

## WATER RELATIONS IN AN INSECT, *THERMOBIA DOMESTICA*

### II. RELATIONSHIPS BETWEEN WATER CONTENT, WATER UPTAKE FROM SUBSATURATED ATMOSPHERES AND WATER LOSS

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#### INTRODUCTION

The apterygote *Thermobia domestica* takes up water from subsaturated atmospheres (Beament, Noble-Nesbitt & Watson, 1964; Noble-Nesbitt, 1969). The desiccation of this species for 3 days over dry  $\text{CaCl}_2$  at  $37^\circ\text{C}$  while starved results in a loss of some 20-25% of the fresh body weight, mainly due to water loss. However, subsequent rehydration for 1 day of such treated insects while still starved at the same temperature but exposed to 83% R.H. results in the attainment of approximately the pre-desiccation weight (Noble-Nesbitt, 1969; Okasha, 1971). The water content is reduced by desiccation, but following rehydration not only does it rise to its pre-desiccation level, but it exceeds the latter, primarily due to the depletion of dry matter caused by starvation and secondarily due to water uptake from the atmosphere (Noble-Nesbitt, 1969; Okasha, 1971).

In the previous paper (Okasha, 1971) it was suggested that water uptake in *Thermobia* does not take place in order that the 'normal' water content can be restored, but in order that the insect can reach a particular volume and that the latter is characteristic of the physiological state of the insect. It is the aim of the present paper to demonstrate beyond doubt the occurrence of water uptake from the atmosphere by firebrats with an already elevated water content, and to show that in general the absolute value of the water content cannot be regarded as a limiting factor in the process of water uptake. This paper also describes the results of experiments designed to investigate the relationship between water content and the rate of water loss.

#### MATERIALS AND METHODS

The materials and methods used in the present paper are those described in the previous one (Okasha, 1971). For clarity, a few points will be mentioned briefly. Desiccation was effected by placing individual insects in desiccators containing granular dry  $\text{CaCl}_2$ . For rehydration or for hydrating conditions, the desiccators contained a saturated solution of KCl (83% R.H.). Both the stock culture and the experimental insects were permanently kept at  $37^\circ\text{C}$ . Water content was determined by drying insects to constant weight at  $56^\circ\text{C}$ . All the experimental insects,

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with the exception of those designated as 'normal' or untreated controls, were denied food from the start to the end of the experiment. To allow for differences in weight and consequently in surface area of insects, the formula of Beament *et al.* (1964) as used by Noble-Nesbitt (1969) was adopted. This involved determining the rate of water loss/standard insect (30 mg)/h according to the following:

$$\left( \frac{30 \text{ mg}}{\text{actual weight of insect in mg}} \right)^{\dagger} \times \frac{\text{actual loss in weight in mg}}{\text{length of desiccation period in h}}$$

## RESULTS

### *Water uptake by insects with an already experimentally elevated water content*

Long-term starvation in *Thermobia* causes a progressive increase in the water content (Okasha, 1971; see also Fig. 2). The following experiment was undertaken to test the earlier conclusion that an insect with an elevated water content can still take up water vapour during rehydration after having been desiccated, even though its water content immediately after desiccation and prior to rehydration might have been at least equivalent to, if not higher than, that of 'normal' untreated insects (Okasha, 1971).

A large number of insects were starved for 3 weeks. Each insect was weighed at the start of the experiment, kept by itself in hydrating conditions and was then weighed after the starvation period. The survivors (53 out of 80) were then divided into three groups:

*Group A.* Each insect was dried for water-content determination.

*Group B.* Each insect was desiccated for 3 days, weighed again and then dried for water-content determination.

*Group C.* Each insect was desiccated for 3 days (as were those in group B), weighed, rehydrated for 1 day, then weighed again before being used for water-content determination.

The results of this experiment are summarized in Table 1, which shows that after 3 weeks of starvation insects of groups A, B and C reached about 83% of their initial weight, i.e. there are no apparent differences between the three groups in this respect. The water content after starvation only reached  $75.6 \pm 0.7\%$  of the last wet weight (group A). In this work it is assumed that the water content of insects of group B immediately before desiccation was similar to that of insects of group A following the 3-week starvation period. It is also assumed that the water content of insects of group C immediately after desiccation and prior to rehydration was similar to that of insects of group B just before they were sacrificed after desiccation. The water content of insects starved for 3 weeks then desiccated for 3 days while still starved ( $72.2 \pm 0.9\%$ , group B) was markedly higher than that of untreated insects. Despite this, when similarly treated insects were put in rehydration (group C), water uptake still proceeded and the insects increased their weight whilst further increasing their water content. The latter was  $76.3 \pm 0.9\%$  of the last live weight in this group (Table 1).

