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WATER BALANCE IN THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD). EXCHANGES OF WATER WITH THE ATMOSPHERE

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INTRODUCTION

Watson (1967) has shown that the synonym *Lepismodes iniquilinus* Newman has priority over *Thermobia domestica* (Packard), but to avoid confusion in the present context the junior synonym as used previously is retained throughout this paper.

The ability of *Thermobia* to take up water from subsaturated atmospheres down to 45% relative humidity (R.H.) at 21° C. has been demonstrated in an earlier paper (Beament, Noble-Nesbitt & Watson, 1964). The ability to absorb water vapour from subsaturated atmospheres has also been demonstrated in a few other insects, notably in *Tenebrio* larvae (Buxton, 1930; Mellanby, 1932; Locke, 1964), *Chortophaga* larvae (Ludwig, 1937), *Xenopsylla* prepupae (Edney, 1947; Knülle, 1967*a*) and larvae (Knülle, 1967*a*) and *Arenivaga* larvae and adult females (Edney, 1966), and in several acarines, notably in ticks (Lees, 1946–8, 1964; Browning, 1954; Belozerov & Seravin, 1960; Camin, 1963; Knülle, 1966) and mites (Knülle, 1962, 1965, 1967*b*; Knülle & Wharton, 1964; Solomon, 1962, 1966; Wharton & Kanungo, 1962; Kanungo, 1963, 1965). This aspect of insect water relations has been reviewed recently in two articles by Beament (1964, 1965).

From these other studies have emerged some principles which may be of general application not only to the ability of arthropods to absorb water vapour from subsaturated atmospheres, but also to their ability to restrict transpiratory water losses and to regulate their body-water content. The aim of the present investigation is to examine how far these principles apply in the case of *Thermobia*. Some of the main results have been reported briefly elsewhere (Noble-Nesbitt, 1968*a*).

MATERIALS AND METHODS

Stock laboratory cultures of *Thermobia domestica* (Packard) were maintained at 37° C. and in 83% R.H. The insects were fed with a mixture of 'Baby Wheat', 'Baby Oats' and dried yeast.

For experiments involving the effects of temperature, a water-jacketed incubator was used to maintain constant temperature during the experiments. Atmospheres of constant relative humidities were maintained within desiccators placed in the incubator, using saturated solutions of the appropriate salts (O'Brien, 1948; Winston & Bates, 1960). The insects were weighed on a balance reading to 0·1 mg. For this purpose, the insects were briefly removed from the experimental conditions.

Experiments involving continuous weight recording were conducted on a Cahn RG Electrobalance connected to a suitable potentiometric recorder. In some of these experiments the insect was placed in an open-topped aluminium-foil container which rested on the stirrup of the electrobalance. The humidity in the weighing chamber was maintained by the appropriate salt solution or drying agent in a container placed in the weighing chamber.

In other experiments the suspension of the weighing stirrup was lengthened to allow the insect container to hang below the electrobalance in a special chamber attached to the underside of the electrobalance. Humidity was maintained by the appropriate salt solution or drying agent in a container placed in the chamber. Ports in the sides of the chamber allowed the introduction of gas mixtures, which could be used to change the atmosphere in the chamber. The position of one of the ports was arranged so that a tube could be inserted to allow the rapid flushing-out of the insect container. A magnetically driven fan within the chamber allowed equilibrium to be set up rapidly in the apparatus. It was turned off during measurements.

The statistical treatments applied to the experimental data were those given in Bailey (1959). In the text, figures given after means are the standard deviations of the individual measurements. Where values are related to a standard insect, this refers to an insect weighing 30 mg., the multiplicand (30 mg./observed wt.)$^{\frac{2}{3}}$ being used to correct for differences among the weights of the insects, and making allowance for differences in surface area (cf. Beament *et al.* 1964).

RESULTS

1. *The time-course of water uptake*

In the experiments of Beament *et al.* (1964), uptake of water vapour in previously desiccated *Thermobia* was measured 1 day after confinement in constant relative humidity. Full recovery from prior desiccation occurred only in the higher humidities tested. From these results it was impossible to determine the behaviour of the uptake mechanism subsequent to full recovery, or to decide whether full recovery would be achieved in all humidities in which some uptake was recorded in the first day of exposure, given sufficient time. In other arthropods equilibrium is usually achieved when full recovery from prior desiccation has occurred (Mellanby, 1932; Lees, 1946; Edney, 1966). In the larva of the oriental rat flea, however, the equilibrium level reached depends upon the humidity to which the insect is exposed (Knülle, 1967*a*). The experiments described in this section were designed to determine whether equilibrium is reached, where no further net uptake occurs. Experiments described in later sections deal with other aspects of the uptake of water from the atmosphere.

Insects were taken and placed individually in clean glass vials, weighed, and then exposed to dry air over dry calcium chloride at 37° C. for 3 days, after which they were weighed again before being transferred to the test humidity and temperature. At suitable intervals the insects were removed briefly to be weighed. Desiccated insects exposed to 63% R.H. at 23° C. regained most of their lost weight within 2 days. Subsequently, further weight gain did not occur, the insect maintaining a weight close to its initial, pre-desiccation value (see Fig. 1). 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. There is no apparent danger to the firebrat that it may take in too much water at high humidities, and it is not likely to be confined to humidities close to the critical equilibrium humidity in which no gain or loss occurs (45 % R.H. at 21° C). Once equilibrium is reached further small losses in weight occur. That these losses are attributable to starvation will be shown below.

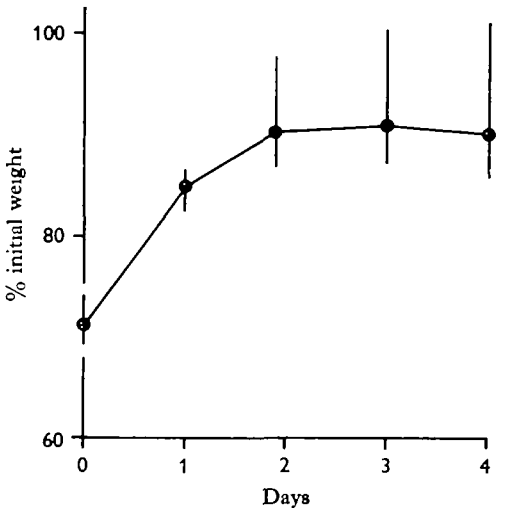

Fig. 1. Uptake of water by *Thermobia* from an atmosphere of 63 % R.H. at 23° C. The insects were desiccated for 3 days in dry air at 37° C. beforehand. Values expressed as % pre-desiccation weight against time in days after transference to the test humidity and temperature. The solid lines join the mean values. The vertical lines show the spread of the individual measurements.

2. *Maintenance of body-water content*

Measurements of weight change described above and those to be described below show that the insect maintains its body-weight constant in the higher humidities, and that previously desiccated insects increase in weight only until their normal weight is attained. Following death, water is lost rapidly and the weight of the insect falls (see Figs. 5, 6). In life, at these humidities, the insect is in a steady-state condition, and it is to be expected that deviation from this normal condition would be compensated by an alteration in the uptake mechanism tending to return the insect to its steady-state condition.

To test this, individual insects were placed on a continuously recording electrobalance, in controlled humidities, and their weights were continously monitored. These experiments were conducted in the normally lighted laboratory at room temperature. Recovery from desiccation in high humidity occurred at a constant rate, almost until full recovery. During the late stages of recovery small gains and losses occurred alternately as the pre-desiccation weight was approached. Once the firebrat was fully hydrated again, and in undesiccated insects, slight oscillations, approximately $\pm 0{\cdot}1$ % of the total weight of the insect, continued, indicating the dynamic nature of the equilibrium and the constant resetting of the uptake mechanism as the equilibrium

position was overshot. This 'hunting' action is characteristic of steady-state conditions controlled by a feed-back mechanism.

During the course of these experiments it was noted that generally uptake was inversely related to the locomotor activity of the insect. When first placed on the pan of the balance in the humidity chamber and whilst the humidity was stabilizing, the insect remained active and ran around in its container. This activity was recorded by the balance as an increase in the 'noise' level of the trace. The insect often became inactive after uptake commenced (see Fig. 2*a*), and uptake continued during periods of inactivity, when the insect remained still. Uptake usually, though not invariably,

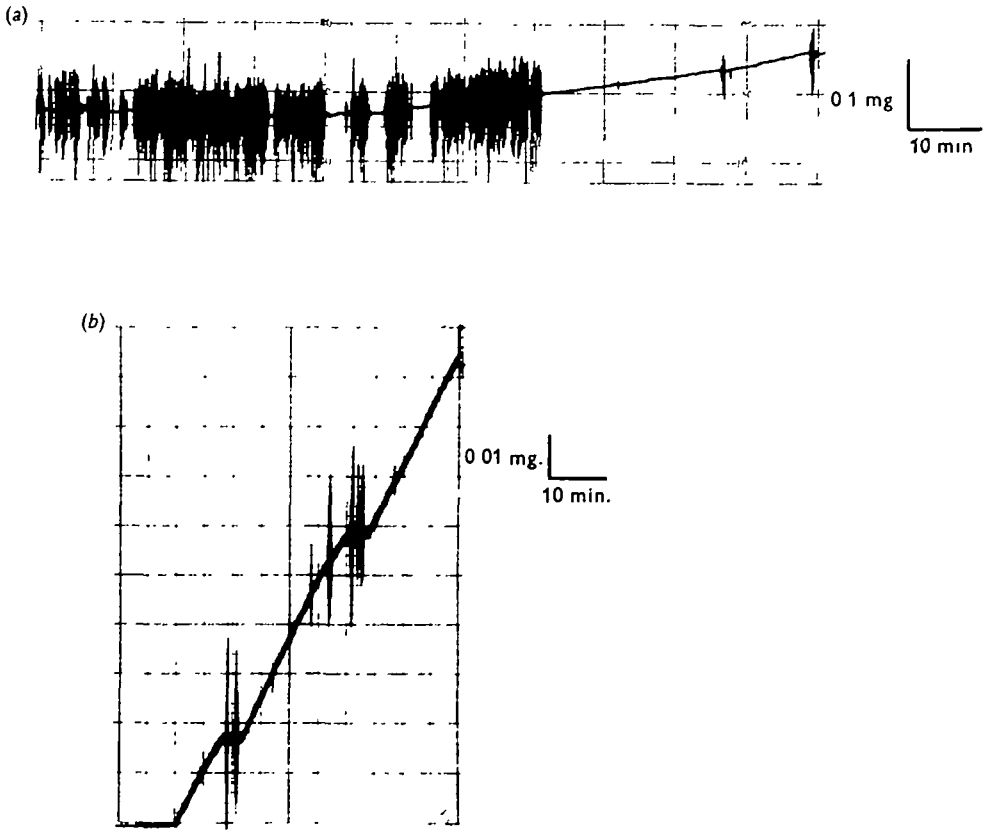

Fig. 2. Continuous weight recordings of *Thermobia* during uptake of water. (*a*) weight recording showing commencement of uptake by a previously desiccated insect in 75 % R.H. at 21° C. after transference to the test humidity and temperature. Uptake commenced within 45 min. Locomotor activity, shown by rapid vertical excursions of the trace, was great beforehand, but decreased markedly after the commencement of uptake. (*b*) weight recording showing interruption of uptake in 83 % R.H. at 22° C. by short bursts of locomotor activity. Shown are two short (5 min.) plateaus, coincident with locomotor activity.

