

THE LONGEVITY OF THE FASTING BED-BUG
(*C. LECTULARIUS* L.) UNDER EXPERIMENTAL
CONDITIONS AND PARTICULARLY IN RELATION
TO THE SATURATION DEFICIENCY LAW OF
WATER-LOSS

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(With 11 Figures in the Text)

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I. INTRODUCTION

THE data in this paper were collected primarily to determine how long bed-bugs (*Cimex lectularius*) were likely to remain alive in an empty English house under extreme circumstances: whether they are capable of moulting and breeding at the end of a long period of starvation is another problem and, although essential to a full knowledge of the ecology of the insect, it has not yet been studied.

The physiological problems associated with the survival of bugs in relation to climate were of secondary importance when this work started, although it was hoped at the outset that the quantity of data aimed at might be sufficient to enable some general conclusions to be drawn. Thus, the many physiological problems which arise have not been pursued experimentally largely because of the precedence given to the original requirements of the research in this and in other aspects of the life of the bed-bug.

I have suggested some hypotheses: but they all require to be tested by direct experiment. It must be sufficient for the time being to point out where data are lacking.

II. METHODS

Atmospheric humidity was controlled usually with sulphuric acid-water mixtures. Only with adult bugs kept at approximately 90 % R.H. was the control effected with glycerine-water solutions.

All immature stages were kept on the voile at the one end of 2 × 1 in. tubes. These tubes, capped with voile at each end, were suspended vertically over dilute acid in 250 c.c. jars which were closed with corks (Fig. 1). Rubber bungs were not used on account of their possible toxic effects (Mellanby & Buxton, 1935).

Adult bugs were confined, singly or in pairs, in 2 × 1 in. tubes with voile on both ends in battery jars with an air stream. Dilute sulphuric acid was used to maintain relative humidities between 5 and 10 % and glycerine-water mixtures for approximately 90 % R.H. The method and apparatus is described elsewhere (Johnson, 1939*b*).

The stock from which experimental bugs and eggs were obtained was reared at 23° C. on rabbit with one feed per instar. The stock came originally from Beckenham, England, in 1927 and has since then been kept at 23° C. in mass culture. Insects in experiments were inspected, usually at intervals of 3–6 days, when dead bugs were removed. The host in the experiments was rabbit, unless otherwise stated. Variations in temperature are shown in the tables in the text. Other details of technique are discussed on pp. 266–267.

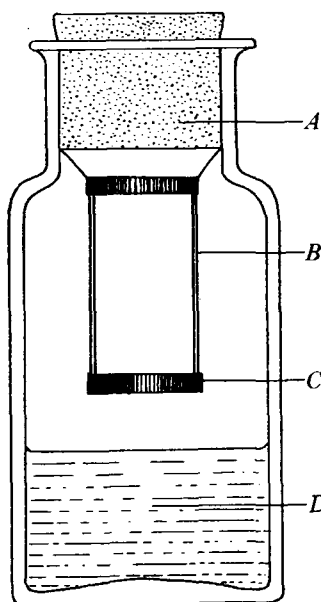


Fig. 1. 250 c.c. jar with 2 × 1 in. tube, voile on both ends, in which 1st instars were kept. A, cork; B, 2 × 1 in. glass tube; C, adhesive tape securing voile; D, sulphuric acid-water mixture.

III. EXPERIMENTAL RESULTS AND DISCUSSION

(1) *Longevity in relation to temperature, atmospheric humidity and the rate of water-loss*

General observations.

The effects of temperature and atmospheric humidity on the longevity of the fasting first instar bed-bug are seen in Fig. 2. The following points are evident:

(1) At constant temperatures of 7 and 15° C. and no doubt at the intermediate ones too, the relationship between mean length of life and saturation deficit is hyperbolic over the humidity range used. As the expected results in

Table 1 and the fitted lines in Fig. 2 show, hyperbolas fit the data very well at these temperatures. At temperatures above 15° C., however, the relationship between longevity and saturation deficit becomes increasingly more linear. Table 2 which lists the deviations from the fitted lines (linear and hyperbolic), shows this clearly.

Table 1. *Observed and expected mean length of life and the maximum observed length of life of unfed 1st instars of C. lectularius at constant temperatures and humidities. All the values date from hatching*

The expected mean lengths of life are the values on the best fitting hyperbolas obtained from the data by the method of least squares and the linear regression of log mean against log saturation deficit (Fig. 2).

Insects were inspected at intervals: therefore, a maximum length of life of, for example, 80–83 days, is shown as 80–3 in the last column.

° C.	R.H. %	Sat. def. mm.	Mean lengths of life (days)		Stand. dev. of obs.	No. bugs used	Maximum obs. length of life (days)
			Obs.	Exp.			
7.0° ± 0.8° (44.6° F.)	6	7.05	53.0	52.0	14.83	108	80–3
	27	5.50	60.4	57.1	18.12	78	103–6
	56	3.30	65.0	69.3	22.80	138	111–4
	90	0.75	123.5	121.2	41.36	127	210–3
15.0° ± 0.2° (59.0° F.)	6	12.03	46.6	47.5	17.42	81	106–9
	27	9.34	52.5	52.1	9.67	82	74–7
	56	5.60	64.0	63.2	14.49	132	102–5
	90	1.30	108.4	109.1	31.37	109	183–6
18.5° ± 0.4° (65.3° F.)	6	15.0	24.2	27.4	4.83	66	38–40
	28	11.6	32.4	30.6	5.12	59	42–4
	57	6.9	42.8	38.2	13.04	71	71–4
	90	1.6	66.4	70.9	20.23	50	132–5
25.0° ± 0.3° (77.0° F.)	7	22.10	13.3	15.6	2.09	106	20–2
	29	16.90	19.5	17.8	2.63	79	28–30
	58	10.0	29.1	23.6	6.04	87	44–6
	90	2.40	45.7	49.7	11.95	85	68–71
28.0° ± 0.3° (82.4° F.)	7	26.4	11.3	13.7	2.17	30	14–6
	29	20.10	15.4	15.2	2.47	32	20–2
	58	11.9	22.2	18.7	4.66	49	32–4
	90	2.9	31.1	32.2	6.56	88	44–6

(2) The effects of humidity on length of life are greater at the lower than at the higher temperatures: and this is more noticeable when the humidity is high. Numerical expression can be given to this effect by the ratio

$$\frac{\text{difference between longevity at two different saturation deficits}}{\text{longevity at the higher saturation deficit (i.e. minimum longevity)'}}$$

which expresses the proportionate effects of humidity at different temperatures. Table 3 gives some values for this ratio and over some ranges of saturation deficit the effect of humidity on longevity is increased by over 100 % by a fall of 9.5° C., from 28 to 18.5° C. This greater effect at low temperatures is at first puzzling: and it involves an apparent contradiction when we notice that the rates of water-loss are more affected by the same humidity change at the *high* temperatures (Table 9, Fig. 9). An explanation for this is given later (p. 246).

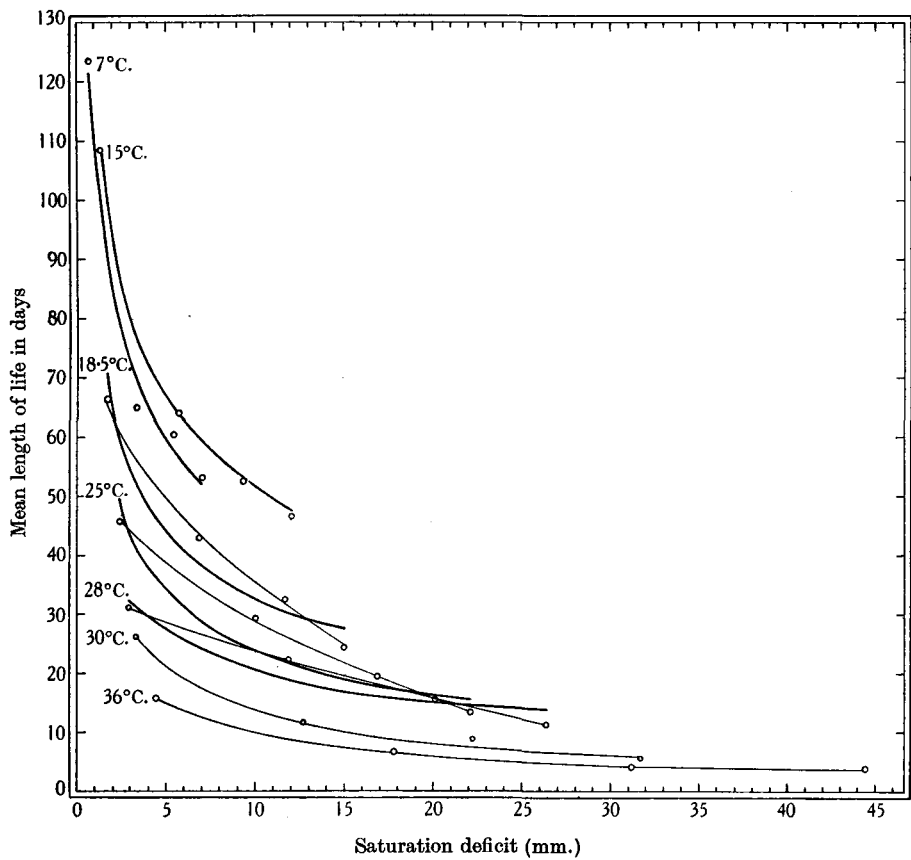


Fig. 2. Mean length of life of unfed 1st instars of *C. lectularius* in relation to temperature and humidity (Table 1). Thick lines are hyperbolas fitted to observed data by the method of least squares. Thin lines are fitted visually to the observed data. Graphs at 30 and 36° C. are from Mellanby's (1935a) data.

Table 2. Values of S (observed-expected)² for straight lines and hyperbolas fitted to the longevity-saturation deficit data for unfed 1st instars of *C. lectularius* (Table 1)

Comparison between hyperbola and straight line can be made at any temperature. The smaller the value S (obs.-exp.)² the closer the fit.

° C.	Hyperbola	Linear
28	19.67	1.72
25	54.25	12.03
18.5	55.88	32.29
15	2.08	266.66
7	35.21	595.18

Table 3. *Ratio $\frac{\text{difference in mean longevity}}{\text{minimum mean longevity}}$ for ranges of saturation deficit 3-7, 7-12 and 3-12 mm. at various temperatures. The longevities have been found, approximately, by interpolation on the observed graphs in Fig. 2*

° C.	Mean longevity in days at sat. def.			Differences in mean longevity			Differences in mean longevity Minimum mean longevity		
	3 mm.	7 mm.	12 mm.	3-7 mm.	7-12 mm.	3-12 mm.	3-7 mm.	7-12 mm.	3-12 mm.
28	31	27	22	4	5	9	0.15	0.23	0.41
25	44	35	26	9	9	18	0.26	0.35	0.69
18.5	59	42	31	17	11	28	0.40	0.35	0.90
15	83	59	46	24	13	37	0.41	0.28	0.80
7	71	53	—	18	—	—	0.34	—	—

(3) At equal saturation deficits the insects live longer at the lower than at the higher temperatures down to about 15° C. where the maximum length of life occurs. Below 15° C. length of life becomes shorter again.

