

WATER RELATIONS IN AN INSECT, *THERMOBIA DOMESTICA*

I. WATER UPTAKE FROM SUB-SATURATED ATMOSPHERES AS A MEANS OF VOLUME REGULATION

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INTRODUCTION

It is now well established that a few arthropods take up water from subsaturated atmospheres (Edney, 1957) and that the apterygote *Thermobia* exhibits such a property (Beament, Noble-Nesbitt & Watson, 1964; Beament, 1964; Noble-Nesbitt, 1969). A list of the insects and references to other arthropods which have been found to have this faculty is given in Noble-Nesbitt (1969).

The mechanism underlying water uptake from subsaturated atmospheres is not as yet fully understood. In *Tenebrio* larvae, the first well-documented case, neither the production of metabolic water as suggested by Buxton (1930) (see Mellanby, 1932) nor the uptake through the tracheal system as suggested by Mellanby (1932) can be accepted as the possible mechanisms (Beament, 1964). The production of hygroscopic faeces which are ingested after being in contact with the humid air has also been eliminated as a possible mechanism (Wigglesworth, in Beament, 1964; Noble-Nesbitt, 1970). The integument has been implicated in the uptake mechanism (for a review of the earlier literature see Edney, 1957). In his thorough article Beament (1964) suggested a model of a water pump based essentially on the reorganization of a lipid barrier in the epicuticle, on the degree of hydration of the cuticular proteins which is controlled by the epidermis and on the epicuticular pores. Following the same line, Noble-Nesbitt (1969) described a pore-model in *Thermobia*, stating that a lipid barrier is complete during transpiration and disrupted during uptake and that pores in the correct size range exist in the outer epicuticle. However, recently Noble-Nesbitt (1970) showed that blockage of the anus of previously desiccated *Thermobia* results in the cessation of water uptake. He therefore concluded that the rectum is the site of uptake. Further, he stated that preliminary work on *Tenebrio* supports this theory.

The present paper reports on the results of some experiments performed on *Thermobia* in an attempt to throw more light on the nature of the uptake mechanism and a different theory concerning the inhibition of uptake by anus blockage is proposed here.

MATERIALS AND METHODS

Cultures of *Thermobia domestica* were maintained at 37 °C and 83 % R.H. as described by Noble-Nesbitt (1969). This relative humidity was achieved by using a saturated solution of KCl. Experimental insects were isolated individually in clean

glass vials and placed in desiccators either containing anhydrous CaCl_2 where desiccation was involved or a saturated solution of KCl where rehydration was needed. Unless otherwise stated all the experiments were carried out at 37°C .

Centrifugation was conducted using a MSE minor Type I centrifuge at full speed (c. 3000–3500 rev/min) for 20 min. The insects were accurately weighed immediately before and after centrifugation.

Dry weight determinations were carried out by drying insects which had been accurately weighed (to the nearest 0.1 mg) to a constant weight; this was achieved after 3 days at 56°C .

Unless otherwise stated, in all the experiments that involved desiccation, rehydration or hydrated controls the insects were starved. However, in one experiment which involved repeated desiccation and rehydration of insects, food was allowed; in this case the vials containing the diet were placed permanently in the CaCl_2 or KCl desiccators and the insects were transferred from one type of desiccator to the other as desired, without having to transfer the vials themselves.

In order to experiment upon insects at a specific stage in the moulting cycle, insects isolated in glass vials that contained food were checked every 2 h for ecdysis (since the exuviae are eaten soon after ecdysis). Those which had moulted were set aside, labelled, allowed to feed and were kept at 83 % R.H. until needed.

RESULTS

Effects of various forms of stress on water uptake

One approach in trying to discover the mechanism of water uptake is to interfere with it. It was thought that various kinds of stress might be illuminating in this context. Although the present experiments gave negative results, nevertheless they

Table 1. *Effects of centrifugation either before or after 3 days desiccation on body weight loss and on subsequent rehydration*

Treatment	Mean final weight as % of initial weight \pm s.e.				
	After centrifugation	After desiccation	After centrifugation	After rehydration	
				1 day	2 days
Centrifugation before desiccation	92.6 \pm 1.3 (15)	70.9 \pm 2.0 (6)	—	105.3 \pm 5.2 (5)	102.3 \pm 5.3 (5)
Centrifugation after desiccation	—	72.3 \pm 0.8 (20)	68.9 \pm 0.8 (20)	94.2 \pm 2.0 (6)	101.5 \pm 3.5 (6)
Starved controls	92.4 \pm 1.6 (18)	—	—	95.4 \pm 1.6 (12)	95.7 \pm 0.8 (8)

Figures in parentheses indicate the number of insects.

are reported here so that such factors can be excluded from those that might inhibit water uptake in *Thermobia*. The effects of centrifugation either before or after desiccation on water loss and on the subsequent ability to rehydrate are shown in Table 1. Control experiments were carried out and this involved centrifugation and keeping the starved insects in hydrating conditions.