stopped when the insect became active and walked around its container, even for short periods (see Fig. 2*b*). Longer periods of activity occurred at longer intervals, often during the night and especially in the fully, or almost fully, recovered insect, when actual loss of weight was usually recorded. This loss was subsequently made good. These results confirm the active nature of the uptake mechanism. Presumably the

metabolic resources of the insect are channelled according to the needs of the insect (Noble-Nesbitt, 1967). That this is a feature under the control of the insect is shown by the fact that there is not an invariable inverse relationship between uptake and locomotor activity.

Further evidence of the active nature of the mechanism accrues from the effect of carbon dioxide anaesthesia, which is considered more fully below. This treatment prevents further uptake. Recovery from anaesthesia is followed by a resumption of uptake (see Fig. 11*b*).

3. *The effect of temperature on uptake*

Firebrats tested at room temperature resorb water from the atmosphere in humidities above 45% R.H., but in culture their optimum conditions involve a temperature of 37° C. and a humidity of 83% R.H., which provides a saturation deficit very close to the value experienced by them at the critical equilibrium humidity at room temperature

Table 1. *Rate of gain or loss of water by previously desiccated insects*

Relative humidity (%)	Weight gain or loss in first day of exposure at 23° C *n*	23° C mg. ± S.D.	28° C *n*	28° C mg. ± S.D.	33·5° C *n*	33·5° C mg. ± S.D.	37° C *n*	37° C mg. ± S.D.
43	5	−0·3 ± 0·3	5	−0·9 ± 0·5	5	−1·3 ± 0·6	5	−1·3 ± 0·3
63	4	+3·2 ± 0·2	4	+3·3 ± 0·3	5	+4·8 ± 0·5	5	+4·0 ± 0·5
75		—		—		—	4	+4·7 ± 2·4
83		—		—		—	5	+6·7 ± 1·9

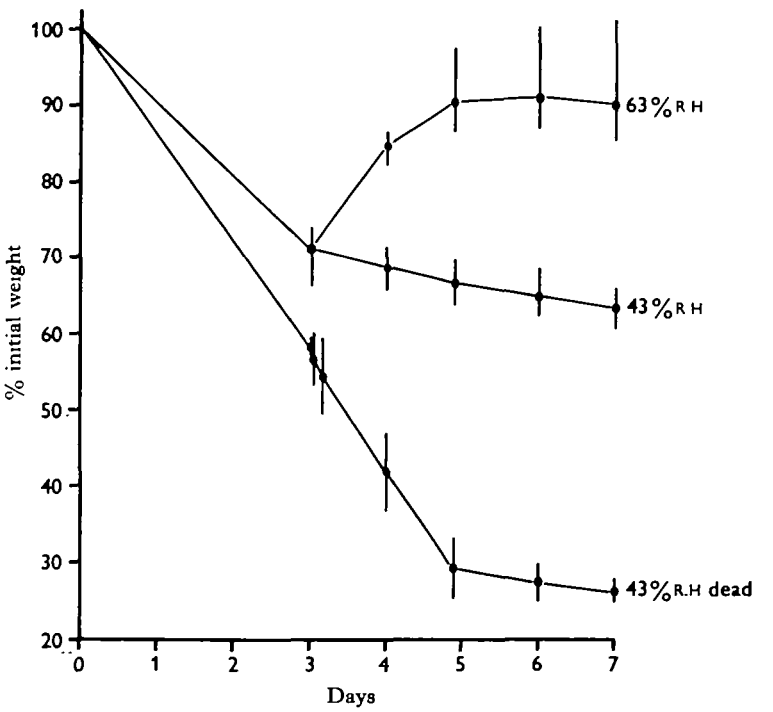

Fig. 3. Gains and losses of water by living and dead *Thermobia* in 63% R.H. and 43% R.H. at 23° C. following 3 days of dessication in dry air at 37° C. in the living state. Solid circles are mean values and the vertical lines show the spread of the individual measurements.

(Beament *et al.* 1964). This posed the question as to whether the insects in their optimum culture conditions were nearly at the balance point between uptake and loss. Experiments were conducted to test the ability of the insects to absorb water from various humidities at various temperatures.

The first series of experiments were conducted in 43% R.H. and 63% R.H. at 23, 28, 33½ and 37° C., the procedure being the same as for the experiments described in §1 above. In addition, in some instances insects were killed by exposure for 1 min. to carbon dioxide followed by 1 min. of hydrogen sulphide immediately after desiccation

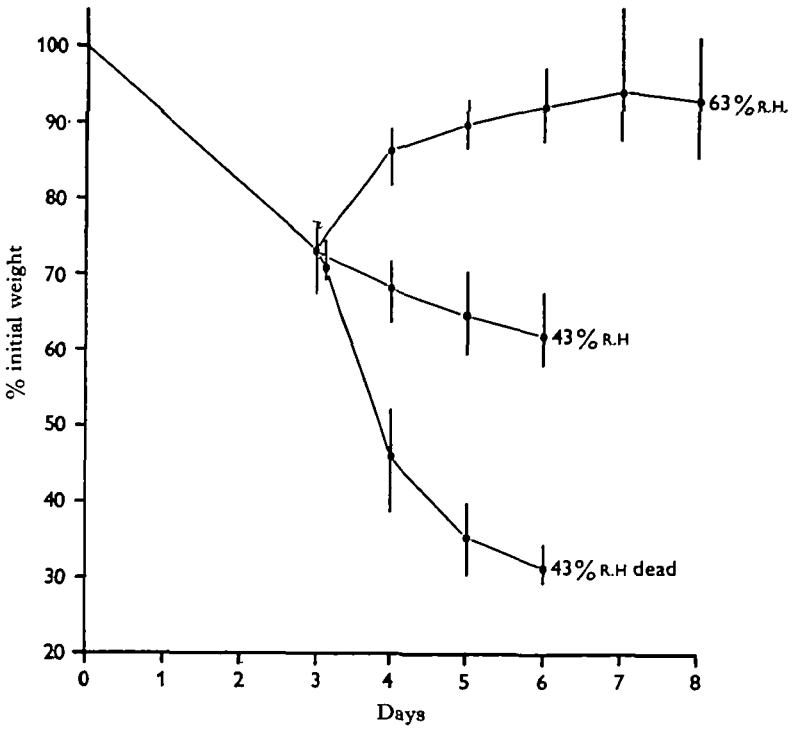

Fig. 4. Gains and losses of water by living and dead *Thermobia* in 63% R.H. and 43% R.H. at 28° C. following 3 days of desiccation in dry air at 37° C. in the living state. Symbols as for Fig. 3.

and before transference to the test conditions. The results for living insects in 63% R.H. at 23° C. have already been given above (Fig. 1). These results, together with the results for the other temperatures and humidities are incorporated in Figs. 3–6. At all temperatures, in the living insects, water is resorbed in 63% R.H. until the same equilibrium level is reached, but is lost in 43% R.H. This loss is greatly exceeded in the dead insects. In Table 1 the rates of gain or loss in the living insects are given, expressed as the rate at which water is gained or lost per standard insect during 24 hr. following transference to the test conditions. The rates are higher at the higher temperatures. At 43% R.H. this reflects partly the greater activity of the water molecules and partly an increase in cuticular permeability at the higher temperatures; at

63 % R.H., it is possible that the uptake mechanism is also working faster at the higher temperatures.

These results show that as the temperature is raised the uptake of water occurs faster and continues until equilibrium is reached, which is achieved earlier therefore, even above the critical temperature of approximately 30° C. (Beament *et al.* 1964). Further, at 63 % R.H. and 37° C. the saturation deficit (17 mm. Hg) is considerably in excess of that experienced by the insects held at 45 % R.H. at room temperature (10 mm. Hg), indicating that in the firebrat the lowest 'critical' humidity for equilibrium to be established is related to the degree of saturation of the atmosphere and not to the absolute value of the saturation deficit.

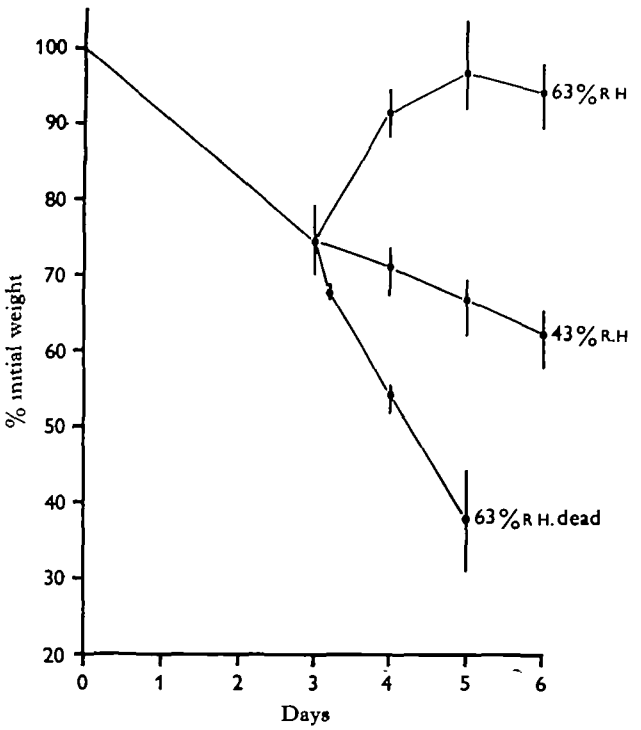

Fig. 5. Gains and losses of water by living and dead *Thermobia* in 63 % R.H. and 43 % R.H. at 33·5° C. following 3 days of dessication in dry air at 37° C. in the living state. Symbols as for Fig. 3.