### *Effect of degree of hydration of the body on the rate of water loss*

In the previous experiment it was noticed that insects with a higher water content (group B, Table 1) do not lose more weight during desiccation than do those insects with a lower water content. For example, the insects of group B in this experiment†

Table 1. *The effect of desiccation and subsequent rehydration on the water content of long-term starved insects*

Group	Treatment	No. of insects	Final live weight (as % of initial live weight) ± S.E.	Water content (as % of final live weight) ± S.E.
A	3 weeks starvation	15	83.8 ± 0.9	75.6 ± 0.7
B	3 weeks starvation	19 (after starvation)	83.8 ± 0.7	—
	+ 3 days desiccation	16 (after desiccation)	69.6 ± 1.1	72.2 ± 0.9
C	3 weeks starvation	19 (after starvation)	83.0 ± 1.5	—
	+ 3 days desiccation	16 (after desiccation)	68.8 ± 1.7	—
	+ 1 days rehydration	14 (after rehydration)	81.9 ± 1.9	76.3 ± 0.9
—	Untreated controls*	10	—	66.1 ± 0.5

\* From Okasha (1971, see Table 4).

Table 2. *The effect of desiccation for 3 days on the rate of water loss in insects previously starved for different periods*

Starvation period before desiccation	No. of insects	% loss in wet weight during desiccation ± S.E.	Rate of water loss mg H <sub>2</sub> O/standard insect/h ± S.E.	Estimated H <sub>2</sub> O content before desiccation (%)*
None	40	23.6 ± 0.5	0.0648 ± 0.0016	66.1
1 week	9	20.2 ± 1.9	0.0975 ± 0.0096	69.3
2 weeks	8	18.4 ± 1.4	0.0925 ± 0.0081	73.0
3 weeks	7	20.8 ± 2.3	0.0875 ± 0.0105	75.5
4 weeks	9	24.3 ± 1.6	0.1063 ± 0.0061	76.9
5 weeks	6	21.5 ± 1.9	0.0991 ± 0.0096	79.5

\* Read from curve in Fig. 2.

lost  $17.6 \pm 0.9\%$  of their fresh weight during 3 days of desiccation; the corresponding figure in group C is  $19.7 \pm 1.2\%$ . The loss in weight caused by a similar treatment in one experiment that involved insects taken directly from their feeding vials was  $23.6 \pm 0.5\%$  of the pre-desiccation weight (Table 2). Thus the rate of water loss is seemingly even higher in the latter insects, but weight loss due to defecation would be expected to be somewhat higher in those which were not starved before desiccation. However, the weight of faeces voided during the first 3 days of starvation, whether in desiccating or hydrating conditions, is only about 1% of the weight of the insect.

The result of a more critical investigation of the rate of water loss during the desiccation of insects with various levels of water content are shown in Table 2. If water loss is expressed as a percentage of the loss in wet weight, it will be noted that there does not seem to be any detectable effect of the degree of hydration of the body on the rate of water loss. This appears to hold true whether the insects are taken directly from their feeding vials or are starved for up to 5 weeks prior to desiccation. When water loss is expressed on the basis of loss in mg/standard insect/h, thus taking account of differences in the weights of insects, it becomes clear that the rate is lowest in the insects with the lowest water content (those which were not starved before desiccation). However, despite the tremendous variation in the pre-desiccation water content in the starved insects, depending on the length of the starvation period, the rate of water loss does not seem to be correlated with the water content (Table 2).

