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WATER RELATIONS IN AN INSECT, *THERMOBIA DOMESTICA*

III. EFFECTS OF DESICCATION AND REHYDRATION ON THE HAEMOLYMPH

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INTRODUCTION

The desiccated firebrat, *Thermobia domestica*, can absorb water from subsaturated atmospheres (Beament, Noble-Nesbitt & Watson, 1964; Noble-Nesbitt, 1969, 1970). The exact mechanism of uptake and its control are still obscure. It has been shown that the water content generally is not a limiting factor in the control of the mechanism, but the body volume is apparently a crucial factor (Okasha, 1971, 1972). An investigation into what happens inside the body of the firebrat due to desiccation and to rehydration seemed worthwhile. The haemolymph being an important tissue since it bathes all the other tissues was chosen for this purpose. Although the results of the present work do not shed any light on how water is extracted from subsaturated air, nevertheless, they are reported here since they describe some of the physiological changes associated with induced variations in the water relations of this species.

MATERIALS AND METHODS

For the methods of culturing, desiccation, rehydration, water-content determination and of experimenting upon insects of a known age with respect to the moulting cycle, see Okasha (1971, 1972); see also Noble-Nesbitt (1969).

The concentrations of Na^+ and K^+ were determined using untreated haemolymph. The latter was collected under liquid paraffin and a known volume was pipetted into 5 ml of double de-ionized water contained in a clean capped plastic vial. The volume of the sample ranged from 0·5 to 1·9 μl, depending on the size and state of the insect. In some cases haemolymph obtained from more than one insect had to be pooled since it was difficult to get sufficient amounts from small or desiccated insects. The concentrations of Na^+ and K^+ were measured in the same sample on an E.E.L. flame photometer using standard solutions of NaCl and KCl. There was no interference between these two ions within the range of concentrations discussed in this work. The results are expressed as the concentration of Na^+ or K^+ in mM (referring to mM/l).

For the estimation of the volume of haemolymph the weighed insect was submerged in liquid paraffin in a Petri dish half-filled with paraffin wax. The neck membrane was pierced with a dissecting needle and gentle pressure was applied to extract as

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much as possible of the haemolymph, which was collected as one droplet. Care was taken to avoid any contamination with the gut contents. The diameter of the droplet was measured using a calibrated ocular micrometer and the volume was then calculated. The calculated volumes of droplets of water determined by this method were in good agreement with the actual known volumes. Under the conditions of this work droplets of haemolymph or water acquired what appeared to be an approximately spherical shape. Insects of varying weights were used in each treatment. The volume of obtainable haemolymph (V) was plotted against the weight of the insect (W) and a regression line representing the mean V/W ratio and constrained to pass through the origin was drawn.

The concentrations of Na^+ and K^+ in the faeces were determined in the following way. Soon after collection the faecal samples were placed in clean glass vials at 56 °C for 2 or 3 days. Each sample, taken from several insects, was then examined under a stereomicroscope to remove any exuvial remains. After weighing, the samples were placed in clean platinum boats which were left for 24 h in a muffle furnace at 650 °C. The ash of each sample was dissolved in 5 or 10 ml of double de-ionized water and the ionic concentrations were determined as described above. The same procedure was adopted for the determination of Na^+ and K^+ concentrations in whole insects except that samples were left for 4 h in the furnace and the ash of each insect was dissolved in either 50 or 100 ml of double de-ionized water. Insects were dried to constant weight at 56 °C before being placed in the furnace.

Haemolymph Cl^- concentration was determined according to the first potentiometric method of Ramsay, Brown & Croghan (1955). A sample of untreated haemolymph measuring 1·2 μl was pipetted in 1 ml of double de-ionized water contained in a clean capped plastic vial. From this, 0·5 ml was titrated against a solution of $AgNO_3$ of known concentration. A solution of NaCl of known concentration was used as a standard.

The method of Rosen (1957) was used for the determination of the concentration of free α-amino acids in the haemolymph. The sample of haemolymph measured 1·95 μl and blanks without haemolymph were run with each experiment. The optical density of the reaction mixture was measured on a Hilger and Watts MK_2 spectrophotometer against distilled water as a reference. Leucine/isoleucine of a known concentration was run as a standard. The results are expressed as mg amino N/100 ml haemolymph.

RESULTS

Haemolymph Na^+ and K^+ during the moulting cycle

Before trying to discover the effects of dessication or rehydration on the haemolymph it was necessary to establish whether there are any changes in the concentrations of Na^+ and K^+ during the various stages of the moulting cycle. The results shown in Fig. 1 indicate that there are no drastic changes in the concentration of either ion. It will be recalled that the water content of whole insects remains relatively constant during the period between two ecdyses (Okasha, 1972), although the feeding activity varies a great deal (Watson, 1967).

It was therefore considered safe to use insects of unknown ages with respect to the moulting cycle in the experiments to be described below.

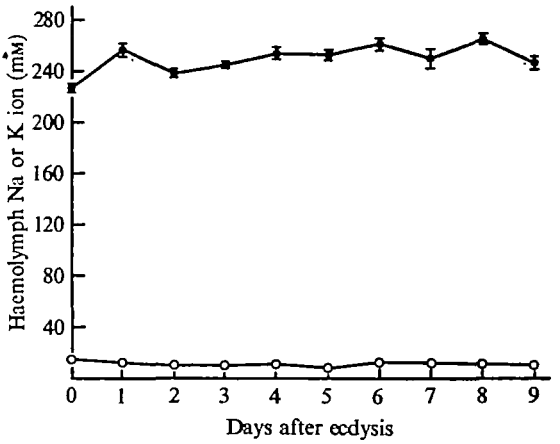

Fig. 1. Concentrations of Na^+ and K^+ in the haemolymph during the moulting cycle. ●, Na^+; ○, K^+; vertical lines, standard error. The scale used was too small to allow the plotting of the standard error for K^+.

Haemolymph Na^+ and K^+ following desiccation, rehydration, and starvation

Insects were desiccated for various periods and then the haemolymph was assayed for Na^+ and K^+. Rehydration was allowed for 1 day only. Control insects were kept in hydrating conditions without food. The insects were weighed before and after the application of a particular treatment to ensure that the treatment brought about its expected effects (in terms of body weight losses or gains).

