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DEVELOPMENT, HATCHING AND MORTALITY OF THE EGGS OF *CIMEX LECTULARIUS* L. (HEMIPTERA) IN RELATION TO CLIMATE, WITH OBSERVATIONS ON THE EFFECTS OF PRECONDITIONING TO TEMPERATURE

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(With 13 Figures in the Text)

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I. Introduction

This study of the relations of bed-bug eggs to climate is part of a wider programme on the ecology of the insect. But owing to its peculiarities, the egg stage may be considered separately; for eggs cannot move, and the added complexities associated with a motile organism capable of choosing an environment and of influencing its length of life by active movement (Mellanby, 1938) are absent.

Other workers (Bacot, 1914; Hase, 1917, 1930; Mellanby, 1935; Geisthardt, 1937) who have worked with *Cimex* eggs have experimented usually at temperatures above 13° C. (55·4° F.) which is the lowest constant temperature for complete development with hatching. In England, however, room temperatures are often between 0 and 13° C. for many months at a time (Johnson, 1938); and it is important to study eggs within this range since high egg mortalities undoubtedly occur when it is prolonged (Fig. 1).

The recent evidence that the threshold for movements of *C. lectularius* depends on the temperature at which bugs have been kept previously (Mellanby, 1939*b*) has necessitated a consideration of possible effects of acclimatization on the mortality and hatching of the eggs. Some experiments in this field are described below; these may have an ecological significance although their physiological bases are unknown. For this work is primarily ecological, and much of the detail described here has been merely incidental to the attempt to discover two important facts, namely, the longest possible time an egg can survive and the lowest possible temperature at which it can hatch.

II. Methods

The stock of insects came to us from Beckenham, England, in 1927, and ever since has been mass-cultured on rabbit at 23° C. Adults for experimental work were kept in $2 \times 1\frac{1}{2}$ in. tubes (males and females in approximately equal numbers) at 23 and 14–16° C. and were given an opportunity to feed twice a week and once a week respectively. Eggs were gathered every day from these bugs, and the very few unfertilized eggs which were laid were rejected. The eggs were detached from the blotting paper on which they had been deposited: this involved no mortality, as the following results of an experiment with eggs of the same batch show:

	No. of eggs	% hatch at 23° C., 90 % R.H.
Attached eggs	292	95·9
Detached eggs	325	95·7

Eggs were kept during an experiment in 2×1 in. tubes with voile over both ends. These tubes were placed either in small battery jars or in small bottles (150 c.c.), and the humidity of the air was controlled in the bottles by the appropriate mixture of sulphuric acid and water and in the battery jars by an

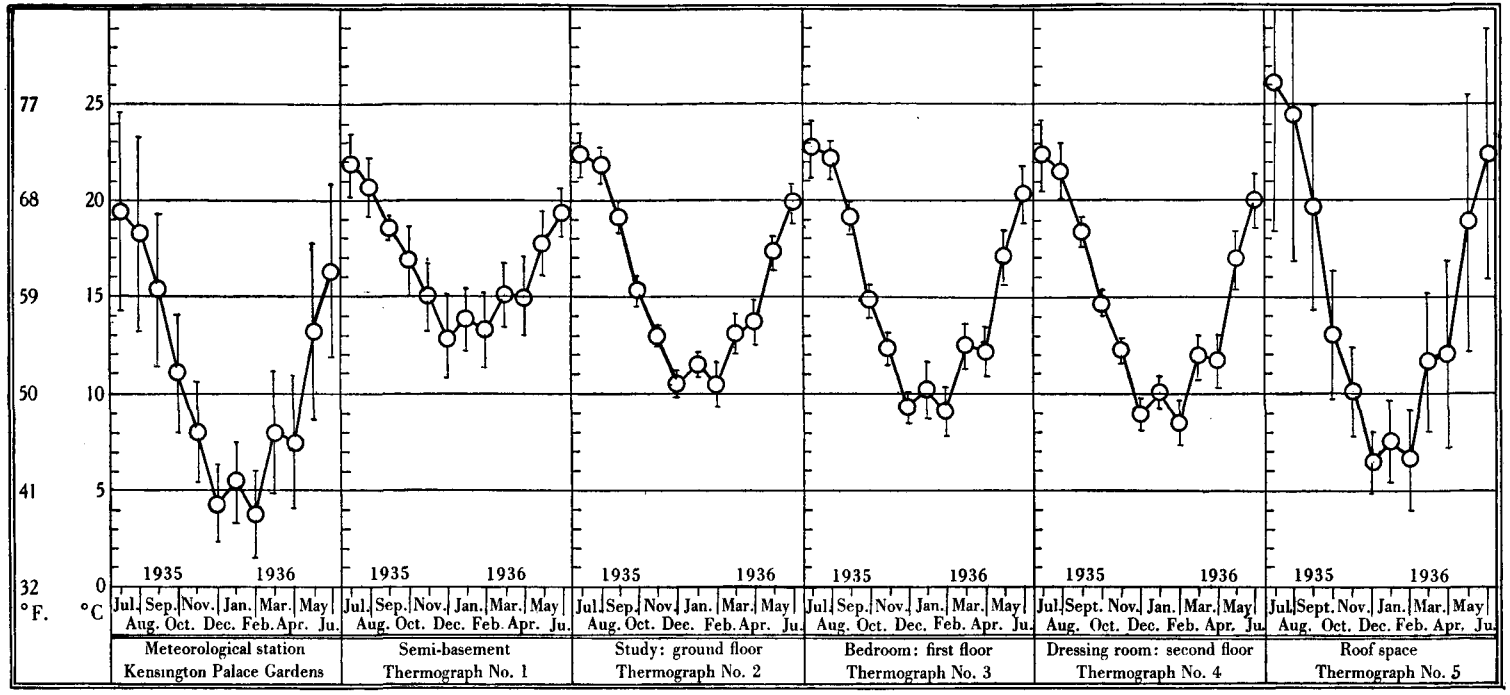

Fig. 1. Thermograph records from rooms of a house in Kensington, London, expressed as mean monthly temperatures and mean maximum and mean minimum monthly temperatures. Circles indicate mean monthly temperatures and vertical lines through the circles mean maximum and mean minimum monthly temperatures. I am indebted to the Cambridge University Press for permission to reproduce this figure from the *Journal of Hygiene* (see Johnson, 1938).

air stream humidified with a glycerine-water mixture for 90 % R.H. (Johnson, 1939) and an acid-water mixture for 5–10 % R.H.: the acid spray was removed by a glass-wool and cellulose filter. Battery jars were used for 5–10 and 90 % R.H. at the temperatures of 7–8, 10, 14, 18, 23 and 27·5° C. In other cases the small closed bottles were used with acid-water mixtures, but since these were usually opened at intervals of several days the humidity control was adequate. Corks and not rubber bungs were used with closed bottles in order to avoid toxic effects (Mellanby & Buxton, 1935). Humidities of air streams were checked at intervals of 3–4 days, and values given in the following tables are means with a variation of about ± 2 % R.H.

The experiments with eggs from 14 to 16° C. were usually done with relatively small numbers of eggs, for few eggs are laid at this temperature. In the mortality and survival experiments many eggs died while hatching. These have been counted as having died, not hatched.

All thermometers used were tested against an accurate standard thermometer.

III. The rate of development of eggs

(1) *The rate of development and the period between feeding and oviposition of the parent*

Hase (1917) noticed that eggs dissected from female bugs frequently contained embryos in an advanced stage of development. He suggested that variation in the duration of the egg stage was partly due to eggs being in different stages of development when laid, and his data showed that eggs dissected from a female took longer to reach the hatching time than those laid normally. His temperature control was not exact, however (22–26° C.), and the variations in duration of the egg stage could possibly have been due to temperature differences.

My own results (Table 1) support Hase's explanation. In fact, on one occasion an egg actually commenced to hatch within an exceedingly starved bug. If starved female bed-bugs are fed and then kept at 23° C. they start to lay eggs on the 5th or 6th day after the blood meal and will continue to oviposit for a period of 8–10 days without another feed. The later the eggs are laid under these conditions the sooner will they hatch, and although the differences in duration from day to day are slight they are often statistically significant and show the same trend. The data in Table 1 show an average daily decrease in the length of the egg stage of about 2·3 % up to the 12th day from the female's blood meal: this difference is the same at both the incubation temperatures used. The coefficients of variation show strikingly that the eggs which are laid late possess a very much greater variation in the time required to hatch than eggs laid soon after the meal. The variation in this time is very considerable with individual eggs; the longest and shortest observed times from oviposition to hatching were 3 and 14 days at 23° C. and 22 and 52 days at 14° C.

Table 1. *Mean duration in days from oviposition to hatching and standard deviation, of* C. lectularius *eggs laid at daily intervals after females were fed once. Females kept for oviposition at* 23° C (73·4° F.):90 % R.H. *Results for several independent egg batches pooled*

Eggs incubated at ...	23° C., 90% R.H.			14° C., 90% R.H.		
Interval blood meal to oviposition days	Mean time oviposition to hatching days	No. hatched	S.D.	Mean time oviposition to hatching days	No. hatched	S.D.
5	9·52	33	0·663	44·55	29	2·373
6	9·13	115	0·520	43·32	77	2·015
7	8·82	163	0·656	41·78	98	2·371
8	8·67	205	0·843	40·64	70	2·474
9	8·60	150	1·015	39·05	58	4·075
10	8·11	99	1·221	36·40	30	5·675
11	8·28	40	1·025	36·32	25	5·725
12	7·90	39	1·253	37·32	25	4·193
13	7·58	26	1·594	—		
14	5·75	12	2·740			

The observations in Table 1 may be explained by assuming that the later the eggs are laid after a meal the more advanced is embryonic development; for the embryos can develop inside the female before the eggs are laid, since fertilization occurs in the ovarioles (Cragg, 1920). At present these facts appear to have little ecological significance but they must be taken into consideration when experiments are being planned. The variability in survival of eggs when exposed to heavy naphtha fumigation (Gough, 1938) may be due to the use of eggs of different ages associated with the time of laying after the blood meal.

(2) *The rate of development at various constant temperatures and humidities*

Periods from oviposition to hatching of *C. lectularius* eggs kept at constant temperatures are given in Table 2. All eggs were laid at 23° C. (73·4° F.) and placed at the experimental temperatures within the first 24 hr. from oviposition. Under these conditions no eggs hatched at 37° C. (98·6° F.) nor at temperatures below 13° C. (55·4° F.). The times for development shown in Table 2 are not considered to have serious errors due to the factors discussed in the preceding section, since the stock females which laid the eggs were fed twice weekly, thus ensuring a rapid oviposition rate.

The present work confirms the observations of Mellanby and of Geisthardt that a wide range of humidity is without effect on the duration of the egg stage. Several experiments were made at 99–100 % R.H. (with the eggs kept above distilled water) and in those not upset by a growth of mould no lag in development at this high humidity, as reported by Clark (1935) for *Rhodnius* eggs, was observed.

To study the relation of temperature and rate of development the developmental times for different humidities have been pooled at each temperature (Table 3, Fig. 2). Geisthardt records a slight retardation in duration of the

Table 2. *Duration of the egg stage with standard deviation of* C. lectularius *from oviposition to hatching at various constant temperatures and humidities. Eggs laid at* 23° C.; 90 % R.H.