The results show that centrifugation for as long as 20 min, although fatal to a very

high percentage of insects, does not seem to interfere with the ability of those which survive the treatment to rehydrate. In some insects it was noticed that although rehydration had taken place during the first 24 h after transfer to KCl desiccators it was only partial and more or less complete rehydration occurred 24 h later. Perhaps in such insects there was some injurious effect caused by centrifugation.

Table 2. *Effect of desiccation at 45 °C for different periods on loss in body weight and on subsequent rehydration at 37 °C*

Series	Treatment	Mean final weight as % of initial weight \pm S.E.	
		After desiccation	After rehydration
A	16 h desiccation at 45 °C + 24 h rehydration at 37 °C	84.9 \pm 3.2 (19)	97.0 \pm 4.5 (19)
B	24 h desiccation at 45 °C + 24 h rehydration at 37 °C	79.2 \pm 0.5 (19)	95.8 \pm 0.8 (19)
C	31 h desiccation at 45 °C + 24 h rehydration at 37 °C*	74.5 \pm 1.0 (17)	96.0 \pm 1.8 (15)

Figures in parentheses indicate the number of insects.

* These are the same insects used in B. Their last weight after rehydration was considered as the initial weight in C.

Table 3. *Effects of submerging or burning desiccated insects on subsequent rehydration at 37 °C*

Treatment	Final weight as % of initial weight \pm S.E.		
	After desiccation	After submergence	After rehydration
3 days desiccation at 37 °C + 1 h submergence in H ₂ O + 1 day rehydration	80.3 \pm 0.4 (10)	80.7 \pm 0.5 (10)	95.7 \pm 2.3 (10)
1 day desiccation at 45 °C + burning + rehydration*	After desiccation	After 1 day rehydration	After 2 days rehydration
	80.7	96.2	100.8
	79.0	101.8	109.0
	82.4	85.0	90.0

Figures in parentheses indicate the number of insects.

* Figures for the only three insects out of ten that survived are given individually.

It was also found that exposure of insects kept over dry CaCl₂ to 45 °C for up to 31 h did not have any detectable effect on subsequent rehydration at 37 °C (Table 2). It is possible that a higher temperature or a longer exposure period might have an effect. Likewise, submerging desiccated insects for 1 h in distilled water or burning the body surface with an electrically heated wire (more severe injury than the cuticular abrasion of Beament *et al.* 1964) did not inhibit water uptake (Table 3).

Effect of repeated desiccation and rehydration on water uptake

To test whether the ability to take up water would be affected by repeated desiccation and rehydration the following experiment was undertaken. Twenty insects, ten

of each sex, were weighed accurately, kept individually in clean glass vials and desiccated for 3 days. The insects were then weighed, rehydrated for 1 day and then weighed again. These insects were subjected to five alternate such cycles of desiccation and rehydration. A similar number of each sex was used as a control. The control insects were starved for an equivalent period (20 days), weighed whenever the experimental insects were weighed, and were kept permanently at 83 % R.H. The results of this experiment are shown in Fig. 1. This figure suggests the following conclusions.

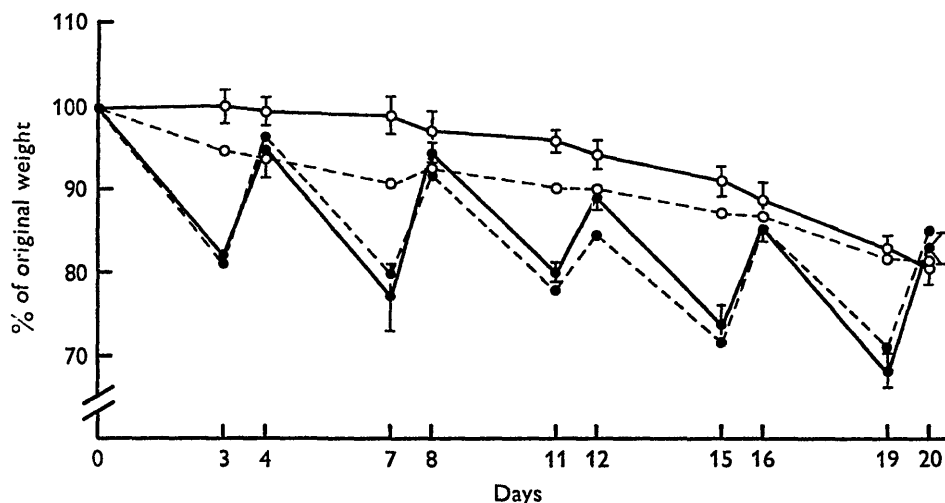


Fig. 1. Effects of 5 alternate cycles each consisting of 3 days desiccation followed by 1 day rehydration on the fresh weights attained by starved insects. Black circles represent experimental insects, open circles hydrated controls, continuous lines females, discontinuous lines males and vertical lines the standard error. For clarity the standard error in the case of males is not shown as it is of the same order as in females.