It became evident from the results of several experiments that desiccation, desiccation followed by rehydration, and starvation exert little or no effect on haemolymph concentrations of Na^+ and K^+. The data presented in Table 1 summarize pooled results in the case of some treatments. Although insects desiccated for 3 days appear to have the highest Na^+ concentration ($239{\cdot}1 \pm 7{\cdot}0$ mM/l) shown in Table 1, this value is in close agreement with concentrations obtained from untreated insects (see Fig. 1). Those insects desiccated for 2 days and then rehydrated exhibit the lowest Na^+ concentration ($185{\cdot}2 \pm 4{\cdot}8$ mM/l). In the case of K^+ the highest concentrations are those shown by insects desiccated for 2 days and the lowest by insects desiccated for 3 days then rehydrated. However, on several occasions almost the same values for Na^+ and for K^+ concentrations were obtained whether the insects were subjected to desiccation, to desiccation followed by rehydration, or to starvation alone. The use of different batches of insects, individual variations within the same batch and experimental errors might partly account for the variations amongst the different treatments shown in Table 1. But the possibility that desiccation results in a slight increase in the concentration of these ions, whereas rehydration causes a slight reduction cannot be entirely excluded. The N^+/K^+ ratio is similar to that characteristic of most insects.

Changes in haemolymph volume induced by desiccation, rehydration or starvation

During the course of the previous experiments it proved very difficult to collect sufficient haemolymph for analyses from desiccated insects. This necessitated studying the effects of various factors on the volume of the haemolymph.

Table 1. *Effects of desiccation, of desiccation followed by rehydration and of starvation on the concentrations of Na^+ and K^+ in the haemolymph*

Treatment	No. of determinations	Concentration (mM/l ± S.E.)		Na^+/K^+ ± S.E.
Untreated	19	219·2 ± 4·1	15·7 ± 1·1	14·9 ± 0·8
1 day desiccation	34	216·4 ± 6·0	16·5 ± 0·7	13·9 ± 0·7
2 days desiccation	22	209·2 ± 3·1	18·7 ± 1·4	12·5 ± 0·9
3 days desiccation	42	239·1 ± 7·0	16·9 ± 0·9	14·7 ± 0·6
2 days desiccation and 1 day rehydration	7	185·2 ± 4·8	16·0 ± 2·1	12·4 ± 1·1
3 days desiccation + 1 day rehydration	45	187·1 ± 1·6	10·3 ± 0·4	19·3 ± 0·7
3 days starvation in hydration	13	198·0 ± 7·0	12·5 ± 0·9	16·7 ± 1·1
4 days starvation in hydration	21	214·3 ± 1·8	14·2 ± 0·9	16·1 ± 0·8
5 alternate cycles of 3 days desiccation + 1 day rehydration	21	190·7 ± 2·4	14·7 ± 1·1	14·4 ± 1·1
20 days starvation in hydration	13	191·7 ± 5·2	18·6 ± 1·8	11·3 ± 0·9

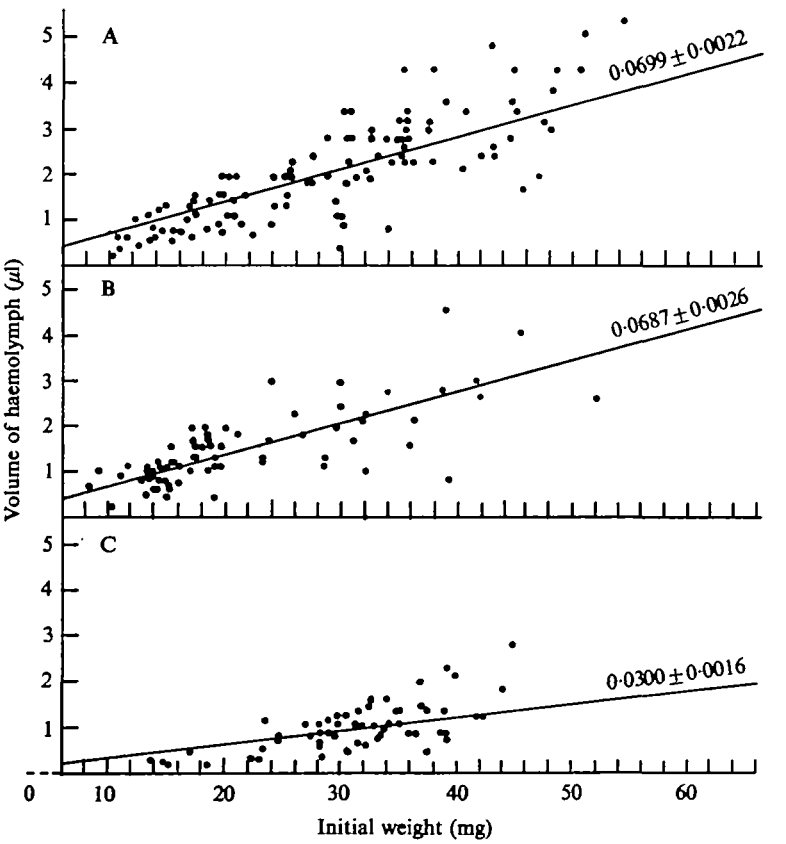

Fig. 2. Effects of desiccation and of rehydration on the volume of haemolymph. Each point represents one individual and values above each regression line show the mean V/W ± the standard error. A, Untreated controls; B, 3 days desiccation and 1 day rehydration; C, 3 days desiccation.

The results represented in Fig. 2 illustrate the relationship between the weight of the insect and the volume of obtainable haemolymph. It will be seen that in untreated insects the volume increases with the increase in body weight; the mean V/W is $0{\cdot}0699 \pm 0{\cdot}0022$ (Fig. 2A). If the insects are desiccated for 3 days (Fig. 2C) the volume of obtainable haemolymph is drastically reduced, and it seems reasonable to assume that this reflects a drastic decrease in the actual haemolymph volume. The mean V/W is only $0{\cdot}0300 \pm 0{\cdot}0016$. Following the rehydration of such desiccated insects the volume rises to a level comparable to that of untreated insects; the mean V/W in the rehydrated group is $0{\cdot}0687 \pm 0{\cdot}0026$ (Fig. 2B).

