ± 5·6	+12
	Thorax	106·7 ± 5·2	105·4 ± 2·7	129·2 ± 9·9	+22·6
	Abdomen	144·3 ± 16·3	138·0 ± 16·4	185·5 ± 52·7	+34·4
	Total body	124·9 ± 8·8	124·8 ± 8·3	157·7 ± 30·8	+26·4
K^+	Head	266·2 ± 27·5	352·1 ± 30·3	350·9 ± 42·4	−0·3
	Thorax	292·2 ± 16·4	352·9 ± 28·4	366·1 ± 26·5	+3·7
	Abdomen	390·7 ± 117·7	591·5 ± 103·4	767·7 ± 150·1	+29·8
	Total body	332·6 ± 60·0	473·6 ± 68·4	538·0 ± 90·8	+13·6

For each group $n = 5$.

Table 3. *Concentrations of Na^+ and K^+ in the haemolymph of adult male* P. americana *in different states of hydration*

State of hydration	Na^+ (μ-mol/ml) Mean ± S.E.	K^+ (μ-mol/ml) Mean ± S.E.	Na^+/K^+	n
Normal	127·7 ± 4·0	8·3 ± 0·5	15·4	8
Dehydrated	137·7 ± 3·1	11·7 ± 1·3	11·8	6
Rehydrated 1 day (with food)	90·1 ± 3·9	7·5 ± 1·5	12·0	6
Rehydrated 7 days (without food)	104·0	8·3	12·5	2
Hydrated	99·8 ± 6·5	7·9 ± 2·8	12·6	4

normally fed animals there was only a small volume of fluid, or none at all, and none was seen in any of the dehydrated animals. From a comparison of the concentrations of ions per unit weight of dry tissue, it can be seen that the greatest increases in the mean Na^+ content were found in the midgut caeca, Malpighian tubules and hindgut, while for K^+ the biggest increases were found in the midgut caeca and Malpighian tubules. For these tissues dehydrated animals were generally found to show a wider range of ion concentrations than the hydrated and normally fed animals. The salt content of the salivary glands was found actually to decrease with dehydration.

Changes in the water and Na^+ and K^+ content of the coxal muscle tissue are shown in Table 5. In severely dehydrated animals the large decrease in the water content of the tissue, associated with a decrease in Na^+ per unit dry weight, is probably partly a reflexion of a decrease in the amount of haemolymph between the bundles of muscle fibres. Although tissues were well blotted on filter paper before analysis, some contamination with haemolymph would be unavoidable and much greater in well-hydrated animals than in the severely dehydrated animals where no free haemolymph was observed around the muscles. Rehydration increases the water content of the muscles again and the Na^+ returns to the level found in the tissue from normally hydrated animals. Severe dehydration did not significantly alter the total K^+ content of the muscle.

Table 4. *Na^+ and K^+ in tissues of the gut of adult male* P. americana *in various states of hydration*