(i) The property of water uptake does not seem to be affected by alternate desiccation and rehydration. However, during the course of the experiment it became obvious that some insects failed to rehydrate. Meanwhile such insects lost considerably more weight than expected during the subsequent desiccation cycle; this was usually associated with the existence of remains of exuviae. These observations led to the belief that when a particular insect failed to gain in weight after transfer from desiccation to rehydration it was about to moult. Further experiments performed to test this possibility showed that this was so (see Figs. 3, 4).

(ii) The final weight, expressed as a percentage of the initial weight, attained by insects in both groups was approximately the same.

(iii) There was no apparent sexual difference in either (i) or (ii).

A consideration of the dry matter, expressed as a percentage of the final fresh weight, in insects subjected to 5 cycles of desiccation and rehydration showed that it was reduced to 22.1 ± 0.6 in males and 21.4 ± 0.6 in females. The corresponding figures in the case of the controls were 21.9 ± 1.0 and 20.8 ± 0.4 respectively. These values once more indicate that there is no sexual difference either in the experimental or in the control insects. If the values for males and females are pooled, the dry matter in the experimental insects reaches 21.7 ± 0.4 % of the last live weight; the corresponding figure in the controls is almost identical, being maintained at 21.4 ± 0.5 % (Table 4).

The dry matter in untreated insects which are allowed access to food is 33.9 ± 0.5 % of their wet weight. Thus in both groups used in this experiment there was a substantial rise in the water content associated with the depletion of the dry matter. These results strongly suggest that the loss in dry matter is a result of starvation only.

In his investigation on the same species Noble-Nesbitt (1969) calculated that in insects hydrated and starved for 4 days and in those starved but desiccated for 3 days then rehydrated for 1 day there was a loss of 16 % and 24 % of their initial dry matter respectively. The data shown in Table 4 do not substantiate these apparent differences. It was found that there were no differences in the total loss in dry matter in insects used in the previous experiment; the loss in dry matter in insects starved and hydrated for 20 days was 48.9 ± 1.7 % of the initial dry matter. The corresponding figure in those subjected to 5 alternate cycles of desiccation and rehydration (where loss in dry

Table 4. *Effects of repeated desiccation followed by rehydration, and of starvation on the final fresh weight and the dry weight*

Treatment	No. of insects	Final fresh weight	Final dry weight
		as % of initial weight \pm S.E.	as % of last live weight \pm S.E.
Hydrated and starved for 20 days	♂ 10	81.5 ± 1.6	21.9 ± 1.0
	♀ 10	80.6 ± 1.9	20.8 ± 0.4
	Both 20	81.1 ± 1.2	21.4 ± 0.5
5 cycles of desiccation and rehydration (starved)*	♂ 10	85.4 ± 1.4	22.1 ± 0.6
	♀ 10	83.1 ± 2.1	21.4 ± 0.6
	Both 20	84.1 ± 1.3	21.7 ± 0.4
Hydrated and fed for 20 days	♂ 10	117.8 ± 1.5	34.2 ± 0.3
	♀ 10	119.4 ± 3.1	33.6 ± 0.9
	Both 20	118.6 ± 1.7	33.9 ± 0.5
5 cycles of desiccation and rehydration (fed)*	♂ 5	97.1 ± 1.5	29.2 ± 1.9
	♀ 9	100.6 ± 4.2	30.8 ± 1.0
	Both 14	99.3 ± 2.7	30.2 ± 0.7

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

matter would be expected to be more dramatic than in the starved controls according to Noble-Nesbitt's results) was 47.3 ± 1.4 % of their initial dry matter. Further, the calculation of Noble-Nesbitt that during starvation the insect loses dry matter to the extent of 1.4 % of its initial weight per day and 2.1 % per day during desiccation and rehydration (over a period of 4 days) does not hold true either, according to the present results. From Table 4 it is clear that the final fresh weight (as a percentage of the initial weight) maintained by the insects in either the alternate cycles of desiccation and rehydration or in the starved hydrated controls is approximately the same (see also Fig. 1). However, the distinction must be made that the present results concern long-term effects compared with the short-term results of Noble-Nesbitt. Thus the difference in results might lie in the length of the period of experimentation; it is possible that over longer periods of starvation the rate of loss both in fresh weight and in dry weight is in some way greatly reduced. This view can be supported by the finding that the final fresh weight (as a percentage of the initial fresh weight) is the same whether insects are starved for either 3 or 5 weeks (Table 6). However, this does not explain the greater loss in dry matter following desiccation and rehydration as compared with starvation for an equivalent period as suggested by Noble-Nesbitt.