Table 3. *The effect of desiccation for 1 day on the rate of water loss in insects at various stages in the moulting cycle*

Days after moulting when desiccated	No. of insects	% loss in wet weight during desiccation $\pm$ S.E.*	Rate of water loss mg H <sub>2</sub> O/standard insect/h $\pm$ S.E.	Estimated H <sub>2</sub> O content before desiccation (%) $\pm$ S.E.†
0	20	11.0 $\pm$ 1.0	0.1248 $\pm$ 0.0115	66.6 $\pm$ 0.8
1	10	10.2 $\pm$ 0.6	0.1133 $\pm$ 0.0053	69.1 $\pm$ 1.1
2	10	9.5 $\pm$ 0.7	0.1104 $\pm$ 0.0121	67.4 $\pm$ 1.2
3	10	9.3 $\pm$ 0.5	0.1129 $\pm$ 0.0080	66.7 $\pm$ 2.0
4	10	8.7 $\pm$ 0.5	0.1192 $\pm$ 0.0129	66.3 $\pm$ 1.3
5	10	8.5 $\pm$ 0.3	0.1404 $\pm$ 0.0127	65.2 $\pm$ 1.4
6	10	8.1 $\pm$ 0.7	0.1150 $\pm$ 0.0125	66.6 $\pm$ 0.7
7	10	7.3 $\pm$ 0.4	0.1104 $\pm$ 0.0095	66.0 $\pm$ 0.9
8	10	6.9 $\pm$ 0.7	0.0954 $\pm$ 0.0116	66.9 $\pm$ 1.3
9	10	6.5 $\pm$ 0.9	0.0917 $\pm$ 0.0143	67.4 $\pm$ 0.8
10	10	7.9 $\pm$ 0.9	0.0112 $\pm$ 0.0110	66.8 $\pm$ 0.7

\* Results of same insects shown in Fig. 3 in Okasha (1971).

† Results of another set of insects, of course, involving ten insects in each treatment, except at 8 and 10 days after ecdysis when seven and eight insects were used respectively.

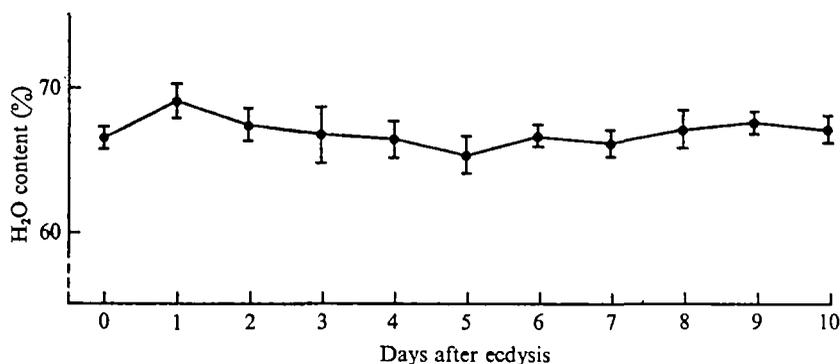


Fig. 1. Water content during various stages of the moulting cycle. Each point represents the mean of values obtained from 7-10 insects and the vertical lines represent the standard error.

One important factor which should be taken into consideration is the developmental state of the insect. For instance, the loss in wet weight due to 24 h of desiccation progressively decreases from a maximum of 11.0  $\pm$  1.0% when the insects are taken 0-2 h after ecdysis to a minimum of 6.5  $\pm$  0.9% of the original weight when taken 9 days after ecdysis. If, however, the data are converted to allow for differences in the weights of the insects, it will be seen that the corresponding figures for the rates of water loss are 0.1248  $\pm$  0.0115 and 0.0917  $\pm$  0.0143 mg H<sub>2</sub>O/standard insect/h respectively. It does not seem from the converted data presented in Table 3 that there are any pronounced differences in the rates of water loss during the various stages of the moulting cycle (cf. Okasha, 1971). It is interesting to point out that the water content of insects taken at daily intervals over the period 0-10 days after ecdysis does not vary a great deal either (Table 3, Fig. 1).

From the present results it cannot be certainly concluded whether or not the rate of water loss is limited by the water content. This will be considered in the Discussion.