4. *The effect of atmospheric humidity on uptake*

Firebrats regain lost water when held in humidities above 45 % R.H. at room temperature, but continue to lose water to the atmosphere in humidities lower than this. During the first 24 hr. of recovery, most water is regained in the higher humidities (Beament *et al.* 1964). In the present series of experiments, further humidities at 37° C. were used to test the effects of varying atmospheric humidity on the uptake of water from the atmosphere.

In addition to the 43 and 63 % R.H. results dealt with in § 3 above, humidities of 75 and 83 % R.H. were used. The full results are given in Fig. 6 and Table 2. The rates of uptake or loss over the first day in the test conditions following the initial pre-

desiccation period are given in Table 1. Loss of weight is recorded in insects held in 43% R.H., but gains occur in the higher humidities.

Although the rate of attainment of equilibrium is greater the higher the humidity, there are no significant differences between the equilibrium levels eventually attained at the different humidities of 63% R.H. and above (*t* test; P > 0·1 for 63% *vs.* 75%; 63% *vs.* 83%; and 75% *vs.* 83%).

The levels reached in these insects do not differ significantly overall from the levels reached in control insects subjected to identical regimes, but with the substitution of

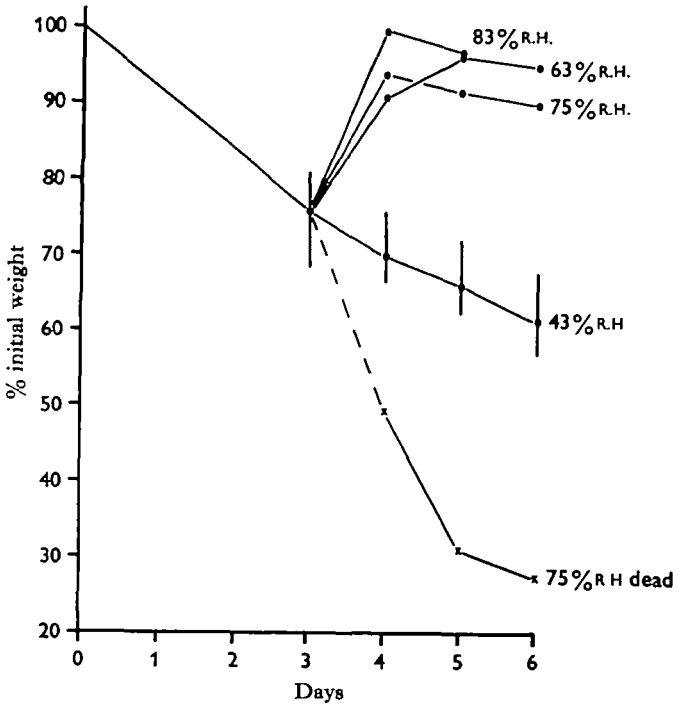

Fig. 6. Gains and losses of water by living and dead *Thermobia* in various humidities at 37° C. following 3 days of desiccation in dry air at 37° C. in the living state. Symbols as for Fig. 3. Spread lines are omitted from values for 63, 75 and 83% R.H. for clarity, but are similar to those given for 43% R.H. Crosses show readings from one individual at 75% R.H. which died naturally during the 4th day (indicated by broken line).

Table 2. *Final weights reached by insects exposed to different humidities at 37° C following prior desiccation or hydration*

Pre-treatment	Relative humidity	*n*	Final wt. as % initial wt. ± S.D.
Desiccated	43	5	66·0 ± 3·5
	63	5	96·1 ± 0·7
	75	4	91·5 ± 7·0
	83	5	100·0 ± 6·8
	63, 75, 83 combined	14	96·1 ± 6·1
Hydrated	43	5	81·9 ± 4·5
	63	5	89·2 ± 6·1
	75	5	96·0 ± 2·2
	83	5	94·5 ± 7·0
	63, 75, 83 combined	15	93·3 ± 5·3

3 days in high humidity for the 3 days of pre-desiccation (t test; $P > 0{\cdot}1$). This indicates that the level attained at equilibrium is equivalent to the initial state corrected for losses with time resulting from starvation (see also § 5 below.).

There is apparently no evidence of a lower equilibrium level for lower humidities as found in the oriental rat flea larva by Knülle (1967*a*).

5. *Regulation of the water content of the insect*

In the experiments described above, the loss or gain in weight was taken as a measure of the gain or loss of water. The following experiments were conducted to test the accuracy of this approximation, and to trace the effects of starvation over the periods of the experiments.

Table 3. *To show the effects of starvation, desiccation and rehydration on the total weight, dry weight and water content of insects at* 37° *C.*

Treatment	Duration (days)	n	Total weight as % initial wt. ± S.D.	Water content as % final wt. ± S.D.	Dry weight as % initial wt. ± S.D.
None—controls	0	8	100	65·5 ± 2·5	34·5 ± 2·5
4 days' starvation	4	4	90·5 ± 6·3	67·8 ± 2·9	29·0 ± 3·1
3 days' desiccation	3	8	73·7 ± 7·4	60·1 ± 3·1	29·6 ± 3·0
3 days' desiccation + 1 day rehydration	4	4	93·0 ± 1·7	71·8 ± 1·9	26·2 ± 1·7

A. *The water content of normal insects*

Insects were taken from optimum culture conditions and weighed, then killed with carbon dioxide and hydrogen sulphide. They were then dried to constant weight at 57° C. The results are shown in Table 3 and Fig. 7. The loss in weight was 65·5 ± 2·5%. Though this may represent in part volatile constituents of the body other than water (Edney, 1966; Knülle, 1967*a*), it is probably reasonable to take this loss in weight as representing the water content of the insect at death. The insect thus contains some 34% dry matter and some 66% water. These values are quite normal for most arthropods (Rapoport & Tschapek, 1967).

B. *The water content of treated insects*

(i) *The effects of starvation.* Insects were isolated from normal culture conditions, weighed and then placed without food in 83% R.H. at 37° C. Weights were taken daily. After 4 days they were weighed, then killed with carbon dioxide and hydrogen sulphide. They were then dried to constant weight at 57° C. The results are shown in Table 3 and Fig. 7. At the time of death, these insects contained 67·8 ± 2·9% water; their total weights were 90·5 ± 6·3% of their initial weights; and their dry weight was the equivalent of 29·0 ± 3·1% of their original weight when taken from the culture. Compared with the normal insects, this represents a loss of approximately 16% of their original dry matter. Over the period of the experiments, therefore, starvation can be expected to account for a loss in weight of approximately 1·4% of the initial weight each day due to loss of dry matter. If the proportion of water remained constant, then this loss ot dry matter would result in a total loss of weight (dry matter and water) of approximately 16% of the original weight. In fact the total weight loss, at 9·5 ± 6·3% of the original weight, is somewhat less than this, the proportion of water in the starved

body apparently being higher than in control insects, though the present data do not show significant differences between the starved and the control insects (t test; $P > 0{\cdot}1$). These results, however, suggest that there may be some measure of compensation for loss of dry weight (and, therefore, potentially of volume) by a greater degree of hydration. This should be shown more clearly with greater losses in dry weight, resulting from longer periods of starvation, or from greater demands placed on the insect, for example, during desiccation and subsequent rehydration (see §(iii) below).

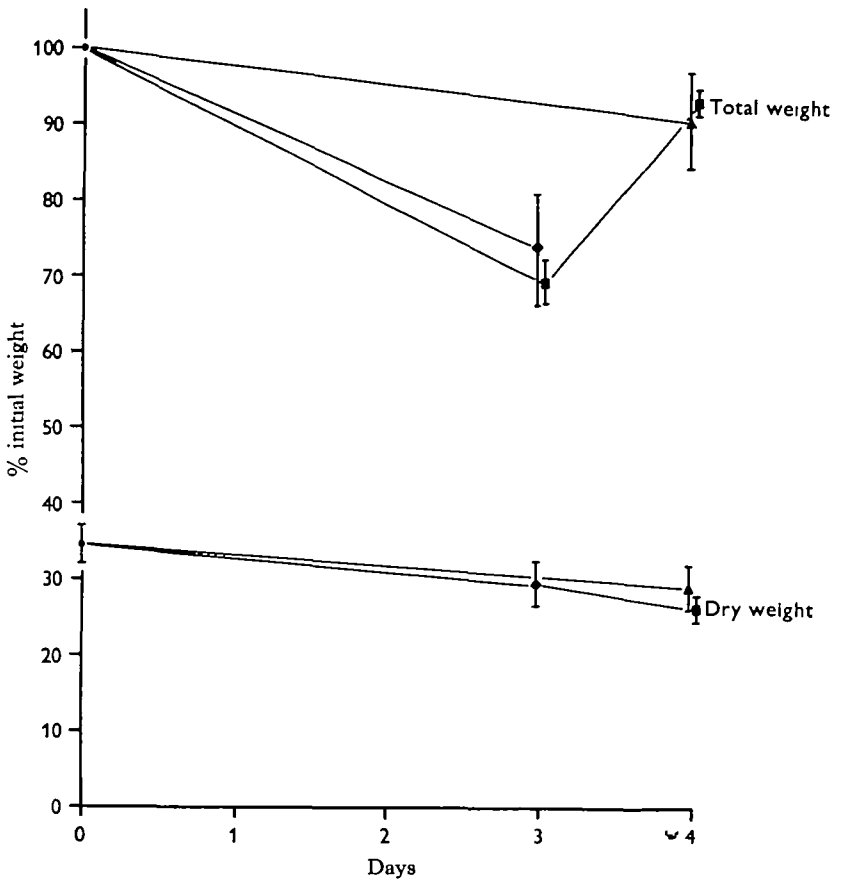

Fig. 7. Wet and dry weights of *Thermobia* following various treatments. ● Fed controls, taken direct from feeding tubes kept at 83 % R.H. and 37° C. ▲ Starved for 4 days in 83 % R.H. at 37° C. ◆ Starved and desiccated for 3 days in 1 % R.H. at 37° C. ■ Starved and desiccated for 3 days in 1 % R.H. at 37° C. followed by 1 day of starvation and rehydration in 83 % R.H. at 37° C. Mean weights are plotted; the vertical lines show the standard deviations of the individual measurements.