On the other hand, in the presence of food the dry matter in insects subjected to 5 cycles of desiccation and rehydration is definitely lower than in the hydrated controls. In the experimental group the dry matter was reduced to 30.2 ± 0.7 of the final weight attained by the time the experiment was terminated, whereas the corresponding figure in the controls, designated here as 'normal' insects, was $33.9 \pm 0.5\%$ (Table 4). Although this might seem to substantiate the results of Noble-Nesbitt mentioned above, care must be taken in interpreting the results of the present experiment. This is because a comparison of the final fresh weight attained by insects used here reveals that while there is a progressive increase in weight in the control group, the weight of the desiccated and rehydrated group remains at a level approximately equal to the initial pre-desiccation weight (Fig. 2, Table 4). This might reflect an impairment of

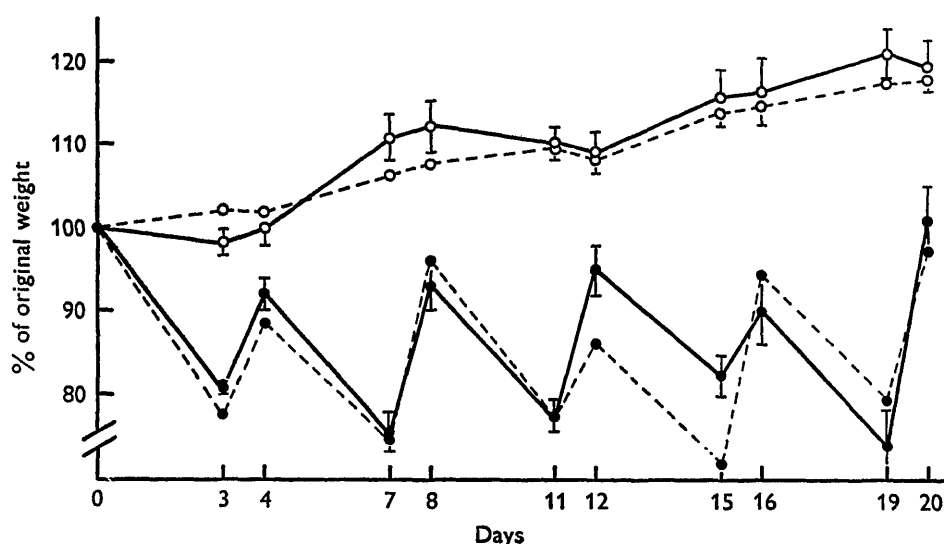


Fig. 2. Effects of 5 alternate cycles each consisting of 3 days desiccation followed by 1 day rehydration on the fresh weights attained by insects that were allowed food. Symbols as in Fig. 1.

feeding caused by repeated desiccation and rehydration rather than a direct loss of dry matter, i.e. partial starvation, which in turn results in a partial loss in dry matter. In addition, it is not known how repeated desiccation and rehydration would interfere, if at all, with growth and development to the next instar.

Effect of starvation on dry matter

The results described so far do not indicate whether shorter periods of experimentation would result in different rates of loss in dry matter during starvation as compared with that during starvation combined with desiccation and rehydration. As noted above, Noble-Nesbitt (1969) had not shown whether the differences were significant or not. It was obviously desirable in the light of the above results to repeat his experiments, but using larger numbers of insects. This was done as follows.

Insects, an equal number of each sex, were accurately weighed and desiccated for 3 days, after which they were accurately weighed again. One half of the insects was dried at 56°C for water-content determinations and the other half was rehydrated

for 1 day before drying at 56 °C. An equal number of each sex was kept at 83 % R.H. and starved for either 3 or 4 days. These latter insects served as controls. The results are summarized in Table 5, which includes the pooled data obtained from both sexes in each treatment, since there was no obvious sexual difference, as mentioned earlier.

From Table 5 it is quite clear that the percentage loss in dry matter from the estimated initial dry matter is almost exactly the same in insects starved for 4 days and in those desiccated for 3 days then rehydrated for 1 day. Thus it can be concluded that desiccation followed by rehydration in *Thermobia* does not result in any loss in dry matter additional to that caused by starvation.

Table 5. *Effect of 3 days desiccation followed by 1 day rehydration, and of starvation on the final fresh weight and the dry weight*

Treatment	No. of insects	Final fresh weight as % of initial weight ± S.E.	Final dry weight		Loss in dry matter as % of initial dry matter ± S.E.*
			As % of last live weight ± S.E.	As % of initial weight ± S.E.	
3 days desiccation	20	76.6 ± 0.8	38.3 ± 0.8	29.4 ± 0.8	13.4 ± 2.2
3 days starvation	20	94.3 ± 0.8	30.5 ± 0.6	28.8 ± 0.7	15.1 ± 1.9
3 days desiccation + 1 day rehydration	20	100.3 ± 2.0	27.9 ± 0.7	27.7 ± 0.6	18.2 ± 1.7
4 days starvation	20	94.9 ± 1.0	29.0 ± 0.8	27.6 ± 0.8	18.7 ± 2.3

* Assuming that each insect had 33.9 % of its initial fresh weight as dry matter (see Table 4) at the start of the experiment.