			Tissue					
		State of hydration	Foregut	Salivary glands	Midgut caeca	Midgut ventriculus	Malpighian tubules	Hindgut
H_2O (%)		Hydrated	79·56 ± 1·87	93·07 ± 2·91	82·81 ± 0·86	80·36 ± 0·86	72·67 ± 0·88	80·96 ± 0·91
		Normal	75·37 ± 2·05	79·71 ± 1·42	74·82 ± 3·26	77·24 ± 2·50	68·30 ± 1·90	81·61 ± 1·06
		Dehydrated	74·17 ± 0·92	66·99	73·05 ± 5·12	74·17 ± 1·41	68·40 ± 1·55	77·41 ± 1·85
Na^+	μ-mol/g wet wt.	Hydrated	41·2 ± 2·3	22·1 ± 6·3	49·8 ± 5·2	50·8 ± 4·5	74·1 ± 5·9	43·9 ± 4·4
		Normal	42·4 ± 4·4	41·6 ± 5·3	58·7 ± 9·1	50·5 ± 7·1	73·8 ± 6·9	49·2 ± 6·9
		Dehydrated	53·3 ± 4·2	74·1	97·4 ± 16·1	70·3 ± 7·5	107·4 ± 3·3	78·5 ± 12·6
	μ-mol/g dry wt.	Hydrated	209·2 ± 15·0	327·9 ± 12·9	223·8 ± 42·7	213·0 ± 17·9	275·7 ± 30·2	218·2 ± 21·8
		Normal	169·1 ± 15·4	206·6 ± 26·1	211·4 ± 34·3	216·4 ± 36·6	237·1 ± 27·7	277·6 ± 34·7
		Dehydrated	203·6 ± 18·8	183·6	327·7 ± 70·3	294·8 ± 28·6	342·3 ± 20·1	372·0 ± 62·0
K^+	μ-mol/g wet wt.	Hydrated	57·0 ± 4·1	47·8 ± 15·0	139·7 ± 16·3	116·1 ± 10·8	136·7 ± 7·6	88·8 ± 3·4
		Normal	61·9 ± 5·4	84·9 ± 6·3	152·9 ± 20·6	118·7 ± 12·2	167·5 ± 10·8	96·0 ± 10·2
		Dehydrated	76·7 ± 4·2	150·3	256·7 ± 33·7	113·4 ± 8·6	238·5 ± 22·3	126·4 ± 12·0
	μ-mol/g dry wt.	Hydrated	283·5 ± 12·4	733·2 ± 85·3	589·1 ± 74·5	475·7 ± 24·1	508·4 ± 51·5	443·5 ± 22·0
		Normal	248·9 ± 21·7	405·9 ± 23·1	479·4 ± 56·7	507·0 ± 66·6	537·7 ± 50·1	538·1 ± 41·7
		Dehydrated	291·9 ± 20·0	452·0	838·1 ± 130·1	551·5 ± 56·8	758·1 ± 75·4	580·9 ± 51·8

All values are for means ± S.E. $n = 8$ for hydrated animals, 9 for normal, and 10 for dehydrated animals except for salivary glands, where $n = 2$.

Table 5. *Water content and Na^+ and K^+ concentrations in the coxal muscle of adult male* P. americana *in different states of hydration*

		Na^+		K^+		
State of hydration	H_2O (%)	μ-mol/g wet wt.	μ-mol/g dry wt.	μ-mol/g wet wt.	μ-mol/g dry wt.	*n*
Normal	75·82 ± 1·79	24·9 ± 2·3	106·8 ± 14·1	94·9 ± 3·2	399·9 ± 24·5	6
Dehydrated 4–6 days	72·69 ± 1·17	24·4 ± 2·1	89·0 ± 11·0	95·0 ± 4·1	340·9 ± 17·8	9
Dehydrated 13 days	55·87 ± 9·49	22·0 ± 3·2	53·6 ± 6·2	158·9 ± 12·4	400·5 ± 33·3	4
Rehydrated 1 day (without food) after 6 days' dehydration	82·04 ± 1·39	18·6 ± 1·1	107·7 ± 11·6	85·8 ± 3·0	501·4 ± 33·4	6
Rehydrated 13 days (with food) after 13 days' dehydration	76·29 ± 1·75	22·9 ± 1·7	96·9 ± 2·1	75·8 ± 5·2	327·5 ± 39·8	
Hydrated	79·73 ± 1·29	21·4 ± 2·1	110·2 ± 12·6	78·6 ± 2·8	402·2 ± 29·7	9

Analyses of the ventral nerve cord did not give an accurate picture of changes within the nervous tissue itself. The fat bodies of the cockroaches used in these experiments were extensive, and concentrations of Na^+ and K^+ in the fat bodies were almost always considerably higher than in nervous tissue. In some parts of the nerve cord the nervous tissue was completely surrounded by fatty tissue, so that any slight changes in ion content of the nervous tissue would be masked by changes in the fat body. Similarly, it was impossible to completely separate the testes from the associated fat body, and so analyses of this tissue were not carried out throughout this series of experiments. Some analyses were made of the male accessory organs (utriculi majores, utriculi