This difference in position of the graphs for 7 and 15° C. is apparently quite real, for if the two hyperbolas are converted into straight lines by plotting log longevity against log saturation deficit, the lines are significantly different in position. For at the point at which they are nearest together the difference between the values for log longevity at 7 and 15° C. is 0.0474 and the standard error of the difference is 0.00006.

The results for fasting adults show a similar relationship (Table 4, Fig. 3). Geisthardt (1937) figures a decrease in maximum survival times at temperatures below 15° C.

It is interesting to note that the eggs of *C. lectularius* kept at temperatures

Table 4. *Mean and maximum length of life in days of adult C. lectularius, mated and unmated and fed once on rabbit (Fig. 3)*

Maximum length of life of, for example, 291-8, means that death occurred between 291st and 298th day.

Longevity dates from feeding—4 days, at 23° C., after moulting.

° C.	% R.H.	Male				Female			
		No. of bugs	Mean length of life	S.D.	Maximum length of life	No. of bugs	Mean length of life	S.D.	Maximum length of life
		Mated							
7	10	48	208.8	37.04	291-8	24	228.4	29.14	306-12
	90	23	219.5	71.66	382-90	23	285.6	104.94	460-9
13	10	21	186.8	48.64	256-63	22	179.0	49.04	235-42
	90	22	338.3	85.46	464-71	21	359.7	109.41	562-72
18	10	22	98.6	28.18	148-53	21	90.69	22.51	139-41
	90	19	151.5	64.35	257-64	19	143.05	51.31	222-7
23	10	22	40.3	13.02	57-60	22	38.32	12.94	67
	90	25	85.2	22.87	135-7	25	69.4	25.97	126-8
Unmated									
7	90	21	222.8	113.61	518-31	21	317.21	139.10	550-3
23	90	20	86.6	22.4	126-31	18	120.7	44.5	181-5

below the developmental-hatching threshold ($13^{\circ}\text{C}.$), at constant saturation deficits, live longest at $12\text{--}13^{\circ}\text{C}.$ and longevity also decreases as the temperature falls to $0^{\circ}\text{C}.$ (Johnson, 1939*a*). $13\text{--}15^{\circ}\text{C}.$ is thus a critical temperature for longevity and also for development with hatching; moulting and oviposition also stop at temperatures immediately below $13^{\circ}\text{C}.$

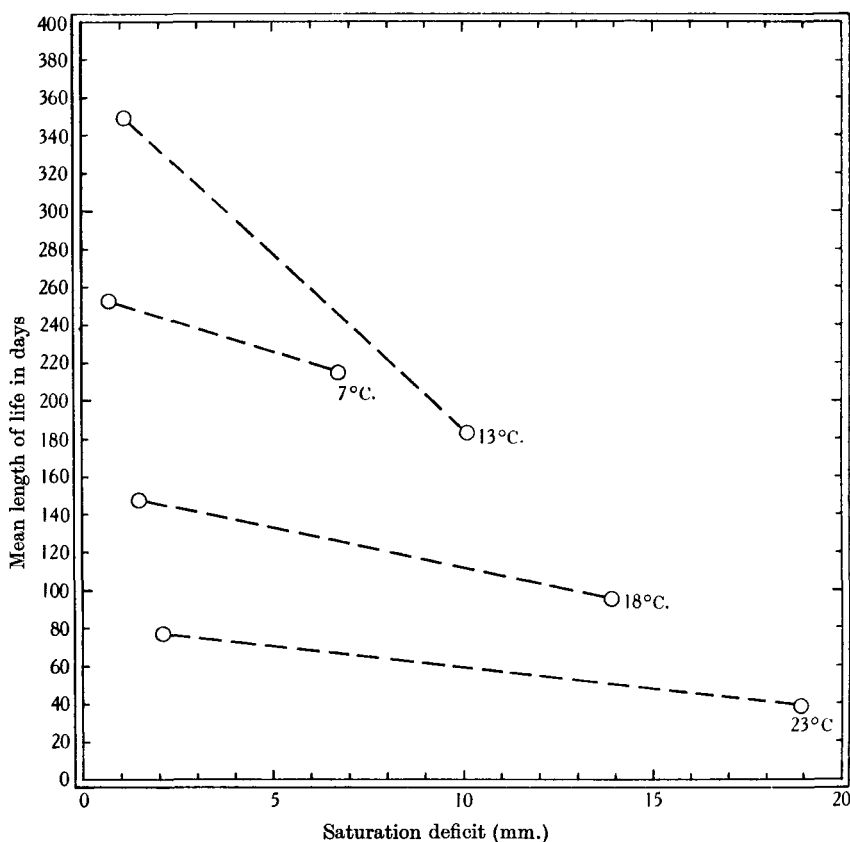


Fig. 3. Mean length of life of mated adults of *C. lectularius* (males and females together), fed once on rabbit, in relation to temperature and humidity. Longevity dates from feeding. Broken lines merely join the two observations at each temperature and do not indicate length of life at intermediate humidities (Table 4).

Longevity in relation to water-loss.

In the interpretation of these results certain ideas associated with Dalton's law of water-loss at once suggest themselves. After glancing for a moment at Fig. 9 in order to understand the relationship between rate of water-loss and saturation deficit with *C. lectularius*, it will be advisable first to consider a purely hypothetical case where, at a constant temperature, the length of life of an insect is controlled by water-loss alone and that death occurs

when a critical quantity of water has been lost. Then, if at a constant temperature,

A. The rate of water-loss is directly proportional to saturation deficit (linear relationship, Table 5, Fig. 4, A).

B. Since longevity depends only on the amount of water lost, the quicker the loss the shorter the life. And longevity is inversely proportional to the rate of water-loss (hyperbolic or inverse of linear relationship).

Table 5. *Hypothetical data for rate of water-loss at different temperatures and saturation deficits, and the corresponding reciprocal curves representing longevity (see Fig. 4)*

Rate of water-loss = b (saturation deficit).			
Longevity = constant $\left(\frac{1}{\text{rate of water-loss}}\right) = \frac{\text{constant}}{b \text{ (saturation deficit)}}$.			
Sat. def. units	Rate of water-loss		
	$^{\circ}\text{C} \dots x$ $b \dots 1$	$x+1$ 2	$x+2$ 4
0	0	0	0
1	1	2	4
2	2	4	8
3	3	6	12
4	4	8	16
5	—	10	20
6	—	—	24
	Longevity		
	x 1	$x+1$ 2	$x+2$ 4
	∞	∞	∞
	1.0	0.50	0.25
	0.50	0.25	0.13
	0.33	0.17	0.08
	0.25	0.13	0.06
	—	0.10	0.05
	—	—	0.14

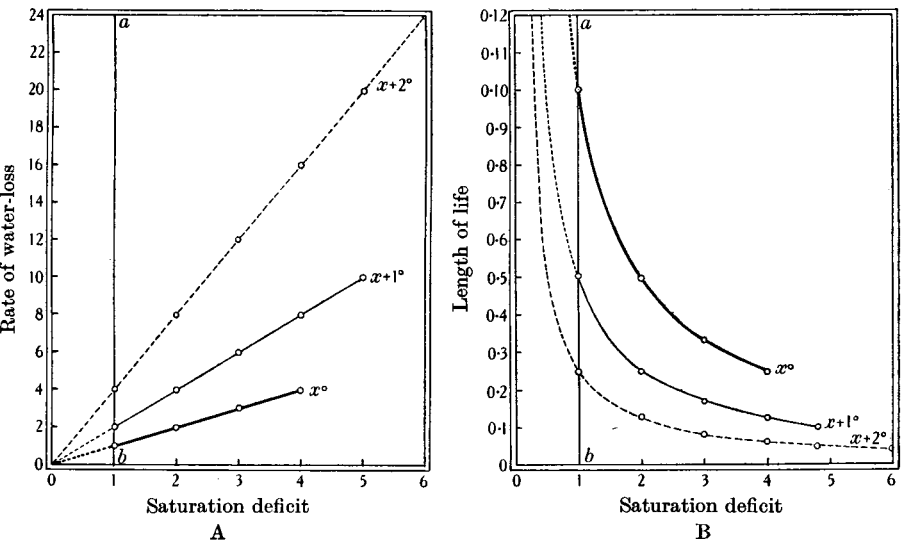


Fig. 4. Illustrating a hypothetical case where longevity is limited only by water-loss over a temperature range x° to $x+2^{\circ}$. A, rate of water-loss is directly proportional to saturation deficit at each temperature (linear); B, longevity (represented by $1/\text{rate of water-loss}$) is inversely proportional to saturation deficit (hyperbolic). Graphs to the left of vertical line, ab , are theoretical, for with the bed-bug rate of water-loss is not linear over the whole saturation deficit range; and with longevity insects are not immortal (Table 5). Cf. also Figs. 5 and 9.

It follows that

C. Longevity will also be inversely proportional to saturation deficit (hyperbolic relationship, Table 5, Fig. 4, B).

For the sake of simplicity let us assume that the rate of water-loss at a constant temperature is linear even at very high humidities: also that since death is due only to water-loss, at 0 mm. saturation deficit the insects live for an exceedingly long and for an equal length of time at all vital temperatures. In completely dry atmospheres the insects will live for different lengths of time at various temperatures since the saturation deficits will be different.

Then the curves for longevity against saturation deficit will be the same shape and will have the same positions relative to each other as the reciprocals of the curves for rate of water-loss against saturation deficit (Fig. 4, A, B).

The longevity curves are, in such a case, hyperbolas which are virtually complete along the ordinate since they extend to the same distant point on it. The higher the temperature the closer to the ordinate and abscissa do these curves lie and their reciprocal curves for the rate of water-loss are correspondingly steeper. The effect of a humidity change *from 0 mm. saturation deficit* will, therefore, be greatest at the highest temperature both on longevity and on the rate of water-loss.

In nature, however, we do not deal with complete hyperbolas but only with portions of them, for bed-bugs are affected by temperature at 0 mm. saturation deficit and accordingly survive for different lengths of time in a saturated atmosphere: or the experiments may not give data for the relative humidities near 100 %—as with the *Cimex* work. Such graphs will, therefore, appear as those to the right of the vertical line *ab* in Fig. 4, B. Thus the effects of a humidity change, although greatest at higher temperatures on the rate of water-loss, will be greatest at *lower* temperatures on longevity. Thus, with *Cimex*, if (1) water is lost at a rate directly proportional to saturation deficit at constant temperatures, and (2) longevity is limited mainly by water-loss, the apparent inverse effects of humidity change on the longevity of this insect are explained.