Table 6. *Effects of starvation and of allowing food to starved insects on the final fresh weight and the dry weight*

Treatment	No. of insects	Final fresh weight as % of initial weight ± S.E.	Final dry weight as % of last live weight ± S.E.
1 week starvation	40	90.9 ± 0.8	28.4 ± 0.9*
1 week starvation, offered food 3 days	14	100.7 ± 2.0	33.0 ± 0.8
1 week starvation, offered food 7 days	12	102.8 ± 1.9	33.2 ± 1.0
3 weeks starvation	19	79.4 ± 2.1	24.7 ± 1.0†
3 weeks starvation, offered food 3 days	6	97.2 ± 2.4	29.1 ± 1.1
3 weeks starvation, offered food 7 days	6	101.7 ± 4.7	32.9 ± 2.4
5 weeks starvation	12	79.2 ± 1.5	18.5 ± 0.4

* Mean of 14 insects only. † Mean of 7 insects only.

The results presented in Table 6 show how the depletion in dry matter (and simultaneously the rise in water content) in starved insects depends on the length of the starvation period. The effect of starvation would be even more pronounced if the results are expressed as the percentage of dry matter with respect to the initial weight. It is interesting to note here that although starvation for 3 or 5 weeks results in insects maintaining the same final fresh weight (as a percentage of the initial weight), longer starvation causes more depletion in the dry matter. However, the figure representing the dry-matter content in insects starved for 3 weeks shown in Table 6, being 24.7 ± 1.0 % of the last fresh weight, is somewhat higher than the values reported in Table 4 for insects starved for 20 days only, probably due to the smaller number of insects

used in the determination of the former figure. Nevertheless, it is quite clear that 5 weeks starvation resulted in a tremendous rise in the water content (being $81.5 \pm 0.5\%$ of the last wet weight). In other words, the dry matter was reduced to 14.7% of the initial fresh weight, as compared with about 34% in 'normal' insects.

The results of experiments which showed that allowing previously starved insects access to food changes their water balance are included in Table 6. These results indicate that, after feeding, starved insects attain their pre-starvation weight and this is associated with a rise in their dry matter, i.e. a reduction of their experimentally elevated water content. It seems that the feeding for a week of insects previously starved for 3 weeks is approximately sufficient to restore the 'normal' dry weight/wet weight ratio.

Water uptake during the moulting cycle

The above-mentioned observation concerning the possibility of the cessation of water uptake during the later stages of the moulting cycle was confirmed by the following experiment.

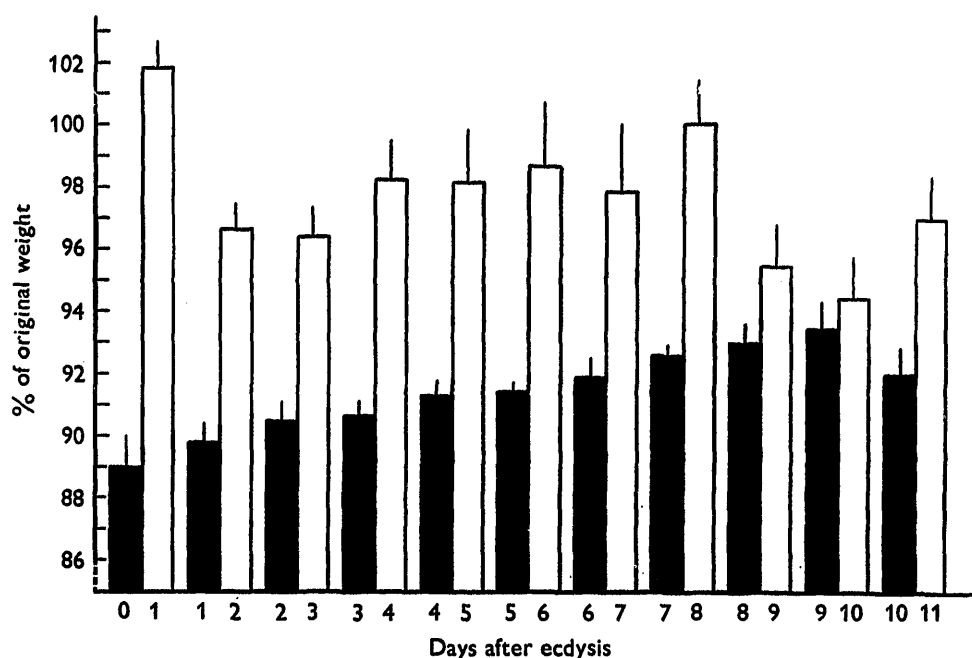


Fig. 3. Effects of 1-day desiccation followed by 1-day rehydration on the fresh weight of insects taken at various stages in the moulting cycle. Black columns represent weights following desiccation, white columns weights following rehydration, and vertical lines on tops of columns indicate the standard error. Ten insects were used for each treatment.

A group of ten insects was taken each day during the period 0–10 days after ecdysis. The insects of each group were accurately weighed, placed in clean glass vials and desiccated while being starved for 24 h. Then the insects were weighed, rehydrated for another 24 h, still starved, after which time they were accurately weighed again. The results of this experiment are represented in Fig. 3.