Table 6. *Na^+ and K^+ in the integument (abdominal sternites 3+4) of adult male* P. americana *in different states of hydration*

State of hydration	Na^+		K^+	
	μ-mol/g wet wt.	μ-mol/g dry wt.	μ-mol/g wet wt.	μ-mol/g dry wt.
Hydrated	67·9±11·8	153·6±25·8	86·7±10·7	205·9±41·8
Normal	84·4±7·5	214·4±31·7	97·4±12·1	238·2±26·9
Dehydrated	78·9±13·2	203·4±40·1	89·1±12·4	223·0±26·5

Table 7. *Na^+ and K^+ in the fat body tissue of adult male* P. americana *in different states of hydration*

State of hydration	Na^+		K^+			
	μ-mol/g wet wt.	μ-mol/g dry wt.	μ-mol/g wet wt.	μ-mol/g dry wt.	Na^+/K^+	*n*
Normal	133·2±17·6	210·4±29·3	852·2±43·7	1309·9±62·0	0·163±0·023	12
Dehydrated 4–6 days	153·5±26·7	233·1±51·7	609·5±103·4	831·1±124·4	0·328±0·076	9
Dehydrated 13 days	158·2±32·8	200·9±36·9	510·8±32·8	666·0±80·5	0·314±0·072	5
Rehydrated 1 day (without food) after 6 days' dehydration	106·8±9·0	169·1±16·2	840·9±59·1	1311·8±71·5	0·134±0·052	12
Rehydrated 13 days (with food) after 13 days' dehydration	40·3±12·3	63·2±19·8	175·4±22·5	282·6±54·9	0·232±0·062	4
Hydrated	120·8±17·1	250·9±40·4	777·5±62·9	1606·5±207·8	0·172±0·033	6

minores and the conglobate gland) but the Na^+ and K^+ content of these tissues from dehydrated and hydrated animals were not significantly different from normal.

Various parts of the integument (protergite and abdominal tergites and sternites) were analysed for Na^+ and K^+ content after the superficial adhering tissues had been wiped away with absorbent paper. The mean Na^+ content of animals which had been on a water-only diet was found to be lower than normal, but dehydration caused little change from the normal Na^+ or K^+ content. Values obtained for abdominal tergites 3+4 are shown in Table 6. The Na^+ and K^+ concentrations in the protergite and abdominal sternites were found to be similar to those in the abdominal tergites.

The highest concentrations of Na^+ and K^+ present in any tissue, and the greatest variability, were found in the fat body (Table 7). As has already been mentioned, the change in the organic matter of the tissue make it impossible to calculate absolute changes in the Na^+ or K^+ concentration caused by dehydration or starvation. Also, subsequent work has shown that drying fat body tissue at 105 °C causes a greater weight loss from the tissue than does drying at room temperature over phosphorus pentoxide. Therefore, it is possible that the tissue-drying procedure used in these experiments caused the loss of some volatile lipids from the tissue, which could be a source of error in comparing the dry weights of the fat body tissue from fed and starved animals. In spite of these difficulties in interpretation of the ion content of the fat body, it can be seen that dehydration causes an increase, and rehydration a decrease, in the mean Na^+/K^+ ratio of the tissue. Exceptionally low Na^+ was found in the fat

bodies of animals which had been rehydrated after being desiccated. The highest K^+ values were found in animals which had been provided with water, but no food, and K^+ decreased with dehydration.

DISCUSSION

The ability of *Periplaneta americana* to withstand periods of desiccation is due to many factors. Water loss from the body surface is kept to a minimum by low cuticular permeability (Ramsay, 1935; Beament, 1958), and Treherne & Willmer (1975) have suggested that the permeability of the integument may be under hormonal control. Animals may be able to limit evaporation from the tracheal system by control of spiracular opening, as has been demonstrated for tsetse flies in different humidities (Bursell, 1957). However, although Hazelhoff (see Jordan, 1927) showed that the degree of opening of spiracles of *Periplaneta* could be varied, *Periplaneta* has never conclusively been shown to control its spiracular openings in response to changes in humidity. Ramsay (1935) observed that temperature, humidity and wind velocity affected evaporation from the tracheal system in a way similar to their effect on purely physical systems.