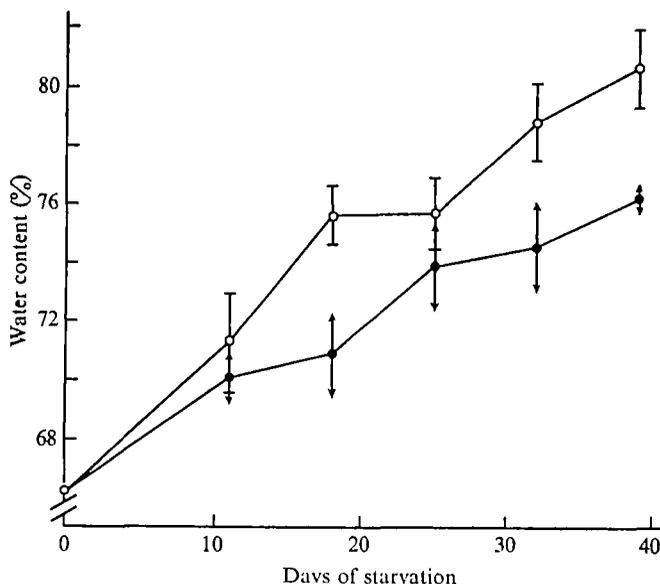


Fig. 2. Water content of insects starved for various periods before desiccation and subsequent rehydration, and of starved hydrated controls. ○, Insects starved in hydrating conditions. ●, Insects starved in hydrating conditions for 1, 2, 3, 4 or 5 weeks, then desiccated for 3 days and rehydrated for 1 day. Each point represents the mean of values obtained from 6–9 insects and the vertical lines represent the standard error.

#### *Water content and the control of the uptake mechanism*

To test whether or not the water content is a limiting factor in the control of the uptake mechanism the following experiment was performed.

A large number of insects were starved in hydrating conditions. At the start of the experiment each insect was weighed and kept by itself. Groups of insects, each consisting of 7–10 insects, were desiccated, then rehydrated at various intervals after the onset of starvation. Each insect was weighed immediately before and after 3 days' desiccation, then after 1 day rehydration, and then its water content was determined. On the day of rehydration of each desiccated group, insects that had been starved in hydration for an equivalent period were weighed and then their water content was determined. These latter insects served as controls. The results of this experiment are shown in Figs. 2–4.

In Fig. 2 the values of the water content of insects starved for various periods then subjected to desiccation and subsequent rehydration are represented together with those of the control insects. It is clear that the increase in the water content of the controls is directly proportional to the length of the starvation period. The water content of the experimental insects after rehydration was never higher than that of the controls at any given state of starvation, but in fact seems to be somewhat lower. Previous experiments showed that there is no detectable difference in the water content between insects subjected to desiccation and subsequent rehydration and controls that are starved for equivalent periods in hydrating conditions (Okasha, 1971).

The results shown in Fig. 3 illustrate that there are no obvious differences in the fresh weights reached either by the experimental groups of insects before their desicca-

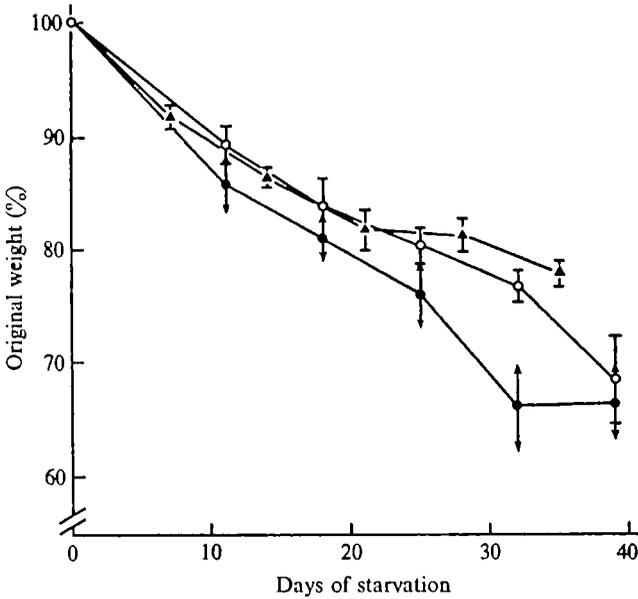


Fig. 3. Changes in body weight of insects starved for various periods before desiccation and subsequent rehydration, and of starved hydrated controls. O, Insects starved in hydrating conditions. ▲, Insects starved in hydrating conditions before they were subjected to desiccation. ●, The same insects after 3 days' desiccation and 1 days' rehydration. Each point represents the mean of values obtained from 6-9 insects and the vertical lines represent the standard error.

tion or by the control groups. However, the experimental groups reached somewhat lower weights after rehydration compared with control groups at the same state of starvation. This is particularly pronounced in the case of insects starved for longer periods. The relative extent of weight losses and gains following desiccation and rehydration are shown in Fig. 4, which shows that in insects starved for either 4 or 5 weeks prior to desiccation the mean increase in weight due to rehydration was far less pronounced than in groups of insects starved for shorter periods. The percentages of insects that failed during rehydration to gain 50% or more of the loss in weight recorded after desiccation are also included in Fig. 4, which indicates that a much higher proportion of the insects starved for a long period failed to rehydrate fully. It is not known whether this is a direct consequence of the developmental stage of the insect (water uptake ceases temporarily towards the later stages of the moulting cycle; see Okasha, 1971) or whether it is due to severe starvation. This will be considered in the next section.