Dehydration causes a reduction in haemolymph volume whereas rehydration results in an increase in many other insects, e.g. in female larvae of *Chortoicetes terminifera* (Djajakusumah & Miles, 1966), in nymphs of *Arenivaga* sp. and of *Periplaneta americana* (Edney, 1968), and in adult males of *P. americana* (Wall, 1970). From these examples only *Arenivaga* is known to take up water from subsaturated atmospheres (Edney, 1966, 1968).

The results obtained from the rehydrated insects shown in Fig. 2 do not necessarily mean that starvation has no effect on the volume of haemolymph. This is because (i) the volume of obtainable haemolymph in Fig. 2 is plotted against the initial weight; the fact that the latter was slightly higher than the post-rehydration weight might conceal any starvation effects; and (ii) the experimental period (4 days) might be too short to allow the detection by such a crude technique of any possible changes in haemolymph volume caused by starvation. However, when the volume was plotted against the final rather than the initial weight (Fig. 3) essentially the same results were obtained (cf. V/W ratios in Figs. 2 and 3).

If there is any increase in haemolymph volume due to starvation, it should be manifested in severely starved insects. Fig. 4 shows the results obtained from female insects starved for 3 weeks. Although there is almost no difference between starved and untreated insects when the volume is plotted against the initial weight (mean $V/W = 0{\cdot}0684 \pm 0{\cdot}0024$ and $0{\cdot}0699 \pm 0{\cdot}0022$ respectively), there is a substantial difference if volume is plotted against the final weight. In this latter case the mean V/W for the starved insects is $0{\cdot}0802 \pm 0{\cdot}0028$ (Fig. 4C). These results strongly suggest that in starved females the absolute amount of haemolymph found in the insect before the onset of starvation remains more or less constant, despite the loss in body weight. Prolonged starvation therefore results in insects with a higher proportion of their wet weight as haemolymph, assuming that the density of the haemolymph does not change drastically during starvation.

The results of a similar experiment designed to assess the effect of severe starvation on males are shown in Fig. 5. It will be noted from a comparison between Figs. 4A and 5A that, weight for weight, the male appears to have a lower haemolymph volume than the female. This does not hold true if starved insects are compared (cf. Figs. 4B and 5B or Figs. 4C and 5C) since the V/W ratio is approximately the same in starved insects of both sexes. The values for untreated males may be too low, but it must be mentioned that the mean V/W shown in Fig. 5A is the average of two different experiments. In the first experiment a mean V/W ratio equal to $0{\cdot}0581 \pm 0{\cdot}0027$ was obtained, and in the second the corresponding figure was $0{\cdot}0509 \pm 0{\cdot}0017$. Both these values are somewhat lower than in the case of untreated females. However, it must

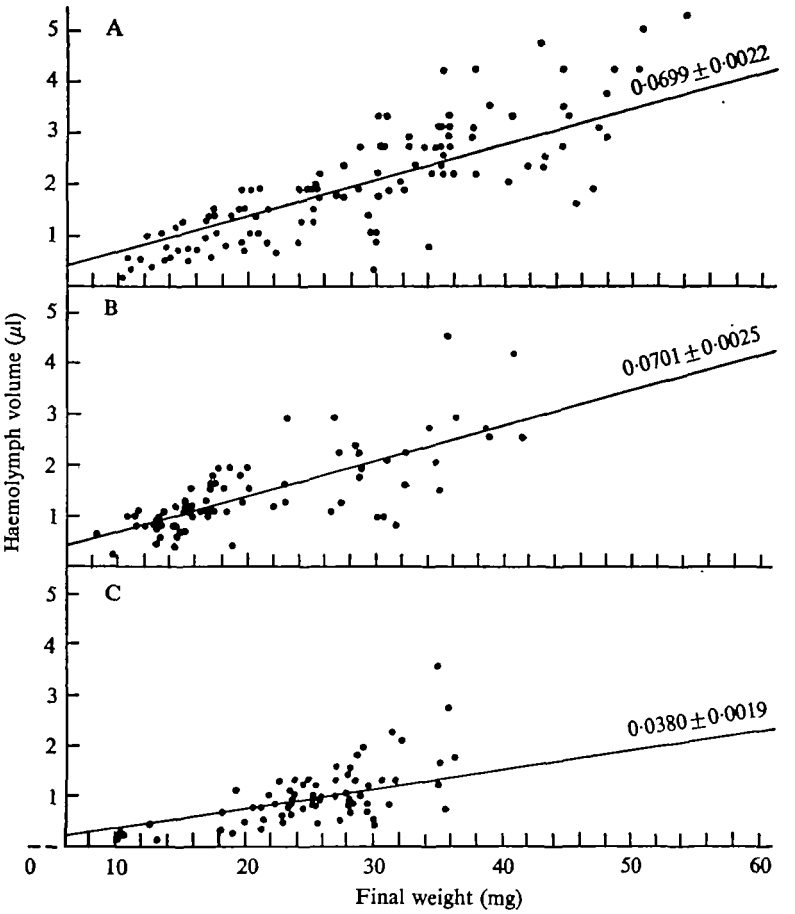

Fig. 3. The same as in Fig. 2 except that the volume of haemolymph is plotted against the final and not the initial fresh weight of the insect.

be emphasized that a less inaccurate method would be needed to substantiate the presence of a sexual difference in this respect. Any blood dilution method, however, using amaranth for example (Wheeler, 1963) or [^{14}C]inulin (Wharton, Wharton & Lola, 1965) would have been very difficult if not impossible in practice in the case of *Thermobia* because of the small size of the insect and its extreme fragility.

From these last experiments it can be concluded that the volume of haemolymph in a starved insect of either sex is higher than in a feeding insect of a similar weight.