Fig. 3 clearly indicates that in those insects which were desiccated at 8 and 9 days after ecdysis the mean weight attained after rehydration is lower than on any other

day. In fact the difference between the mean desiccated and rehydrated weights in these two groups is by far the lowest. The percentages of insects that totally failed to rehydrate during the various stages of the moulting cycle are shown in Fig. 4. It is known that adult females undergo ecdysis every 9–10 days Rhodendorf & Watson, 1969). Fig. 4 also indicates that 50% of the insects taken for desiccation at either 8 or 9 days after ecdysis failed to rehydrate (they were then 9 and 10 days after ecdysis respectively). Due to individual differences, a few insects moulted earlier than this, and it was also evident that such insects failed to gain any weight while in rehydration and before they moulted.

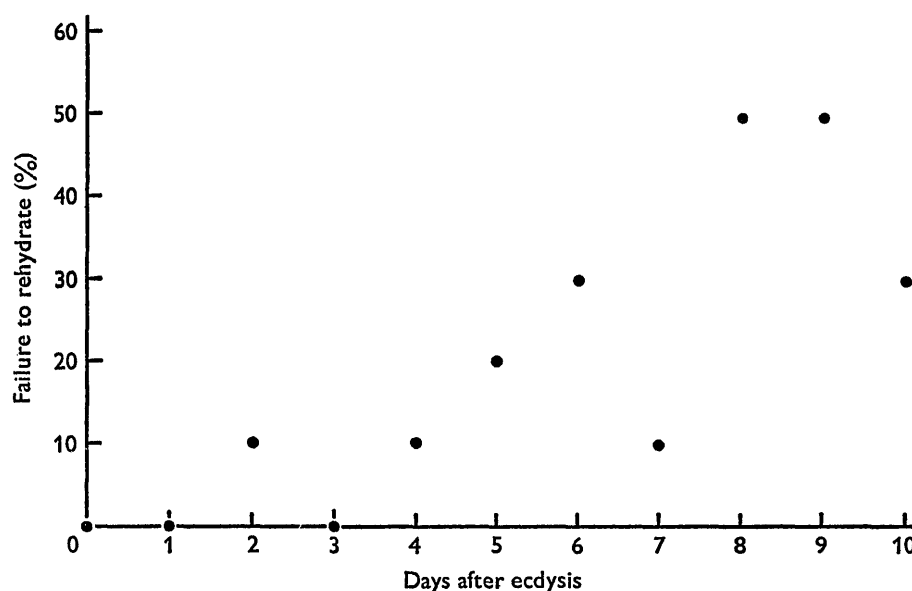


Fig. 4. Percentage of insects that totally failed to rehydrate during the various stages of the moulting cycle. Insects were desiccated for 24 h on the days shown in the graph and were rehydrated for the following 24 h.

The reason for the relatively higher degree of rehydration in insects desiccated at 0 days after ecdysis and rehydrated 1 day later (Fig. 3) is probably due to the great decrease in their initial weight during the period between ecdysis and being weighed prior to desiccation (0–2 h after ecdysis). This was repeated with insects only 0–1 h after ecdysis when they were first weighed, and essentially the same results were obtained.

Another point worth mentioning here is the apparent differential loss in weight during desiccation in the different stages of the moulting cycle. The results represented in Fig. 3 show that there is a progressive slight increase in the mean final weight (expressed as percentage of the initial weight) after desiccation during the period 0–9 days after ecdysis. This does not necessarily mean that the insects become progressively more waterproof during the period between 2 ecdyses and hence that less water is lost as the insect proceeds to the next instar. It might rather be correlated with the increase in weight in a particular instar during the moulting cycle (Watson, 1967), presumably without a parallel change in surface area. The finding that there is no continuous increase in weight throughout the instar in celibate males or females

but the increase ceases after the middle of the instar (Watson, 1967) does not totally support this explanation. However, this explanation might be true since the situation is complicated, concerning the results shown in Fig. 3, by the fact that not all the insects moulted at the same time. In this connexion it might be relevant to recall the work of Beament *et al.* (1964) on the permeability of the cuticle in *Thermobia*, as determined by the rate of water loss from insects killed at different stages of the moulting cycle. They found that there is no significant increase in permeability as the insect progressed towards the next instar when the rate of water loss was carried out at room temperature (19–26 °C), but when performed at 34 °C the insects were more permeable on the 5th and 7th day than on the 1st day after ecdysis.

From this experiment it is concluded that towards the end of the moulting cycle water uptake somehow ceases in previously desiccated *Thermobia*. The significance of this conclusion will be dealt with later. Incidentally it is interesting to mention here that if a droplet of liquid water is placed on the integument of a cockroach it disappears in times varying from 10 min to 2 or 3 h, depending on the site of the application of the droplet, which becomes completely waterproofed on its external surface. When a cockroach whose two cuticles have separated prior to ecdysis is used, the droplet shrinks so slowly that its disappearance could be accounted for by evaporation (Beament, 1964). Although the mechanism of uptake of such droplets might be different in the cockroach, nevertheless this example clearly illustrates the cessation of uptake towards the end of the moulting cycle. In *Arenivaga* nymphs and adult females, but not in adult males, water vapour is absorbed from and above 82.5 % R.H. over a wide range of temperatures (Edney, 1966). In agreement with the present work, Edney stated that nymphs preparing to moult do not regain weight after having been desiccated, but that soon after the moult is over the process begins again. However, the daily changes in weight during rehydration of previously desiccated nymphs (Fig. 4 in his work) show that there are no increases in the weight of individual insects during the periods 2–3 days prior to and 1–2 days following ecdysis. In *Thermobia*, the temporary arrest of uptake during the moulting cycle seems to be of a much shorter duration, although the precise time (prior to ecdysis) and the duration of arrest are not known.