An important point emerges from the present results: when different groups of insects were starved for various lengths of time, then were exposed to exactly the same conditions of desiccation and rehydration, water uptake ceased at a particular stage in each group. By the end of the process of uptake (assuming that full rehydration takes place in only 1 day as reported by Noble-Nesbitt, 1969) each group had a characteristic level of water content (Fig. 2) and a characteristic weight, and consequently volume (Fig. 3), that depended on the degree of starvation. Put another way, it appears that at any given state of starvation the previously desiccated insect takes up water until either a particular body volume which is associated with a particular water con-

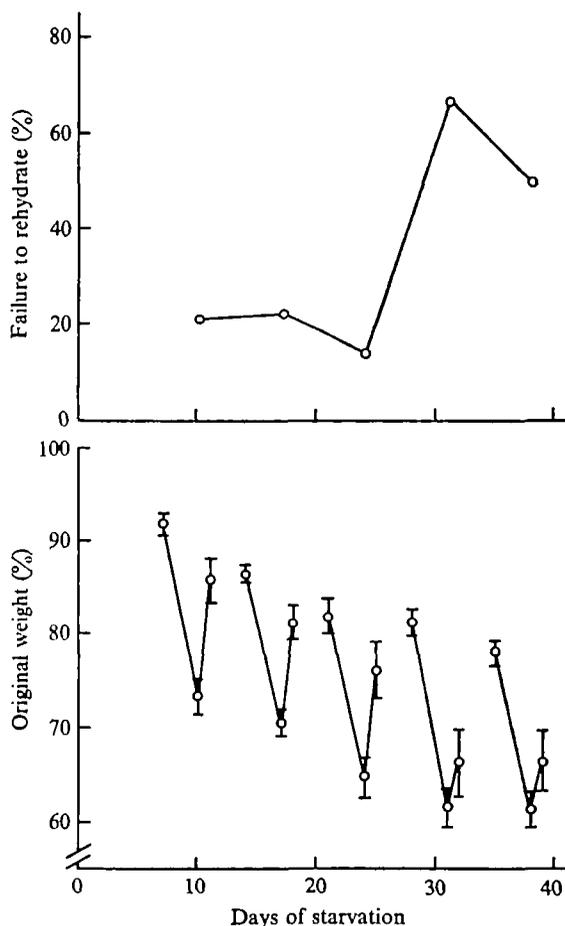


Fig. 4. Upper. Effect of the length of the starvation period before desiccation on the percentage of insects failing to gain in rehydration 50% or more of the weight loss recorded after 3 days' desiccation.

Lower. Changes in body weight after 3 days' desiccation and after 1 days rehydration of insects starved for 1, 2, 3, 4 or 5 weeks before desiccation. Each point represents the mean of values taken from 6-9 insects and the vertical lines represent the standard error.

tent is reached, or until a particular water content that is paralleled by a specific body volume is attained. For the purposes of the present discussion it is irrelevant whether it is the former or the latter explanation which controls the reaching of equilibrium in the process of uptake. But what is relevant here is that in general terms the water content cannot be regarded as a limiting factor in the control of the uptake mechanism, thus arresting uptake once full rehydration is achieved. However, it can be regarded as such for any given state of starvation under specific environmental conditions.

#### *Impairment of the uptake mechanism by severe starvation*

In this experiment insects were subjected to 24 days of starvation in hydrating conditions. The surviving insects (22 out of 30) were then desiccated for 3 days (five died during desiccation) and this was followed by 1 day in rehydration. Weights were recorded when necessary. The results are shown in Table 4.