Ionic regulation during desiccation and rehydration

Despite the pronounced changes in the volume of haemolymph induced by desiccation or by rehydration (Figs. 2, 3) the concentrations of Na^+ and K^+ in the haemolymph remain more or less constant under these conditions (Table 1). An attempt to discover some of the factors involved in regulating the ionic concentration in the haemolymph was made by conducting the following experiments.

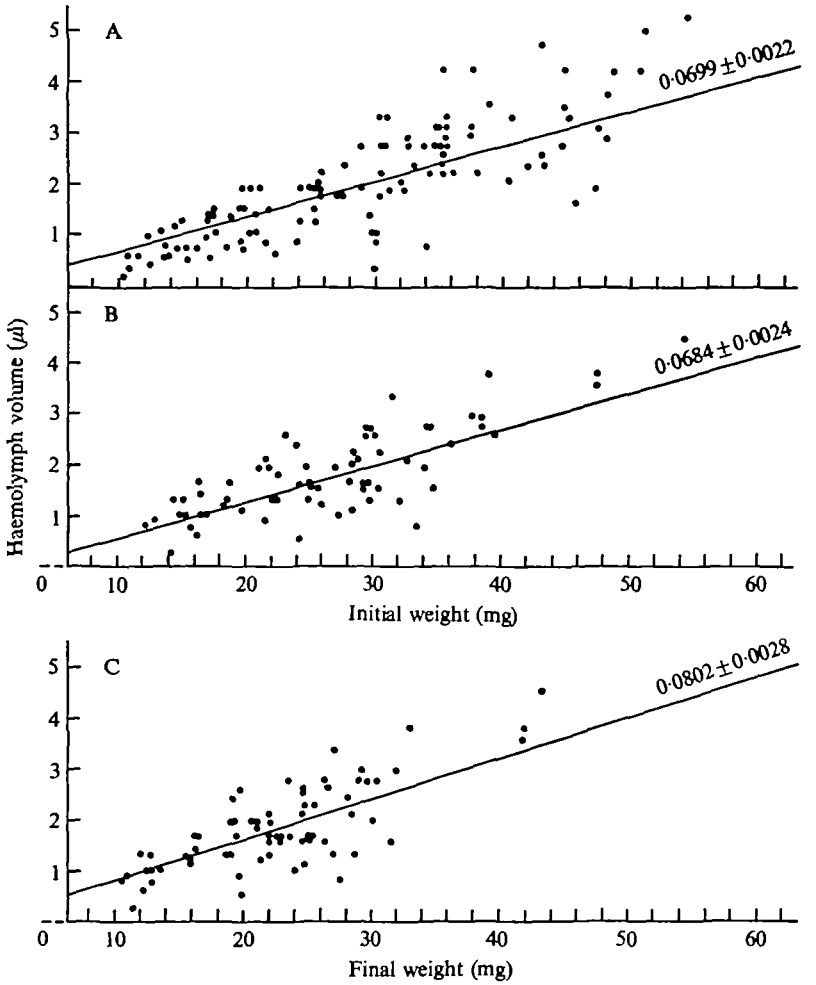

Fig. 4. Effect of starvation on the volume of haemolymph in female insects. Details as in Fig. 2. A, Untreated controls; B, females, starved for 3 weeks (plotting against initial fresh weight of the insect); C, same females except that plotting is against final fresh weight.

1. *Concentrations of Na^+ and K^+ in the faeces*

The possibility that 'excess' ions in the haemolymph under conditions of water stress are voided with the excreta was investigated. Faecal samples collected from desiccated insects were analysed together with controls collected from insects starved in hydrating conditions. Each sample consisted of faeces produced by 4 or more insects. The results are presented in Table 2. It must be borne in mind that the ionic composition of faeces produced by insects that are allowed food might be different from the results discussed here.

From Table 2 it is obvious that the concentrations of both Na^+ and K^+ in faeces produced by desiccated insects vary greatly, thus making it difficult to compare them with the controls. However, the Na^+/K^+ ratio in three different experiments in which the insects were subjected to the same length of desiccation is fairly consistent, the ratio ranging from $0{\cdot}77 \pm 0{\cdot}11$ to $0{\cdot}91 \pm 0{\cdot}17$ (Table 2). It seems therefore that