°C.	% R.H.	Duration in days: Mean	Duration in days: S.D.	No. hatched
37 ±0·6	—	—	—	0
35 ±0·3	—	4·56	0·73	68
34 ±0·3	7	4·59	0·51	74
	90	4·42	0·49	86
30 ±0·2	7	5·07	0·33	45
	75	4·54	0·54	48
	99–100	4·90	0·78	42
27·5±1·0	7	5·95	0·31	93
	75	5·94	0·31	90
	99–100	5·94	0·35	96
23 ±0·1	0–1	9·16	0·79	74
	7	8·87	1·22	90
	75	9·18	0·68	96
	90	9·00	0·95	162
	99–100	9·53	0·92	87
17·8±1·0	7	20·18	0·65	77
	75	20·16	0·97	86
	90	21·69	1·56	161
	99–100	20·65	0·86	51
16·1±0·1	7	29·23	2·46	161
	75	29·17	2·70	178
	90	28·65	2·87	341
14 ±0·1	90	40·66	4·29	412
13·1±0·3	90	49·24	4·19	17
13 ±0·1	13	—	—	0
	74	—	—	1
	90	39·00	—	1
	99–100	—	—	0

Table 3. *The duration of the egg stage and its reciprocal (developmental units) for* C. lectularius *eggs. Data as in Table* 2 *but values for the various humidities pooled at each temperature. Column five gives values for the thermal constants (day-degrees), i.e.* $(T-t)\,D$, *where* D *is the duration of the egg at temperature* T *and* t *is the developmental-hatching threshold,* 13° C. (*see Fig.* 2)

°C.	°F.	Mean duration days	$\frac{1}{\text{Duration}}$ dev. units	Thermal constant: the threshold taken as 13° C.
35	95·0	4·56	0·219	100·32
34	93·2	4·50	0·222	94·50
30	86·0	4·83	0·207	82·11
27·5	81·5	5·94	0·168	86·13
23	73·4	9·12	0·110	91·20
17·8	64·0	20·89	0·048	100·27
16·1	61·0	28·92	0·035	89·65
14	57·2	40·66	0·025	40·66
13	55·4	48·67	0·021	0

egg stage at 35° C. as compared with eggs at 33° C. I have found no significant retardation at these temperatures: unfortunately, it is not possible to test the significance of Geisthardt's values, and even so, the difference may be due to eggs having been laid at different times after the blood meal.

Table 3 and Fig. 2 show the relation of temperature to the reciprocal of the duration of the egg stage after oviposition. This is the temperature-velocity curve, and between 18 and 30° C. it appears to be approximately linear. Straight lines could, however, be drawn satisfactorily through all of the four points or through the three at each end of this range and a slightly different slope obtained for each graph. Moreover, the thermal constant is very irregular and does not show even a constant trend.

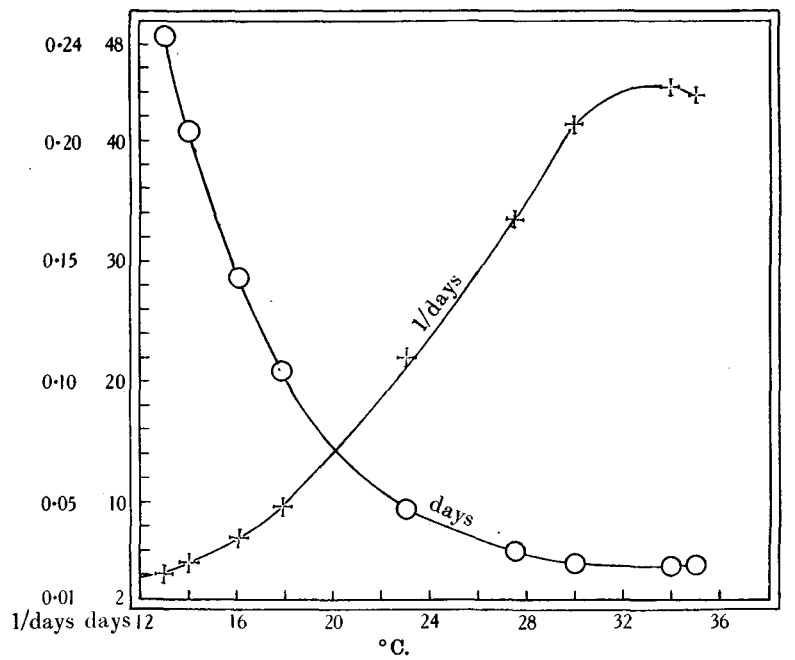

Fig. 2. The duration of the period from oviposition to hatching of *C. lectularius* eggs at constant temperatures and the reciprocals of these values plotted against temperature. Data in Table 3. ○ = duration; + = reciprocal.

(3) *Effects of some alternating temperatures on the rate of development*

Only two experiments were made with alternating temperatures: 90 % R.H. was used at all temperatures.

(1) Eggs were placed alternately at 23 and 13·1° C. for 24 hr. at each temperature until the maximum number had hatched. The transfer from one temperature to the other was abrupt, not gradual.

(2) A similar experiment was made with alternating temperatures of 27·8 and 17·5° C.

In the experiment at $23 \rightleftharpoons 13{\cdot}1$° C. control eggs taken from the same batch as the experimental eggs were kept at a constant temperature of 17·9° C. For the experiment at $27{\cdot}8 \rightleftharpoons 17{\cdot}5$° C. controls were kept at 23° C., but it is better

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to take an interpolated value at 22·7° C. from the curve in Fig. 2, since the means of the fluctuating temperatures did not keep exactly at 28 and 18° C. as originally intended at the start of the experiment.

The results of the experiments are:

	Mean duration	Mean duration at	
	days	23°	13·1°
(1) 23⇌13·1° C.	14·12	7·49	6·63
Control of 17·9° C.	21·69		
(2) 27·8⇌°17·5° C.	8·45	4·66	3·79
Expected at 22·7° C.	9·70		

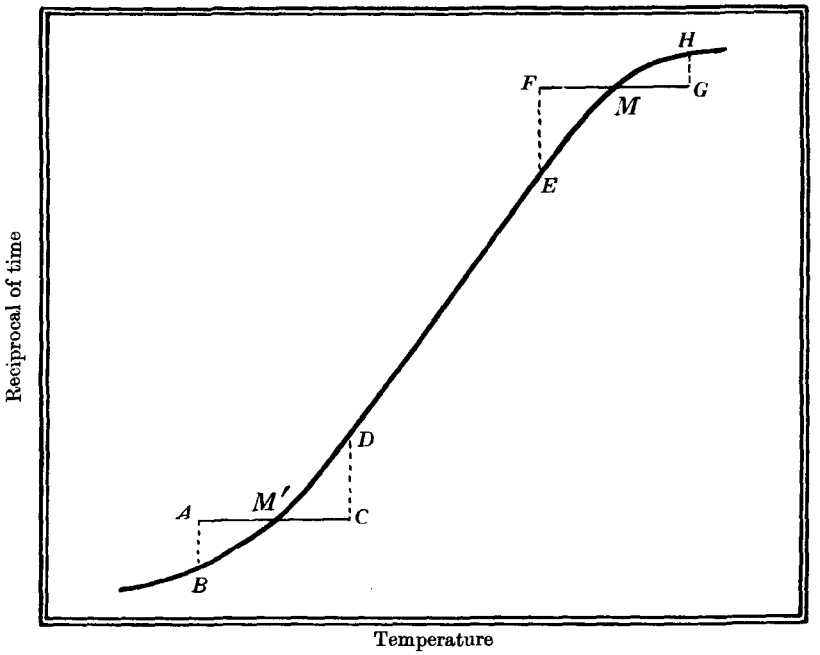

Fig. 3. A hypothetical non-linear temperature velocity curve which illustrates how an equal temperature fluctuation above and below a mean value can account for an acceleration or a retardation of developmental time. With the temperature fluctuation of *MG* and *MF* about the mean *M*, *EF* is greater than *GH* and, therefore, the fluctuation towards the lower temperature has a greater effect than the fluctuation towards the higher. Retardation of developmental speed compared with the speed at *M* is the result. With the temperature fluctuation of *M′A* and *M′C* about the mean *M′*, *DC* is greater than *AB* and, therefore, the fluctuation towards the higher temperature has a greater effect than the fluctuation towards the lower. The result is a shortening of developmental time compared with the time at *M′*.

There is thus a decrease in the time from oviposition to hatching when the temperature alternates between the limits used, compared with the time taken to hatch at a constant temperature midway between the alternating temperatures.

Now such a decrease at alternating temperatures may be due to two causes: either (1) an acceleration of development due solely to the temperature fluctuations above the mean producing a relatively greater effect than the fluc-

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tuations below the mean. This would happen with the non-linear temperature-velocity curve of *C. lectularius* (Fig. 3); or (2) an acceleration of development apart from (1) above, due to the temperature fluctuation itself.

A temperature-velocity curve may or may not possess a well-marked linear portion over a range of median temperatures. If it does not, or if it is curved over the range to be considered, the usual procedure of temperature summation is modified in the following way (Sanderson, 1910; Ludwig & Cable, 1933).

The amount of development which takes place in one day at any temperature is expressed by the reciprocal of the time for the completion of the whole stage at that temperature. Thus when the same individuals of a developmental stage, e.g. the egg, complete their development at a number of different temperatures, the sum of the products (reciprocal of time for complete development at a temperature × time spent at the particular temperature) for each of the different temperatures should be unity.

Actually this method tends to confuse development itself with time for development, and the expression "percentage of development" (Ludwig & Cable, 1933) has no meaning except as percentage of the time for complete development at one temperature. Thus the assumption is tacitly made that the effects of temperature on different phases of development within the developmental stage under consideration are the same as for the whole stage. That this is almost certainly not so is suggested by the work of Ludwig & Cable, who found differences in speed of development at the same alternating temperatures, according to whether the higher or the lower temperature was administered first (see also next subsection).

However, I have used this method with the data for *C. lectularius*. The calculations appear below:

$23 \rightleftharpoons 13{\cdot}1°$ C.

Amount of development at 23° C. represented by 7·49 (0·110)	=0·824
Amount of development at 13·1° C. represented by 6·63 (0·022)	=0·146
Complete development at $23 \rightleftharpoons 13{\cdot}1°$ C. represented by	0·970
Development of control at constant temperature 17·9° C. represented by 21·69 (0·049)	=1·063
Expected development at 18·0° C. (constant) by interpolation 20·25 (0·050)	=1·013

$27{\cdot}8 \rightleftharpoons 17{\cdot}5°$ C.

Amount of development at 27·8° C. represented by 4·66 (0·177)	=0·825
Amount of development at 17·5° C. represented by 3·79 (0·046)	=0·174
Complete development at $27{\cdot}8 \rightleftharpoons 17{\cdot}5°$ C. represented by	0·999
Expected development at 22·7° C. (constant) by interpolation = 9·7 (0·107)	=1·038

The values within brackets in the above calculations, except for that at 23° C., and the time 9·7 days at 22·7° C., were found by interpolation from the graphs in Fig. 2.

Thus with alternating temperatures unity is not quite attained, and the control and expected values at constant temperatures are slightly greater than unity: these departures from unity are within the limits of experimental error.

Therefore, it cannot be said that the developmental time for *C. lectularius* eggs is affected by the alternating temperatures used here, apart from effects due to the non-linearity of the temperature-velocity relationship. This conclusion is in agreement with the views of Ludwig & Cable (1933) for *Drosophila* pupae, that, if the alternate temperatures are both between the threshold and the optimum values (as they are in my experiments), then neither acceleration nor retardation of developmental time takes place apart from the effects associated with non-linearity of the temperature-velocity relationship.

Ludwig & Cable unfortunately do not explain how they have calculated the total time spent by the *Drosophila* pupae at each of the alternating temperatures. Slight differences in these values may be obtained when methods which do not involve weighting are used. Such differences will affect the approximation to unity (and the "percentage of development") for the alternating temperatures. I have adopted the following method for estimating the time spent at each temperature.

27·8⇌17·5° C.

99 eggs to start with: 92 hatched

Days exposure	°C. daily means	No. hatched	No. exposed throughout	No. exposed to 27·8° C.	No. exposed to 17·5° C.
1	27·4	—	92	92	—
2	17·8	—	92	—	92
3	27·5	—	92	92	—
4	17·1	—	92	—	92
5	27·7	—	92	92	—
6	18·0	—	92	—	92
7	27·8	22	92	92	—
8	17·7	10	70	—	70
9	28·1	57	60	60	—
10	17·1	2	3	—	3
11	28·2	1	1	1	—
	Weighted total time			429	349
	Mean time (weighted) spent at each temp.			4·66	3·79

Weighted total time, e.g. at 27·8° C. = 92 *eggs for (times)* 4 days) + 60 eggs for (times) 1 day) + 1 egg for (times) 1 day) = 429

$$\text{Mean time (weighted) spent at each temp.} = \frac{\text{Weighted total time}}{\text{Total no. of eggs exposed.}}$$

Amount of development represented by

4·66 days at 27·8° C. = 4·66 (0·177) = 0·825
3·79 days at 17·5° C. = 3·79 (0·046) = 0·174
8·45 days at both temps. = 0·999
Control: 9·70 days at 22·7° C. = 9·7 (0·107) = 1·038

(4) *Effects of exposure to low temperatures on the rate of subsequent development*

If eggs, newly laid at 23° C., are kept at constant temperatures below 13° C. they do not hatch. It is probable, however, that partial development occurs below 13° C. which may possibly be detected by comparing the hatching time of eggs incubated at 23° C. from oviposition with those brought to 23° C. from a prolonged exposure to a low temperature. Then if some development had occurred at the low temperature, the time for subsequent development at

23° C. would very likely be shorter than that of the control. This method neglects all possible acclimatization effects due to a long exposure to a low temperature, and these effects may perhaps be either a lengthening or a shortening of the time to hatching quite irrespective of any development having taken place.