Table 4. *The effect of severe starvation before desiccation on the subsequent ability to rehydrate\**

Insect no.	Live weight (mg)				Ability to rehydrate
	Immediately before starvation	After 24 days of starvation	After 3 days in desiccation	After 1 day in rehydration	
8	34.4	31.8	27.0	30.5	+ve
9	40.2	37.7	31.7	34.6	+ve†
11	45.5	42.3	34.8	40.8	+ve
12	45.5	40.8	35.2	34.5	-ve
13	28.6	25.2	19.7	19.8	-ve
14	30.5	25.3	19.4	20.0	-ve
16	36.1	31.4	21.2	23.7	-ve
17	41.2	34.3	27.4	30.8	+ve†
18	43.3	39.2	31.8	30.8	-ve
19	34.8	27.1	21.2	21.2	-ve
20	38.1	31.6	23.0	25.0	-ve
21	43.7	36.3	29.1	33.1	+ve
24	32.1	27.3	22.3	24.3	-ve
25	34.2	27.1	21.7	23.5	-ve
28	34.4	30.1	22.6	23.0	-ve
29	38.5	36.3	29.5	31.4	-vs
30	45.0	40.3	32.6	36.7	+ve

\* The ability to rehydrate successfully is defined here as the ability to gain after 1 day in rehydration 50% or more of the loss in body weight recorded during the 3 days' desiccation period.

† Insects number 9 and 17 are considered as having successfully rehydrated although the gain in rehydration was just slightly under 50%.

Table 5. *The effect of severe desiccation on the ability to rehydrate*

Treatment	No. of insects	Final weight as % of initial weight			
		After desiccation		After rehydration	
		Range	Mean $\pm$ S.E.	Range	Mean $\pm$ S.E.
5 days desiccation + 1 day rehydration	8	63.4-69.9	67.7 $\pm$ 0.7	76.1-95.6	88.3 $\pm$ 2.5
6 days desiccation + 1 day rehydration	10	70.3-79.7	74.0 $\pm$ 1.1	64.5-102.0	86.0 $\pm$ 3.2
8 days desiccation + 1 day rehydration	9	61.4-66.1	64.1 $\pm$ 0.6	74.4-93.4	83.4 $\pm$ 1.9

It will be seen that only 6 out of 17 insects succeeded in rehydrating. The remaining insects obviously failed to rehydrate in a normal manner. Although there is some increase in weight in 7 out of these 11 insects after rehydration, such an increase is by no means pronounced as in insects not subjected to a long period of starvation. Thus it is concluded that about 65% of the insects in the present experiment failed to rehydrate, a figure which compares very well with the results shown in Fig. 4.

From this and from the results shown in Fig. 4 it seems unlikely that the failure to rehydrate in such starved insects can be attributed wholly to the physiological state of the insect in relation to the moulting cycle, although the latter of course is an important factor. It is strongly suggested, therefore, that severe starvation somehow either inhibits or interferes with water uptake by previously desiccated firebrats. The

mechanism underlying such an inhibition or impairment is not as yet clear. It is possible that the concentration of some substance(s) necessary for the uptake mechanism to take place effectively is depleted below a particular concentration in severely starved insects. Another possibility is that full rehydration in most insects is not achieved after only 1 day. Unfortunately, rehydration for longer periods was not tested. If a longer rehydration period is required in such a case, it would be because of a lag period and/or a decreased efficiency of the uptake mechanism rather than due to a possible excessive water loss in severely starved insects with elevated water contents (see Discussion).

The results illustrated in Fig. 4 suggest that starvation for periods of up to 3 weeks before desiccation and subsequent rehydration does not seem to interfere with the uptake mechanism. The finding that some 20% or so of insects starved for 1, 2 or 3 weeks failed to rehydrate (Fig. 4, upper graph) is most probably due to the stage of such insects in so far as the moulting cycle is concerned. However, when the starvation period prior to desiccation was extended beyond 3 weeks, the percentage of insects failing to rehydrate was increased, as shown in Fig. 4 (see also Table 4). Whether longer periods of starvation would be reflected in a higher proportion of insects failing to rehydrate remains to be tested. Also it would be interesting to discover whether the inhibition or impairment of the uptake mechanism manifested in severely starved insects is reversible.

#### *Effect of excessive water loss on the uptake mechanism*

According to Edney (1966) nymphs and adult females of the cockroach *Arenivaga* sp. take up water from 82.5% R.H. and above. He found that ten nymphs succeeded in rehydrating when desiccated to a mean loss equal to 31.8% of their original weight, whereas ten other nymphs failed to recover when the mean loss was 36.3%. The possibility that *Thermobia* might fail to rehydrate following severe desiccation was tested in the following experiments.