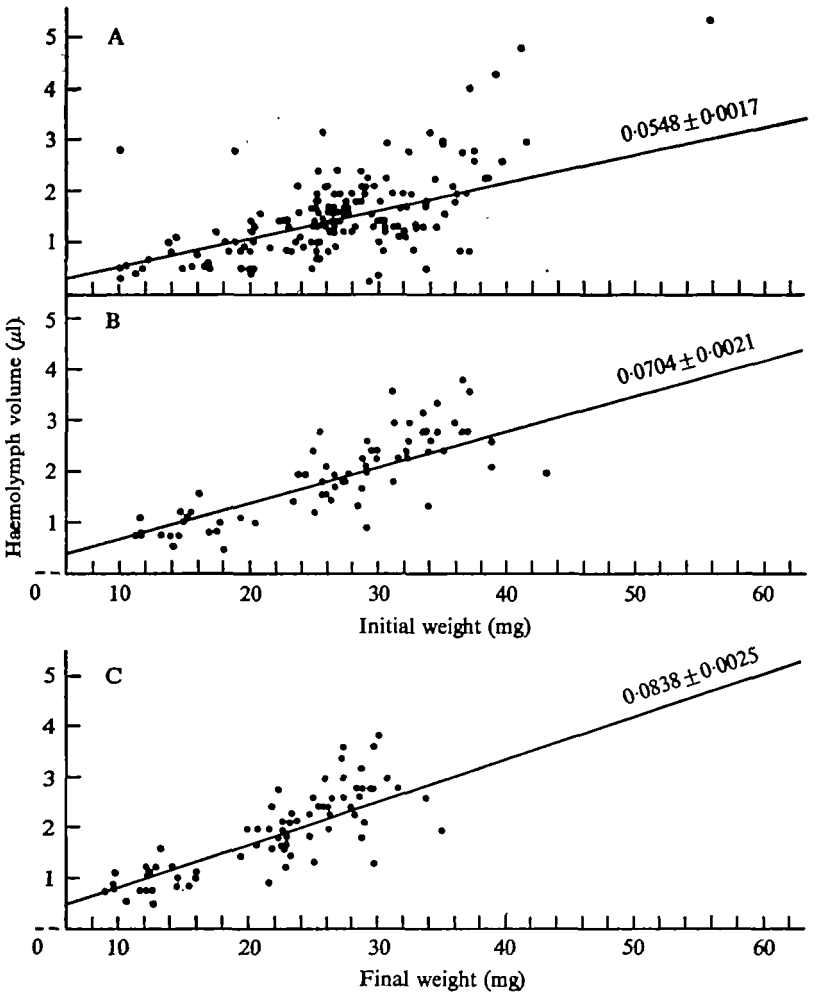

Fig. 5. Effect of starvation on the volume of haemolymph in male insects. Details as in Fig. 2. A, Untreated controls; B, males starved for 3 weeks (plotting against initial fresh weight of the insect); C, same males except that plotting is against final fresh weight.

Table 2. *Concentrations of Na^+ and K^+ in faecal samples collected from starved insects subjected either to desiccation or to hydrating conditions*

Treatment	No. of insects used	No. of samples collected	Mean weight of faeces (mg/insect/day)	Concentration (μg ion/mg faeces) ± S.E.		Na^+/K^+ ± S.E.
3 days desiccation	40	10	0·0817	10·59 ± 0·95	14·58 ± 0·85	0·91 ± 0·17
3 days desiccation	40	7	0·0683	4·58 ± 0·61	6·46 ± 0·63	0·80 ± 0·18
3 days desiccation	40	10	0·0817	5·77 ± 0·59	8·64 ± 1·3	0·77 ± 0·11
3 days hydration	36	6	0·0713	2·64 ± 0·42	16·17 ± 1·02	0·17 ± 0·03
4 days hydration	25	5	0·0890	2·78 ± 0·28	16·47 ± 1·10	0·17 ± 0·02
7 days hydration	40	6	0·0495	2·90 ± 0·48	12·63 ± 1·84	0·24 ± 0·04

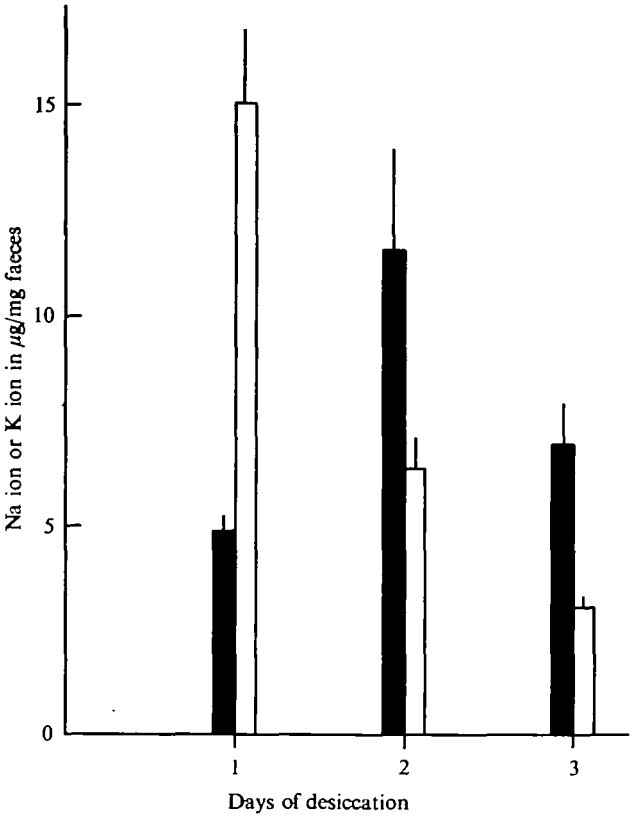

Fig. 6. Ionic concentrations of faecal samples collected daily from insects subjected to desiccation. Black columns, Na^+; white columns, K^+; vertical lines, standard error.

these variations are most probably due to errors in determining the weights of faecal samples; each sample weighed about 1 mg or even less whereas the accuracy of the balance was $\pm 0{\cdot}1$ mg. Conversely, there is some consistency in the concentrations of Na^+ and K^+ in the controls. A comparison of the desiccated and the control groups reveals that the Na^+/K^+ ratio is increased by a factor of around 3–5 by desiccation. Whether this is a consequence of the voiding of more Na^+ in the faeces, or of the retention of K^+ by the insect, or of both processes, will be considered later.

To investigate the time course of changes in the ionic composition of faeces samples were collected daily for 3 days from a large number of individuals. Each sample consisted of faeces collected from 10 or more insects. The samples were assayed as usual together with the controls, and the results are represented in Figs. 6 and 7 and in Table 3.

From Fig. 6 it is clear that the concentration of Na^+ rises to reach its maximum by the end of the second day of desiccation, whereas that of K^+ progressively decreases over the experimental period. In the controls (Fig. 7) the concentration of Na^+ is very low and probably does not change much while that of K^+ again decreases continually. Taken together, Figs. 6 and 7 indicate that Na^+ concentration is increased by desiccation, especially after the 2nd and 3rd days of the treatment. These figures