The results from the mortality experiments in § VI below have therefore been analysed from this point of view. Hase (1930) has recorded that an exposure to a low temperature (2° C.) delayed the time of hatching on subsequent incubation at 25° C. In my experiments eggs were laid at 23° C. and put immediately at various temperatures between 0 and 13° C. (32·0 and 55·4° F.) for varying periods of time, after which they were incubated at 23° C. and 90 % R.H. and the mean time until hatching was recorded. Controls from the same batch of eggs (i.e. from the same females on the same day) were kept at 23° C. and 90 % R.H. from oviposition. In comparing exposed with control eggs one other factor in addition to a possible acclimatization effect must be borne in mind.

After a prolonged exposure to a temperature below 13° C. some eggs died. It is conceivable that those which died would have taken longer to develop at 23° C. than the survivors. Such a selection would give a shorter hatching time even if no development had occurred. If, however, the variation in hatching time is due to eggs being in different degrees of development when laid, the selection of weaker eggs would have the opposite effect; for, as shown on p. 156, eggs with older embryos are more easily killed by cold, and thus the subsequent hatching time would be prolonged rather than shortened.

However, in spite of these reservations, the results are set out in Table 4 and Fig. 4.

The results for the different mean temperatures may be summarized as follows:

1·0–1·1° C. (33·8–34° F.): At 5 % R.H. all differences between exposed and control eggs indicate a shortening, while at 89 % R.H. they suggest a lengthening of the subsequent developmental time. When the results of the two humidities are combined, there is no evidence that any development occurs even after a 28-day exposure.

4·1–4·3° C. (39·4–39·7° F.): Differences between control and exposed eggs indicate the possibility of a slight development after about 3 weeks' exposure. In two cases, 28–30 and 44 days' exposure, the differences are statistically significant.

7·7–7·8° C. (45·9–46·0° F.) and 9·8° C. (49·6° F.): There is some evidence that a very slight amount of development takes place at these temperatures.

11·7° C. (53·1° F.): A considerable amount of development presumably takes place at this temperature; it is evident with every exposure after the seventh day.

In the above results several statistically significant differences between developmental times for control and exposed eggs exist, which favours the

Table 4. *Duration and standard deviation in days of the egg stage of* C. lectularius *at* 23° C., 90 % R.H. *after exposures to low temperatures. Controls at* 23° C., 90 % R.H. *from oviposition.* (*See Fig.* 4)

Exposure days	No. hatched	Duration at 23° C.	S.D.	No. hatched	Duration at 23° C.	S.D.
	1·1° C., 5 % R.H.			1·0° C., 89 % R.H.		
7	18	10·06	1·90	20	9·50	0·50
14	11	9·56	0·88	18	9·20	0·71
21	4	8·25	0·44	2	9·50	0·51
28	1	8·00	—	1	11·00	—
Control	80	9·56	1·11	72	9·33	0·67
	4·3° C., 5 % R.H.			4·1° C., 89 % R.H.		
9	24	8·79	1·00	23	9·48	0·65
16	24	8·92	1·00	22	9·50	0·78
23	13	8·54	1·15	13	9·46	0·56
28	6	8·00	2·00	—	—	—
30	—	—	—	4	9·50	0·50
35	—	—	—	2	9·00	—
44	—	—	—	2	8·00	—
Control	36	8·80	1·10	73	9·42	0·71
	7·8° C., 9 % R.H.			7·7° C., 90 % R.H.		
7	19	9·32	0·66	21	8·76	1·27
14	21	9·05	0·95	30	9·00	0·89
21	13	7·77	0·70	24	8·38	0·76
28	14	8·71	0·59	28	9·00	0·26
35	2	8·00	—	22	8·82	2·17
42	—	—	—	2	9·00	1·00
			Controls			
	7, 14, 35 days			7 and 14 days		
	18	9·50	0·50	20	9·45	0·74
	21 days			21 and 35 days		
	13	8·62	1·15	19	8·79	0·77
	28 days			28 days		
	17	9·35	1·94	12	9·75	0·60
				42 days		
				28	9·36	0·97
	9·8° C., 6 % R.H.			9·8° C., 89 % R.H.		
7	28	7·61	1·01	16	8·69	0·98
14	32	7·59	0·96	26	8·62	1·00
21	24	7·58	0·45	30	8·43	0·89
28	10	7·80	0·60	16	8·38	0·78
35	—	—	—	5	8·00	1·10
Control	51	7·76	1·26	74	9·19	0·74
	11·7° C., 5 % R.H.			11·7° C., 89 % R.H.		
7	27	8·26	0·64	29	7·72	0·83
14	13	7·23	1·19	28	7·57	0·50
21	12	6·75	1·54	18	7·11	0·74
27	9	6·67	0·47	—	—	—
29	—	—	—	7	6·43	0·50
30	6	6·17	0·37	—	—	—
33	4	6·00	0·22	—	—	—
35	—	—	—	11	5·73	1·14
42	—	—	—	4	5·50	0·50
			Controls			
	7, 27, 30 days			7 days		
	44	8·80	0·62	19	8·26	1·02
	14, 21, 33 days			14 days		
	38	8·37	0·81	48	8·81	0·60
				21 days		
				18	9·06	0·70
				29, 35, 42 days		
				27	8·74	0·75

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view that slight amounts of development occur below 12° C. and particularly in the early stages, i.e. for the first 7 to 9 days' exposure. At 4° C., however, longer exposures seem to be necessary for these initial stages to proceed. It is the general trend of these differences which should be considered rather than the individual differences, since the numbers of eggs are sometimes

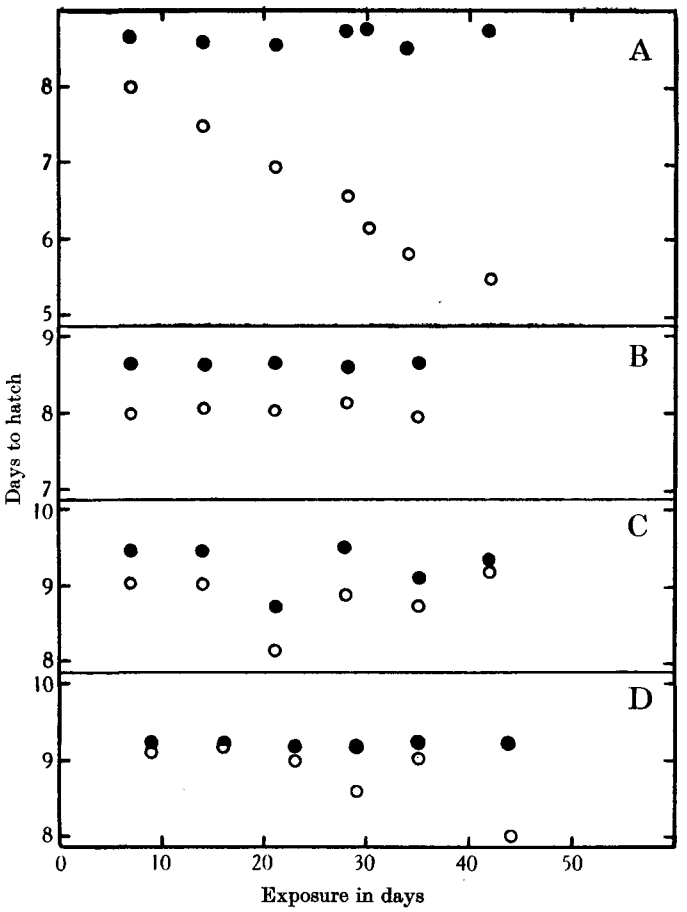

Fig. 4. Diagram to illustrate the shortening of developmental time of *C. lectularius* eggs incubated at 23° C. after exposure to low temperatures for various periods of time. Data in Table 4 pooled for different humidities. ○ Exposed eggs. ● Corresponding controls, not exposed but kept continuously at 23° C. A, 11·7° C. B, 9·8° C. C, 7·7–7·8° C. D, 4·1–4·3° C.

very small and the possibility that partial development had occurred before oviposition cannot be entirely dismissed, in spite of the fact that controls from the same batches were always used.

Thus, although the method employed is crude, it is possible that slight development occurs even at 4° C. after very long exposures and humidity

appears to have no consistent effect at the low temperatures. There is no evidence that, over the temperature range used, an exposure to a low temperature consistently *increases* the subsequent duration of the egg stage as claimed for *Cimex* by Hase (1930). It must be mentioned that Hase exposed eggs to 2° C. and reincubated them at 25° C., and that in my experiments at 1° C. there is apparently a lengthening of the hatching time after 28 days in 89 % R.H. which is statistically significant. Hase's results cannot be analysed statistically, and the difference he recorded is probably due merely to the variation in hatching time of different egg batches. Conclusions that development has occurred which are drawn from the appearance of the embryo (Hase, 1930; Omori, 1938) are unreliable, since even eyespots may be present immediately after oviposition; the view that after prolonged exposure to 2° C. a considerable percentage of the eggs develop but are unable to hatch (Hase in Uvarov, 1931) is probably due to this fact. In *Cimex* no acceleration of development after an exposure to a low temperature occurs as with *Melanoplus* (Parker, 1930) in which an exposure to 0° C. results in a greater subsequent acceleration than exposure to 8° C.

These data for *Cimex* can be used to illustrate the difficulties and uncertainties associated with the theory of thermal summation. For, although after exposure of the eggs to 13 and 11·7° C., subsequent times for development until hatching at 23° C. are shorter than the time for complete development at 23° C. from oviposition, they are longer than would be expected from thermal summation calculated from the temperature-velocity relationship for complete development (Table 3, Fig. 2). Data for 11·7° C. are given in Table 4. The results for 89 % R.H. will be considered. The data at 13° C., 0 % R.H. are as follows:

Mean duration from oviposition to hatching of seventy-four control eggs at 23° C. and 0 % R.H. = 9·16 days.

Mean duration from oviposition to hatching at 23° C. and 0 % R.H. after previous exposure to 13° C. and 0 % R.H. were

After 9 days at 13° C.,	40	eggs hatched in	7·60	days.
,, 17	,,	31	,,	6·36	,,
,, 25	,,	21	,,	5·50	,,

Then, if we suppose that the amount of development which occurs during exposure to a certain temperature is represented by (the number of days exposed) × (the reciprocal of the time for complete development at that temperature), the sum of these products for the period at the low temperature and the subsequent incubation at 23° C. should be unity. If the sum is greater than unity then the time taken to complete the development at 23° C. is longer than would be expected. The sums of the products at the temperatures under consideration are given in Table 5. At 23° C. reciprocals of control times were taken; at the low temperatures reciprocals were found from the temperature-velocity curve in Fig. 2; the value for 11·7° C. was found by extrapolation, since no eggs complete development at this temperature.

Table 5. *Thermal summation of time spent by* C. lectularius *eggs at* 11·7 *and* 13·0° C. *and later at* 23° C.

Duration at low temperature days	Sum of products (no. days at a temp.) × (reciprocal of time for complete development at that temp.) for low temperatures and for 23° C. Low temperatures, °C.	
	11·7	13·0
7	0·97	—
9	—	1·03
14	1·08	—
17	—	1·06
21	1·16	—
25	—	1·13
29	1·22	—
35	1·25	—
42	1·35	—
Controls at 23° C. throughout	0·91–1·00	1·01

We can see from Table 5 that the sums of the products are increasingly greater than unity the longer the exposure to the low temperature has been. Therefore the times necessary to complete development at 23° C. are longer than would have been expected. There are three possible explanations for this and all are exceedingly difficult to verify. They are as follows:

(1) Partial development may have occurred at first at the low temperature, and the eggs remained exposed to it for some time subsequently during which development was in complete abeyance. Thus (exposure time) × (reciprocal for developmental time) would represent more development than had actually occurred.

It is unlikely that this has happened, since Fig. 4 suggests that, within the exposure times used at 11·7° C., development is continuous.

(2) There may be a true retardation of development at the higher temperature due to the effects of the exposure to the lower temperature.