Let us consider the data for longevity with unfed 1st instars of *C. lectularius* in relation to the rate of water-loss from adults (Fig. 9).

If longevity were governed entirely by the rate of water-loss between 10 and 90 % R.H. in a manner somewhat similar to the hypothetical case discussed above, then the curves for $1/\text{rate of water-loss}$, and for longevity, against saturation deficit should be similar in shape and in position relative to one another at the same temperatures. If the reciprocals of the curves for Mellanby's data on the rate of water-loss with saturation deficit for fed adults (Fig. 5) are compared with the longevity curves for unfed 1st instars the similarity is distinct, although Mellanby used slightly different temperatures in his experiments. The two sets of curves are, however, slightly different in shape and in relative position.

Differences in shape.

The best way of obtaining the degree of correlation which exists between longevity and rate of water-loss is obviously by direct experiment. This, however, has not been done for *Cimex* and we must fall back on an indirect method.

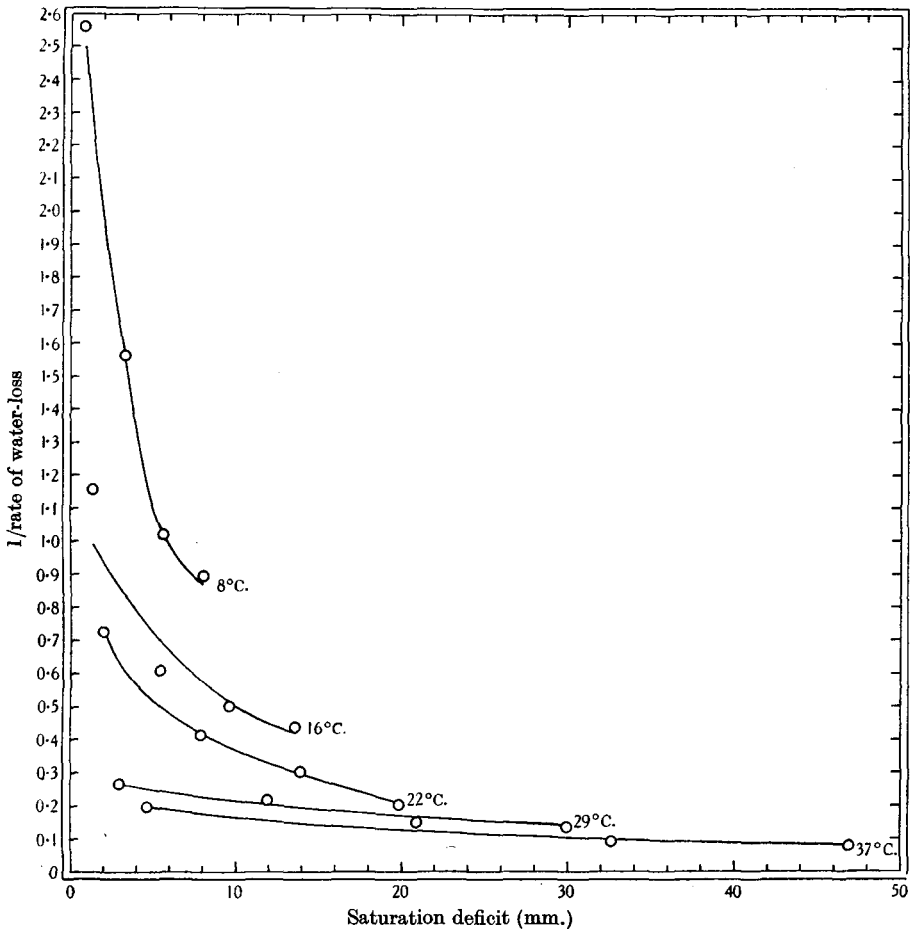


Fig. 5. Reciprocal curves of rate of water-loss in relation to temperature and humidity for the fasting adult *C. lectularius*. If longevity was limited only by water-loss and the rate of water-loss was directly proportional to saturation deficit at constant temperatures the curves of longevity (or of $1/\text{rate of water-loss}$) with saturation deficit would be hyperbolic. Lines in figure are hyperbolas fitted to Mellanby's (1932) data. (See Table 9 and also Fig. 9.)

If a perfect correlation exists between (1) saturation deficit (S) and rate of water-loss (R), and between (2) saturation deficit (S) and $1/\text{longevity}$ ($1/L$), then $1/L$ will be perfectly correlated with R .

It would be exceedingly difficult to prove that in such a case longevity depended on rate of water-loss although no doubt this would be true.

Therefore, if the correlation coefficients of *R* against *S* and of *1/L* against *S* are very high indeed (*r* = 1 when correlation is perfect), we would be justified in concluding that *1/L* and *R* are also very highly correlated. Table 6 gives such values for the correlation coefficients. For all temperatures between 7 and 36° C. the coefficients for *R* on *S* and for *1/L* on *S* are above 0.95 and are all significant according to Fisher's Table VA (Fisher, 1936).

Table 6. *Correlation coefficients for $\frac{1}{\text{longevity of 1st instars}}$ and for rate of water-loss for fed adults (from Mellanby's data, 1932) with saturation deficiency at various temperatures. Four pairs of observations in each case except at 37° C. where there are only three*

° C.	$\frac{1}{\text{longevity of 1st instars}}$	Rate of water-loss (% original wt.). From data by Mellanby (1932)
	Unfed	
7	0.9532	—
8	—	0.9963
15	0.9911	—
16	—	0.9670
18.5	0.9886	—
22	—	0.9905
25	0.9761	—
28	0.9682	—
29	—	0.9753
*30	0.9894	—
*36	0.9658	—
37	—	0.9999

* Longevity from Mellanby, 1935*a*.

Thus assuming that adults and unfed 1st instars lose water in an identical manner it can be concluded that, within the limits of experimental error and with due regard to the very small number of points on each graph, *1/L* is also almost perfectly correlated with *R* at all constant temperatures between 7 and 36° C.

But we have already seen that longevity in relation to saturation deficit is hyperbolic only between 7 and about 15° C., and that above 15° C. the relationship is more linear. Therefore, we would expect *1/L* to be completely correlated with *S* (and therefore with *R*) only below about 15° C. and that at higher temperatures the correlation would be less perfect since *1/L* on *S* is not perfectly linear. The exceedingly high observed values of *r* in Table 6 are perhaps more reliable for temperatures below 15° C.: the discrepancies between *r* and the fitted lines of *L* against *S* above 15° C. are no doubt due to the small number of points on the graphs. With only four points a deceptively high correlation is possible: nevertheless, even above 15° C. their values are significant and even if they are too high the correlation between *1/L* and *S* must be very close. For *R* against *S*, however, the correlation coefficients are consistent with the experimental data, for perfectly fitting straight lines are obtained (Table 9). This indicates that the departure of the relationship of *L*

to S from the hyperbola at higher temperatures is not due to an alteration in the mechanism controlling water-loss from the insect but to additional factors.

There are at least two such physiological factors:

(1) We suppose that since rate of water-loss and longevity are almost perfectly correlated at each temperature between 15 and 7° C., rate of water-loss is the only limiting factor controlling longevity below a certain maximum value at each temperature. This may be due to the relatively slow rate at which food is utilized, or at which water is withdrawn from it, at these temperatures. Above 15° C. food reserves are utilized more rapidly and water-loss ceases to be the only limiting factor in longevity.

(2) At high temperatures and relative humidities metabolic water may accumulate more rapidly than it is eliminated and an excess of internal water may be produced with harmful effects on the insect (Buxton, 1932; Mellanby, 1935*b*).

With the present data it is not possible to tell if either or both of these factors operate. But such data as exist can be discussed.

When a bed-bug is starved for a long time, the water-loss from the insect causes a reduction in the volume of body fluids. This creates a relatively higher pressure inside the sac-like midgut which becomes filled with air through the proboscis and bulges into the legs and antennae—in fact develops hernia (Wigglesworth, 1931). Now at high temperatures (20–28° C.) the blood-meal is quickly digested and the midgut, which empties of the blood-meal fairly rapidly, becomes air-filled and is withdrawn into the legs, where it ruptures: thus digestion and water-loss proceed simultaneously, and probably both play important parts in affecting longevity. But at low temperatures (15 to 7° C.) digestion is extremely slow, and many weeks, sometimes many months, pass before the blood-meal loses its redness and much of its bulk. Yet, shortly before death occurs at 7 and 15° C., the midgut develops hernia and bursts: usually the blood-meal is still quite red and bulky, although enough may have been digested to promote moulting. This indicates that digestion and utilization of the blood-meal or, more precisely perhaps, a withdrawal of water from it, is impeded although water-loss from the body fluids proceeds and, therefore, probably remains the master factor in longevity at these low temperatures.

That the withdrawal of water from the blood-meal rather than the actual digestion of the food matter (protein) limits survival seems probable. For the hyperbolic nature of the curves at 7 and 15° C. suggests that water-loss is limiting. Yet if this was the only factor the curve for 7° C. would be above that for 15° C. as Fig. 5 shows. We can suppose, therefore, that, if blood-meals are of equal sizes, although the rate of water-loss is higher at 15 than at 7° C., there must be more water available at the higher temperature for longer life to occur.

It has been mentioned above that 13° C. is the approximate threshold for moulting: and it may be that failure to absorb food at this temperature is responsible for cessation of moulting. 13° C. is at any rate a critical temperature

in respect to the longevity as well as with respect to other processes and the causes are perhaps the same.

If these suppositions are true, the actual curves for longevity against saturation deficit may bear the following relationships (Fig. 6, A, B) with the hypothetical curve where water-loss was the only limiting factor, at least below a certain optimum humidity for survival.