Twenty insects were weighed and then desiccated. When weighed after 5 days (18 survivors) only those insects that lost 30% or more of their original weight (eight insects) were transferred to hydrating condition; the rest were desiccated for 1 more day and then put in rehydration. Each group was rehydrated for 1 day. The results of this experiment presented in Table 5 indicate that insects desiccated for 5 days with a mean loss of  $32.3 \pm 0.7\%$  of their original weight or for 6 days with a mean loss of  $29.8 \pm 1.1\%$  can rehydrate in an apparently normal manner when placed in a humid atmosphere. (Table 3 shows that the percentage loss in weight is halved at 9 days after ecdysis as compared with 0 days after ecdysis, although the rate of water loss after allowing for differences in body weight is approximately the same.)

In another experiment 20 insects were desiccated for 8 days and the survivors were rehydrated for 1 day. The results (Table 5) show that when these insects were desiccated to a mean loss of  $35.9 \pm 0.6\%$  of the initial weight they succeeded in taking up water. It remains a possibility, however, that more severe desiccation might interfere with the uptake mechanism. That a few insects in each group shown in Table 5 failed to rehydrate either fully or partially cannot necessarily be attributed to the length of the desiccation period, since this is a phenomenon which has been observed fairly frequently in insects desiccated for shorter periods.

## DISCUSSION

The results presented in this paper provide further and direct evidence which supports the earlier conclusion that in *Thermobia*, if an insect with an experimentally elevated water content is subjected to desiccation and subsequent rehydration, water uptake from the atmosphere still occurs. On the one hand, uptake takes place even when the insect *does not* seem to need water, if the need is to be judged by the water content. On the other hand, net uptake from the atmosphere apparently ceases when a particular body volume is reached, and this is characterized by a specific water content. In this context it must be repeated that the physiological state of the insect, in so far as starvation is concerned, is crucial.

A comparison of the increased water content caused by starvation in *Tenebrio molitor* larvae (Buxton, 1930; Mellanby, 1932) and in *Thermobia* seems worth while. In the former species Buxton suggested that at 90% R.H. combined with 23 or 30 °C the starved larva might produce more metabolic water than it can liberate into the atmosphere. In fact, Mellanby (1932) confirmed that *Tenebrio* larvae become quite dropsical in 14 days at 37 °C, combined with 70% R.H., which is even lower than the critical relative humidity below which there is no net uptake of water from the atmosphere in this species. In this connexion Mellanby stated that 'In hot moist air their water balance is upset, as they are unable to get rid of their water of metabolism sufficiently rapidly. In very moist air they even take up water, and this upsets their internal conditions still further.' If this is correct for *Tenebrio*, it certainly does not seem to apply in the case of *Thermobia*. In the latter species the experimentally elevated water content caused by starvation coupled with uptake from a humid atmosphere during rehydration (e.g. group C of the first experiment) does not seem to be manifested in any visible ill health. Moreover, it does not seem to result in any detectable harmful effects on the internal environment, at least in so far as the ionic composition of the haemolymph is concerned (Okasha, 1972). It must be admitted that after prolonged starvation at 83% R.H. and 37 °C the mortality rate increases and that this is associated with an elevated water content. The cause of death in such cases is not known, but it may well be due to the depletion of reserves rather than to overhydration or 'water intoxication'. The possibility of *Thermobia* producing water of metabolism for the control of its water relations has not been explored, but it might be relevant to recall here that in starved insects the loss in dry matter in hydrating conditions is approximately the same as that in desiccating conditions at the same temperature (Okasha, 1971). This strongly suggests that *Thermobia*, in contrast to *Tenebrio*, does not have to burn more food reserves in very dry air as compared with moist air; but more detailed studies in *Thermobia* are necessary before such an assertion can be substantiated.