Metabolic water, obtained from the oxidation of reserve foodstuffs, is an important source of water for a variety of other animals with no water to drink but, as was shown in the experiments reported here, metabolic water by itself is not sufficient to maintain water balance in *Periplaneta* and the total water content per cent in dehydrated animals is lower than in hydrated ones.

Laird, Winston & Braukman (1972) showed that removal of salivary reservoirs reduced the osmoregulatory capacities of the cockroach *Leucophaea* and they suggested that water lost during desiccation might be replaced by swallowing the saliva, which has a very low osmotic pressure. A limited source of water for the general metabolic pool in dehydrating *Periplaneta* is probably taken up from the reservoirs of the salivary glands, which may contain 0·1 ml of fluid in well-hydrated animals (Sutherland & Chillseyzn, 1968), but the fluid in these reservoirs is soon used up. In the present study, reservoirs were always found to be empty after an individual had been without water for a few days and under the same experimental conditions animals were found to be able to withstand desiccation for at least 2 weeks.

The experiments reported in this paper confirm the finding of Edney (1968) and Wall (1970) that when *Periplaneta* is dehydrated, even though the haemolymph volume is markedly reduced, the major ions (and thus the osmotic pressure) of the haemolymph show only a slight increase in concentration, indicating that some ions have been removed from the haemolymph during the period of dehydration. After adult males have been dehydrated, as well as a decrease in haemolymph volume, there is a slight decrease in the water content of the various body tissues and after 13 days' dehydration the decrease in the water content and the associated increase in the osmotic pressure in the tissues may be so great as to disrupt the normal functioning of the tissue. This was particularly noticeable in one individual which was dehydrated for 13 days. By the twelfth day of dehydration it was hyperactive and by the thirteenth day its movements were unco-ordinated and 'spastic', symptoms such as one sees before prostration in cockroaches which have been poisoned with DDT. When its muscle tissue was analysed it was found to contain only 21% water, compared with a

mean value of 56% for other individuals which had been dehydrated for the same length of time and 76% for normally hydrated animals. The muscle contained 60·7 μ-mol Na^+/g tissue wet weight and 433·3 μ-mol K^+/g, both values being considerably higher than those found in any other individual.

Several factors other than the state of hydration are likely to affect the concentrations of ions in the haemolymph. Changes in cations brought about by changes in diet have been reported (Tobias, 1948; Pichon & Boistel, 1963; Pichon, 1963, 1970). In the present study the K^+ values obtained for the haemolymph of normal animals were considerably below those given by Tobias and Pichon & Boistel, but are similar to those published by Van Asperen & Van Esch (1956) and Heit *et al.* (1973). It is likely that these differences are due to differences in diets. Pichon (1970) also observed variation in haemolymph composition for samples taken from different points on the same individual and one sample may affect subsequent samples (Brady, 1967*b*; Pichon, 1970). The number of haemocytes in the circulation is very variable and, as more than 50% of the whole haemolymph K^+ may be sequestered in the haemocytes of some specimens (Brady, 1967*a*), changes in the number of haemocytes in the haemolymph will cause variations in the K^+ concentration. An attempt was made to analyse serum samples as well as whole haemolymph, to see if changes in haemolymph K^+ were due to changes in haemocytes, but it was found impossible to achieve consistent separation of haemocytes and serum analyses were not continued. Removal of haemocytes from circulation during dehydration, and a subsequent increase in the number of circulating haemocytes when the haemolymph increases in volume again, could result in the K^+ concentrations in haemolymph from dehydrated and hydrated animals being similar, even if the serum K^+ had changed.