(3) As Wigglesworth (1939) has pointed out, the principle of thermal summation involves the assumption that the accelerating effects of a temperature are the same on each separate phase of embryonic development as on the whole development. If the first phases of development in the cases under consideration are slower than is to be expected from the temperature-velocity curve, then a longer time for completion of development at 23° C. would be necessary than is estimated by thermal summation, even if no true retardation occurred. The fact that development is arrested at some temperatures suggests that the effects of temperature on the rate of development of all phases of embryonic growth are not uniform.

If this is true, then extrapolation to temperatures at which only partial development takes place is not justified, and the same applies to the reciprocal values at the threshold temperature and at temperatures just above it, where many organisms do not complete their development. And in fact, the

indiscriminate use of day-degrees at any temperature is, therefore, of doubtful value for estimating amounts of partial development only.

It cannot be too strongly stressed that even at temperatures at which development is completed by all the individuals, one day-degree represents only an average amount of development in terms of time occurring in one day and perhaps cannot be used safely for particular phases in embryonic growth.

IV. Thresholds for hatching and development

(1) *Definitions*

The term "threshold" is used in the sense of Shelford (1929) who writes: "The threshold of development is that intensity or amount of any factor immediately above which development begins to be perceptible in amount."

Uvarov (1931) states that "each partial process of development and the effects of external factors on it must be studied separately", where "partial process" means a well-marked stage in a life-history, e.g. the egg, larva or pupa.

If we consider the development of the egg of *C. lectularius* in this light, as previous workers with the insect have done, then the lowest constant temperature at which complete development from oviposition to the conclusion of the hatching process will occur is 13° C. This cannot rightly be called the developmental threshold, however, according to Shelford's definition, since we have seen that a slight amount of development may occur below 13° C., although the embryo fails to hatch. Neither is 13° C. the hatching threshold, for if eggs are incubated till just before eclosion they will hatch at 8° C. (46·4° F.).

I propose, therefore, to call the constant temperature of 13° C., the *developmental-hatching threshold* as distinct from the *hatching threshold* and the *developmental threshold*.

(2) *Factors affecting the developmental-hatching threshold*

Alternating temperature. It seemed possible, in view of the acceleration in development associated with alternating temperatures, that eggs might be induced to hatch at a slightly lower mean temperature than 13° C. if fluctuations in temperature occurred. An experiment with fifty eggs at 90% R.H. was, therefore, made with a temperature with a slow mean daily fluctuation of $\pm 2{\cdot}2°$ about a mean of 11·5° C. This range was chosen on account of its similarity to the temperature fluctuations likely to be found in rooms in English houses. None of the eggs hatched, however, although the experiment was continued for 80 days. It seems, therefore, that mean temperatures are probably a good guide to the possibility of hatching in nature.

Atmospheric humidity. Table 6 indicates the effect of humidity at the developmental-hatching threshold.

Table 6. *Hatching of* C. lectularius *eggs laid at* 23° C. *and then placed at a constant temperature of* 13 ± 0·1° C. *and various humidities*

% R.H.	No. eggs used	No. eggs hatched	No. died hatching	Control from same egg batches: at 23° C., 90 % R.H. No. used	No. hatched	% hatched
99–100	100	0	0	31	29	93·5
90	100	1	1	79	76	96·2
75	226	1	11	119	114	95·8
13	160	0	0	110	105	95·5

Although the numbers hatched are very small, there is an indication that the humidity has a slight effect. The range 75–90 % is the most favourable to hatching among those investigated: this is reflected more particularly in the number which died during the hatching process, than in those which emerged successfully. Embryos which die while hatching usually manage to emerge from the chorion but are unable to withdraw the hind legs from the embryonic cuticle.

The temperature at which eggs are laid. An experiment was made by taking a culture of adults from 23° C. which had ceased to lay eggs owing to lack of food, feeding and then placing them at approximately 15 ± 1·0° C (59·0° F.) and 90 % R.H. In this way eggs were obtained which had been formed and laid by insects at 15° C.

A batch of such eggs laid at 15° C. was divided into two lots, one of which was placed at 15° and the other at 23°, with 7 % R.H. in each case. The batch at 23° C. served as a control for the viability of the eggs. At the same time a batch of eggs laid at 23° C. was placed at 15° in the same container, a Kilner jar, as the eggs laid at 15° so that the two batches from the different temperatures were subjected to identical conditions throughout the experiment. A control for the eggs laid at 23° C. was kept at 23°, and the experiment continued for 3 months. Table 7 shows the result.

Table 7. *Percentage hatch of eggs at* 15 ± 1·0° C., 7 % R.H., *eggs laid at* 23 ± 0·1° C. *and at* 15 ± 1·0° C., 90 % R.H. *Control eggs laid at* 15 *and* 23° C. *from the same batches as the experimental eggs were kept at* 23° C., 7 % R.H.

Temperature at oviposition °C.	No. of eggs used	% hatch		% died hatching	No. used	Control hatching time days	% hatch
15	35	2·9	diff. statis. sig.	17·1	44	7·9	86·4
23	70	67·1		17·1	60	8·1	88·3

Thus, most of the eggs laid at 15° C. fail to hatch at that temperature, although the control shows that the batch was a good one. The developmental-hatching threshold at 7 % R.H. for eggs laid at 23° C. has not been ascertained precisely (see Table 9), but it undoubtedly lies very close to 13° C. It is, therefore, somewhat lower for eggs laid at 23° C. than for eggs laid at 15°. This is in all

probability a direct effect of temperature; other variables in the experiment must, however, be mentioned. First, although the relative humidity at which eggs were laid (90 %) was the same at 15° and at 23° C., the saturation deficiencies were slightly different (1·3 and 2·1 mm.). The effect of this should be negligible, since large saturation deficiencies acting for a long time have only a slight influence on egg mortality (see Table 12), and this difference existed for less than 1 day. Secondly, the eggs laid at 23° and 15° C. were put into the 15° container within the first 24 hr. after oviposition, by which time the eggs laid at 23° C. may have been in a slightly later developmental stage than the eggs from 15°; although the controls hatched in 7·9 and 8·1 days at 23° C. for eggs laid at 15° and 23° respectively. As far as we know, however, from experiments described on pp. 156, 157, the older the eggs, at any rate beyond the 4th day at 23° C., the more susceptible they are to death from exposure to low temperatures. This would, however, tend to favour hatching from 15° C. There is little probability that the eggs laid at 15° C. were in a more advanced stage of development than those laid at 23° C. at the start of the experiment, since the controls hatched in almost the same time. The only other element of uncertainty lies in the possibility that in the very early stages of embryonic development eggs are much more easily killed at 15° C. than in the final stages; for the experiments on the effect of age in relation to mortality at low temperatures were made with eggs with a minimum mean age of 12 hr. But in this case, eggs laid at 15° C. could scarcely be expected to hatch at 23° C. successfully, whereas, in fact, they do.

But whatever uncertainty exists of the actual mechanism responsible for this effect of the ovipositional temperature on mortality at 15° C., the observations still have an ecological importance; and it can be said that eggs laid at 15° C. in nature will suffer an almost total mortality at that temperature if the humidity is low, although if laid at higher temperatures they could be more successful under the same conditions. In these short-term experiments there is thus no evidence that eggs become adapted to low temperatures. It is very desirable that this experiment should be repeated and at other humidities; especially since the exact thresholds at 7 % R.H. for eggs laid at 23° C. and at 90 % R.H. for eggs laid at 15° C. are not precisely known. In a future experiment great care should be taken in order to obtain eggs in the same state of embryonic development; therefore, only the first eggs laid after the blood meal should be used.

(3) *The effect of the temperature during development on the hatching threshold*

It was pointed out in a previous section that if eggs are incubated until nearly ready to hatch, they can be placed at temperatures well below 13° C. (the developmental-hatching threshold) and they will hatch successfully. Kirkpatrick (1923) noted the same phenomenon with *Oxycarenus* eggs. I call the lowest temperature at which the hatching process itself occurs the hatching threshold.

It is very difficult to determine the exact hatching threshold accurately. For in the first place no sharp break occurs between the last stages of development and the commencement of hatching: and it is difficult to estimate when two batches of eggs are in exactly the same condition if comparative experiments are to be made. Even if an arbitrary point in the later stages of development is selected for use it is difficult to procure two egg batches both developed exactly to this point. The procedure in my experiments was as follows.

Eggs were obtained at 23° C. and 90 % R.H. and were incubated until 20–30 % of the original number had hatched. The eggs remaining unhatched were then placed at low temperatures and the hatching recorded every few days. It was thought that the hatching threshold might be affected by the temperature at which eggs had been incubated till the arbitrary point had been reached and experiments were, therefore, made with the primary incubation at 15, 18 and 23° C.

Eggs laid at 23° C., 90 % R.H. over a period of 4 days by a stock of females were split up into three batches; these were placed respectively at 23° C. ($\pm 0{\cdot}1$), 18° C. ($\pm 0{\cdot}2$) and 15° C. ($\pm 1{\cdot}0$) and at 90 % R.H. Controls from each batch were kept at 18° C., 90 % R.H. An attempt was made to remove eggs to the low temperatures when the same percentage had hatched in each batch at the three above temperatures, but as this proved very difficult it was not always possible to do it. For when hatching is once started a few minutes or half an hour may result in an extra 10 % hatch above that desired. This fact, however, indicates that a considerable proportion of the eggs in the different batches are in a very similar stage of development even if the exact proportion hatched in each batch is not the same.

It would be possible to estimate comparable stages of development by using day-degrees. But apart from theoretical considerations on the accuracy of the method, it was not very practical. For it is not easy to get the large numbers of eggs required for this experiment in a single batch less than 24 hr. old, and even this involves an error of ± 12 hr. at the end of development—a far greater error than with the method used here.

To continue, however, with the details of the method used. After approximately the required proportion of eggs had hatched at each temperature the remaining unhatched eggs were placed quickly into very small, voile-capped tubes which were already at the required low temperatures. Each tube was suspended in a 3×1 in. tube, with an acid-water mixture at the bottom giving a 90 % R.H., fixed into a vacuum flask filled with water at the low temperature to be used; the flasks were kept most of the time in an incubator running at 9·5–10° C. Quite a satisfactory temperature control was obtained by occasionally placing the vacuum flasks with the eggs at a higher or lower temperature until the correct temperature was again assumed (see Table 8 and Fig. 5). A very strict watch on temperature was kept during this procedure.

The eggs were inspected on the day after their insertion into the flasks. Only rarely did an egg hatch during this first 24 hr. and, therefore, possibly

before the experimental temperature had been attained; for some slight rise above this was inevitable, although the whole apparatus was brought to the correct temperature before the eggs were inserted. These hatches do not, however, affect the conclusions. Inspection was subsequently made at intervals of 3–5 days, but the eggs were not removed from their tubes and the latter extracted only for a few seconds. Table 8 gives the results of the experiments.

The experiments summarized in Table 8 are beset with difficulties, some of which have already been mentioned. There is another variable in the experiment, however, which it seems cannot be eliminated satisfactorily.

The dispersion of hatching times around a mean value are different for eggs kept at 23, 18 and 15° C. and are in inverse relation to the temperature (Fig. 6).

Let us consider a certain percentage hatch in three batches of eggs at these three temperatures which we attempt to obtain before the unhatched eggs are exposed to the low temperatures. This percentage is represented by *a*, *b* and *c* for 23, 18 and 15° C. in Fig. 6. The different slopes of the three curves at this value indicate that more eggs will be ready to hatch at the same temperature from the eggs incubated at 23° C. than with those from 18° C.; and more will be ready from 18° C. than from 15° C. Thus, although the same percentage hatch may have occurred in each of the three batches, the numbers hatching at the low temperatures will be biased in favour of those which received the higher preliminary incubation. The extent of this error will be very difficult to estimate. But if no differences in percentages hatched at low temperatures from the three groups exist, it cannot be supposed that there has been no

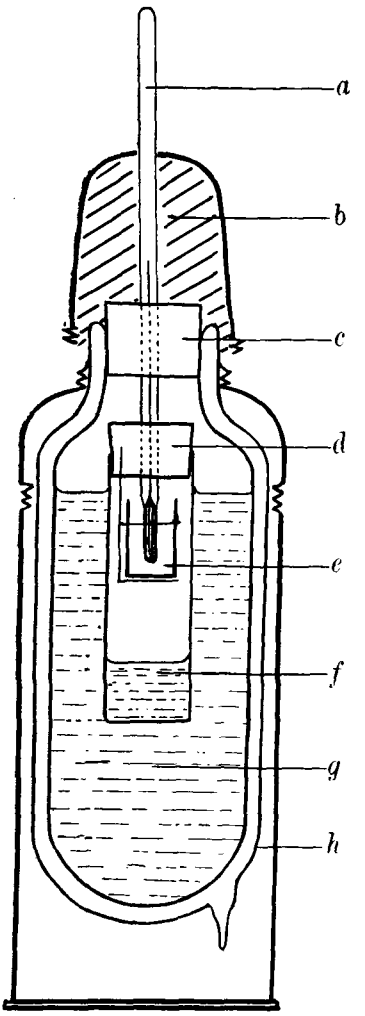

Fig. 5. Apparatus in which eggs were exposed to temperatures near the hatching threshold after having been incubated until nearly ready to hatch at higher temperatures. *a*, thermometer; *b*, cotton-wool inside the metal cap of the thermos flask; *c* and *d*, corks; *e*, glass container for eggs fixed to cork by wire; *f*, sulphuric acid-water mixture; *g*, water at constant temperature; *h*, wall of vacuum flask.