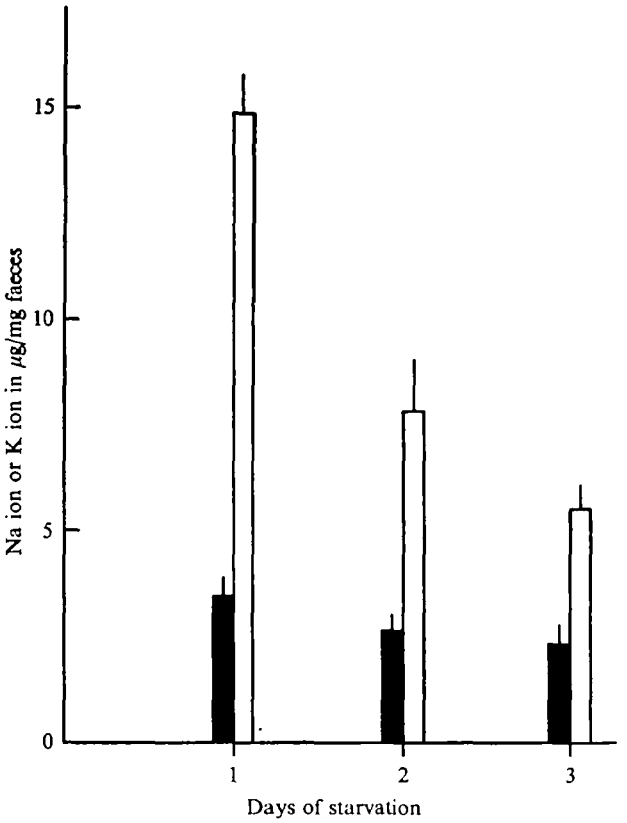

Fig. 7. Ionic concentration of faecal samples collected daily from insects starved in hydrating conditions. Black columns, Na^+; white columns, K^+, vertical lines, standard error.

Table 3. *Ionic content of faeces collected daily from starved insects subjected either to desiccation or hydrating conditions*

Samples collected after	No. of samples	Mean weight of faeces in mg/insect	Na^+/K^+ of daily sample ±S.E.	Average μg ion voided/insect		Na^+/K^+ of total amounts of faeces
				Na^+	K^+	
Treatment: desiccation (100 insects used)						
1st day	10	0·1250	0·34 ± 0·03	0·5975	1·8738	0·67
2nd day	8	0·0611	2·58 ± 0·76	0·7002	0·3819	
3rd day	5	0·0420	2·48 ± 0·51	0·2898	0·1247	
Total	—	0·2281	—	1·5875	2·3804	
Treatment: hydration (140 insects used)						
1st day	13	0·1343	0·23 ± 0·03	0·4553	1·9971	0·27
2nd day	10	0·0714	0·35 ± 0·04	0·1885	0·5582	
3rd day	11	0·0693	0·43 ± 0·06	0·1615	0·3791	
Total	—	0·2729	—	0·8053	2·9344	

also suggest that K^+ concentration hardly changes during desiccation, although it might be somewhat lower in the 3rd day samples than in the controls.

Assuming that weight recording of faecal samples is reliable, then the data presented in Table 3 show that there is no appreciable difference in the weight of excreta

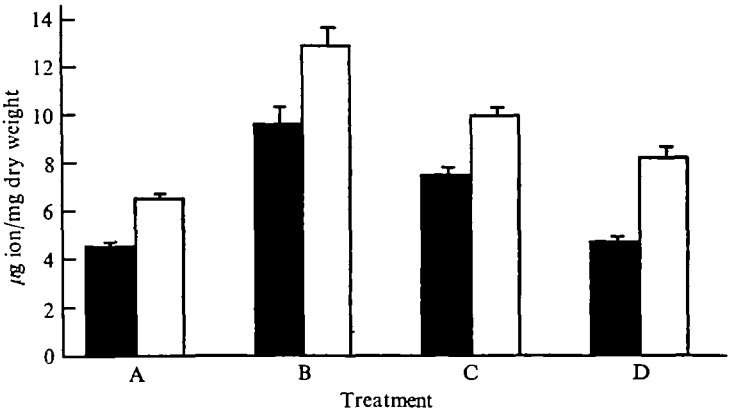

Fig. 8. Concentrations of Na^+ and K^+ in the ash of whole insects on a final dry body weight basis. Black columns, Na^+, white columns, K^+; vertical lines, standard error. A, untreated controls; B, starved for 20 days in hydration; C, 5 alternate cycles each consisting of 3 days desiccation and 1 day rehydration and starvation; D, 5 alternate cycles each consisting of 3 days desiccation and 1 day rehydration and food.

voided per insect per day in either treatment over the experimental period (see also Table 2). During 3 days of desiccation an insect produces on average 0·2281 mg faeces containing 1·5875 μg Na^+ and 2·3804 μg K^+. An insect in the control group produces 0·2729 mg of faeces containing 0·8053 μg Na^+ and 2·9344 μg K^+. Allowing for the small difference in the weight of faeces it can be concluded that the amount of Na^+ voided per insect during 3 days of desiccation is approximately double that of the controls and that the amount of K^+ is nearly the same in the 2 groups over the experimental period. The Na^+/K^+ ratio changes profoundly during the 2nd and 3rd days of desiccation (Table 3). It is worth noting that the overall Na^+/K^+ ratios in both the desiccated and control groups in this experiment are comparable to those shown in Table 2.

2. *Concentrations of Na^+ and K^+ in whole insects*

Compared with the Na^+ content of the whole insect the extra Na^+ voided due to desiccation would be negligible. Indeed, there was no measurable difference in the concentration of either Na^+ or K^+ in the whole body of insects desiccated for 3 days compared with starved hydrated controls. However, when the insects were subjected to five alternate cycles each consisting of 3 days desiccation and 1 day rehydration, a different pattern was obtained.

In Figs. 8 and 9 the results of Na^+ and K^+ determinations in the body of insects subjected to different treatments are illustrated. It was found that there are no obvious sexual differences in the concentration of either ion under any of the treatments discussed here and consequently only the combined data derived from both sexes are shown here.

On a final dry weight basis it will be seen that the concentrations of Na^+ and K^+ in insects subjected to repeated desiccation and rehydration while starved (Fig. 8C) are lower than those in starved hydrated controls (Fig. 8B). In addition, the concentrations of Na^+ and K^+ in insects subjected to these two treatments are considerably higher than those characteristic of untreated insects (Fig. 8A). The results obtained

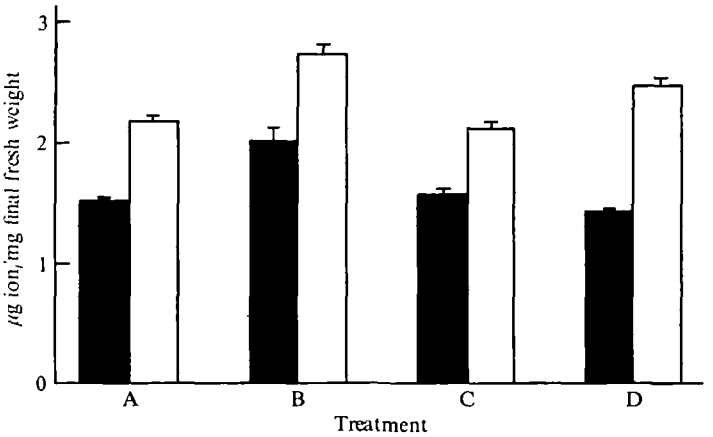

Fig. 9. The same as in Fig. 8 except that concentrations are expressed on the basis of the final fresh body weight.