The only effect of desiccation on the haemocytes of *Periplaneta* which has been reported was by Taylor (1935), who observed that there was a large increase in the relative number of amoebocytes (= cystocytes – Jones, 1962) in animals deprived of moisture. It is interesting that at ecdysis, another physiological state where the blood volume changes markedly, an increase in the percentage of cystocytes has also been observed (Wheeler, 1963).

One aspect of ion and water balance which has been previously investigated is the regulation brought about by the Malpighian tubule/rectal system. The primary urine in *Periplaneta* is slightly hyperosmotic to the haemolymph (Wall, 1970; Wall, Oschman & Schmidt, 1975) and, on an average, contains more K^+ than Na^+ (Wall, 1970). In the rectum there is differential reabsorption of ions and a greater reabsorption of water in dehydrating animals than in hydrated ones (Wall, 1967; Wall and Oschman, 1970). The low water content of faecal pellets in dehydrating animals, as compared with hydrated ones, which they observed was also noted in this current work. The rate of secretion by the Malpighian tubules and reabsorption by the rectum is under hormonal control and, although this control is not yet completely understood, it appears to involve at least two hormones (see e.g. Wall & Ralph, 1962; Cazal, 1965; Cazal & Girardie, 1968; Goldbard, Sauer & Mills, 1970; Keeley, 1975). Although an investigation of these mechanisms is beyond the scope of this work, it was noted in this study that Malpighian tubules of dehydrated animals had higher than normal Na^+ and K^+ per unit dry weight and the hindgut of dehydrated animals contained higher than normal Na^+.

Probably because of higher temperature and/or lower humidity, the rate of weight loss in dehydrating animals in this study was higher than that found by Wall. A loss of about 20 mg per animal per day found here, compared with 10–12 mg per animal per day found by Wall, would mean that the degree of desiccation of animals which were dehydrated for 4–6 days in the present study should be comparable to that of individuals which Wall kept without water for 8 days. Although the amount of K^+ excreted by dehydrating animals was found to be comparable with the value obtained by Wall for animals which had been subjected to a similar amount of desiccation (cf. 4·8 μ-mol K^+ during 5 days' dehydration found in the present work and 4·2 μ-mol found by Wall for animals which had been dehydrated for 8 days), the Na^+ excreted by animals in the present experiments was much higher than that found by Wall (cf. 3·2 μ-mol with 0·4 μ-mol Na^+). This discrepancy is not surprising since the ion content of the faeces will be greatly affected by the ion content of the food, especially during the initial period of dehydration when more material is defaecated than at later stages of dehydration, and it is unlikely that the food given to the cockroaches by Wall would have the same ion content as the rat pellets with which the cockroaches were fed in the present experiments. (The rat pellets contained about 100 μ-mol Na^+/g and 180 μ-mol K^+/g.)

To calculate what proportion of the ions removed from the haemolymph is accounted for by excretion, one must have an estimate of the blood volumes in both hydrated and dehydrated animals. Haemolymph volumes for normally hydrated adult male *Periplaneta* quoted in the literature show a wide range – see Table 8. Because of the difficulty in sampling haemolymph from dehydrated animals it is impossible to use any of the standard dye or inulin dilution methods for haemolymph volume determinations and Wall (1970) is the only author who has attempted to obtain an estimate of the haemolymph volume in dehydrated adult cockroaches. After dehydration at room temperature and humidity, she removed the haemolymph by swabbing the animals' tissues with absorbent tissue and estimated that in animals which had been dehydrated for 8 days the haemolymph volume was about 9·6%. Edney (1968) found similar decreases in haemolymph in *Periplaneta* nymphs dehydrated at 0% R.H., but Yeager & Munson (1950) reported that the blood volume of nymphs was still 19% of the live weight even after 35 days' starvation and dehydration. In this study the swabbing method was used to obtain estimates of haemolymph volumes of 6 adult male cockroaches and the mean values were 19·1% for hydrated and 11·0% for dehydrated animals. Using these approximations for the blood volumes, it is possible to make a rough estimate of the amount of Na^+ and K^+ removed from the haemolymph during dehydration.