Since humidity probably has no effect on the metabolic rate (Mellanby, 1936) food would tend to become limiting only when water-loss was slow, i.e. at low saturation deficits (Fig. 6, A). It is possible that even in the individual bug, and more probably where numbers of bugs are considered, that food and water do not always act independently on the organism and the actual and hypothetical curves may coincide when water and food are equally limiting, and the graph is the relatively straight part of the hyperbola. If only food is

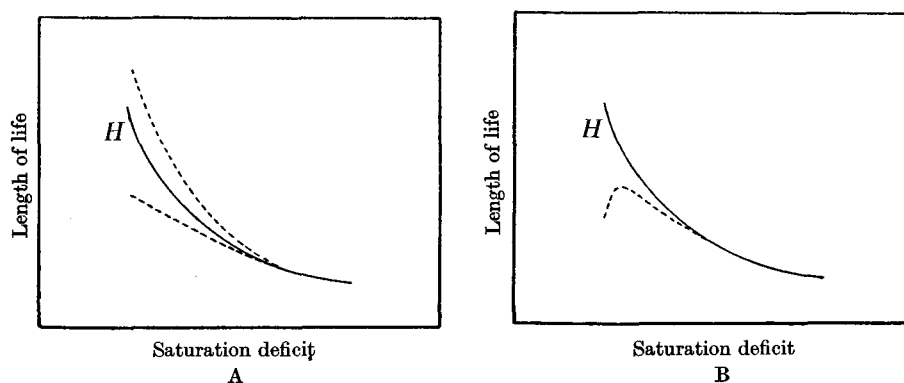


Fig. 6. Illustrating longevity in relation to saturation deficit at a constant temperature. A, curve *H* is a hyperbola where longevity depends only on water-loss the rate of which is directly proportional to saturation deficit. Dotted lines are more linear and suggest that other factors besides water-loss (e.g. food supply) affect longevity. B, curve *H* as in Fig. A. Dotted line indicates longevity when influenced adversely at high humidities by, perhaps, toxic effects of an excess of water in the insect.

limiting and humidity is without effect on metabolic rate then the longevity curve would be parallel to the abscissa.

The accumulation of an excess of metabolic water would no doubt also result in the types of curve shown in Fig. 6, B.

The position of a hypothetical curve at a constant temperature where water-loss alone is limiting to longevity is not known in *Cimex*, and so it cannot be said precisely where the departure from the hyperbolic relationship of longevity to saturation deficit at the higher temperatures occurs. But from the high values for the correlation coefficients between $1/L$ and S it is probable that the departure takes place at the higher humidities for at the lower ones humidity is probably the important limiting factor.

There is, moreover, another possibility, which may apply to the perfectly fitting hyperbolas at 7 and 15° C. as well as to curves at higher temperatures.

If, as seems probable (particularly from Kirkpatrick's (1923) data for the bug *Oxycarenus*), the longevity curve is shaped as in Fig. 7 (curve *A*), the optimum humidity may be below 90 % R.H. and longevity at 90 % R.H. although lying on a perfect hyperbola in conjunction with values at 60, 30 and 7 % R.H. may do so accidentally, not because rate of water-loss but because the accumulation of an excess of internal water may be the limiting factor (Fig. 7, *B*).

This point can be settled only by obtaining data at many more humidities at each constant temperature.

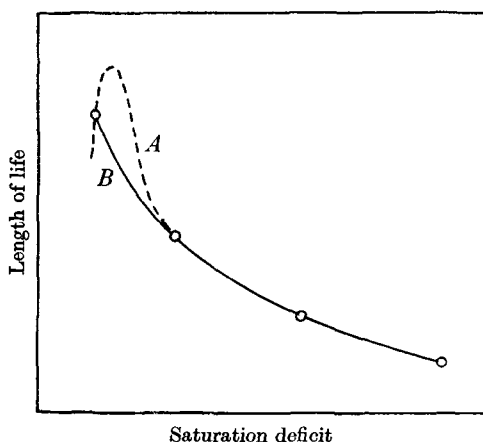


Fig. 7. Illustrating a possible relationship of longevity to saturation deficit at a constant temperature as exhibited by data for unfed 1st instars of *C. lectularius* (Table 1). Curve *B* is a hyperbola which, owing to insufficient data, may lead to the conclusion that water-loss alone limits longevity; whereas actually water-loss is limiting only at the higher saturation deficits while at high humidities food and an excess of internal water are the limiting factors (curve *A*).

Differences in the positions of curves.

The positions of the longevity-saturation deficit curves along the ordinate—in other words, the length of life at a constant saturation deficiency but at different temperatures—may depend on water-loss alone or upon other factors: if water-loss alone determined the maximum length of life, then, apart from the obviously exceptional range close to 100 % R.H., the position of the longevity curves in relation to each other and to temperature would be identical with the relative positions for the curves for the reciprocal of rate of water-loss to saturation deficiency at different temperatures (Fig. 5). In the absence of direct experiment which would relate the rate of water-loss at different temperatures to longevity we cannot tell how close to this relationship the longevity curves are. We could obtain the correlation coefficients for $1/L$ against temperature and for R against temperature at the same saturation deficits and, if both were exceedingly high, suppose that at this saturation deficit and different temperatures L was highly correlated with R and probably determined by it. But obviously this will not apply to longevity below 15° C.; for there maximum

length of life is probably determined by the capacity to utilize food or the water contained in it (p. 249).

At 5 mm. saturation deficit the correlation coefficients of rate of water-loss and of $1/\text{longevity}$ with temperature (results for 7° C. omitted in r for $1/\text{longevity}$) are 0.9678 and 0.9236 respectively: they are both highly significant. The values of R and $1/L$ used in the calculations were obtained from regression equations for R and for $1/L$ against saturation deficit. This suggests that the relative positions of the longevity curves between 15 and 36° C. is almost that which would be expected if longevity was due entirely to rate of water-loss, below a certain maximum depending on the amount of water in the bugs, over the whole temperature range used. The accuracy of this conclusion must, however, be accepted with caution since the number of temperatures on which the correlation coefficients are based is so small.

(2) *The effects of a single feed on longevity*

The probable effects of food as a limiting factor in survival have been partly discussed in the preceding section. We must now compare the survival of fed with unfed bugs, i.e. bugs with different amounts of food and water available. It is convenient to compare fed with unfed 1st instars. Tables 1 and 7 and Figs. 2 and 8 supply the data. We assume that the same amount of blood has been taken by each experimental batch.

Table 7. *Observed mean and maximum lengths of life of 1st-2nd instars of C. lectularius at constant temperatures and humidities. The bugs were fed once, as 1st instars, on rabbit 2 days after hatching. Length of life dates from hatching (Fig. 8)*

The numbers used refer to those insects which died as 2nd instars, except at 7° C. when moulting did not occur and all remained as 1st instars.

Temperature variations as in Table 1.

° C.	R.H. %	Sat. def. mm.	Mean length of life (days)	Stand. dev.	Maximum length of life (days)	No. of bugs used
7	6	7.05	98.6	43.40	155-160	44
	90	0.75	195.6	111.98	340-350	36
15	6	12.03	98.3	33.45	150-155	13
	90	1.30	223.6	59.90	355-360	33
25	7	22.10	23.8	2.59	28	46
	90	2.40	53.8	10.89	66-68	37

Is the shape of the longevity-saturation deficit curve different with fed and unfed 1st instars?

From Fig. 8 and Table 8 it is evident that at the higher temperatures (25° C. and above) the actual amount of increase in longevity of fed bugs is about the same at high as at low humidities. Mellanby's (1935*a*) figures for longevity at 36° C. show discrepancies, but it is probable that lethal effects due to heat alone were operating at this high temperature. Thus the curves for

unfed and fed bugs run parallel at the higher temperatures and are, therefore, not altered in shape. We can conclude, therefore, that the extra food lengthens life but that, at each temperature, the same factors limit survival as with the unfed bugs.

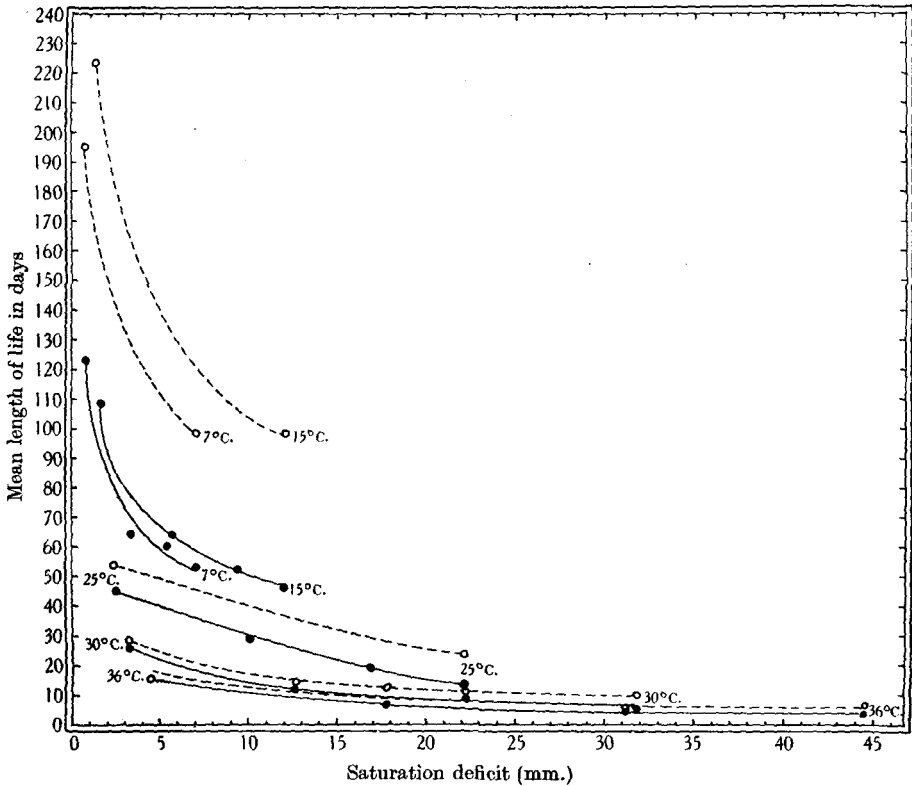


Fig. 8. Mean length of life of unfed and fed 1st instars (fed 1st = unfed 2nd instar at 15° C. and above) of *C. lectularius* in relation to temperature and humidity. All lines fitted visually (Tables 1 and 7). Data at 30 and 36° C. from Mellanby (1935a).

○---○, Fed. ●—●, Unfed.

Table 8. Increase in survival time in days of fed 1st instar *C. lectularius* from times for unfed 1st instars (Fig. 8). Data at 30 and 36° C. from Mellanby (1935a)

° C.	R.H. %				
	0	6-7	30	60	90
7	—	45.6	—	—	72.1
15	—	51.7	—	—	115.2
25	—	10.5	—	—	8.1
30	4.28	—	2.48	2.87	2.43
36	2.78	—	1.42	5.79	-0.21

At the lower temperatures, however (15° C. and below), the absolute increase in longevity is greater at the higher humidity. This indicates that the curves for fed bugs are different in shape from those for unfed bugs. But we

have already suggested that, since the longevity-saturation deficit curves are hyperbolic at 7 and at 15° C., water-loss is most probably the limiting factor in longevity between 6 and 90 % R.H.—in other words that from 6 to 90 % the food reserve does not appear to be fully utilized. Now since the 90 % R.H. value for longevity for fed bugs is relatively higher than the 6 % value, as compared with the value for unfed bugs, the curve for fed bugs is not hyperbolic and the limiting factor at 90 % R.H., at least, is probably food; the proportion of water to food in fed bugs would indeed probably be higher than in the unfed ones.