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Table 8. *The effect of the temperature during development on the hatching threshold of* C. lectularius *eggs*

Eggs laid at 23° C., 90 % R.H.; incubated till hatching commenced at 23, 18 and 15° C. and 90 % R.H. The unhatched eggs then put at low temperatures of approximately 10, 9 and 8° C. and 90 % R.H. All eggs in first experiment from the same batch collected over a 4-day period. In the second experiment there are two different batches. Italicized figures are percentages hatched corrected for mortalities in controls. Figures above italics are the actual (uncorrected) percentages hatched.

The actual highest and lowest recorded temperatures are given after each mean for approximately 10, 9 and 8° C.: the mean variations are considerably less than these. Temperatures read twice daily for 3–5 weeks. Controls kept at 18° C., 90 % R.H. from oviposition.

The percentage hatch corrected for mortality in control is obtained as follows:

x eggs put at preliminary temperature:, y % hatch: z % hatch in control: z–y % of x viable to start with at low temperature: from which is calculated *number of viable* eggs put at low temperature.

Then (no. hatched at low temperature/no. viable at low temperature) × 100 = corrected percentage hatch at low temperature (italicized figures below).

Temperature preliminary incubation °C.	% hatched	No. remaining put at each low temperature	% hatch of remaining eggs put at low temperatures (mean °C. below)			Control No. used	Control % hatched
			First experiment				
23 ± 0·1	18·2	47	10·1 ± 0·2	9·0 + 1·0 − 0·5	—	48	91·7
			36·2	12·8	—		
			49·3	*17·4*	—		
18 ± 0·2	20·8	56	10·1 + 0·4 − 0·2	9·2 + 0·6 − 0·3	8·2 + 1·7 − 1·2	56	89·3
			48·2	35·7	21·4		
			70·3	*52·1*	*31·3*		
15 ± 1·0	32·7	48	9·9 + 0·6 − 0·4	9·1 + 0·4 − 0·2	8·1 + 0·9 − 0·6	43	81·4
			22·9	2·1	6·3		
			47·0	*4·3*	*12·8*		
			Second experiment				
23 ± 0·1	28·8	52	10·1 ± 0·5	9·1 + 0·9 − 0·4	7·7 ± 0·7	52	90·4
			26·9	1·9	0		
			43·8	*3·1*	—		
15 ± 1·0	25·5	30	10·0 ± 0·5	8·9 + 0·4 − 0·2	7·7 ± 0·7	18	83·3
			26·7	10·0	6·7		
			45·5	*17·0*	*11·4*		

effect of "acclimatization". If a higher percentage hatch from a lower preliminary temperature, however, one can assume that some "acclimatization" effect has occurred.

The figures in Table 8 can now be discussed.

The lowest threshold for hatching yet found with the eggs of *C. lectularius* is 8° C. (46·4° F.). At this temperature the two experiments with the preliminary incubation of 15° C. show very similar percentage hatches; there is, however, a much higher percentage hatch from 18° C. (31·3 % compared with 12·8 and 11·4 %), and the differences are statistically significant although the numbers hatched are small. It is possible of course that the difference may be due to the error discussed in the preceding paragraph. This error cannot, however, account for the failure of eggs to hatch after a preliminary incuba-

tion at 23° C. Thus both 15 and 18° C. appear to be more favourable temperatures than 23° C. for preliminary incubation of eggs before they hatch at 8° C.

Consider now the incubations at 9° C. Eggs from 18° C. have a higher percentage hatch than eggs from either 23 or 15° C., and the differences are always statistically significant. Moreover, preliminary incubation at 23 and at 15° C. produces similar hatches—17·4 and 4·3 % and 3·1 and 17·0 % for 23 and 15° C. in the first and second experiments respectively: the former percentages are significantly different, the latter are not. It appears, therefore,

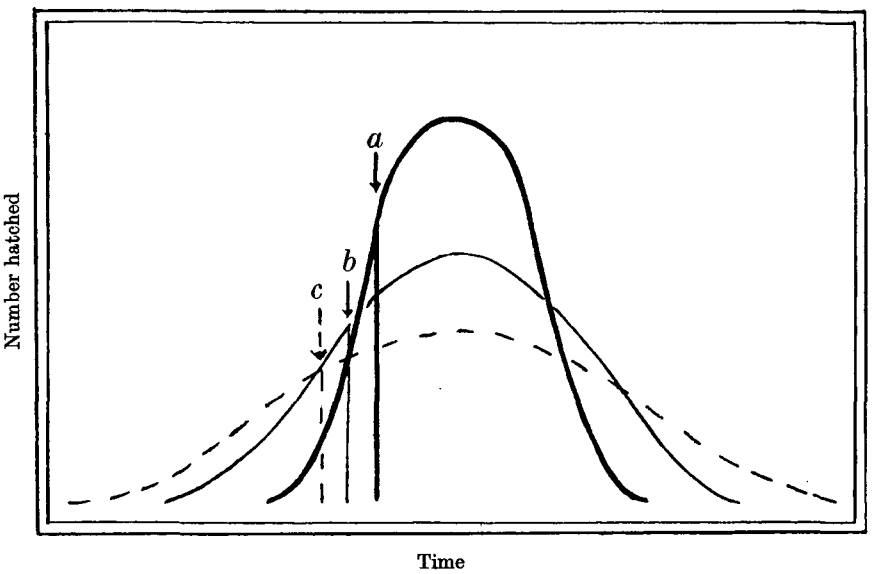

Fig. 6. Diagram to illustrate the dispersion of hatching times of eggs kept at three different temperatures. Three frequency distributions of the same area represent the same number of eggs which hatch at the three different temperatures: the lower the temperature the larger the scatter. Perpendiculars at *a*, *b* and *c* each cut off an equal area from their respective distributions. Therefore the points *a*, *b* and *c* on the curve indicate when the same percentage hatch has occurred at each temperature: but if at this point all the eggs are put at the same temperature, then a greater proportion will be ready to hatch from *a* than from *b*, and a greater proportion from *b* than from *c*, in the same time.

that at this temperature there is little to choose between eggs incubated at 23 and 15° C. in their ability to hatch at 9° C., but that those from 18° C. are much more successful; this would somewhat strengthen the conclusion that this difference was not due to the variation in hatching times about the mean values at the three preliminary incubation temperatures.

With the incubations at 10° C. there is again no evidence that preliminary incubation at 23 and at 15° C. affect subsequent hatching. In fact the proportions hatched are remarkably close and with no statistically significant differences if the corrected values are taken: these are 49·3 and 47·0 %, 43·8 and 45·5 % from 23 and 15° C. respectively in the two experiments. But, as

with the exposures to 8 and 9° C. eggs first incubated at 18° C. hatch more successfully at 10° C. than those first incubated at 23 or 15° C., and the differences are statistically significant.

Thus in spite of all obvious considerations, and particularly the error associated with varying degrees of dispersion in hatching at the three preliminary temperatures, there appears to be a slight adaptation of the hatching process to low temperatures: 18° C. appears to be a more favourable temperature than 23° C. and possibly also more favourable than 15° C. for subsequent hatching of the eggs at temperatures near the hatching threshold. It may be possible to demonstrate that the hatching threshold is below 8° C., particularly if a more favourable temperature than 18° C. can be found for preliminary incubation and if larger numbers of eggs are procured which are nearer to eclosion when placed at the threshold temperature.

(4) *Variation in threshold temperatures during development*

With eggs laid at 23° C. the lowest constant temperature at which development with hatching will occur is 13° C.; but the actual hatching process and presumably also the final stages of development will occur at 9° C. It seems at first, therefore, that 13° C. is the lowest temperature at which certain penultimate processes of development can proceed. But this may not be so, for although complete development is not possible if the eggs are kept at 12° C. from oviposition it may be the prolonged time associated with development at this temperature rather than the temperature itself which causes development to cease, e.g. a critical amount of water may be lost after such a prolonged exposure. If the eggs were incubated at a higher temperature until the processes which stop at 12° C. (when applied constantly) were reached it may be possible to induce further development at 12° C. or even at a still lower temperature. We have seen that this can be done with the hatching process at the threshold. Mellanby (1935) showed that a similar phenomenon occurs at the upper hatching limit: for eggs kept constantly at 37° C. will not hatch although hatching at 37° C. occurs if the eggs are previously incubated for 2 days at 30° C.

Thus the temperatures at which certain developmental processes cease probably cannot be considered apart from the conditions under which previous development has taken place. In the determination of developmental thresholds this must be taken into consideration.

V. SURVIVAL OF EGGS AT CONSTANT TEMPERATURES ABOVE THE DEVELOPMENTAL-HATCHING THRESHOLD

If a stock of bed-bugs is kept at 23° C. with males and females in approximately equal numbers so as to ensure a constantly high proportion of fertilized females, then there is usually a slight mortality in the eggs which are laid, even

if kept at optimal temperatures, quite apart from the unfertilized or "taube" eggs which appear when the numbers of sperms are exhausted. The cause of this mortality is unknown, for the eggs have a normal appearance and usually undergo some development. Table 9 gives the percentage hatch of eggs, all of which looked normal within 24 hr. after oviposition, when laid and incubated at 23° C.

Table 9. *Percentage hatch of* C. lectularius *eggs at various constant temperatures and humidities above the developmental-hatching threshold. All eggs laid at* 23° C., 90 % R.H. *Variations of temperatures as in Table* 2. *Percentage hatch not corrected for control mortality.* (*See Fig.* 7)

°C.	°F.	% R.H.	% hatch	No. eggs used	S.E.	Sig. tests + = sig., − = not sig.	Controls 23° C., 90 % R.H.
13·1	55·5	99–100	0	100			
		90	1·0	100	1·00	−	From same batches
		75	0·4	226	0·42		
		13	0	60			Over 90 % hatch
		7	0	100			
14·0	57·2	90	58·9	699	1·86		Over 90 % hatch
15·0	59·0	7	67·1	70	5·62		88·3 % hatch
16·0	60·8	90	91·0	100	2·86	−	Over 90 % hatch
		75	89·5	200	2·17	+	
		7	80·5	200	2·80	+	
18·0	64·4	90	89·0	181	2·33	−	
		75	86·0	100	3·47	+	
		7	77·0	100	4·21	−	
23·0	73·4	99–100	87·9	99	3·28	−	
		90	93·1	1786	0·60	+	
		75	97·0	99	1·71	+	
		7	90·9	275	1·73	+	
28·0	82·4	99–100	96·0	100	1·96	−	
		75	90·0	100	3·00	−	
		7	93·0	100	2·55	−	
30·0	86·0	99–100	85·8	148	2·87	−	
		75	91·3	309	1·60	−	
		7	90·0	50	4·24	−	
34·0	93·2	90	86·0	100	3·47	+	
		7	74·0	100	4·39	−	
34·5	94·1	99–100	63·2	114	4·52	+	Over 90 % hatch
		75	87·8	115	3·05	+	
		7	70·2	67	5·59		
35·5	95·9	75	90·0	50	4·24	+	
		7	32·0	50	6·60	+	Over 90 % hatch
37·0	98·6	low	0	100			Over 90 % hatch

(1) *The effect of temperature and humidity during incubation*

Consider, in Table 9 and Fig. 7, the eggs which were laid at 23° C. and incubated at various temperatures. As the temperature rises above 13° C. the optimum range is approached very quickly and it extends from about 16° C. (60·8° F.) to beyond 30° C. (86·0° F.). At temperatures above 30° C. mortality sets in very quickly when the humidity is low till at 37° C. no eggs hatch. At the higher humidities of 75–90 % the optimum range of temperature

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is wider, particularly at the high temperatures, and extends to about 34–35° C.