(ii) *The effects of dehydration.* Insects were isolated from normal culture conditions, weighed and then desiccated over dry, granular calcium chloride at 37° C. for 3 days without food. They were then weighed again and killed with carbon dioxide and hydrogen sulphide. They were then dried to constant weight at 57° C. The results are shown in Table 3 and Fig. 7. At the time of death, these insects contained $60{\cdot}1 \pm 3{\cdot}1$ % water; their total weights were $73{\cdot}7 \pm 7{\cdot}4$ % of their initial weights; and their

dry weights were 29·6 ± 3·0% of their initial weights. During desiccation, their total weight loss was therefore 26·3 ± 7·4% of their initial weights. Their dry weight as compared with that of the normal control insects represents a loss of approximately 14% of their original dry matter over the 3-day period of desiccation and starvation, or of approximately 1·6% of their initial weight per day.

(iii) *The effects of dehydration followed by rehydration.* Insects were isolated from normal culture conditions, weighed and then desiccated over dry, granular calcium chloride at 37° C. for 3 days without food, and reweighed daily. After the 3 days of

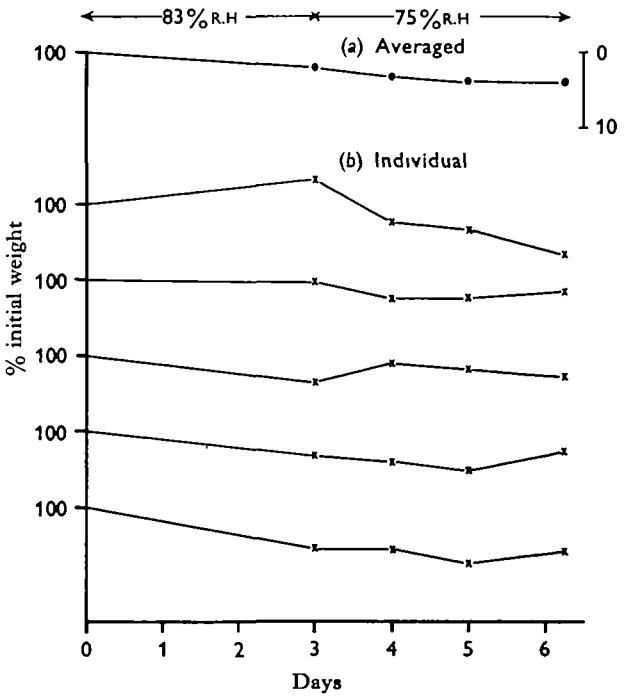

Fig. 8. Effect on total body weight of transferring *Thermobia* from 83% R.H. to 75% R.H. at 37° C. Circles show mean weights (*a*), crosses individual weights (*b*).

desiccation the insects were transferred to 83% R.H. at 37° C. for rehydration for 1 day, after which they were weighed again, then killed with carbon dioxide and hydrogen sulphide. They were then dried to constant weight at 57° C. The results are shown in Table 3 and Fig. 7. At death, these insects contained 71·8 ± 1·9% water; their total weights were 93·0 ± 1·7% of their original weights; and their dry weights were 26·2 ± 1·7% of their initial weights. Compared with normal insects this represents a loss of approximately 24% of their original dry matter over the 4-day period of the experiment, or of approximately 2·1% of their initial weight per day.

In these insects the loss of body weight caused by starvation is partially offset by a higher hydration. The proportion of water in the body at the end of the experiment was higher than in normal untreated insects (*t* test; $P < 0{\cdot}01 > 0{\cdot}002$). This confirms that during starvation the body weight (and therefore volume) is maintained by retention of a higher proportion of water.

The results of these experiments also confirm that starvation accounts for the normal overall weight loss found in all insects used for experiments over a period of a few (1–8) days.

6. *Equilibrium body-water content at different humidities*

The experiments described above indicate that the body-water content returns to the same level irrespective of the recovery humidity, provided that this is above the critical equilibrium humidity, and allowing for the effects of starvation. This was further tested by transferring insects at 37° C. from 83% to 75% R.H. to see if any change in weight associated with the setting up of a new equilibrium level would

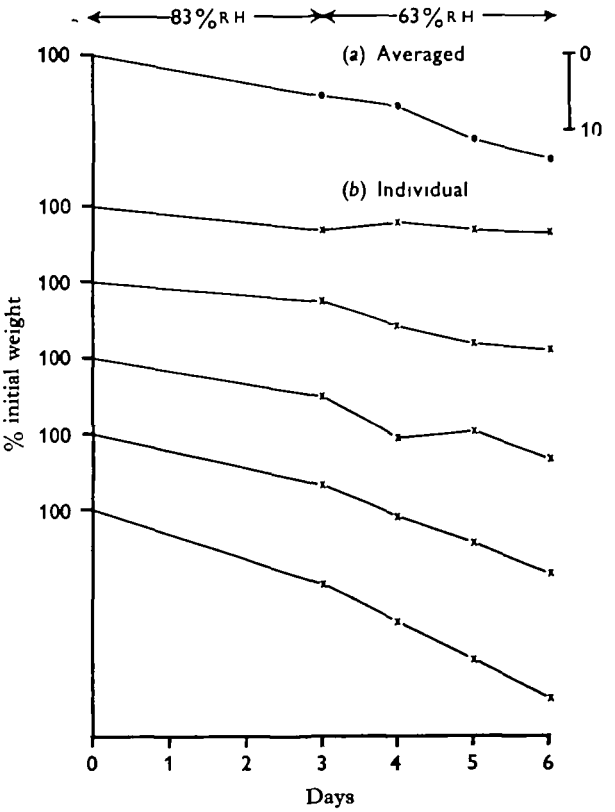

Fig. 9. Effect on total body weight of transferring *Thermobia* from 83% R.H. to 63% R.H. at 37° C. Circles show mean weights (*a*), crosses individual weights (*b*).

occur. Five insects were tested and the average values of weight against time are plotted in Fig. 8*a*. This shows that the time course of weight change is the same under the two regimes (*t* test; $P > 0{\cdot}1$, for the rates of weight loss observed under the two regimes), and no abrupt discontinuity is seen at the time of transference. This is shown clearly in Fig. 8*b*, where the weights of the individual insects are plotted against time. The overall steady losses in weight can be attributed to the effects of starvation. The equilibrium body-water content does not appear to change with the ambient humidity experienced by the insect (cf. Knülle, 1967*a*).

This conclusion is further substantiated by the results of a similar experiment in which insects at 37° C. were transferred from 83 to 63% R.H. (see Fig. 9). Again, the time course of weight change continues uninterruptedly for every insect when transferred from one regime to the other (*t* test; $P > 0{\cdot}1$, for the rates of weight loss observed under the two regimes).

7. *Restriction of water loss in lower humidities*

Dead insects lose water in all humidities and temperatures tested (1, 20, 30, 43, 63, 75 or 83% R.H. at 21, 23, 28, 33·5 or 37° C.; see Figs. 3–6). We have seen that living insects can gain water in the higher humidities. Retention or resorption of water in the living insect in the higher humidities is clearly dependent upon the metabolism of the insect.

Below the critical equilibrium humidity water is lost from the living insect to the atmosphere and the insect's weight decreases. Living insects kept at 37° C. and in 1% R.H. lose weight at the rate of $0{\cdot}10 \pm 0{\cdot}02$ mg./hr./standard insect ($n = 83$). However, dead insects lose water at an increased rate. At 34° C. and 0% R.H. the rate of water loss from dead insects is 0·28–1·36 mg./hr./standard insect (from Beament *et al.* 1964). The living insect is therefore restricting its water loss. It may be expected that the same mechanism as that which allows net uptake in the higher humidities would provide this restriction in the lower humidities (Lees, 1947; Edney, 1957; Beament, 1961; Winston & Nelson, 1965; Knülle, 1967*a*). The following experiments were carried out to investigate this possibility in *Thermobia*.

Table 4. *Rates of water loss from living insects held in low humidities at* 37° C

Relative humidity (%)	*n*	Rate of weight loss over 24 hr. period (expressed as mg./mm. Hg/hr./standard insect ± S.D.) Assuming water potential at exchanging surfaces ≡ 50% R.H.	Assuming water potential at exchanging surfaces ≡ 100% R.H.
1	5	$0{\cdot}0046 \pm 0{\cdot}0006$	$0{\cdot}0023 \pm 0{\cdot}0003$
20	5	$0{\cdot}0059 \pm 0{\cdot}0005$	$0{\cdot}0022 \pm 0{\cdot}0002$
30	5	$0{\cdot}0081 \pm 0{\cdot}0014$	$0{\cdot}0023 \pm 0{\cdot}0004$
43	5	$0{\cdot}0228 \pm 0{\cdot}0041$	$0{\cdot}0028 \pm 0{\cdot}0005$

A. *Rates of loss from living insects in low humidities*

If the uptake mechanism is involved in the restriction of water loss in low humidities, it should maintain the water potential at the exchanging surfaces of the insect at a value equivalent to the critical equilibrium humidity. If this critical equilibrium humidity is assumed to be 50% R.H. at 37° C. then losses to the atmosphere should be proportional to the water potential gradient between the exchanging surface at 50% R.H. and the atmosphere.