Are the relative positions of the longevity-saturation deficit curves different with fed and unfed 1st instars?

If no change in relative positions occurs, i.e. if, in relation to temperature at a constant saturation deficit, longevity is still almost perfectly correlated with rate of water-loss, we should *not*, as appears at first sight, expect the same amounts of increase in longevity at each temperature. For at lower temperatures the same increase in amount of available water will be utilized more slowly and with a consequently greater effect on longevity than at higher temperatures. Thus temperatures which are associated with longer survival for unfed bugs should also be associated with greater increase of survival after feeding. This is in fact what we find (Table 8 and Fig. 8); the data are not, however, sufficiently complete to attack the problem arithmetically in order to see if the correlation of survival with rate of water-loss is equally good for fed and unfed bugs.

At 7° C. we notice that the increase in longevity is not so great as at 15° C., and that the relative positions of curves for fed bugs at 7 and 15° C. are similar to those for unfed bugs. Once again it may be argued that, as with unfed bugs, not all the available water can be utilized at 7° C. but that, apart from this, water-loss limits longevity except perhaps at the very high humidities (p. 249).

These conclusions differ somewhat from those drawn by Mellanby (1935*a*) on the effects of feeding on longevity. He worked only at 30 and 36° C. and noted that survival times in dry air were increased but that in "moist air, however, the survival time was not significantly altered". Mellanby also considered that at 90% R.H. unfed bugs died from starvation and not from desiccation but that at the lower humidities desiccation was the principal cause of death. Thus since no increase in survival times was noticed at 90 % he concluded that "*C. lectularius* nymphs do not survive starvation (in distinction to desiccation) any better after feeding".

But if Mellanby's figures are examined, it can be seen (Table 8) that approximately the same amount of increase in survival times does occur at all humidities at 30° C. although at 90 % R.H. the difference is not significant; and that although the smallest increase at 36° occurs at 90 % the greatest occurs at 60 % R.H. But at 36° C. the results are erratic and probably upset by the high temperature. The results at 30° C. agree quite well with mine at 25° C. and

reasonably well with those at 7 and 15° C. Thus Mellanby's conclusion that there was no increase in survival at the high humidity was probably incorrect although it is indeed probable that food and not water is the limiting factor at 90 % R.H. But alternatively it may also be due to toxic effects of an excess of water in the tissues. Incidentally with *C. rotundatus* Mellanby found that feeding increased survival time even at high humidities.

Thus summarizing the effects of a blood-meal on survival it may be assumed that at low relative humidities the effect of food is to increase the water supply which is probably the limiting factor in survival, but that at high humidities either an excess of water curtails longevity or that food becomes the most important limiting factor even at low temperatures where with unfed bugs water alone appears to be limiting.

(3) *The rate of water-loss and the law of saturation deficiency*

I have made no experiments on the loss of weight or of water with fasting bed-bugs. But some results obtained over a wide range of temperature and humidity have been published (Mellanby, 1932). An analysis of these data is necessary since certain of Mellanby's conclusions require modification.

In the first place, nearly all the weight lost by a fasting bed-bug can be attributed to water-loss. This has been demonstrated indirectly by Mellanby: he showed that protein is the principal food reserve and this breaks down to CO₂, water and uric acid. Since uric acid is retained by the bug and the weight of carbon lost with CO₂ is nearly balanced by the oxygen taken in to produce metabolic water, water-loss alone remains to diminish weight.

I have calculated the percentage loss of weight per day (rate of water-loss) from Mellanby's data and have plotted this against the saturation deficit of the atmosphere (Table 9, Fig. 9). The expected rates of water-loss and the regression coefficients for the best fitting straight lines are given in Tables 9 and 10 respectively.

It can be seen that over the range from 0 to 90 % R.H. the relationship is linear at each constant temperature: the agreement between observed and expected results is very close. It can, therefore, be concluded that the rate of loss in weight, or the rate of water-loss, is directly proportional to the saturation deficit of the atmosphere at constant temperatures between 8 and 38° C. and between 0 and 90 % R.H. This result is, of course, subject to the possible errors of technique described by Ramsay (1935*a, b*) but these are discussed later (p. 261).

This was, however, not precisely Mellanby's conclusion. He stated that "the rate of loss of water is not strictly proportional to the saturation deficiency" although it nearly was so (p. 426).

Mellanby also concluded that "although the rate of loss of water was greatest in dry air, the rate of loss was relatively greater in moist air when the saturation deficiencies are compared" (p. 428). He concluded that the insects

Table 9. *The observed and expected rates of loss of weight (loss of water) from fasting adult C. lectularius at various temperatures and humidities. Data from Mellanby (1932)*

Expected rates are calculated from the linear regression equation for the best fitting straight line for the observed data (Fig. 9).

° C.	% R.H.	Sat. def. mm.	Rate of loss of weight (% of original wt. per day)	
			Observed	Expected
37	0	46.9	13.00	13.18
37	30	32.7	10.57	10.44
37	90	4.7	5.00	5.04
29	0	30.0	7.35	7.58
29	30	21.0	6.75	6.26
29	60	12.0	4.60	4.94
29	90	3.0	3.70	3.62
29	98	0.6	2.00	Not used in calculation
22	0	19.8	4.80	4.60
22	30	13.9	3.26	3.52
22	60	7.9	2.41	2.42
22	90	1.9	1.38	1.31
16	0	13.6	2.27	2.41
16	30	9.6	1.99	1.95
16	60	5.4	1.64	1.47
16	90	1.3	0.86	1.01
8	0	8.0	1.12	1.15
8	30	5.6	0.98	0.90
8	60	3.2	0.64	0.65
8	90	0.8	0.39	0.40

tended to conserve water in dry air but that they cannot prevent all evaporation because of their great proportion of surface area to volume.

These conclusions were reached as follows:

If two objects are losing water under different conditions at rates directly proportional to the saturation deficit, then

$$\frac{\text{loss of water}}{\text{saturation deficit}}$$

should be constant. If the period of the experiment is different in each case, then

$$\frac{\text{loss of water}}{\text{saturation deficit}} \times \frac{1}{\text{time}}$$

should be constant.

Mellanby calculated the values of

$$\frac{\text{loss of water}}{\text{saturation deficit} \times \text{time}},$$

which is the same as

$$\frac{\text{rate of water-loss}}{\text{saturation deficit}},$$

for the bugs kept under different climatic conditions. The values were not constant but varied, both with temperature and with different humidities

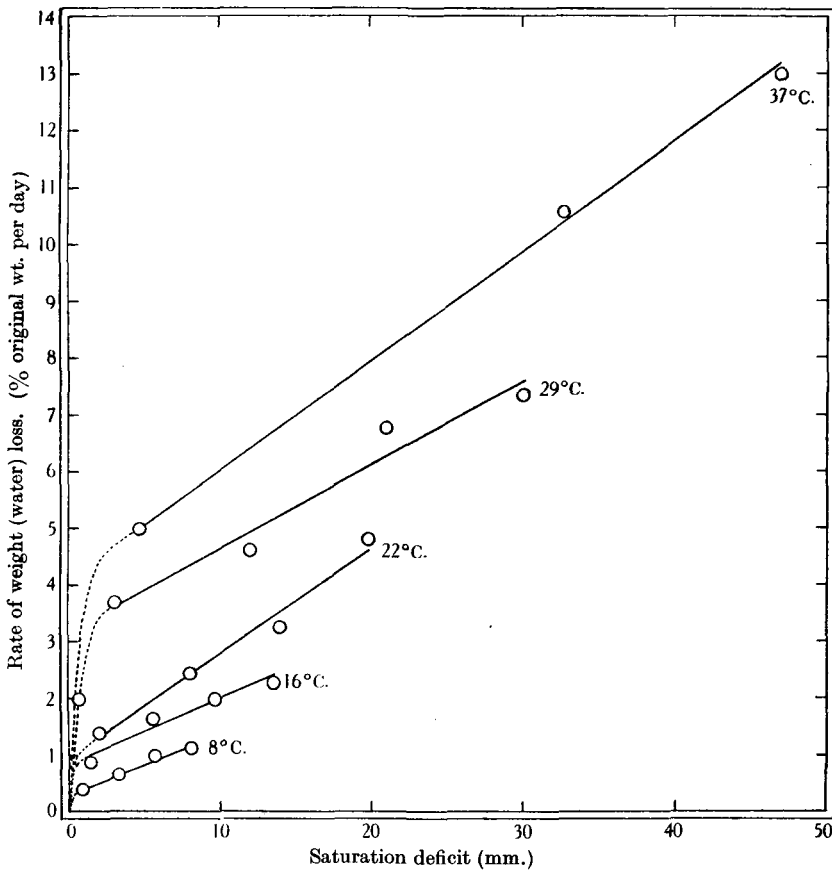


Fig. 9. Rate of loss of weight (loss of water) of fasting adult *C. lectularius* in relation to temperature and humidity. Unbroken lines are linear regression lines fitted to data of Mellanby (1932). Dotted lines fitted visually.

Table 10. *Regression coefficients, b , and their variances for rate of water-loss against saturation deficit at various constant temperatures. Calculated from data by Mellanby (1932)*

The regression coefficients express the effect of humidity on rate of water-loss at the various temperatures. The effect is greater at the higher temperatures (Fig. 9).

° C.	b	$v(b)$
37	0.1929	0.0000008
29	0.1463	0.000618
22	0.1838	0.000239
16	0.1142	0.000459
8	0.1051	0.000306

at the same temperature (Table 11). It was because these values were highest at the higher humidities that the conclusion was reached that the loss of water was relatively greater at high than at low humidities. Therefore, the rate of water-loss was not considered to be directly proportional to the saturation deficit even at constant temperatures.