Total Na^+ and K^+ excreted have been calculated by summing the mean values for dry weights of faecal pellets and ion concentrations during 6 days of dehydration. Values used for blood volumes are 19% for normally hydrated animals and 11% for dehydrated ones. As the animals did not eat during dehydration, the overall Na^+ and K^+ balance can be summarized as in Table 9. Less than one-third of the Na^+ removed from the haemolymph during 6 days' dehydration is accounted for by excretion, and the amount of K^+ excreted is much greater than that which would have to be removed from the haemolymph alone. This suggests that during dehydration Na^+ must have been taken up by some other tissue(s) in the body, as was hypothesized by Wall (1970)

Table 8. *Blood volumes of adult male* P. americana

Method	Per cent of total wet weight Mean ± S.E.	Reference
Amaranth dye	27·5 ± 1·87	Yeager & Munson, 1950
Chloride	15·3 ± 1·21	Yeager & Munson, 1950
[^{14}C]Inulin	36·3 ± 0·68	Wharton, Wharton & Lola, 1965
Swabbing	18·0	Wall, 1970

Table 9. *Na^+ and K^+ balance during 6 days' dehydration of an average-sized adult male* P. americana

	Weight (mg)	Blood volume (μl)	Na^+ μ-mol/ml	Na^+ Total μ-mol	K^+ μ-mol/ml	K^+ Total μ-mol
Normally hydrated	1000	190·0	128	24·3	8	1·5
Dehydrated	820	90·2	138	12·4	12	1·1
Removed from haemolymph	—	—	—	11·9	—	0·4
Excreted	—	—	—	3·4	—	5·0
*Increase in tissues of the gut	—	—	—	1·4	—	1·8
Balance to be sequestered	—	—	—	7·1	—	−6·4

* This approximation was obtained by summing the mean values for tissue weight times the ion concentration difference between normal and dehydrated animals.

and Pichon (1963, 1970), and that K^+ must have been removed from some tissue(s). However, as has already been mentioned, the K^+ content of the Malpighian tubules actually increased with dehydration. Also, dehydration caused a marked increase in the K^+ content of the midgut caeca.

The large increase in K^+ in the midgut caeca in dehydrated animals is interesting because Sauer & Mills (1969) found that in a *Periplaneta* midgut ventriculus *in vitro* preparation there was generally a small but variable net flow of K^+ from the haemolymph side to the lumen, and this flow was inhibited by DNP. Sauer, Mills and co-workers investigated ion movements across only the epithelium of the ventriculus and not the caeca. O'Riordan (1969) suggested that there was a linked Na–K pump in the basal region of the epithelium of *Periplaneta* midgut ventriculus. In a study on the larvae of the freshwater mosquito *Aedes aegypti* and the saline species *A. detritus*, Ramsay (1950) found that in both species the caecal fluid was invariably hypertonic to the haemolymph in all media, while the midgut fluids in both species were approximately isotonic with the haemolymph. Harvey & Nedergaard (1964) found that in isolated midgut from the larvae of *Hyalophora cecropia* there was an electrogenic pump moving K^+ across the epithelium from the haemolymph side to the lumen, and that this K^+ movement accounted for at least 87% of the measured short-circuit current. An increase in the K^+ concentration on the blood side, at least in the range 2–64 mM, caused an increase in the short-circuit current (Nedergaard & Harvey, 1968). The increase in K^+ in the midgut caeca during dehydration which was found in this work might be brought about by an active pumping of K^+ from the haemolymph

to the lumen or, alternatively, it might be caused by the accumulation of K^+ from food which had been passed into the caeca and not absorbed during the dehydration period.

The fat body was the only tissue in which dehydration caused a decrease in the mean K^+ concentration. However, the K^+ concentrations in the fat body were very variable. The amount of K^+ in the fat body is correlated with the urate concentration (Mullins & Cochran, 1974; Tucker, 1977) and variability in K^+ is at least partly a reflexion of differences in the amount of urate accumulated in individuals.