DISCUSSION

The results obtained from the present investigation indicate that in its water uptake from subsaturated atmospheres *Thermobia* is not simply replenishing water lost through desiccation to attain the dry weight/wet weight ratio prevailing under the culture conditions used here. Although it is generally assumed that such a ratio is more or less constant under certain environmental conditions, in *Thermobia* it changes a great deal according to the availability of food. Thus 5 weeks of starvation results in an extremely hydrated insect compared with a feeding one kept under the same temperature and humidity conditions. However, the absolute amount of water present in the insect varies little during starvation, whether or not this is coupled with desiccation and rehydration, as shown in Table 7. The values in Table 7 compare well with the water content of 66.1 ± 0.5 % in insects taken directly from culture conditions (see Table 4). Of course, this does not apply in the case of insects desiccated for 3 days, where both the water content and the final fresh weight are greatly reduced.

Also in the case of insects desiccated for 3 days and then rehydrated for 1 day, the absolute amount of water (Table 7) seems to be higher than in the 4-day starved controls; this is attributed to the fact that in the former the fresh weight after rehydration slightly exceeded the pre-desiccation weight (Table 5). Such 'overshooting' occurs fairly frequently during the first stages of rehydration.

Although the data of Noble-Nesbitt (1969) concerning the water content in insects starved for 4 days and in untreated controls kept under the same conditions did not show significant differences, he was able to show that such differences existed between those desiccated for 3 days then rehydrated for 1 day and the untreated controls. He thus substantiated his suggestion that during starvation the body weight and

Table 7. *Calculated final amounts of water in mg/100 mg initial fresh weight of insects subjected to various treatments**

Treatment	Water (mg)	Treatment	Water (mg)
3 days desiccation	47.3 ± 0.7	1 week starvation	64.5 ± 1.2
3 days starvation	65.6 ± 0.7	5 weeks starvation	64.5 ± 1.1
3 days desiccation + 1 day rehydration	72.5 ± 1.9†	5 cycles of 3 days desicca- tion + 1 day rehydration	64.2 ± 1.6
4 days starvation	67.1 ± 1.1	Starved controls (20 days)	63.6 ± 0.8

* Data are derived from those summarized in Tables 4, 5 and 6.

† This is a relatively high value since the insects in this case attained a weight slightly exceeding their original weight (see Table 5).

therefore the volume is maintained by the retention of a higher proportion of water. The present work confirms beyond any doubt that this occurs equally in insects whether subjected to desiccation followed by rehydration, or to starvation, and that it occurs to the same extent over the same length of time. Thus his conclusion that a higher degree of hydration should be shown more clearly as a result of longer periods of starvation (longer than 4 days in his experiments) is confirmed here, but the suggestion that greater dry-weight losses should also result as a consequence of 'greater demands placed on the insect, for example, during desiccation and subsequent rehydration...' is not. There is in fact no indication that repeated cycles of desiccation and rehydration impose greater demands on the dry matter of the insect than does starvation only. Since the dry-weight losses will, at least in part, be attributable to the metabolic requirements of the insect, this result would suggest that net uptake of water from the atmosphere by the firebrat imposes no additional strain on the metabolism of the insect as measured by loss in dry matter.

If an insect with an experimentally elevated water content is desiccated, it can be shown that it would still take up water during subsequent rehydration, although its water content immediately after desiccation might be approximately 'normal', i.e. equivalent to the water content of insects taken directly from their feeding vials. This can easily be postulated from the results summarized in Fig. 1. To illustrate this point let us assume that

(i) by the end of the 4th rehydration (day 16 in Fig. 1) the insects had on average the modest estimation of 73 % of their final fresh weight as water (their water content 4 days later was actually 78.3 ± 0.4 % of their final fresh weight by then, see Table 4);

(ii) the experimentally measured loss of 18.6 ± 0.9 % of their fresh weight at day 16

during the 3 days of desiccation that followed was exclusively of water without any loss in dry matter.

Then it follows that the water content in these insects (both sexes combined together) on day 19 (Fig. 1) is

$$\frac{73 - 18.6}{81.4} \times 100 = 66.8\%$$

of the final weight immediately after desiccation.