Table 4. *Effects of repeated desiccation and rehydration and of starvation on Na^+/K^+ in the ash of whole insects*

Treatment	No. of insects	$Na^+/K^+ \pm$ S.E.
Untreated	20	0·70 ± 0·02
Starved (20 days) in hydration	14	0·73 ± 0·03
5 cycles of desiccation* + rehydration + starvation	17	0·75 ± 0·02
5 cycles of desiccation* + rehydration + food	14	0·59 ± 0·02

* Each cycle consisted of 3 days desiccation followed by 1 day rehydration.

from insects subjected to repeated desiccation and rehydration but in the presence of food are also included (Fig. 8D). In this latter case the Na^+/K^+ ratio is apparently lower than in other treatments (for detailed values see Table 4). Whether this is due to the elimination via the faeces of more Na^+ than K^+ during repeated desiccation, to selective intake through the diet of more K^+, or to both processes, is not clear.

The tremendous differences in concentrations shown in Fig. 8 between untreated insects and those starved either in hydration or in desiccation and rehydration must be attributed to the depletion of dry matter by starvation. This is supported by the finding that the differences are not as great when the results were computed on a final fresh-weight basis (Fig. 9). In fact, there is no measurable difference between untreated insects (Fig. 9A) and those starved in hydration (Fig. 9B) when the results were calculated on an initial fresh-weight basis (where applicable). But it must be pointed out that repeated desiccation and rehydration of starved insects (Figs. 8C, 9C) causes the loss of both Na^+ and K^+ as compared with the starved hydrated controls (Figs. 8B, 9B). Previous work has shown that the depletion of dry matter in these two treatments occurs almost exactly to the same extent (Okasha, 1971). The only route for the loss of Na^+ and K^+ would be through the faeces. Unfortunately no attempt was made to investigate the ionic content or concentrations in faeces produced by such treated insects. It is of some interest, however, to note that Na^+ and K^+ concentrations in the haemolymph of insects subjected to repeated desiccation and rehydration do not differ from those of the hydrated controls (Table 1).

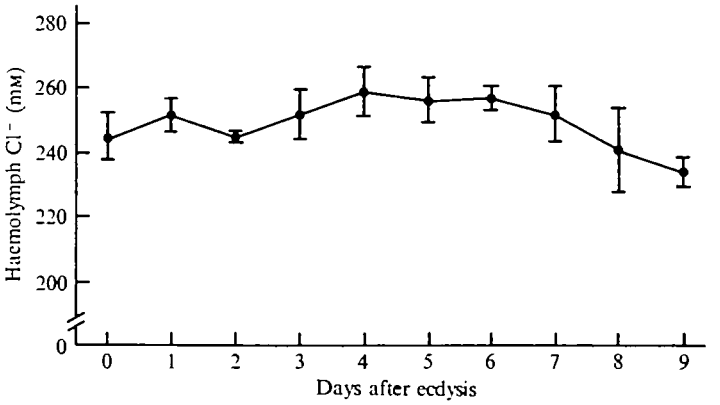

Fig. 10. Concentration of Cl^- in the haemolymph during the moulting cycle. Vertical lines, standard error.

Table 5. *Effects of desiccation, desiccation followed by rehydration and of starvation on the concentration of chlorides in the haemolymph*

Treatment	No. of determinations	Concentration (mM/l ± S.E.)
Untreated	4	268·2 ± 5·6
3 days desiccation and starvation	6	274·3 ± 6·3
3 days starvation in hydration	9	219·4 ± 2·9
3 days desiccation and 1 day rehydration and starvation	5	250·7 ± 8·0
4 days starvation in hydration	5	225·3 ± 6·4
5 alternate cycles of desiccation and rehydration in starvation	12 ♀,	200·9 ± 3·2
	11 ♂,	202·1 ± 5·0
	together	201·5 ± 2·7
Starved (20 days) in hydration	10 ♀,	239·0 ± 8·0
	5 ♂,	214·7 ± 6·6
	together	230·9 ± 6·2

3. *Concentrations of Cl^- and free amino acids in the haemolymph*

In view of the previous findings attention was directed towards other important constituents of the haemolymph; chlorides and free α-amino acids only are considered here.

The results illustrated in Fig. 10 show that Cl^- concentration in the haemolymph remains more or less constant during the moulting cycle and therefore insects of unknown ages were used. The data presented in Table 5 suggest that starvation for up to 20 days in hydrating conditions results in a slight reduction of Cl^- concentration. On the other hand, desiccation appears to cause a slight increase which counterbalances starvation effects. Thus in insects desiccated for 3 days while starved the concentration is almost the same as that in untreated insects. Rehydration seems to reduce the concentration of intermediate levels. For some reason the concentration in insects subjected to repeated desiccation and rehydration is fairly low compared with that of the starved hydrated controls. According to Edney (1968) the concentrations of chlorides in the haemolymph of both *Arenivaga* and *Periplaneta* nymphs are not significantly different before and after dehydration.