Groups of insects were subjected to identical regimes before transference to the test conditions. They were fed in individual vials, then starved for 1 day in clean vials, at 37° C. and 83% R.H. They were then transferred, in clean vials, to the test humidity, still at 37° C. Weights were monitored during the pre-treatment as well as during the actual test.

The rates of weight loss after making an allowance for the continued operation of the uptake mechanism are given in Table 4 and Fig. 10. These values are far from constant, indicating that the continued operation of the uptake mechanism does not

explain the low rates of water loss. If, however, the rates are calculated without making any such allowance for the continued operation of the uptake mechanism, a much more constant value is obtained (Table 4; Fig. 10), indicating that water loss is proportional to the full saturation deficit of the atmosphere, i.e. as though the water potential at the exchanging surfaces is at a value equivalent to that of haemolymph. The low rates of water loss appear to be due to a permeability barrier.

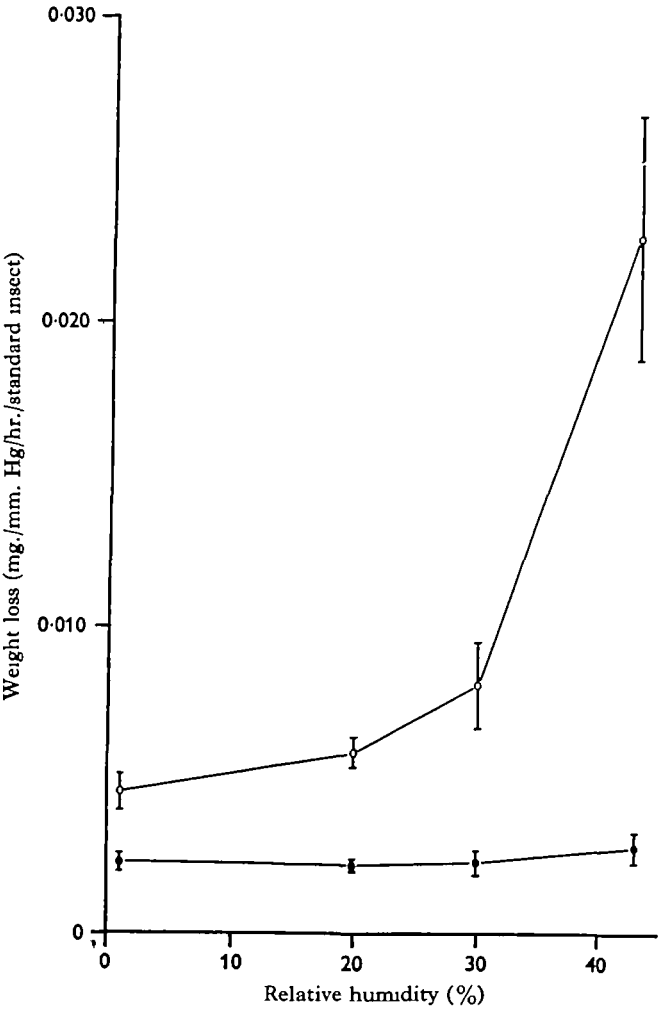

Fig. 10. Rate of water loss from *Thermobia* in low humidities at 37° C. (i) assuming that a water potential equivalent to 50 % R.H. is maintained at the exchanging surfaces of the insect (open circles). (ii) assuming a water potential equivalent to that of haemolymph at the exchanging surfaces of the insect (solid circles). Mean rates are plotted; the vertical lines show the standard deviations of the individual values.

B. *Rates of loss from dead insects*

After death, rates of water loss are consistently higher than the values obtained from living insects (see Table 5). For the rates obtained in 43 % R.H. the difference could be accounted for if the active uptake mechanism is lost on death, but is maintained in

operation in the living insect, as shown in Table 5. Following the results reported in the previous paragraphs, it is evident that this is a consequence of the proximity of 43% R.H. to the critical equilibrium humidity, causing the rates expressed in this manner to appear high. Rates measured in the lower humidities do not follow this pattern. For instance, the rate obtained for living insects desiccated at 37° C. and 1% R.H., even when expressed in this manner (see Table 4), is still less than the rates obtained at lower temperatures and 43% R.H. for previously desiccated dead insects (see Table 5), or for dead insects at the same temperature and humidity (see also below). It is important to note that this possibility does not provide an adequate explanation in all cases for the greater losses seen in dead insects.

Table 5. *Rates of water loss from insects in various physiological states at different temperatures*

State of insect	R.H. at which rate determined (%)	Temperature at which rate determined: *n*	23° C	*n*	28° C	*n*	33·5° C
(*a*) Rates expressed as mg./hr./standard insect ± standard deviation							
Dead. Taken direct from culture							
Initial rate	43	5	0·37 ± 0·13	5	0·96 ± 0·26	5	1·04 ± 0·51
Rate after initial 20% wt. loss	43	5	0·19 ± 0·04	5	0·44 ± 0·05	5	0·42 ± 0·11
Dead. Desiccated before killed	43	2	0·16 ± 0·03	4	0·35 ± 0·14	4	0·15 ± 0·05
Living. Dessicated before tested	43	5	0·01 ± 0·01	5	0·04 ± 0·02	5	0·06 ± 0·02
(*b*) Rates expressed as mg./mm. Hg/hr./standard insect ± standard deviation							
Dead. Taken direct from culture							
Initial rate	43	5	0·031 ± 0·011	5	0·060 ± 0·016	5	0·047 ± 0·023
Rate after initial 20% wt. loss	43	5	0·016 ± 0·003	5	0·028 ± 0 003	5	0·019 ± 0 005
Dead. Desiccated before killed	43	2	0·013 ± 0·002	4	0·022 ± 0·009	4	0 007 ± 0·003
Living. Desiccated before tested	43	5	0·001 ± 0 001	5	0·002 ± 0·001	5	0·003 ± 0 001
Assuming water potential at exchanging surfaces ≡ 50% R.H.	43	5	0·010 ± 0·007	5	0·019 ± 0·010	5	0·021 ± 0 009

C. *Effects of cuticular abrasion and repair*

From the experiments of Beament *et al.* (1964), it is apparent that isolation of living insects in individual vials allows repair of cuticular abrasion to occur. The condition of the cuticle is likely to be poorer in insects living under normal culture conditions than in insects kept individually in vials. Transpiration in insects freshly drawn from culture conditions is likely to be of major importance, therefore. This is confirmed by the results of experiments using insects taken direct from normal culture conditions (see Table 5).

Freshly killed insects held in an atmospheric humidity of 43% R.H. initially lost 0·031 ± 0·011 mg./mm. Hg/hr./standard insect at 23° C.; 0·060 ± 0·016 mg./mm. Hg/hr./standard insect at 28° C.; and 0·047 ± 0·023 mg./mm. Hg/hr./standard insect at 33·5 °C. These rates are in excess of the maximum rates given by Beament *et al.* (1964) for insects previously kept individually in vials and tested at room temperature or 34° C. and 0% R.H., in moving air. When some 20% of the total body weight had been lost, the rates of loss fell to 0·016 ± 0·003 mg./mm. Hg/hr./standard insect at 23° C.; 0·028 ± 0·003 mg./mm. Hg/hr./standard insect at 28° C. and 0·019 ± 0·005 mg./mm. Hg/hr./standard insect at 33·5° C., confirming that the transpiration rate

falls as the total body-water content falls (cf. Beament, 1961). These rates, however, are still high.

Insects which had been desiccated for 3 days at 37° C. and 1% R.H. in the living state, during which time they had been kept in individual vials and lost 20–25% of their total weights, then subsequently killed and desiccated at 43% R.H., showed weight losses of 0·013 ± 0·002 mg./mm. Hg/hr./standard insect at 23° C.; 0·022 ± 0·009 mg./mm. Hg/hr./standard insect at 28° C.; and 0·007 ± 0·003 mg./mm. Hg/hr./standard insect at 33·5° C. These values are lower than the losses recorded for dead insects taken direct from culture conditions, after they had lost their initial 20–25% of their body weight. This indicates that some repair process probably occurred during the 3 days of isolation during desiccation in the living state. The values so obtained are nearer to the values obtained in control insects by Beament *et al.* (1964). They are still in excess of the values obtained from living insects desiccated at 37° C. and 1% R.H., which may also undergo repair during their 3 days of isolation.

Table 6. *Rates of water loss from insects at different times after commencement of desiccation in* 1% R. H. *at* 37° *C, expressed as mg./standard insect* ± *standard deviation*

State of insect	*n*	0–12 hr.	12–24 hr.	24–36 hr.	36–48 hr.	48–72 hr.
(*a*) Living						
Total loss in period	5	1·7 ±0·2	0·9 ±0·3	1·0 ±0·1	0·9 ±0·2	2·0 ±0·6
Loss/hr.	5	0·14 ±0·02	0·08 ±0·03	0·08 ±0·01	0·08 ±0·02	0·08 ±0·03
Loss/mm. Hg/hr.	5	0·0030±0·0003	0·0016±0·0005	0·0018±0·0002	0·0016±0·0004	0·0018±0·0005
(*b*) Dead						
Total loss in period	5	2·0 ±0·5	1·8 ±0·6	2·6 ±0·9	3·2 ±0·8	4·5 ±1·6
Loss/hr.	5	0·17 ±0·04	0·15 ±0·05	0·22 ±0·08	0·27 ±0·06	0·19 ±0·07
Loss/mm. Hg/hr.	5	0·0035±0·0009	0·0032±0·0011	0·0046±0·0017	0·0057±0·0014	0·0040±0·0014

It is possible, however, to obtain even from normal culture conditions insects which after being killed transpire less rapidly than as recorded for the above dead insects. A group of five insects desiccated in 1% R.H. at 37° C. lost water at the overall rate of 0·006 ± 0·002 mg./mm Hg/hr./standard insect during the first day. This rate, though fairly low, is still in excess of the rates of loss from living insects.

Dead insects, which had been kept in normal culture conditions, but in individual vials without food for 40 hr. before being killed, when desiccated in 1% R.H. at 37° C. lost water relatively slowly at first, then more rapidly, and finally more slowly again as their water contents were depleted. The rates observed at various times after death are given in Table 6. They are all in excess of the rates of loss recorded for living insects of similar immediate past history under identical test conditions and also given in the table, especially after the initial period, when the rates for the living insects have settled to a remarkably constant and lower value. However, they are somewhat less than those recorded for the insects drawn direct from culture. The implications of the varying rate with time are discussed below.