As already mentioned, it is difficult to decide with certainty whether or not the rate of water loss is limited by the water content. The rate of transpiration in freshly killed *Thermobia* falls as the total body-water content falls (Noble-Nesbitt, 1969). Naturally, this is not surprising since the restrictive water-loss devices are no longer operating in the dead insect. Regarding the living insect, Noble-Nesbitt stated that 'Depletion of the reserves of body water may be expected to tighten spiracular control, but what lowering of the transpiration rate there is can be attributed to the automatic lowering

of the rate with lowered body-water content as seen also in dead insects.' A consideration of the rates of water loss from insects with elevated water contents does not lend support to this statement. This is because the rate of water loss over a period of 3 days in desiccation is almost the same in insects with initial water contents varying from 69.3 to 79.5% (Table 2). However, the finding that rates of water loss in such insects are in excess of that characteristic of insects with an initial lower water content (those which were not starved before desiccation, Table 2) supports the above-mentioned quotation from Noble-Nesbitt. Moreover, the rate of water loss/standard insect/h in insects desiccated for only 24 h at any stage of the moulting cycle (Table 3) is much higher than that recorded for insects desiccated for 3 days (those not starved before desiccation, Table 2), thus indicating a higher rate of water loss during the initial phases of desiccation.

Incidentally, Mellanby (1932) states that the starved larvae of *Tenebrio* 'have to rely on evaporation to get rid of superfluous water, and this arrangement breaks down when the air is too moist'. Even when such an evaporative mechanism breaks down at 70% R.H. the question arises as to why a larva that cannot get rid of superfluous water still takes up water vapour from 90% R.H. It is difficult to understand how an insect which presumably developed the ability to extract water vapour from sub-saturated atmospheres, since it normally lives in hot and dry air as Mellanby mentions, would have to depend for its survival on evaporative water loss under conditions of high humidity and temperature. Surely it would be more economical for the insect under such conditions to spare the energy spent in what is presumably an energy-requiring process (Beament, 1964), rather than to rely on evaporation, which in such a case is inadequate because of the production of metabolic water in addition to water absorbed from the atmosphere. The possibility that *Thermobia* might suffer from excessive water content in hot and very humid air, as is the case in *Tenebrio* larvae, is worth investigating. But as Noble-Nesbitt (1969) points out, 'There is no apparent danger to the firebrat that it may take in too much water at high humidities', a state of affairs which has always been observed at 37 °C and 83% R.H. However, under extreme environmental conditions this might not be the case.

The uptake mechanism is impaired or inhibited in severely starved *Thermobia* (Fig. 4, Table 4) and in severely desiccated *Arenivaga* nymphs (Edney, 1966). In both cases this is associated with a substantial decrease in the fresh body weight. Whether the degree of loss in fresh weight (and consequently body volume) is an important factor in water uptake remains to be investigated. But in the case of *Thermobia* weight loss could be attributed in equal proportions both to depletion in body weight caused by prolonged starvation and to water loss caused by desiccation. It is not therefore a question of a severely depleted water content that impairs the uptake mechanism, although this might be the reason in severely desiccated *Arenivaga*.

The title 'water relations' rather than the more commonly used term 'water balance' has been chosen for the present series of publications. This is because the latter term implies that a balance is struck between losses and gains (Edney, 1957) and it might therefore be assumed that water uptake from the atmosphere would cease as soon as the 'normal' water content or water balance is reached (e.g. see Noble-Nesbitt, 1969). In *Thermobia* it is obvious from the foregoing results that in general terms the absolute value of the proportion of water in the body of the insect is definitely not a limiting

factor in the control of the uptake mechanism. As previously suggested (Okasha, 1971), there is little doubt that water uptake from subsaturated atmospheres in *Thermobia* is concerned primarily with volume regulation. In the present state of knowledge it is impossible to know with certainty the significance of volume regulation and its advantage to the species. This becomes particularly puzzling when it is borne in mind that volume regulation is achieved at the expense of altering the proportion of water in the body, which consequently results in overhydrated, but seemingly very healthy, insects under certain experimental circumstances. Clearly, more information is needed concerning the mechanism of uptake, its significance and its control before such problems can be satisfactorily solved.

## SUMMARY

1. The percentage water content of *Thermobia* can be increased by starvation.
2. After desiccation (3 days in dry air) the water content of starved insects is still above normal; yet such insects will take up water from a subsaturated atmosphere (83% R.H.).
3. The rate of water loss into dry air from starved insects is not dependent upon water content.
4. Both water content and rate of water loss remain constant throughout the moulting cycle.
5. In general terms the uptake mechanism is not dependent upon water content.
6. Severe starvation before desiccation seems to impair or inhibit the uptake mechanism.

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