This last figure, which in all probability is an underestimate, compares fairly well with the determined water content of 'normal' untreated insects ($66.1 \pm 0.5\%$, see Table 4). Despite this, however, when the insects were placed in rehydration on day 19 (Fig. 1), water uptake continued and the water content rose to $78.3 \pm 0.4\%$ on day 20. Obviously the insects in their uptake are regulating so as to attain a certain weight and consequently a certain volume, the latter being characteristic of the degree of starvation. It was on these grounds that it was stated earlier in this discussion that in its water uptake *Thermobia* is not simply replenishing water lost through desiccation. This is in contrast to Noble-Nesbitt (1969), who stated that 'This indicates that uptake of atmospheric water by the firebrat continues only until the body water lost during desiccation is replenished, after which no net uptake occurs. The uptake process is evidently diminished once the body again has its normal water content.' Certainly water uptake continues in previously desiccated then rehydrated firebrats only up to the level characteristic of insects starved in hydration for an equivalent period. The evidence at hand strongly suggests that volume regulation, a phenomenon first described in *Thermobia* by Noble-Nesbitt (1969), is an extremely important factor in the mechanism of water uptake from subsaturated atmospheres.

It can be argued, however, that when the insect absorbs water from subsaturated atmospheres at the expense of raising its water content far beyond the 'normal' value, as in the case of repeated desiccation and rehydration, it is not regulating for volume, but water uptake proceeds so that the insect can maintain a specific constant concentration of some substance(s) in the haemolymph or in some other tissue. That the uptake mechanism does not seem to be a means of maintaining a constant ionic concentration in the haemolymph will be demonstrated later (Okasha, 1971; see also Edney, 1966).

If the theory that volume regulation is the reason for water uptake is correct, it might have far-reaching implications on the mechanism of uptake and its control, at least in *Thermobia*. The conclusion of Noble-Nesbitt (1970) that the rectum is the site of uptake in this species comes into doubt in the light of the theory of volume regulation. This is because anal blockage, the experimental basis of his suggestion of rectal uptake, could equally well result in a nervous inhibition which in turn would inhibit water uptake (assuming that the body surface, as has been generally believed, is the site of uptake, or indeed in conjunction with the rectum which is in any case a continuation of the general body surface). In this respect stretch receptors in the body surface could be important as 'volume detectors', and anal blockage could result in an interference with the nervous message from the stretch receptors to the operative site in the nervous system causing an inhibition of volume regulation and consequently of water uptake from a humid environment. The role of stretch receptors in controlling

vital processes in the life of insects is well known. In *Rhodnius*, for example, the degree of stretching of the abdomen after a blood meal determines whether or not the secretion of the brain hormone and consequently of the moulting hormone takes place in an effective manner (Wigglesworth, 1934), and stretch receptors have been found in the abdominal tergites of the same species (van der Kloot, 1960, 1961) (for a review see Wigglesworth, 1964). It would be interesting to discover whether *Thermobia* has stretch receptors in its body surface.

The cessation of water uptake during the later stages of the moulting cycle (Figs. 3, 4) could be explained according to the present theory by a possible detachment of the stretch receptors from the old cuticle. This theory also provides an explanation for the cessation of uptake once a certain specific volume has been reached, irrespective of temperature or relative humidity provided the latter is at or above 63 % R.H. as reported by Noble-Nesbitt (1969). But it is difficult to perceive the factor(s) that determines a specific volume characteristic of an insect in a given physiological state.

Although Noble-Nesbitt (1970) mentions that there is every indication, from preliminary results, that 'the rectum of *Thermobia* has the complex fine structure expected of a water-absorbing tissue', one should be cautious in interpreting this as additional evidence of rectal uptake of water from subsaturated atmospheres. The rectal epithelium is known to exhibit a reabsorptive faculty, extracting water from the faeces before they are voided, especially in insects living in dry habitats (Wigglesworth, 1953), a characteristic of the rectum mentioned by Noble-Nesbitt in the same publication.

It must be admitted that the theory proposed here regarding the possible nervous inhibition caused by anal blockage lacks conclusive and direct experimental evidence. Attempts to insert fine glass capillary tubes through the anus, which would have allowed a permanent dilatation of the rectum without damaging it and would have allowed free exchange between the rectal tissue and the atmosphere whilst possibly causing nervous inhibition, were unsuccessful. This must await improved techniques, but there is little doubt that water uptake is concerned primarily with volume regulation.

SUMMARY

1. Water uptake is not inhibited by centrifugation, exposure to 45 °C, burning of the integument or by submergence in water for 1 h.
2. Repeated desiccation followed by rehydration does not inhibit water uptake. This, however, results in insects with an extremely high water content.
3. Starvation also results in insects with a very high water content, the latter depending on the length of starvation, and also results in the depletion of dry matter.
4. Desiccation followed by rehydration does not result in any loss in dry matter additional to that caused by starvation.
5. The ability to rehydrate of insects desiccated at various stages of the moulting cycle is described. It is concluded that at the later stages of the moulting cycle water uptake ceases.
6. The results are discussed in the light of current theories of the uptake mechanism. It is suggested that the uptake mechanism is primarily concerned with volume regulation.

7. It is also suggested that anal blockage, which is known to arrest uptake, results in a nervous inhibition bringing about such an effect, rather than the rectum being the site of uptake.

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