Though cuticular repair is certainly a factor to be considered in the restriction of water loss, it does not provide an explanation for the restriction seen between living and dead insects of similar immediate past history.

D. *Control of spiracular opening*

An additional restrictive mechanism based on the control of spiracular opening would not normally be expected in apterygotes (cf. Miller, 1966, for instance). A closing device appears to be present in *Thermobia*, however (Noble-Nesbitt, 1968*b*), but preliminary experiments indicate that this may not be important in the restriction implied by these results (see also next section, and discussion).

The results given in this section show that the living insect, in addition to the effects of cuticular repair and any spiracular closing mechanism, depends largely on a permeability barrier for the low rates of loss of water experienced by it in the lower humidities. The mechanism used in the uptake of water in the higher humidities does not appear to be involved. Further experiments, described in the following sections, were conducted to investigate the nature of this permeability barrier.

8. *Comparison of rates of uptake and loss*

A. *Living insects in high and low humidities*

A comparison of the rates of uptake in high humidities and loss in low humidities at the same temperature in the living insect shows that for similar vapour pressure differences between insect and atmosphere the rate of uptake is greater than the rate of loss, indicating the asymmetrical nature of cuticular permeability (cf. Beament, 1961, 1964, 1965). Similar observations have been made in *Tenebrio* (Locke, 1964) and in the larva of *Xenopsylla* (Knülle, 1967*a*).

At any one temperature the vapour pressure difference tending to remove water from the insect into dry air is greater than that which would be operating to transfer water into the insect from air at 83% R.H., if a water potential equivalent to 50% R.H. is assumed to be maintained at the exchanging surfaces of the insect. The rate at which water is absorbed into the insect, however, is much more rapid than the rate of loss at the lower humidity, as can be seen in Figs. 6 (37° C.) 11 and 12 (22–23° C.). The rates at 37° C. expressed per unit of vapour pressure difference (assuming the exchanging surfaces of the insect are maintained at a water potential equivalent to air at 50% saturation) are 0·0181 ± 0·0051 mg./mm. Hg/hr./standard insect for the uptake and 0·0041 ± 0·0003 mg./mm. Hg/hr./standard insect for the loss; the difference between these values is highly significant (*t* test; $P < 0{\cdot}01 > 0{\cdot}002$). The difference is even greater if during loss the exchanging surfaces are kept at a water potential equivalent to that of haemolymph and not to 50% R.H., the figure for loss being halved.

These results further indicate that the restriction of water loss in the lower humidities is not simply because of a lowering of the water potential at the exchanging surfaces of the insect to a value in equilibrium with the humidity in which neither gain nor loss occurs in the living insect.

B. *Living and dead insects*

The rates of gain of water in the higher humidities in the living insects are more nearly equal to the rates of loss seen in dead insects, for the same vapour pressure difference (assuming the living insect maintains its exchanging surfaces at a water

potential equivalent to 50% R.H. whilst absorbing water, and the dead insect has a surface water-potential equivalent to fully saturated air). At 37° C., the vapour pressure difference operating to transfer water into the living insect in 75% R.H. is then equivalent to that tending to remove water from the dead insect in 75% R.H. In Fig. 6 the gain and loss in weight is plotted for living insects and for an insect which died naturally, all in 75% R.H. at 37° C. The rates are seen to be similar. Expressed in figures, the rates are 0·017 ± 0·009 mg./mm. Hg/hr./standard insect for the living insects and 0·015 mg./mm. Hg/hr./standard insect for the dead insect. These rates agree closely with the values recorded for losses in dead insects (see Table 5), and for gains in living insects, at other temperatures and humidities.

These results suggest that in the living insect a barrier to water loss, but not to uptake, is maintained, and that it is lost at death. In the previous section, we saw that the rates of loss in the dead insects can in some cases increase from initial lower values. It is probable that in these cases death has not immediately fully disrupted the permeability barrier, but that this occurs progressively.

9. *The effects of treatment with carbon dioxide*

From the results of preliminary experiments conducted to test the effects of carbon dioxide, it appears that the barrier to water loss is retained when the living insect is exposed to carbon dioxide anaesthesia. This treatment prevents uptake (see Fig. 11*b*). The active mechanism is presumably blocked. Rapid losses of water do not occur, however. The maximum rate of water loss recorded on the recording electrobalance for an insect held under carbon dioxide anaesthesia in 84% R.H. at 22° C. (room temperature) in a special chamber attached to the electrobalance was 0·01 mg./hr., which is equivalent to 0·004 mg./mm. Hg saturation deficit/hr./standard insect. This latter rate is the same as the rate of loss recorded for the same insect in the unanaesthetized state in 43% R.H. at the same temperature of 22° C. before transference to 84% R.H. (see Fig. 11*a*). The insect showed rapid uptake of water from the atmosphere both before and after the period of carbon dioxide anaesthesia (see Fig. 11*b*). A barrier to water loss is evidently maintained even when the uptake mechanism is blocked by carbon dioxide anaesthesia.

This result was confirmed in insects in dry atmospheres. These experiments were also conducted with the weight of the insect continuously recorded on the electrobalance whilst the insect hung in its open container in the special chamber attached to the electrobalance. Silica gel or calcium chloride was used to keep the air dry during the course of the experiment. Ports in the chamber walls allowed the introduction of dry air, carbon dioxide–air mixtures, or pure carbon dioxide. Carbon dioxide anaesthesia and lower concentrations of carbon dioxide (10%, 20%) in air did not appreciably alter the rates of loss of water from individual insects. In one insect tested at 23° C. the rate of water loss was identical immediately before, during, and immediately after carbon dioxide anaesthesia (see Fig. 12). The rate was 0·002 mg./mm. Hg/hr./standard insect. In another insect tested at 25° C. there was no change in the rate of loss when the insect was treated successively with air, 10% carbon dioxide in air, and 20% carbon dioxide in air. Dry air was used to flush out the chamber between the 10 and 20% exposures. The rate was again 0·002 mg./mm. Hg/hr. oblique standard insect.

These experiments support the view that the uptake mechanism is not involved in

the restriction of water loss in low humidities. They also confirm that the barrier to water loss in the living insect cannot merely be a carbon dioxide-sensitive spiracular closing device in operation.

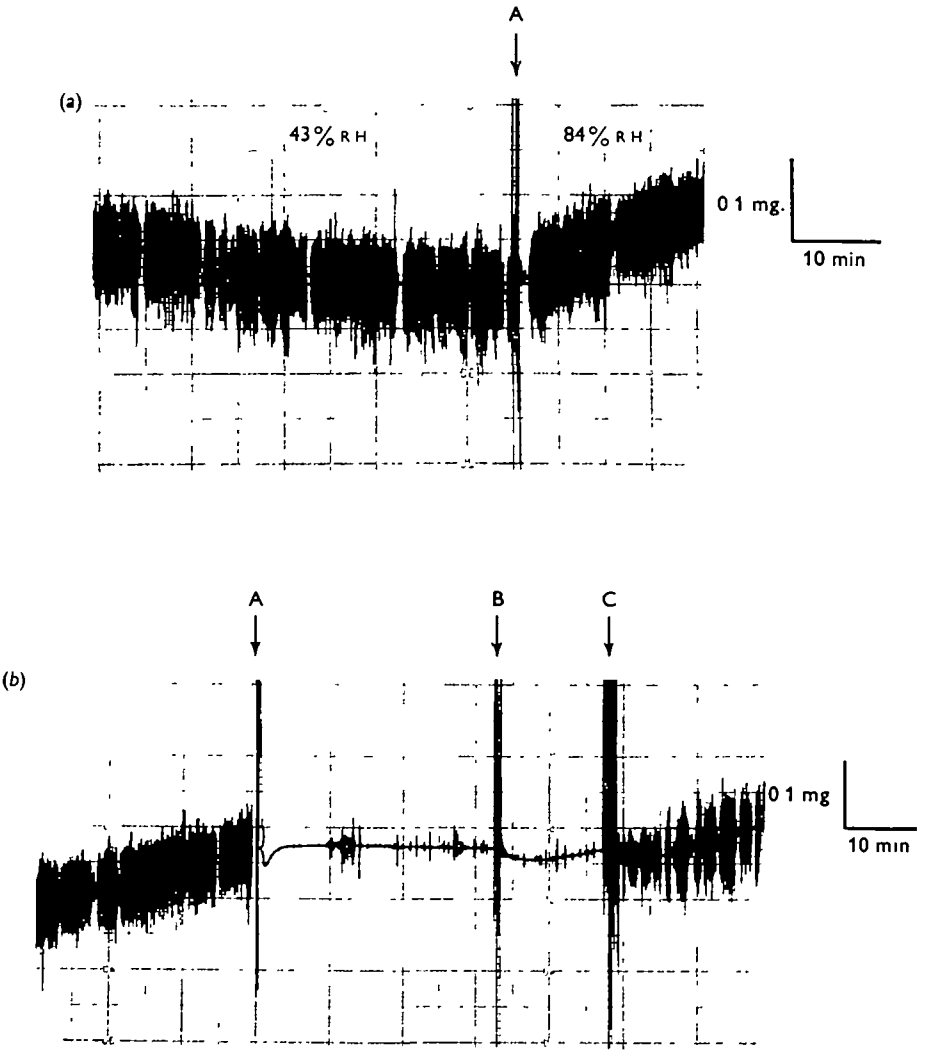

Fig. 11. The effect of atmospheric humidity and carbon dioxide on water exchanges in *Thermobia* at 22° C. Extracts from a continuous recording of weight change. (*a*) overall loss in 43 % R.H. followed by uptake on transference to 84 % R.H. at A. (*b*) Uptake in 84 % R.H. in the same insect interrupted by a short spell of carbon dioxide anaesthesia, during which slight loss occurred. Carbon dioxide was introduced at A, and removed at B–C. Rapid vertical excursions of the trace indicate locomotor activity.