Humidity appears to have a very slight but noticeable effect on mortality within the greater part of the optimum range of temperatures: very low humidities in this range are slightly less favourable, either to the development or to the hatching of eggs, than 90 %. Relative humidities of 99–100 % appear to be less favourable than those of 90 %, at least towards the higher temperatures.

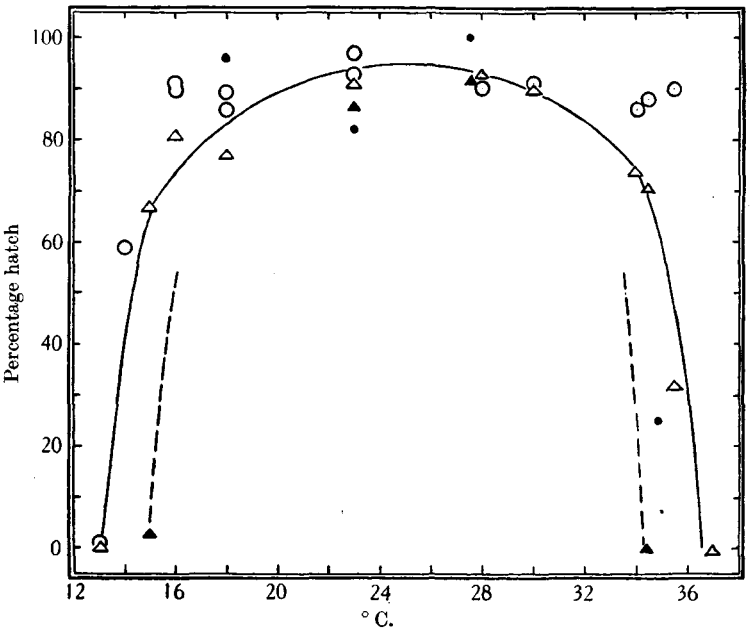

Fig. 7. Percentage hatch of *C. lectularius* eggs laid at 23 and 15° C. and then incubated at various constant temperatures and humidities. Lines drawn by hand through the points for the low humidities. Data in Tables 9 and 10 but batches incubated at 100 % R.H. are omitted from the figure. ○ laid at 23° C., 75–90 % R.H. during incubation. △ laid at 23° C., 7 % R.H. during incubation. • laid at 15° C., 90–95 % R.H. during incubation. ▲ laid at 15° C., 7 % R.H. during incubation.

These results contradict Mellanby's statement (1935) that "below 30° C. the eggs hatched in as great numbers, whatever the humidity", but they agree largely with the results of Geisthardt who states 70 % R.H. to be an optimal humidity from 16·4 to 34·4° C. He claims 100 % R.H. to be optimal between 19·5 and 30·5° C., however, but he gives no data. The effects of humidity are most noticeable at the high temperatures outside the optimal range.

(2) *The effect on mortality of the temperature at which eggs are laid*

For a comparison of the survival of eggs which were laid at 23° C. with others laid at approximately 15° C. the following experiments were made.

Bugs from the same culture as those from which eggs were obtained at

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23° C. were kept without food until egg laying had ceased. They were then fed and, with males and females in about equal numbers, were placed at 15 ± 1·0° C. until oviposition recommenced. The eggs thus obtained were placed at various temperatures (data in Table 10 and Fig. 7). Some of the results obtained have already been discussed in connexion with the effect of the temperature of oviposition on the developmental-hatching threshold (p. 143). This threshold for eggs laid at 15° C. is approximately 2° C. (3·6° F.) higher than for eggs laid at 23° C., and the lowest temperature in the optimum range is about 18° C. as compared with 16° C. for the eggs laid at 23° C. The upper temperature limit for complete development with hatching is at a lower temperature with eggs laid at 15° C. compared with those laid at 23° C. and humidity has, again, a marked effect at these high temperatures.

Table 10. *Percentage hatch of* C. lectularius *eggs at various constant temperatures above the developmental-hatching threshold. Eggs laid at* 15 ± 1·0 and 23 ± 0·1° C., 90 % R.H. *at both temperatures.*

The eggs laid at 23° C. and considered below, were incubated side by side and simultaneously with the corresponding eggs laid at 15° C. Incubations at 23° C., 7 % R.H. are with eggs from same batches as incubations at 15 and 34·4° C. and act therefore as controls. (See Fig. 7.)

	Eggs laid at 15° C.			Eggs laid at 23° C.			Signif. test between
°C. and % R.H. during incubation	% hatch	No. used	S.E. %	% hatch	No. used	S.E. %	hatch for 15 and 23° C.
15 ±1·0, 7 %	2·9	35	2·84	67·1	70	5·62	Signif.
18 ±0·2, 90 %	95·8	192	—	—	—	—	—
23 ±0·1, 90 %	81·8	11	—	—	—	—	—
7 %	86·4	44	5·17	88·3	60	4·15	Not sig.
27·6±0·4, 95 %	100·0	13	—	—	—	—	—
7 %	90·9	11	—	—	—	—	—
34·4±0·7, 7 %	0	35	—	70·2	67	5·59	Signif.
34·8±0·6, 95 %	25·0	20	9·68	See Table 9, not simultaneous			Signif.

Thus eggs laid at 15° C. appear to have a more restricted range of temperature within which they can develop and hatch successfully than have eggs laid at 23° C.; and this restriction occurs at both ends of the range of effective temperatures. Between 18 and 27·6° C. the eggs hatch with equal success whether they are laid at 15 or at 23° C.

As explained on p. 144, eggs laid at 15° C. may be in a slightly different developmental stage from eggs from 23° C. at the start of the experiment, and the possibility that *very* young embryos cannot withstand high and low temperatures as well as older embryos must not yet be dismissed.

In the very large numbers of egg batches with which I have worked, only on rare occasions has no mortality occurred even at optimal temperatures and humidities; this was, apparently, not the experience of Geisthardt, although he gives no tables of figures and plots no points on his graphs. He obtained

eggs at 27° C. however, which may have made the difference. A general conclusion which may be drawn from these experiments is that at temperatures between 18 and 31 or 32° C. the hatch will be between 80 and 95 % whatever the humidity above 7 %, and perhaps at whatever temperature from 15 to 23° C. at which the eggs are laid.

VI. SURVIVAL OF EGGS AT TEMPERATURES BELOW THE DEVELOPMENTAL-HATCHING THRESHOLD

In unheated rooms in England the winter temperatures are usually well below 13° C., the developmental-hatching threshold, for several months at a time (see Fig. 1 and Johnson, 1938), although temperatures below 0° C, (32° F.) are not often sustained for many hours. It is important, therefore, for practical purposes, to know how long eggs in various stages of development, which have been laid at different temperatures, can remain alive and capable of hatching. Knowledge of the mortality among eggs after varying periods of exposure to such subthreshold temperatures is relevant also in studies of bed-bug populations.

(1) *The use of "probits" in the analysis of egg mortalities after various times of exposure*

If several batches of eggs are each given a different period of exposure to a certain lethal temperature a sigmoid curve is obtained when the resulting percentage mortalities are plotted against exposure times. It is not practicable to compare two such mortality curves obtained with different lethal temperatures; but if the percentage mortalities are converted into "probits" by means of a table (Bliss, 1935*a*) the relation between "probits" and length of exposure may be linear (Fig. 8). The usual equations for linear regression may then be used and two curves compared. Thus it is possible to find the "median exposure for death" which is the length of exposure necessary to cause 50 % mortality (probit value for 50 % mortality = 5), and a standard deviation of this value can be obtained which enables significance tests to be made. Similarly, it is possible to test statistically whether the same exposures produce different mortalities at different temperatures.

After the mortalities found by experiment have been converted into probits by Bliss's table, the best-fitting straight line which represents the probit-exposure relationship is found. It is customary to test this line for goodness of fit; that is, to see if the assumption that the probit-exposure relationship is linear is reasonable for the available data. This is done by means of the χ^2 test (Fisher, 1936); the smaller the value of χ^2 the greater is the probability that the relationship is linear.

When the best-fitting straight line has been obtained the regression coefficient, b, will also be known.

Then, the value of any probit Y after an exposure X is

$$Y = \bar{y} + b\,(X - \bar{x}),$$

where $\bar{x}$ and $\bar{y}$ are the mean values of exposure times and probit values respectively for the whole series.

Thus if we wish to know the time at which 50 % mortality occurs (the median exposure for death) then $Y = 5$ and we solve the equation for X.

$$\text{The variance of } X \text{ (i.e. } \sigma^2) = \frac{1}{Sw\,b^2} + \frac{(5-\bar{y})^2}{b^2(Swx^2 - \bar{x}\,Swx)}$$

$$\text{The variance of } Y = \frac{1}{Sw} + \frac{(X-\bar{x})^2}{(Swx^2 - \bar{x}\,Swx)},$$

if the linear relation holds good, i.e. if χ^2 is not significantly different from zero. If χ^2 is significant or the relationship of probit to exposure time is not clearly linear, then the above value for $V(Y)$ is multiplied by χ^2/n, where n = degrees of freedom or the number of exposures made minus 2: w in the above equations is the weighting coefficient.

Thus it is clear that any time (e.g. the "median exposure for death") or any probit along the regression line is the best *estimate* which can be made from the experimental data of the exposure necessary to produce a certain mortality, or of the mortality after a certain exposure.

Now two probit-exposure lines can differ either in position or in slope (Fig. 8). If they differ in position it means that mortality sets in sooner with the one than with the other. If they differ in slope (in which case some parts of the lines will also differ in position) then the scatter of mortality values about the mean is different; the steeper the slope, the smaller the scatter.

It is possible that the median exposure for death may be the same for two series of data; then, the regression lines will either coincide along their whole length or will cross at probit value 5. If the slopes of the curves are significantly different (i.e. if the two values of b differ significantly) but cross at probit 5, then different exposures, other than the median exposure for death, will produce different mortalities in the two cases. This means that although the median exposures (or mean lengths of life) are the same, the scatter about the median value (or mean) is more with one series than with the other (Fig. 8).

The use of probits is fully discussed in papers by Bliss (1935 *a*, *b*) and Irwin (1937).

In experiments described below in which eggs have been subjected to temperatures below 13° C., control eggs from the same batches as the experimental eggs (i.e. eggs laid by the same bugs, at the same time) were kept at 23° C. and 90 % R.H. from oviposition. Not all the eggs in the controls hatch, and there is, therefore, a certain proportion of the experimental eggs which must be considered as dead when the experiment starts. The mortalities in

the experimental batches were, therefore, corrected according to the mortality in the control by the following formula (Bliss, 1935*a*).

$$\text{Percentage mortality (corrected)} = \frac{100\left\{\left(\text{no. used in exp.} \times \frac{\text{no. alive in control}}{\text{no. used in control}}\right) - \text{no. alive in exp.}\right\}}{\text{no. used in exp.} \times \frac{\text{no. alive in control}}{\text{no. used in control}}}.$$

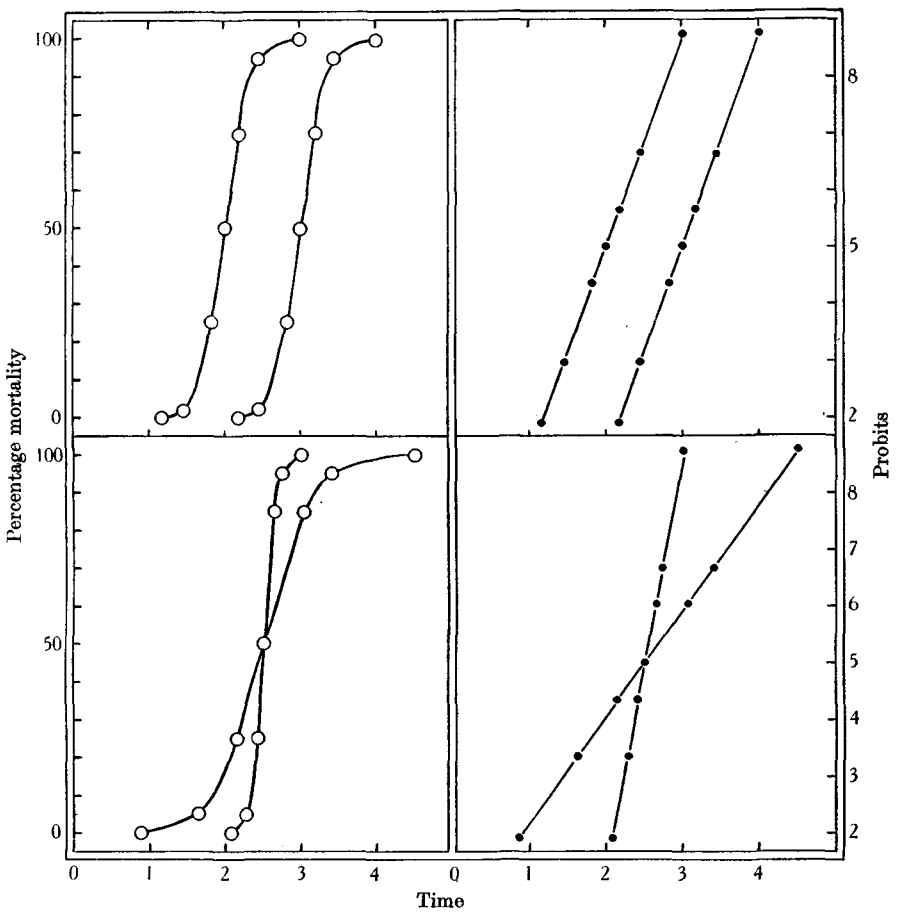

Fig. 8. Graphs illustrating percentage mortalities and the corresponding probit values with an organism subjected to a harmful influence for different intervals of time. ○ % mortality, • probits corresponding to % mortalities in the left-hand graphs. Top: mortality curves which differ in position but not in slope. Bottom: mortality curves which differ in slope but not in position at the median exposure for death (i.e. for 50 % mort.).