Table 11. Values for $\frac{\text{loss of water}}{\text{saturation deficiency}} \times \frac{1}{\text{time of exposure}}$ calculated by Mellanby (1932) for fasting adult bed-bugs

° C.	Days	R.H. %			
		0	30	60	90
37	3	0.27	0.315	—	1.04
29	4	0.24	0.322	0.38	1.25
22	8	0.242	0.233	0.302	0.69
16	14	0.168	0.21	0.306	0.63
8	28	0.15	0.179	0.201	0.49

This argument is fallacious, however. While it is true that if rate/saturation deficit is constant the rate is directly proportional to the saturation deficit, the converse is not true. For if rate/saturation deficit is not constant, as Mellanby

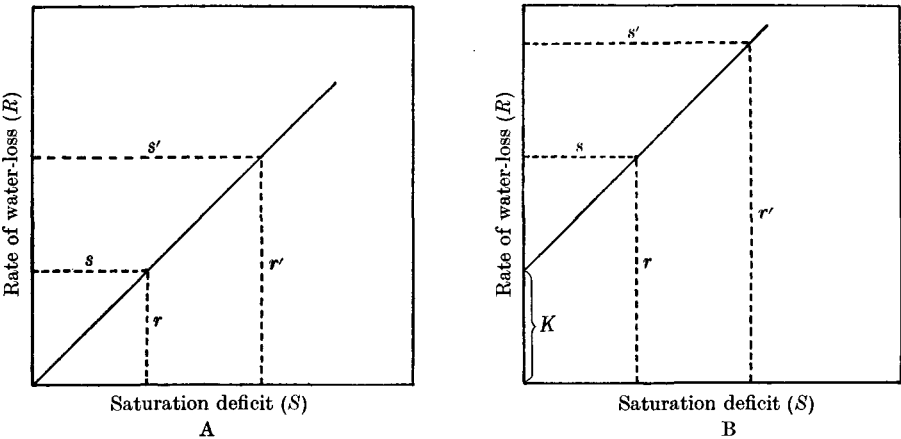


Fig. 10. Illustrating rate of water-loss (R) when it is directly proportional to saturation deficit (S).
(A) $R/S=b$ or $r/s=r'/s'$;
(B) $R/S=K/S+b$ or $R=K+bS$;
and r/s does not $=r'/s'$.

found, then the rate may still be directly proportional to the saturation deficit for the following reasons:

If the rate of water-loss is directly proportional to the saturation deficit, i.e. if rate increases with increase in saturation deficit in a linear manner (Fig. 10, A, B), then the following relationships may arise:

1. $r/s=r'/s'=\text{constant}$,
 $R=bS$

or $R/S=b$ (Fig. 10, A).

Mellanby assumed that this always happened if rate was directly proportional to saturation deficit.

2. r/s does not $=r'/s'$ and is not constant,
 $R=K+bS$

or $R/S=K/S+b$ and as S increases $K/S+b$ diminishes (Fig. 10, B).

So that for R/S to be constant the linear part of the graph of rate against saturation deficit must always go through the origin. At different temperatures however (Fig. 9) the linear graphs between 0 and 90 % R.H. lie one above the other and the expression K varies with temperature. Some of Mellanby's conclusions must, therefore, be modified, and others can be stated.

(1) $\frac{\text{Rate of water-loss}}{\text{Saturation deficit}}$ is not always constant although the rate of water-loss may be directly proportional to saturation deficit.

Rate = $K + b$ (saturation deficit).

But if the graph was linear from 0 to 100 % R.H. and it passed through the origin only then would $K = 0$ and rate = b (saturation deficit) or $\frac{\text{rate}}{\text{saturation deficit}} = b$, which is constant.

(2) The rate of water-loss (i.e. loss in weight) is directly proportional to saturation deficit at constant temperatures between 8 and 37° C. and between 0 and 90 % R.H. at each temperature. The rate of evaporation is, therefore, not relatively higher at 90 % R.H. than at lower relative humidities.

(3) There is then no evidence that the insects tend to conserve water in dry air.

There are no data on the rate of water-loss in the bed-bug above 90 % R.H. except at 29° C., 98 % R.H. given by Mellanby. But, whatever the temperature, it is unlikely that bugs would evaporate water into completely saturated air. Indeed Mellanby records that under those conditions no weight loss was detected. The graphs in Fig. 9 should, therefore, all be continued to meet at the origin at 0 mm. saturation deficit and 0 % per day weight loss. There is, therefore, a departure from linearity at the very high humidities: and at each temperature the graph is steeper above than below approximately 95 % R.H. A similar humidity change, has, therefore, a greater effect on the rate of water-loss above than below 95 % R.H. Perhaps, as Mellanby suggested, the spiracles are open for most of the time in very wet air and closed more frequently at lower relative humidities. It is possible, too, that the loss of weight is not a very accurate measure of the loss of water and that an error, which varies with temperature, probably accounts for the greater steepness of the graphs above 95 % R.H.—also the variation in the temperature of K in the equation $R = K + bS$ may possibly be due to either of these things. For K , it will be realized, indicates the point on the ordinate at which the inflexion of the graph occurs¹.

Another attribute thought to be possessed by the insects, which followed on the original assumption that R/S is always constant, was that a relatively

¹ Since going to press, Dr K. Mellanby tells me that from subsequent observations he imagines that small quantities of clear fluid were sometimes excreted by bugs in his experiments (1932); and that in *Ornithodoros moubata* (Ixodidae) urination is greater at high than at low humidities. If this occurs also in *Cimex* it may account for the nonlinearity of the weight-loss graphs near 0 mm. saturation deficit and therefore for the expression K .

greater rate of water-loss at 90 % R.H. than at lower humidities kept the wet-dry ratio of the insect constant. It was thought that if the loss of weight was directly proportional to the saturation deficit the bugs would become dropsical at 90 % R.H. We have seen, however, from the longevity curves that dropsy probably does occur and that it may account for the departure of these curves from hyperbolas. But if Mellanby's data are analysed it is seen that, although the proportion of dry matter at the end of starvation remains constant at 90 % R.H. for all temperatures, the amounts of water lost are also approximately the same. Since more metabolic water is produced at high than at low temperatures the wet-dry ratio should increase if the same quantities of water are lost. The data do not show this, probably because the amounts of water lost at 90 % R.H. are small compared with the errors in weighing the dried insects.

(4) At any constant saturation deficit between 8 and 37° C., the rate of water-loss from *Cimex* is higher at the higher temperature. This is to be expected from the higher rate of metabolism and presumably with the more frequent opening of the spiracles at the higher temperatures: the rate of diffusion of water vapour away from the bug is also increased (see p. 261).

(5) The graphs for rate of water-loss against saturation deficiency are steeper below 90 % R.H. at the higher than at the lower temperatures. Therefore, at any constant temperature between 8 and 37° C. the effect of the same change in humidity on the rate of water-loss from *Cimex* is greater at the higher temperatures. This is expressed by the regression coefficient *b* (Table 10) which indicates the steepness of the graph.

The effect of temperature on the rate of evaporation from the cockroach has been studied by Gunn (1933) and by Ramsay (1935*b*). Both these authors found that just above 30° C. the rate of evaporation increased suddenly. This increase was attributed by Gunn to an increase in respiratory movements, but Ramsay has shown that increased evaporation occurs from the body surface and evidently coincides with a phase change in the fatty film on the surface.

In *Cimex* there is no very abrupt increase of rate of water-loss at about 30° C. but above approximately 20° C. the curve rises rather more steeply (Table 12, Fig. 11). It is not known if this coincides with evaporation through

Table 12. *The rate of water-loss (% original weight per day) from the adult bed-bug in relation to temperature (Fig. 11). Values obtained by interpolation from Fig. 2 from data by Mellanby (1932)*

° C.	Saturation deficit (mm.)		
	5	8	13
37	5.1	5.7	6.7
29	3.9	4.4	5.1
22	1.9	2.5	3.4
16	1.4	1.8	2.4
8	0.8	1.2	—

the body wall: if so then much evaporation in nature must occur in that way. It is more probable that, from experimental error, the values for 22° C. are too small or those for 29° C. too large, thus causing an apparent sudden increase in rate: the lines for rate of loss against saturation suggest this.

I have discussed what have appeared to me to be the most likely effects of climate and food on longevity. From the statistical point of view the meagre data (particularly for fed 1st instars) have perhaps been strained to form hypotheses: but these seem to be consistent with the data and with what would be expected from physiological principles.

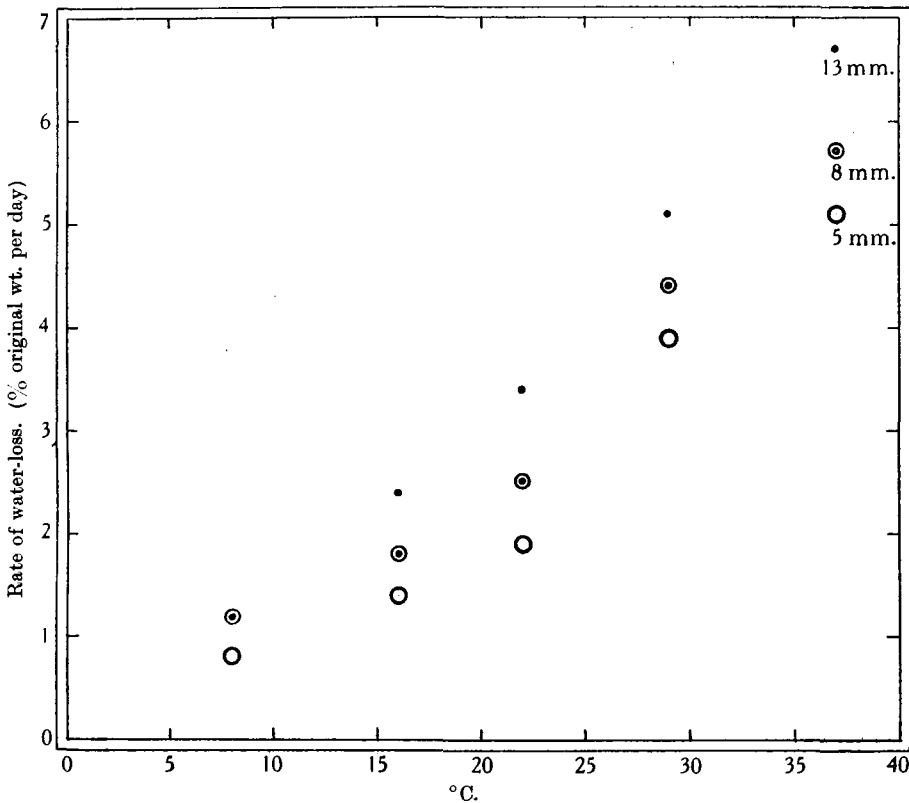


Fig. 11. Rate of water-loss of adult *C. lectularius* in relation to temperature at constant saturation deficits of 13, 8 and 5 mm. (Table 12). Values found by interpolation from Fig. 2.

Mellanby's experiments and my own were carried out with bugs in still air—except for adult longevity which has not been used in the discussion on the saturation deficiency law. Ramsay (1935*a, b*) has shown that results from experiments with still air may contain serious errors, mainly due to the building up of a higher humidity in the immediate neighbourhood of an insect than is reckoned with; but also to the varying degree with which this water vapour may be dispelled by diffusion which is itself strongly influenced by temperature.