DISCUSSION

Net uptake of water from the atmosphere by previously desiccated *Thermobia domestica* occurs only until the 'normal' body water content is re-attained. At this point a balance is struck between losses and gains. The mechanism for uptake is a truly regulatory one. In this respect *Thermobia* is similar to those cases reported for

ticks and mites and for the few insects exhibiting this facility (see p. 745). This similarity is also seen in the independence of uptake to the absolute value of the saturation deficit over a wide temperature range (at least 17–37° C. in *Thermobia*), and its dependence upon the relative humidity of the atmosphere (cf. Beament, 1965).

The proportion of water in the body significantly increases during large dry-weight losses, suggesting that some volume regulation occurs. This feature is also seen in *Arenivaga* nymphs (Edney, 1966), and Mellanby (1932) reported that *Tenebrio* larvae become 'dropsical' during prolonged starvation in high humidities.

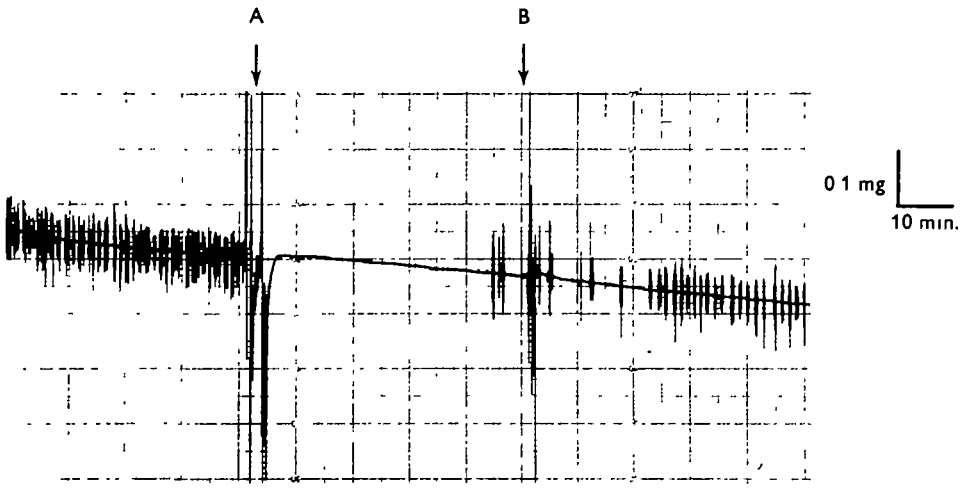

Fig. 12. The effect of carbon dioxide anaesthesia on water loss from *Thermobia* in dry air at 23° C. Continuous record of weight change. Rapid vertical excursions of the trace indicate locomotor activity, which was abolished during the spell of carbon dioxide anaesthesia (A–B), the limits of which are shown by the gross vertical excursions of the trace made when the atmosphere in the experimental chamber was being changed.

The present results with *Thermobia* indicate that the same level of body-water content is reached and maintained in all humidities above the critical equilibrium humidity. More precisely, it can be stated that there are no significant differences between the levels maintained in 63, 75 and 83% R.H. at 37° C. This is different from what is found in the case of flea larvae (Knülle, 1967*a*) and grain mites (Knülle, 1962, 1965), which exhibit a lower water content in the lower humidities at equilibrium. Dependence of the amount of water in the organism on the ambient humidity does not, therefore, seem to be a consequence of a general property of the arthropod vapour pump (cf. Knülle, 1967*a*).

The underlying mechanism providing the driving force for uptake of water from the atmosphere remains largely unsolved, though it presumably derives ultimately from the epidermis (cf. Winston, 1967). Beament (1964, 1965) and Locke (1964, 1965) have suggested mechanisms involving epicuticular pores and lipids for this uptake, and accounting for the more rapid inward movement of water, for the same apparent driving force, than found in the outward direction. Beament's model incorporates a lipid barrier over the surface of water in micro-capillaries which open through the external surface of the cuticle; this barrier is complete during transpiration but is

disrupted during uptake, a meniscus being formed (see Fig. 13*a*, *b*). During uptake an inner 'suction force' withdraws water from the capillaries, pulling the meniscus inwards. The resulting capillarity effect produces a lowered vapour pressure (p_1) at the meniscus surface, according to the relationships which for macroscopic capillaries are

$$\text{R.T. } \ln p/p_1 = \frac{2\gamma V}{r} \tag{1}$$

and

$$P = \frac{2\gamma \cos\theta}{r}, \tag{2}$$

where R is the gas constant, T absolute temperature, p vapour pressure at a plane surface and p_1 at a spherical one, γ the surface tension, V the molar volume of the liquid, r the radius of the capillary (considered as the radius of curvature of the meniscus surface for a fully wetted capillary in the equation (1) and positive for a concave surface), P the capillary pressure and θ the angle of contact.

Water then moves from the atmosphere to the meniscus and into the fluid in the capillary, provided that $p_1 < p_2$ (where p_2 = the vapour pressure of the bulk of the atmosphere). During this phase of the cycle the impedance of the system is low because the lipid layer barrier is interrupted when the fluid is drawn down the capillary. When the suction force falls, the fluid rises in the capillary and the lipid barrier reforms. This gives a high-impedance system, with corresponding restriction in the movement of water across the outer epicuticle. Water can then be drained into the bulk of the insect from the cuticle. The cycle can then be repeated.

This model can be applied to explain the results of the experiments conducted with *Thermobia*, which has micro-capillaries of the correct order of magnitude in its outer epicuticle (Noble-Nesbitt, 1967). During uptake the mechanism could work as outlined above (see Fig. 13*a*); water is absorbed rapidly through a low-impedance system, with the active uptake mechanism giving rise in some way to the lowered water potential necessary to produce the suction force. On the other hand, transpiration from the living insect in an atmosphere of low humidity would occur slowly through a high-impedance system, the suction force no longer being produced, since the active uptake mechanism is no longer in operation (see Fig. 13*b*). The lipid layer barrier is probably maintained in the living insect by continuous production of mobile grease (Beament *et al.* 1964; Noble-Nesbitt, 1967). On death, the most vulnerable part of the lipid layer would be that over the micro-capillaries. At this point there is no firm underlying anchorage for the lipid molecules, and the layer may quickly be disrupted, because of natural erosion and non-replenishment from below, aggravated by the steepening of the meniscus as water is lost more rapidly to the atmosphere. As the barrier is eroded, the high-impedance system will turn progressively into a low-impedance 'open-pore' system comparable with that formed during uptake, but working in the opposite sense (see Fig. 13*c*). The rate of water movement should then be comparable with that found during uptake, for the same driving force. This is precisely what is found in *Thermobia*.

In order for the mechanism to work and allow uptake at 50% R.H., the diameter of the micro-capillaries, as calculated from equations (1) and (2) above, must be 30 Å, for pure water with a contact angle of zero, assuming conditions appertaining to

macrocapillaries hold also for micro-capillaries. In fact, at these small dimensions, the effective diameter of the capillary may be smaller than its actual diameter by a significant amount (cf. Beament, 1965), because of a layer of water of finite thickness adsorbed on to the walls of the capillary, which cannot be ignored as insignificant as in larger-diameter capillaries (Briggs, 1967), and which will more than compensate for any lowering of the surface tension by lipids on the meniscus surface (Beament,

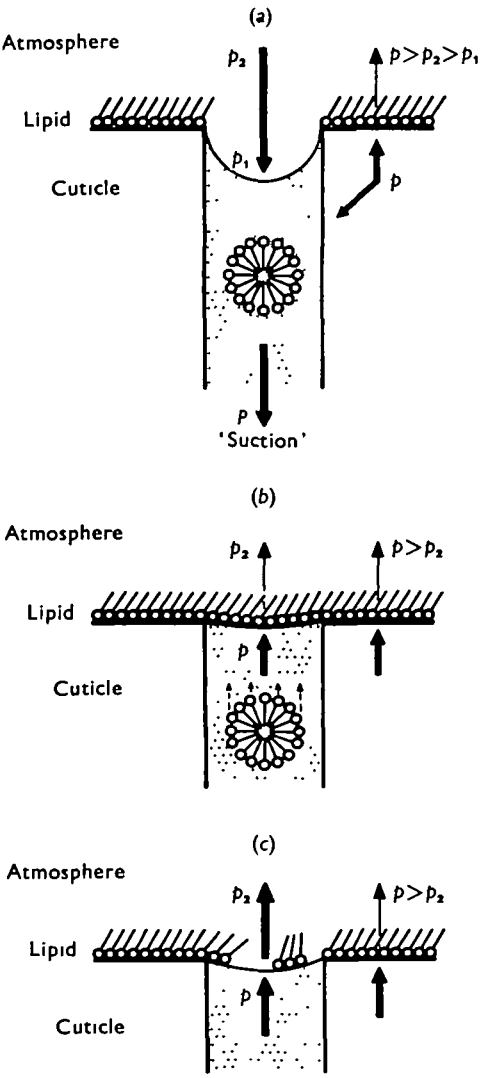

Fig. 13. Diagrammatic representation of pore-model of outer epicuticle accounting for differential permeabilities to water under different conditions (modified after Beament, 1965). For fuller explanation see text. (*a*) The open pore and meniscus formed during uptake in the living insect. (*b*) The complete lipid layer re-formed during loss in the living insect, with continual replenishment from below—a lipid micelle is indicated moving up towards the surface. (*c*) the disruption of the lipid layer over the pore and the steepening of the meniscus during loss in the dead insect. The solid arrows indicate movement of water, the thickness of the shaft giving some indication of the relative rates of flow. The broken arrows indicate movement of the lipid micelles.

1965). Pores approximately 80 Å in diameter have been seen in *Thermobia* epicuticle (Noble-Nesbitt, 1967), whilst in the epicuticle of *Calpodes* at the moult, pores approximately 30 Å in diameter occur (Locke, 1966). There is little doubt that pores in the correct size range do exist in the outer epicuticle.

A consequence of these findings is that restriction of transpiratory water loss does not depend upon the continued operation of the uptake mechanism, but on an efficient permeability barrier. In this respect the firebrat apparently differs from the tick, in which carbon dioxide anaesthesia increases transpiration in low humidities whilst abolishing uptake in high humidities (Browning, 1954). However, it would appear that uptake of water from the atmosphere on the one hand and restriction of cuticular water loss on the other hand are not simply two manifestations of the same general ability of the arthropod in the sense meant by other workers (e.g. Edney, 1957, 1966; Knülle, 1967*a*), at least in *Thermobia domestica*.