Occasionally after a short exposure to a low temperature, more eggs hatched in the experimental batch than in the control; in these cases no correction was made. Otherwise the corrected mortality was used to find the probit

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value in all the following experiments where eggs have been exposed to temperatures below 13° C. In the great majority of cases the value for χ^2 was small and not significantly different from zero, indicating that the probit-exposure regression line could be regarded as linear.

(2) *Mortalities after exposure to temperatures between 1 and 13° C. with eggs of different ages*

Since embryos may be in different stages of development when eggs are laid it is necessary to know if such differences are likely to affect the results of exposure experiments. Observations have, therefore, been made on the effects of exposures to a temperature of 7·7° C. on eggs in different stages of development.

Batches of eggs were incubated at 23° C. (the temperatures at which they were laid) and 90 % R.H. for periods from within 24 hr. up to 7 days after oviposition, and they were then placed at 7·7° C. and 90 % R.H. After various periods they were extracted and incubated at 23° C., 90 % R.H. until the maximum number had hatched.

All the eggs used in this experiment, which were collected and used daily, were laid in the first 2 or 3 days after the females had fed. Controls from the same batches as the experimental eggs all showed more than a 9-day mean for the period from oviposition to hatching at 23°. The experimental eggs can, therefore, all be considered as young and in about the same stage of development when the experiment started. The results are summarized in Table 11 and Fig. 9 except for eggs up to 24 hr. old; data for these are in Table 16.

There appears to be little difference in susceptibility to the low temperature with eggs kept at 23° C. until the fourth day after oviposition. The median exposures for death are not significantly different between eggs with 1, 2 and 4 days' preliminary incubation. The slopes of the probit-exposure regression lines for these eggs are also very close, and the only significant differences which exist are between the two lines for the eggs 4 days old (of which there are two experiments) and between one of these lines and that for eggs within 24 hr. old when exposure commenced. The slopes of the regression lines for eggs with 6 and 7 days' preliminary incubation are less steep (i.e. scatter about the mean is less) than the lines for the eggs with shorter preliminary incubations and the differences are statistically significant. The median exposure for death is significantly shorter with the seventh than with the sixth day preliminary incubation and significantly shorter in both of these than in eggs up to 4 days old before exposure.

Thus the longer the eggs are kept at 23° C. after the fourth day, the more quickly they die when subjected to 7·7° C. This may be due to the advanced condition of the embryo or to an acclimatization effect associated with the longer exposure to 23° C. These results do not agree with those of Geisthardt. He kept eggs at 27° C. till they were nearly ready to hatch, and then subjected

Table 11. *Data on percentage mortalities of* C. lectularius eggs *exposed to* 7·7° C. *and* 90 % R.H. *after preliminary incubation periods at* 23° C. *and* 90 % R.H. *For eggs with up to* 1 *day preliminary incubation see Table* 16. (*See Fig.* 9).

I. Period of exposure to 7·7° C., 90 % R.H. in days.
II. Number of eggs used.
III. Percentage mortality corrected for control mortality.
IV. Regression coefficient and variance (ital.) for probit-exposure relationship, in days.
V. Median exposure for death and variance (ital.), in days.
VI. χ^2 of probit-exposure relationship.

Days' prelim. incub.												
2	I	10	15	20	27	30	35	40	45	49	50	55
	II	38	40	50	48	50	50	50	50	30	30	50
	III	2·8	10·0	28·7	23·4	51·0	62·1	77·4	93·9	100·0	96·6	97·9
	IV					0·0887		*0·000047*				
	V					30·51		*0·6798*				
	VI					10·94						
4	I	14	22	28	35	42	49					
	II	20	25	35	35	40	42					
	III	20·2	40·4	42·3	54·4	78·7	100·0					
	III		0·0677		*0·000095*							
	V		29·10		*2·5069*							
	VI		5·22									
4	I	10	16	21	28	35	42	45	50			
	II	24	21	24	28	30	30	40	37			
	III	12·5	5·1	12·6	28·8	61·5	75·5	89·5	100·0			
	IV			0·0961			*0·000100*					
	V			32·30			*1·2628*					
	VI			4·98								
6	I	10	16	23	30	35	40					
	II	25	30	35	35	40	50					
	III	8·0	0	26·5	87·2	97·2	100·0					
	IV		0·2354		*0·001198*							
	V		25·62		*0·4814*							
	VI		0·90									
7	I	9	10	15	20	25	31	35	40			
	II	50	23	40	40	50	60	90	50			
	III	6·0	56·5	9·5	17·3	52·4	82·8	97·7	100·0			
	IV			0·1380			*0·000120*					
	V			23·39			*0·4302*					
	VI			27·84								

them to a temperature of 0–2° C. for 7 days. 92 % of the eggs hatched compared with 15 % of eggs exposed to 0–2° C. when they were only 24 hr. old. Geisthardt gives no details of the experiment, however, and did not use a control for the young eggs. Omori (1938) found that the percentage hatch on exposure to 0° C. for 14 days is higher with younger than with old eggs, but the reverse was true for 7-day exposures. Definite conclusions for the general behaviour of eggs of different ages to low temperatures cannot, however, be drawn from the experiments of Geisthardt and Omori, since neither of these workers found the time required for a median or mean exposure for death. Variations in the scatter of mortalities about the median time due to inherent differences in the material rather than differences due to the age of the eggs may produce results which appear to be contradictory.

(3) *Survival at temperatures between* 1 *and* 13° C. *when saturation deficiency is constant*

From these experiments eggs were laid at 23° C.: no eggs which were laid later than the third day after the females had fed were used. All eggs were collected within 24 hr. after oviposition and then placed at various low tem-

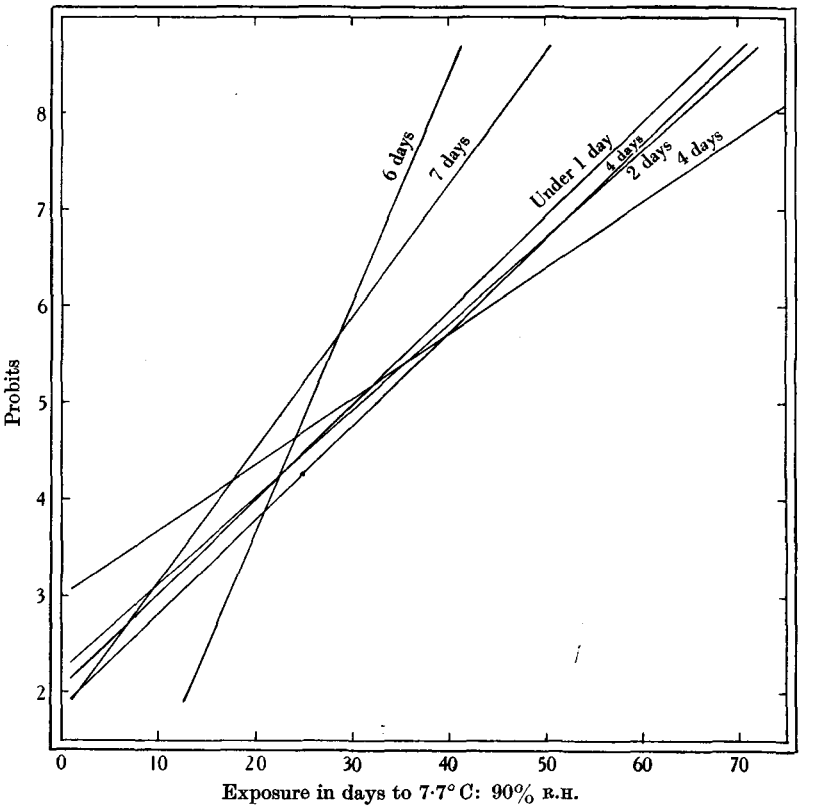

Fig. 9. Regression lines of probits on exposure for eggs of *C. lectularius* in various stages of development exposed to 7·7° C. and 90 % R.H. The eggs were incubated at 23° C. and 90 % R.H. for periods of less than 1 day, and for 2, 4, 6 and 7 days following oviposition: they were then exposed to 7·7° C. for varying periods. The length of the preliminary incubation is indicated at the side of each graph. Data in Table 11.

peratures and different humidities. Samples were extracted after definite periods and incubated at 23° C. and 90 % R.H. A control batch of from 20 to 30 eggs was taken from the same batch as the experimental eggs in each case and used for correcting the experimental mortalities as already explained (p. 155).

Table 12 gives the median exposures for death and their variances for a number of temperature and humidity combinations. Those data which are asterisked are at two similar saturation deficiencies—between 5·1 and 5·8 and

between 2·4 and 2·9 mm. It was considered that saturation deficiency rather than relative humidity should be constant when considering the effects of temperature, since the loss of water from the egg is probably controlled by saturation deficiency. Fig. 10 illustrates the results graphically. At both saturation deficiencies a similar relation of mean survival time to temperature is evident and the lower the temperature between 0 and 12° C. the

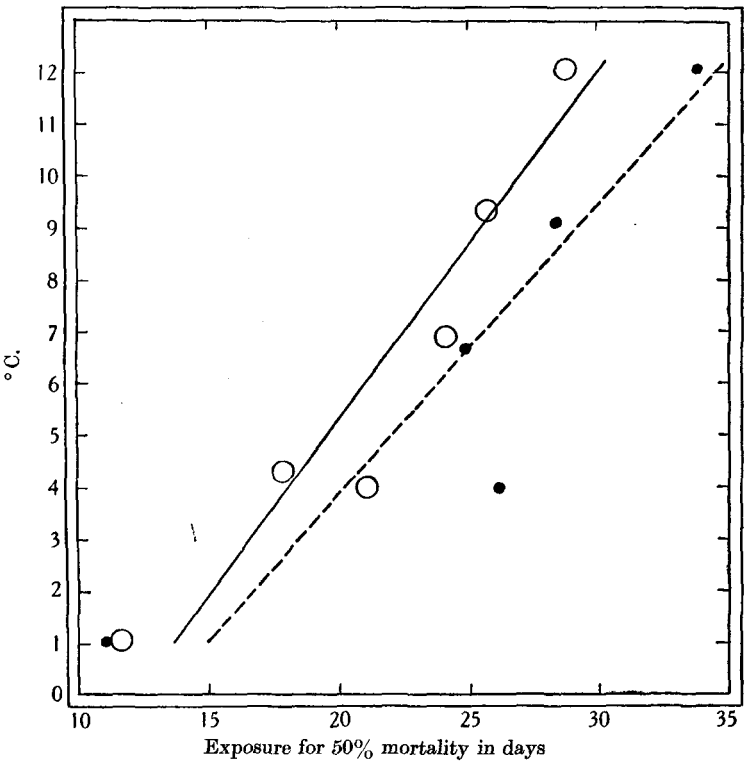

Fig. 10. Relation of the median exposure for death of *C. lectularius* eggs to temperatures between 1 and 12° C. and at constant saturation deficiencies. ● = saturation deficiency 2·4–2·9 mm., $b = 1{\cdot}48$; ○ = saturation deficiency 5·1–5·8 mm., $b = 1{\cdot}77$. The median exposures for death at 1·0 and 1·1° C. are included in the figure, for although the saturation deficiencies are 4·7 and 0·6 mm. respectively humidity appears to have no effect at this temperature. The regression lines are fitted. Data asterisked in Table 12.

shorter is the survival time. If the regression lines for probits and exposures are inspected (Tables 14–19, and Fig. 13) it appears that the differences in the median exposures for death are associated with a displacement of the mortality curves along the time axis rather than with differences in slope of the regression lines. This would mean that the initial mortality is delayed at the higher temperature but that once this has started eggs die at about the same rate. In the asterisked data in Table 12 and Fig. 10 the unweighted regression coefficients for median exposure to death with temperature are 1·77 and 1·48

for low and high humidities respectively. That is, for every 1° C. rise in temperature the median exposure is lengthened by 1·77 and 1·48 days in each case. But the two regression lines do not differ significantly in slope nor in position at any point on them.