It is not starvation which causes a reduction in the fat body K^+ because the highest K^+ values were obtained in animals which had been starved but allowed water. Therefore, low fat body K^+ would seem to be associated with lack of water.

The small increases of Na^+ in the tissues of the gut and the Malpighian tubules account for only a small fraction of the Na^+ which must have been taken up by the body tissues during dehydration. Three other tissues make up a large proportion of the body mass of *Periplaneta* – the muscle, the integument and the fat body. The first two of these did not show any increase in Na^+ after 6 days' dehydration. The Na^+ values obtained for the fat body showed a very wide range. The situation in the fat body is complex and only a partial picture of changes occurring during dehydration and rehydration can be obtained from the present study. As well as changes in Na^+ and K^+, there are large changes in other constituents of the fat body during dehydration. The concentration of urate per unit weight of fat body in dehydrated animals is about 30% higher than in normally hydrated and fed animals, and accounted for 58·8% of the total dry tissue weight, as compared with 45% in normal animals (Tucker, 1977). Also, while an animal is dehydrating it is using reserve food supplies, mainly lipid and glycogen stored in the fat body. Červenková (1960) calculated that, during starvation of 2-month-old adult *P. americana*, 66% of the energy used was obtained from oxidation of lipid, 22% came from glycogen and 11·8% from protein, while Melampy & Maynard (1937) found that starvation caused little reduction in the amount of lipid in mature male *Blattella germanica*. However, the percentage of lipid (1·7% fresh weight) found in the *Blattella* males was much lower than that (29·8% of dry weight) in the *Periplaneta* used by Červenková in her experiments. Gourévitch (1928) found that cockroaches which had been fasted had RQs of 0·69–0·85, again suggesting that energy was being obtained from lipid catabolism. The large increase in the proportion of urate in the fat bodies of dehydrated animals is without doubt at least partly caused by the disappearance of lipid and glycogen from the tissue. Because the relationship between increase in urate (and possibly also other excretory products) and decrease in food reserves is not known, it is impossible to estimate absolute changes in the concentrations of Na^+ and K^+ in the fat body tissue. Also, the degree of contamination of the fat body sample with haemolymph could well vary with the different texture of the tissue found in animals in different physiological states. In *Hyalophora* larvae Jungreis & Tojo (1973) found that haemolymph contamination of the fat body was about 30%.

However, when an animal is dehydrated, the fat body tissue, unlike the other tissues, shows an increase in the Na/K ratio and upon rehydration without food a decrease below the normal Na/K ratio. This is consistent with a hypothesis that Na^+ is sequestered in the fat body when animals are dehydrated, possibly partly in exchange

for K^+, and is then released again to help to form new haemolymph when the animal drinks and the haemolymph becomes diluted.

Because of the diffuse nature of the fat body and its close association with many other tissues, it is impossible to dissect out the entire amount of fatty tissue from an individual. The mean value for fat body dissected out from several animals was 115 mg (wet weight) and the total weight of fatty tissue in an animal will be considerably higher than this. If 7·1 μ-mol Na^+ (see Table 9) were taken up by 150 mg of fat body tissue this would bring about an increase in total Na^+ in the tissue of about 35%. If the range of Na^+ and K^+ values in the fat bodies of normally hydrated animals could be reduced, it should be possible to determine if the fat body does indeed help to regulate the Na^+ and K^+ concentrations in the haemolymph when the haemolymph volume changes. To try to reduce this variance in normally hydrated animals, and also to reduce the concentrations of Na^+ and K^+ in the fat body, some groups of animals have been kept on restricted diets before dehydration. Also, ^{22}Na has been used and serial samples of the fat body have been taken from individuals in some experiments in order to obtain a more accurate picture of the movements of Na^+ between the haemolymph and the fat body. Results of these experiments involving restricted diets, ^{22}Na uptake and serial sampling will be presented in separate papers.

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