On the basis of the present results, it is suggested that control of water loss through the spiracles may not be important in the restriction of water loss in lower humidities, which can be accounted for by the operation of the permeability barrier to the outward passage of water. This suggestion is strengthened by the results reported for the effects of carbon dioxide anaesthesia. The failure also of lower concentrations of carbon dioxide, which may be expected to bring about spiracular opening, to increase transpiration markedly in low humidities argues along similar lines. 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. In these respects *Thermobia* apparently differs from dragonflies, locusts and other insects in which water loss from the tracheal system and its control are of major importance (e.g. Miller, 1964; Loveridge 1968; Wigglesworth, 1965). The exact functioning of the spiracles in *Thermobia* must await further investigation, but it would be surprising if the closing of the spiracles did not pay some part in restricting water loss. It remains a possibility that the spiracle is a low-loss device even under treatment with carbon dioxide up to anaesthetic amounts, and in death.

SUMMARY

1. Uptake of water from the atmosphere by previously desiccated, starved *Thermobia domestica* is related to the % relative humidity, and occurs in and above 63% R.H. between 17 and 37° C. Losses occur in and below 43% R.H.

2. Uptake is often, though not invariably, arrested during even brief periods of locomotor activity.

3. Recovery from desiccation proceeds to the same level of body-water content in all humidities above the 'critical' value. Equilibrium is reached more rapidly in the higher temperatures and humidities.

4. The equilibrium level attained is the same as that found in control insects, and it does not change when the insect is transferred from one high humidity to another.

5. During periods of relatively large dry-weight losses, a greater proportion of water is retained in the body, providing some volume regulation.

6. The living insect restricts its water loss in low humidities by means of a permeability barrier which is disrupted during uptake in high humidities and is progressively lost on death. This restriction is not dependent upon the continued activity of the uptake mechanism.

REFERENCES

BAILEY, N. T. J. (1959). *Statistical Methods in Biology*. London: English Universities Press.

BEAMENT, J. W. L. (1961). The water relations of insect cuticle. *Biol. Rev.* **36**, 281–320.

BEAMENT, J. W. L. (1964). The active transport and passive movement of water in insects. *Adv. Insect Physiol.* **2**, 67–129.

BEAMENT, J. W. L. (1965). The active transport of water: evidence, models and mechanisms. *Symp. Soc. exp. Biol.* **19**, 273–98.

BEAMENT, J. W. L., NOBLE-NESBITT, J. & WATSON, J. A. L. (1964). The waterproofing mechanism of arthropods. III. Cuticular permeability in the firebrat, *Thermobia domestica* (Packard). *J. exp. Biol.* **41**, 323–30.

BELOZEROV, V. N. & SERAVIN, L. N. (1960). Water balance regulation in *Alectrobius tholozani* at different atmospheric humidity. *Medskaya Parazit.* **3**, 308–13.

BRIGGS, G. E. (1967). *Movement of Water in Plants*. Oxford: Blackwell.

BROWNING, T. O. (1954). Water balance in the tick *Ornithodorus moubata* Murray, with particular reference to the influence of carbon dioxide on the uptake and loss of water. *J. exp. Biol.* **31**, 331–40.

BUXTON, P. A. (1930). Evaporation from the mealworm (*Tenebrio*, Coleoptera) and atmospheric humidity. *Proc. R. Soc.* B **106**, 560–77.

CAMIN, J. H. (1963). Relations between host-finding behaviour and life histories in ecto-parasitic Acarina. *Adv. Acarology* **1**, 411–24.

EDNEY, E. B. (1947). Laboratory studies on the bionomics of the rat fleas, *Xenopsylla brasiliensis* Baker and *X. cheopis* Roths. II. Water relations during the cocoon period. *Bull ent. Res.* **38**, 263–80.

EDNEY, E. B. (1957). *The Water Relations of Terrestrial Arthropods*. Cambridge University Press.

EDNEY, E. B. (1966). Absorption of water vapour from unsaturated air by *Arenivaga* sp. (Polyphagidae, Dictyoptera). *Comp. Biochem. Physiol.* **19**, 387–408.

KANUNGO, K. (1963). Effect of low oxygen tensions on the uptake of water by dehydrated females of the spiny rat-mite *Echinolaelaps echidninus*. *Expl. Parasit.* **14**, 263–8.

KANUNGO, K. (1965). Oxygen uptake in relation to water balance of a mite (*Echinolaelaps echidninus*) in unsaturated air. *J. insect Physiol.* **11**, 557–68.

KNÜLLE, W. (1962). Die Abhängigkeit der Luftfeuchte-Reaktionen der Mehlmilbe (*Acarus siro* L.) vom Wassergehalt des Körpers. *Z. vergl. Physiol.* **45**, 233–46.

KNÜLLE, W. (1965). Die Sorption und Transpiration des Wasserdampfes bei der Mehlmilbe (*Acarus siro* L.). *Z. vergl. Physiol.* **49**, 586–604.

KNÜLLE, W. (1966). Equilibrium humidities and survival of some tick larvae. *J. Med. Ent.* **2**, 335–8.

KNÜLLE, W. (1967*a*). Physiological properties and biological implications of the water vapour sorption mechanism in larvae of the oriental rat flea, *Xenopsylla cheopis* (Roths). *J. Insect Physiol.* **13**, 333–57.

KNÜLLE, W. (1967*b*). Significance of fluctuating humidities and frequency of blood meals on the survival of the spiny rat mite, *Echinolaelaps echipninus* (Berlese). *J. Med. Entomol.* **4**, 322–5.

KNÜLLE, W. & WHARTON, G. W. (1964). Equilibrium humitities in arthropods and their ecological significance. *Proc. 1st int. Congr. Acarology, Acarologia* **6**, 299–306.

LEES, A. D. (1946). The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology* **37**, 1–20.

LEES, A. D. (1947). Transpiration and the structure of the epicuticle in ticks. *J. exp. Biol.* **23**, 379–410.

LEES, A. D. (1948). Passive and active water exchange through the cuticle of ticks. *Discuss. Faraday Soc.* **3**, 187–92.

LEES, A. D. (1964). The effect of ageing and locomotor activity on the water transport mechanism of ticks. *Proc. 1st int. Congr. Acarology, Acarologia* **6**, 315–23.

LOCKE, M. (1964). The structure and formation of the integument in insects. In *The Physiology of Insecta*. Ed. by Rockstein, M., vol. III, pp. 379–470. New York: Academic Press.

LOCKE, M. (1965). Permeability of insect cuticle to water and lipids. *Science, N.Y.* **147**, 295–8.

LOCKE, M. (1966). The structure and formation of the cuticulin layer in the epicuticle of an insect, *Calpodes ethlius* (Lepidoptera, Hesperiidae). *J. Morph.* **118**, 461–94.

LOVERIDGE, J. P. (1968). The control of water loss in *Locusta migratoria migratorioides* R. and F. II. Water loss through the spiracles. *J. exp. Biol.* **49**, 15–29.

LUDWIG, D. (1937). The effect of different relative humidities on respiratory metabolism and survival of the grasshopper *Chortophaga viridifasciata* De Geer. *Physiol. Zöol* **10**, 342–51.

MELLANBY, K. (1932). The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L., Coleoptera). *Proc. R. Soc.* B **111**, 376–90.

MILLER, P. L. (1964) Factors altering spiracle control in adult dragonflies· water-balance. *J. exp. Biol.* **41**, 331–43.

MILLER, P. L. (1966). The regulation of breathing in insects. *Adv. Insect Physiol.* **3**, 279–354.

NOBLE-NESBITT, J. (1967). Aspects of the structure, formation and function of some insect cuticles. In *Insects and Physiology*. Ed. by J. W. L. Beament and J. E. Treherne, pp. 3–16. Edinburgh: Oliver and Boyd.

NOBLE-NESBITT, J. (1968*a*). Exchanges of water between the atmosphere and the firebrat, *Thermobia domestica* (Packard) (= *Lepismodes iniquilinus* Newman). *Proc. XIIIth Int. Congr. Entom.* (In the Press.)

NOBLE-NESBITT, J. (1968*b*). A spiracular closing mechanism in the firebrat, *Thermobia domestica* (Packard). (In preparation.)

O'BRIEN, F. E. M. (1948). The control of humidity by saturated salt solutions. *J. Sci. Instrum.* **25**, 73–6.

RAPOPORT, E. H. & TSCHAPEK, M. (1967). Soil water and soil fauna. *Rev. Ecol. Biol. Sol.* **4**, 1–58.

SOLOMON, M. E. (1962). Ecology of the flour mite, *Acarus siro* L. (= *Tyroglyphus farinae* De G.). *Ann. appl. Biol.* **50**, 178–84.

SOLOMON, M. E. (1966). Moisture gains, losses and equilibria of flour mites, *Acarus siro* L., in comparison with larger arthropods. *Entomologia exp. appl.* **9**, 25–41.

WATSON, J. A. L. (1967). Reproduction, feeding activity and growth in the adult firebrat, *Lepismodes iniquilinus* Newman (Thysanura, Lepismatidae). *J. Insect Physiol.* **13**, 1689–98.

WHARTON, G. W. & KANUNGO, K. (1962). Some effects of temperature and relative humidity on water-balance in females of the spiny rat mite, *Echinolaelaps echidninus* (Acarina: Laelaptidae). *Ann. ent. Soc. Am.* **55**, 483–92.

WIGGLESWORTH, V. B. (1965). *The Principles of Insect Physiology*, 6th ed. London: Methuen.

WINSTON, P. W. (1967). Cuticular water pump in insects. *Nature, Lond.* **143**, 383–4.

WINSTON, P. W. & BATES, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–7.

WINSTON, P. W. & NELSON, V. E. (1965). Regulation of transpiration in the clover mite *Bryobia praetiosa* Koch (Acarina: Tetranychidae). *J. exp. Biol.* **43**, 257–69.