The relative positions of the curves for longevity and for rate of water-loss against saturation deficit at the different temperatures may, of course, depend to some extent on the different rates of diffusion of water vapour away from the bugs. But even an adult bed-bug is a relatively small insect and its rate of water-loss is very slow. Mellanby moreover kept his insects singly in tubes: while the results for the minute 1st instars resting on voile only about an inch away from the acid-water mixture controlling humidity would no doubt contain the very minimum of error from the building up of a microclimate. The high and almost identical values for the correlation coefficients for rate of loss and for longevity against saturation deficit (p. 248) with fed adults and with the very much smaller unfed 1st instars suggests that this error may be small.

It is well to remember that mean values have been used in the discussion on longevity and rate of water-loss and a hypothesis has been formed which deals with aggregates, not with individual insects: departures by individual bugs from the average behaviour may be very great indeed.

(4) Longevity in relation to the blood from various hosts

The effect of the blood of different hosts on the longevity of bed-bugs has been discussed in a previous publication (Johnson, 1937): but the survival times of bugs fed on man and on rabbit were not compared. It is necessary to know if there is a great difference between the longevity with the bloods of these two hosts since rabbit, not man, which is the usual host in nature, has been commonly used for the longevity experiments in this paper.

1st-2nd instars (Table 13).

With bugs fed on man (A. H.) and on rabbit there is a slightly longer survival time with man at the higher humidity. No difference occurs at 10 % R.H. when the life is quite short.

Table 13. Length of life of 1st-2nd instars of *C. lectularius* at 25° C., 90 % and 10 % R.H. Eggs were laid and 1st instars were hatched at 23° C. and were fed 3 days after hatching on rabbit and on man (A. H.) at 25° C. Only bugs which moulted into 2nd instars are considered below. Longevity dates from hatching

Rabbit					A. H.				
% R.H.	No. fed	No. moulted	Mean length of life and stand. dev. (days)		No. fed	No. moulted	Mean length of life and stand. dev. (days)		Signif. test
10	50	43	21.73	2.60	70	40	21.55	2.42	Not signif.
90	50	42	52.85	7.86	65	42	60.24	9.87	Signif.

4th-5th instars (Table 14).

Comparison between the survival times of 4th-5th instars when fed on two different men (A. H. and C. J.) show a significantly longer survival on A. H.

than on C. J. This result is in agreement with a previous experiment (Johnson, 1937). The experiment in Table 14 on 4th–5th instars was really undertaken to see if the longer life on A. H.’s blood was due to a larger feed being taken than from C. J. That this is not so is evident; for larger feeds were taken from C. J. yet bugs lived for the shorter time on his blood.

Table 14. *Mean and maximum length of life of 4th–5th instars at 22.5° C., 75 % R.H. and the weight of blood-meal. Bugs were reared on man (A. H. and C. J.) with one feed per instar at 22.5° C. 75 % R.H. Unfed 4th instars were selected and allowed to feed once, to repletion, on A. H. or on C. J. 9 days after moulting. These were allowed to moult and the 5th instars were allowed to starve to death without further food. Longevity dates from feeding of 4th instar*

Group	No. bugs	A. H.				C. J.				
		Mean length of life (days) and s.d.	Mean wt. of unfed 4th instar (mg.)	Mean wt. of blood-meal (mg.)	Maximum length of life (days)	No. bugs	Mean length of life (days) and s.d.	Mean wt. of unfed 4th instar (mg.)	Mean wt. of blood-meal (mg.)	Maximum length of life (days)
A	5	158.2	1.05	4.77	175	4	139.6	1.04	6.91	174
B	5	146.2	1.10	4.37	175	4	123.0	0.93	5.82	128
C	5	141.8	1.15	4.63	178	4	166.6	1.38	6.37	174
D	5	127.4	1.10	4.64	134	4	120.3	1.19	6.01	139
E	4	139.5	1.13	4.81	171	4	146.4	1.25	5.75	168
F	4	130.8	1.09	4.40	147	3	93.0	0.97	5.40	99–105
G	4	126.3	1.21	4.99	134	4	149.1	1.40	6.04	175
H	4	125.5	1.26	5.36	141	3	155.8	1.39	5.41	165–168
J	4	158.8	1.29	5.19	179	3	99.0	1.09	5.68	114
K	4	140.3	1.14	4.31	160	4	101.8	1.15	5.24	111
L	4	139.0	1.23	4.94	157	4	124.6	1.19	4.95	160
M	3	152.3	1.17	4.86	162	2	130.0	1.07	5.53	133
Gen. means and totals	51	140.15 8.99	1.15	4.76	159.4	43	130.17 11.98	1.18	5.79	145.4

Difference of general means for longevity is significant.

Thus, the differences which exist between rabbit-fed and man-fed bugs are not likely to be important in the interpretation of these experimental results to longevity under natural conditions. Other factors, particularly activity, probably outweigh such differences.

(5) *Longevity in relation to the size of the bug and the size of the meal*

It was thought that individual variation in longevity might be correlated either with the weight of the meal taken (bugs were allowed to feed to repletion), the weight of the bug, or the weight of the meal per milligram of bug. But correlation coefficients from each of these with longevity on 4th–5th instars and on fertilized females at 22.5° C. and 75 % R.H. and 23° C. and 90 % R.H. respectively were very small and not significant. With a large amount of

data a significant correlation with the weight of blood taken may be found but it is likely to be exceedingly small with fully fed insects.

(6) *Virginity and survival*

Table 4 gives the data. Females which are given one feed, kept with a male and allowed to lay eggs and then starved till death do not live so long as unmated females fed once. Copulation appears to have no effect on the length of life of the males: for those which were kept unmated and singly in tubes live precisely as long as those kept permanently with a female. These results apply to 23 and 7° C., although the females laid no eggs at 7° C.

This result agrees, as far as the effects of fertilization are concerned, with that of Mellanby (1939). I do not agree, however, that in itself the shorter life of the fertilized bug can be used in argument against Cragg's theory that the sperm has nutrient properties, for, even if these existed, the increased metabolic rate following fertilization may mask nutrient effects of the sperm.

Like Kemper (1930) I find that with mated adults males outlive females except at very low temperatures. With virgin bugs, however, females live longer than males.

(7) *Discussion of discrepancies in the results of various workers*

In making comparisons between the survival times for *C. lectularius* recorded by other workers and those from my experiments I have used only records which state, in some degree, the conditions under which the experiments were made. But even so, it is not easy to draw conclusions from the comparison: for full details of experiments are often not stated. Geisthardt's values, moreover, are maximum not average survival periods, and he gives no indication of the numbers of bugs used nor of the variations in survival times.

1st instar.

The results of Mellanby (1935*a*) have already been discussed: the insects he used were from the same stock as those in my experiments and it is seen from Fig. 2 that Mellanby's data for 30 and 36° C. fit well into my system of graphs. Bacot's (1914) figures at 31 and 32° C. are also reasonably close to mine. But Bacot's data at 24° C. show a much shorter survival period than either Kemper's or my own data at comparable temperatures. At 22° C. bugs lived much longer in Kemper's experiments than is to be expected from my data: and the survival times recorded by Jones (1930) are, too, somewhat longer than in my records.

Adults.

Similar differences exist for adults, but here comparison is more difficult, for the virgin or mated condition (which is not always indicated) has a strong influence on the length of life of fasting bed-bugs. Moreover, in Kemper's work adults were not fed after moulting and Geisthardt's bugs were of unknown age ("Für meine Versuche wurden nur Tiere mittleren Alters verwandt, die aus

den Untersuchungen über die Eiablage stammten") when the experiment started. Moreover he states neither the mean survival nor the proportion of the sexes in his experiments: the latter is important in view of the differences in longevity between males and females. But in spite of these differences, which in general would tend to give shorter survivals than I have recorded, the opposite is generally the case. This applies also to 5th instars, for although the survival times recorded by Kemper are almost identical with those in my experiments (Table 14) Kemper kept his bugs at a lower humidity (40–45 % R.H. compared with 75 % R.H. in my work): his experimental temperature was almost the same as mine (22° against 22·5° C.).

My previous results on the same *Cimex* stock as I have used recently compare quite well with the present data. If the unmated adults (Johnson, 1937, Table 9) are excluded for reasons stated below,¹ the survival times for adults are not widely discrepant if the bugs fed on man are compared with those fed on rabbit (Table 4).

Let us consider the longevity of fed 1st instars (2nd instars unfed). Fed 1st instars (1st–2nd instar) in Kemper's experiment lived 83·65 days at 22° C. and 40–45 % R.H.: these were fed on man. In my previous experiments (Table 13, 1937) at 23·5° C. and 75 % R.H. the survival times on man were 46·23 days (on A. H.) and 37·59 days (on C. J.). Thus Kemper's fed 1st instars were very much longer lived. A direct comparison of Mellanby's results (1935*a*) and mine is possible for we both used the same stock and worked at 30° C., 0 % R.H. My experiments give 19·50 and 19·34 days and Mellanby's 9·96 days. The difference in host blood could scarcely account for this discrepancy, and the tendency is for man-fed bugs to survive longer.

On the other hand if fed 1st (unfed 2nd) instars in Table 7 are compared with the results set out in Table 13 and in my previous paper we see that my own results compare well with each other—e.g. at 25° C. rabbit-fed bugs lived for 23·8 and 53·8 days at 7 and 90 % R.H. respectively (Table 7 this paper), and in another experiment carried out at an interval of several months from the other the survival times were 21·73 and 52·86 days at 10 and 90 % R.H. respectively (Table 13 this paper). In the latter table bugs survived on blood from man (A. H.) 21·55 and 60·24 days at 10 and 90 % R.H. and 25° C. which compares quite favourably with 46·23 at 23·5 and 75 % R.H. in the previous (1937) experiment and when the same host was used.

How are we to account for the discrepancies between the results of various workers? It seems to me that there are several possibilities which may occur and which may each or all influence the results: and that in the present state of knowledge it is difficult to say which is responsible.

¹ In previous experiments (Johnson, 1937, Table 9) I made the reservation that the length of life might have been influenced by the fact that the bugs had wire loops around the pronotum (Mellanby, 1932) and were weighed at intervals. Thus, as well as disturbing them considerably by the weighing, the wire may have caused an abnormal amount of movement. This method was discarded in the other experiments on longevity in the same paper: it was not used at all in later work.

(1) Differences in technique. This involves errors in control of humidity and less so with temperature: also, as Mellanby has pointed out (1938, 1939), differences in the amount of activity will be induced in the insects by different methods of inspection.

(2) Variation in the constitution of the insects used by different workers.

(3) Differences in hosts used and in the actual amounts of blood ingested (if bugs do not feed to repletion) both before and at the start of the experiment. And even if two different hosts of the same species are used (e.g. man) variations in survival times will occur when the conditions of the experiments are otherwise identical (Johnson, 1937).