As for the effects on free amino acids in the haemolymph, only cursory experiments were undertaken and the results are summarized in Table 6. These results suggest

Table 6. *Effects of desiccation and of desiccation and subsequent rehydration on the concentration of α-amino acids in the haemolymph*

Experiment	Treatment	No. of determinations	Concentration ± S.E. (mg amino N/100 ml)
1	3 days desiccation	7	17·3 ± 2·2
	3 days starvation in hydration	9	21·9 ± 3·5
2	3 days desiccation + 1 day rehydration	10	18·3 ± 1·5
	4 days starvation in hydration	7	19·9 ± 1·5
3	3 days desiccation + 1 day rehydration	7	16·5 ± 1·3
	4 days starvation in hydration	9	21·0 ± 2·2

that there are no substantial differences between desiccated, desiccated then rehydrated, or starved hydrated insects. But it is not known whether there are major differences in concentration during various stages of the moulting cycle. In the locust *Chortoicetes*, Djajakusumah & Miles (1966) report that increases in haemolymph proteins due to dehydration are accompanied by decreases in free amino acids and vice versa, and indeed Wall (1970) refers to their finding that the concentration of free amino acids in the haemolymph increases after the desiccated nymphs are allowed access to water. However, their control insects which were kept over distilled water showed progressive increases in amino acid concentration over the 48 h of the experiment and such increases approximate to those of the desiccated then rehydrated insects (see table 1 and fig. 4(*a*) in their work).

DISCUSSION

It is well known that insects can tolerate wide variations in their haemolymph concentrations without apparent ill-effects (Barton-Browne, 1964). The foregoing results show the ability of *Thermobia* to regulate its ionic concentrations under varied environmental conditions. On the one hand desiccation greatly reduces the haemolymph volume and rehydration restores it. On the other hand the concentrations of Na^+, K^+ and free amino acids in the haemolymph remain relatively constant, and chloride concentration fluctuates only slightly. But none of these constituents has been found to undergo changes proportional to the pronounced decreases and increases in the haemolymph volume. Similar findings have been reported in other insects. For example, the osmotic pressure of the haemolymph changes very little after severe desiccation in *Chortoicetes* (Djajakusumah & Miles, 1966) and in adult males of *Periplaneta* (Wall, 1970). Although the osmotic pressure is raised by desiccation and lowered by rehydration in *Periplaneta* and *Arenivaga* nymphs, the measured changes in osmotic pressure are less than they would have been in the absence of osmoregulation (Edney, 1968).

The moving of solutes from the haemolymph during desiccation and back into it due to rehydration has been suggested in all the above-mentioned examples, but none of the authors has shown where the solutes are moved to or from. In her

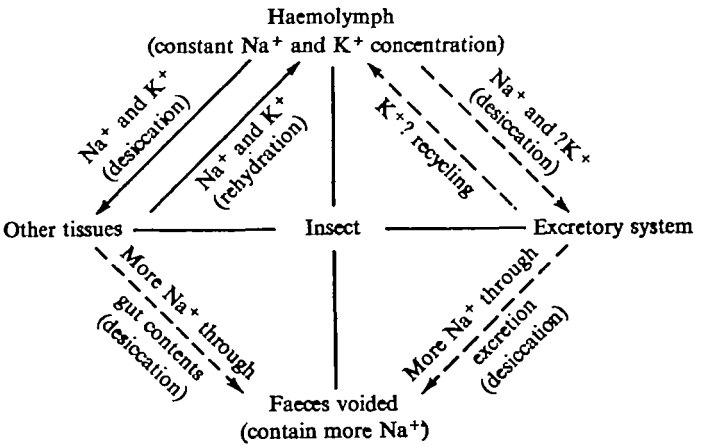

Fig. 11. Diagrammatic representation of the possible mechanisms for ionic regulation during one cycle of 3 days desiccation and 1 day rehydration. Solid arrows indicate likely pathways, broken arrows indicate possible pathways.

detailed study Wall (1970) stated that the Malpighian tubules can remove the 'excess' solutes from the haemolymph during desiccation, but very little Na^+ is excreted in the faecal pellets (only about 3·3% of the NaCl that had to be removed from the haemolymph). Further, she calculated that the crop, midgut, colon and rectum together cannot feasibly provide the major part of the solutes necessary for the formation of new haemolymph in the rehydrated cockroach. The amounts of any solutes that had to be moved from and into the haemolymph in *Thermobia* cannot be reliably estimated and must await a better method of blood-volume determination.

The diagram shown in Fig. 11 summarizes the possible mechanisms that might be involved in the ionic regulation of the haemolymph during one cycle of 3 days desiccation and 1 day rehydration. This does not apply in the case of repeated desiccation and rehydration since this treatment causes the voiding of both Na^+ and K^+. Obviously, the diagram in Fig. 11 is not wholly based on experimental evidence, but partly on speculation and on what is known from other insects. However, it seems certain that desiccation of the firebrat causes the voiding of more Na^+ in the faeces and that above a certain degree of tolerance both Na^+ and K^+ are got rid of, and conceivably other ions in addition.

It is of some interest to know that the elevated water content in severely starved insects (Okasha, 1972) is reflected in an increased haemolymph volume (the absolute amounts of both water and haemolymph initially found in the insect apparently remain unchanged). In adult males of *Periplaneta* starvation causes an increase in total water percentage that is associated with a decrease in haemolymph volume percentage. But in the fed insect although the total water percentage hardly changes with age, the blood volume markedly decreases during the first 4 days (Wharton, Wharton & Lola, 1965). It would be interesting to discover whether there is any particular pattern of haemolymph volume which can be correlated with the constant water content during the moulting cycle in *Thermobia*.

From the work described in this paper nothing can be said as to the mechanism of water uptake from subsaturated air except perhaps that water uptake by the

desiccated firebrat is apparently not concerned with ionic regulation of the haemolymph or at least in so far as the substances studied here are concerned.

SUMMARY

1. The concentrations of Na^+ and K^+ in the haemolymph remain relatively constant during the moulting cycle.

2. Desiccation, desiccation followed by rehydration and starvation exert little or no effect on the concentrations of Na^+ and K^+ in the haemolymph.

3. The volume of haemolymph decreases during desiccation and increases after rehydration.

4. More Na^+ is voided in the excreta during desiccation.

5. Repeated desiccation and rehydration causes the loss of both Na^+ and K^+ from the body.

6. The effects of desiccation and rehydration on the concentrations of chlorides and free amino acids in the haemolymph are described.

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