(4) Conditions under which bugs had been reared previous to the experiment. If, for example, bugs are bred at 28° C. with two weekly feeds adults will lay eggs without a meal after the last moult: but this will not occur if bugs are bred at 23° C. with one feed per instar. Presumably more blood is carried over after the last moult with the former conditions.

(5) Small numbers of insects may give erroneous results although statistical tests are applied, and significant differences are found. For example, the quantities of food ingested during development may be widely different even if the insects are not fed as adults, and the use of very small samples will increase the chances of error.

Mellanby (1938) claims that the differences between the results of Kemper, Geisthardt and Johnson depend on the extent to which the technique of the different workers disturbs the insects, thus causing them to run about and to die quickly, and attributes the very long survival in his experiment to absence of such disturbing factors. But we do not know what methods have been used by the various workers to detect whether an insect was dead. The errors due to disturbance are undoubtedly very important indeed but there are others which, I think, must also be considered. Also I feel that Mellanby's experiments (1938, 1939) may be open to objection on one or more of the variables listed above. Since, in general, my results show shorter survival times than those of other workers, it would be reasonable to suppose that, if Mellanby's view is the correct one, more activity was induced with the bugs in my experiments than in most of those of other workers. I therefore give below the details of the method of inspection which I employed.

Bugs were inspected usually at intervals of 3-6 days. Adults were kept singly or in pairs and 1st instars in batches of 30-50 in 2 × 1 in. tubes with voile on both ends. No paper was put in the tubes and the insects rested on the voile. They thus had comparatively little space on which to move about.

When adults were inspected they were touched with a blunt needle which was pushed up through the voile. If alive they moved a leg or shifted their position by about a millimetre. Bugs were markedly disinclined to move when touched and I never saw one run across the voile as the result of this treatment. It is not easy to tell if an adult is dead merely by looking at it: for after death

the insect may cling to the voile or to paper if it is rough and have the usual appearance of a live insect.

With first instars the tube was held on one side and tapped gently: dead bugs then fell from the voile and could be examined. By experience I know that this method is rarely fallible and it is seldom necessary to touch a bug with a needle to tell if it is alive or dead. Sometimes of course live bugs fall with the dead ones and this over a long period may cause some activity. But usually the handling or tapping of a tube causes the live bugs to tighten their grip. As with adults the 1st instars sat still and were rarely seen to move more than a few millimetres. This applies particularly to temperatures of 15° C. and lower and it is doubtful whether activity became a serious factor in causing error at these temperatures. It was necessary to touch bugs at 7° C. to detect death but this seemed to involve no subsequent activity.

Movement, on the other hand, may have occurred at other times due to stimuli such as switching on a light in the constant temperature room: but bugs are very reluctant to move even when a strong light is turned on them once they have become ensconced in an angle between voile and tube. And the amount of movement at times when bugs were not seen (e.g. at night) is quite unknown—but this applies also to Mellanby's experiment (1938, 1939).

(8) *Some maximum times of survival*

Kemper found that, of all the stages, the 5th instars and the adult males were able to survive starvation longest. His data at 22° C., 40–45 % R.H. in days are

Mean survival (days)	Instars					
	1 unfed	1–2	2–3	4–5	5th adult	
					♂	♀
	46.3	83.7	126.4	141.8	142.6	130.6

My own results show a somewhat similar relationship. Omori (1938) working with *C. lectularius* at 0° C. concluded that fed adults survived longest and could live for more than 175 days while few unfed 1st instars lived more than 150 days. Geisthardt however found that 1st instars hatched at 18° C. lived up to 180 days and attributes the long survival time at this temperature to the fact that the eggs hatched at 18° C. and not at 27° C. In my experiments maximum survivals for unfed 1st instars were 210–13 days at 7° C. and 90 % R.H. and 355–60 days for 2nd instars at 15° C. and 90 % R.H. Bacot (1914) kept bugs fasting in various stages of development in an outhouse for 18 months and induced them to feed after this period.

With adults Geisthardt records 326 days at 14° C., but since the bugs were kept at 22° C. for 2 days before the experiment he estimates that they may have lived for 600 days had they been kept at 14° C. continuously. The longest lived adult in my experiment was a female at 13° C., 90 % R.H. (probably about

optimal for survival): she died between the 562nd and 572nd day after feeding. Although this bug was kept for many months with a male she laid no eggs (13°C . is the ovipositional threshold) so there is some doubt if she was mated: but if so then this period of survival would be by no means the maximum, for virgin bugs are the longer lived. It is conjectural whether it would have fed and oviposited towards the end of the starvation period.

Thus it may be expected that, if a house has remained unoccupied for a long time, 5th instars and adults (particularly unmated females) would be most likely to predominate in the surviving population. But until the effects on longevity of the variables listed on p. 266 have been worked out it is not possible to say what the maximum survival time could be.

IV. SUMMARY

1. With unfed 1st instars the relation between mean length of life and saturation deficit at constant temperatures between 7 and 15°C . at relative humidities between 7 and 90% is hyperbolic. The relationship becomes more linear at higher temperatures.

At constant saturation deficits insects live longer at 15°C . than at lower temperatures. Longevity also decreases with rise of temperature above approximately 15°C .

2. In general, the longevity curves, except for those at temperatures below 15°C ., bear a very similar relation to saturation deficit and to each other as the reciprocal curves for rate of water-loss at the different temperatures.

The influence of climatic factors on longevity at constant temperatures is discussed at length and it is concluded that over much of the temperature and humidity range survival time is limited by water-loss. At the higher humidities it is thought that either food, or perhaps an excessive accumulation of water within the insect, limits survival and causes a departure from the hyperbolic relation of longevity to saturation deficit.

3. The effects on longevity of a single meal are discussed. The principal effect of a blood meal is to increase the time of survival. But the factors which limit survival at different humidities appear to be the same as with unfed bugs, except at high humidities below about 15°C .

4. Mellanby's data on the rate of water-loss from fasting adult bed-bugs is analysed. It is found that the rate of water-loss is directly proportional to saturation deficit at constant temperatures between 8 and 37°C . and between 0 and 90% R.H. Although the rate may always be directly proportional to saturation deficit, the expression $\frac{\text{rate}}{\text{saturation deficit}}$ is not always constant.

Rate = $K + b$ (saturation deficit), where K varies with temperature and b remains constant.

5. Longevity in relation to host blood is discussed. Rabbit blood appears to be slightly less favourable to survival than human blood.

6. If bugs are allowed to feed to repletion, longevity is not correlated with the size of the meal, nor with the weight of the unfed insect.

Virgin females live longer than mated ones, but no effects of mating on survival were noticed with males. Mated males tend to outlive mated females except at very low temperatures: virgin females live longer than unmated males.

7. The results of other workers and the possible causes of some discrepancies are discussed.

8. The maximum survival times of bugs are listed. Adults and 5th instars live longer than other stages. In a house which has remained empty for a long time it is probable that 5th instars and adults, particularly unmated female adults, would predominate in the population.

The longest observed survival was between 562 and 572 days.

I am greatly indebted to Prof. P. A. Buxton for his generous help, to Dr W. J. Martin for advice on statistical matters and to the Medical Research Council who supplied the grant.

REFERENCES

- BACOT, A. W. (1914). The influence of temperature, submersion and burial on the survival of eggs and larvae of *Cimex lectularius*. *Bull. ent. Res.* **5**, 111–17.
- BUXTON, P. A. (1932). Terrestrial insects and the humidity of the environment. *Biol. Rev.* **7**, 275–320.
- FISHER, R. A. (1936). *Statistical Methods for Research Workers*. London: Oliver and Boyd.
- GEISTHARDT, G. (1937). Über die ökologische Valenz zweier Wanzenarten mit verschiedenem Verbreitungsgebiet. *Z. Parasitenk.* **9**, 151–202.
- GUNN, D. L. (1933). The temperature and humidity relations of the cockroach (*Blatta orientalis*). I. Desiccation. *J. exp. Biol.* **10**, 274–85.
- JOHNSON, C. G. (1937). The relative values of man, mouse, and domestic fowl as experimental hosts for the bed-bug, *Cimex lectularius* L. *Proc. zool. Soc. Lond. ser. A*, pt. 1, 107–26.
- (1939a). Development, hatching and mortality of the eggs of *Cimex lectularius* L. (Hemiptera) in relation to climate, with observations on the effects of pre-conditioning to temperature. (In the Press.)
- (1939b). The maintenance of high atmospheric humidities for entomological work with glycerine-water mixtures. (In the Press.)
- JONES, R. M. (1930). Some effects of temperature and humidity as factors in the biology of the bed-bug (*Cimex lectularius* Linn.). *Ann. ent. Soc. Amer.* **23**, 105–19.
- KEMPER, H. (1930). Beiträge zur Biologie der Bettwanze (*Cimex lectularius* L.). I. Über den Einfluss des Nahrungsmangels. *Zeit. morph. Oekol. Tiere*, **19**, 160–83.
- KIRKPATRICK, T. W. (1923). The Egyptian cotton-seed bug (*Oryzaphenus hyalinipennis*), its bionomics, damage, and suggestions for remedial measures. *Min. Agric. Egypt. Tech. Sci. Service Bull.* no. 35.
- MELLANBY, K. (1932). Effects of temperature and humidity on the metabolism of the fasting bed-bug (*Cimex lectularius*), Hemiptera. *Parasitology*, **24**, 419–28.
- (1935a). A comparison of the physiology of the two species of bed-bug which attack man, *Parasitology*, **27**, 111–22.

- MELLANBY, K. (1935*b*). The evaporation of water from insects. *Biol. Rev.* **10**, 317–33.
- (1936). Humidity and insect metabolism. *Nature, Lond.*, **138**, 124.
- (1938). Activity and insect survival. *Nature, Lond.*, **141**, 554.
- (1939). Fertilization and egg production in the bed-bug, *Cimex lectularius* L. *Parasitology*, **31**, 193–9.
- MELLANBY, K. & BUXTON, P. A. (1935). A toxic emanation from rubber. *Nature, Lond.*, **136**, 873.
- OMORI, N. (1938). Experimental studies on the influence of low temperatures upon the common bed-bug (*Cimex lectularius* Linnaeus). First Report: on the influence of a temperature of 0° C. *J. med. Ass. Formosa*, **37**, 1081–1103.
- RAMSAY, J. A. (1935*a*). Methods of measuring the evaporation of water from animals. *J. exp. Biol.* **12**, 355–72.
- (1935*b*). The evaporation of water from the cockroach. *J. exp. Biol.* **12**, 373–83.
- WIGGLESWORTH, V. B. (1931). Effect of desiccation on the bed-bug (*Cimex lectularius*). *Nature, Lond.*, **127**, 307–8.

(*MS. received for publication 21. XI. 1939.—Ed.*)