Table 12. *Exposures for* 50 % *mortality* (*median exposure for death*) *and for* 99·99 % *mortality for* C. lectularius *eggs at temperatures between* 1 *and* 13° C. *and various humidities. Asterisked data are used in Fig.* 10 *for the effect of temperature at similar saturation deficiencies* (*see Figs.* 10–12).

Mean °C.	Mean °F.	% R.H.	Sat. def. mm.	Exposures and variances (ital.) in days: 50 % mortality	99·99 % mortality
1·0±0·8	33·8	89	0·5	11·1 *1·098*	38·7
1·1±0·8	34·0	5	4·7	11·6 *1·199*	39·8
4·1±0·8	39·4	89	0·7	23·8 *1·368*	65·8
4·2±0·8	39·6	70	1·8	22·9 *1·120*	55·6
*4·0±0·8	39·2	60	2·4	26·1 *8·322*	59·2
*4·0±0·8	39·2	15	5·1	21·0 *1·866*	60·5
*4·3±0·8	39·7	5	5·8	17·8 *0·522*	47·4
*6·7±1·0	44·1	65	2·6	24·8 *1·331*	51·8
*6·9±1·0	44·4	29	5·3	24·0 *1·516*	54·5
7·7±1·0	45·9	90	0·8	30·2 *0·744*	68·3
7·8±1·0	46·0	9	7·2	23·9 *0·889*	50·1
*9·1±1·1	48·4	72	2·4	28·4 *1·549*	61·7
*9·3±1·1	48·7	42	5·1	25·7 *1·535*	51·4
9·8±1·1	49·6	89	1·0	25·8 *0·774*	55·0
9·8±1·1	49·6	6	8·5	23·5 *0·922*	52·5
11·7±0·8	53·1	89	1·1	26·7 *1·409*	73·3
11·7±0·8	53·1	5	9·8	19·8 *1·217*	55·8
*12·1±1·0	53·8	73	2·9	33·9 *1·610*	79·8
*12·1±1·0	—	46	5·7	28·9 *1·212*	62·5
12·1±1·0	—	25	7·9	24·4 *0·854*	49·3
12·1±1·0	—	0–1	10·6	23·3 *0·693*	43·6
13·0±0·2	55·4	0–1	11·1	25·7 *1·606*	62·3

For practical purposes in bed-bug control a knowledge of the exposure at which a complete mortality is to be expected is more important than that for the exposure for a 50 % mortality. The exposures which would be expected to produce 99·99 % mortality have, therefore, been found (Table 12). With these as with values for a 50 % mortality the higher the temperature between 1 and 12° C. the longer do eggs survive when saturation deficiencies are constant. The relationship is not so well marked as with median exposures for death and this is undoubtedly associated with the differences in the slopes of the regression lines. For at 100 % mortality deviation from the mean exposure time is maximal and random variations in the material rather than temperature effects are perhaps responsible for such differences in scatter (see p. 157).

(4) *The effect of atmospheric humidity on survival at temperatures between* 1 *and* 13° C.

It can hardly be expected that the effect of humidity between 1 and 13° C. would be very marked, for the greatest saturation deficit is only 11·1 mm. At approximately 12° C. an experiment was made with a wide range of humidity. The curves for median exposure for death against saturation deficiency (Fig. 10) show that the longest survival time is associated with the lowest saturation

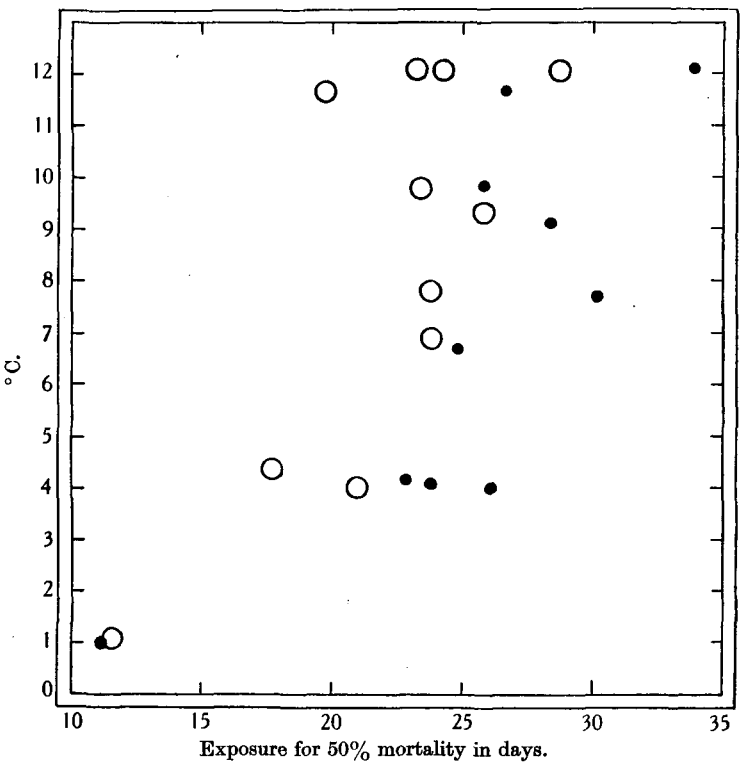

Fig. 11. The relation between the median exposure for death of *C. lectularius* eggs and temperatures between 1 and 12° C. with various humidities. Data in Table 12. ● = saturation deficiency less than 4·7 mm.; ○ = saturation deficiency of 4·7 mm. and more.

deficiency. A relatively wide range of humidity at 1°, however, appears to have no effect on the survival time (Table 12). It is seen from Fig. 10 that over the whole temperature range the lower saturation deficiencies seem to favour survival times, although at 99·99 % mortality this is obvious only at the higher temperatures; for median exposures, however, the regression lines for the two saturation deficiencies differ significantly neither in slope nor in position at any point on them. Fig. 11 includes all the data from Table 12. A line may be drawn which separates, roughly, times for 50 % mortality above and below about 5 mm. saturation deficit. Fig. 12 shows the effect of

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saturation deficit at each of the temperatures studied for the median exposure for death; the higher temperatures and lower saturation deficits favour survival except at 1° C. Even if the evaporative power of the air is measured by (saturation deficit × exposure time) no simple relationship between humidity and survival is apparent over the whole temperature range.

Thus at temperatures immediately below 13° C., as with those above, humidity has a slight but definite effect on survival of *C. lectularius* eggs. The

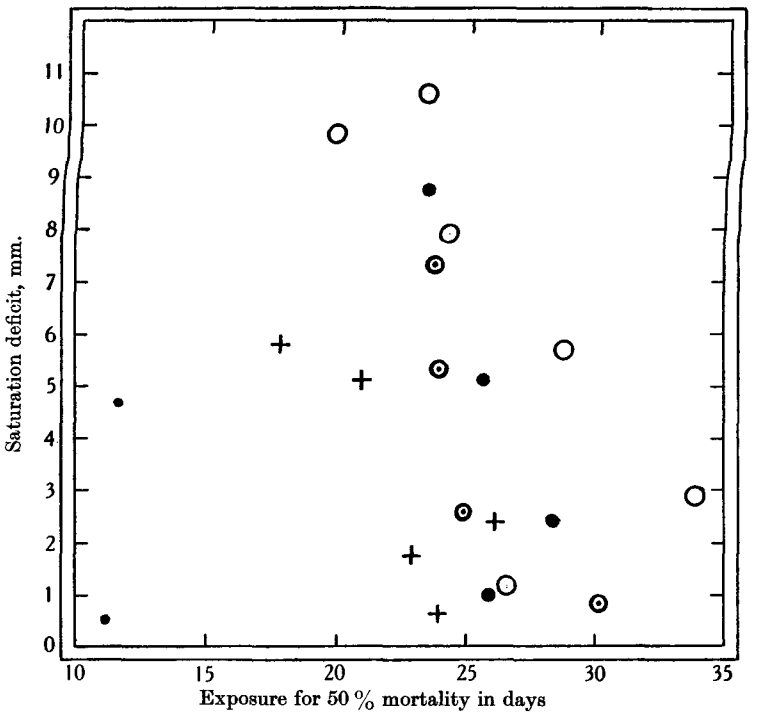

Fig. 12. The relation between median exposure for death of *C. lectularius* eggs and saturation deficiency at temperatures between 1 and 12° C. Data in Table 12.

• 1·0–1·1° C. ● 9·1– 9·8° C.
+ 4·0–4·3° C. ○ 11·7–12·1° C.
⊙ 6·7–7·8° C.

data at 12 and 4° C. suggest that the median humidities at those temperatures—50–80 % R.H.—may be most favourable (Table 12). But the effects, in general, are not sufficiently well marked to allow more precise conclusions to be made.

(5) *The temperatures at which eggs are laid and survival between* 1 *and* 13° C.

As we have already seen, the temperature at which eggs are laid or at which they form inside the female affects the survival above 13° C. and hatching at the hatching threshold. Experiments were made to test the effects of temperature at which eggs were laid on survival below 13° C.

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Eggs were obtained, as explained on p. 143, at 23, 18 and 15° C. and were then exposed to $10 \pm 0{\cdot}2$° C. and 90 % R.H. Samples were extracted after certain intervals and incubated at 18° C. and 90 % R.H. Controls were also kept at 18° C. and 90 % R.H. The results appear in Table 13.

Table 13. *The effect of the temperature of oviposition on the mortality of* C. lectularius *eggs at* 10° C. *and* 90 % R.H. *with subsequent incubation and controls kept at* 18° C. *and* 90 % R.H.

Percentage mortalities in the table are the corrected values.
Figures in brackets are numbers of eggs used.

Days at 10° C. 90 % R.H.	Temperature at oviposition, °C.		
	23	18	15
	% mortality	% mortality	% mortality
13	12·5 (40)	—	—
21	—	—	5·0 (20)
23	25·6 (40)	—	—
24	—	29·3 (11)	—
25	—	50·0 (20)	—
30	64·3 (40)	—	66·8 (30)
33	—	61·8 (32)	—
40	94·1 (40)	—	96·2 (29)
41	—	95·0 (22)	—
Controls	16·0 (50)	Taken as 10 %	For 21 days 12·5 (8) For 30 days 9·5 (21) For 40 days taken as 10 %

Occasionally, owing to the small numbers of eggs obtainable at 18 and 15° C. no controls were taken. It has been assumed that in these cases a 90 % hatch would have occurred in controls had they been kept, and appropriate corrections were made from this basis. In any case, the percentage mortalities are so close in the three series, 23, 18 and 15° C., whether they are corrected or not that the conclusions are the same providing the mortality is due in every case of exposure to 10° C. and not to some other cause; that this is so cannot be seriously doubted.

The results of the experiment indicate, then, that the temperature at which eggs are laid is without effect on the mortality produced by exposures to 10° C., 90 % R.H. followed by incubation at 18° C., 90 % R.H. This result is rather unexpected in view of the definite effect of ovipositional temperature on mortality at the developmental-hatching threshold (p. 143); no explanation is offered to account for the discrepancy in the two results.

(6) *The scatter of mortalities about the median exposure for death—or the slope of the probit-exposure regression line*

I have tried to find evidence of association between the values of the regression coefficient, b, of the probit-exposure relationship and temperature, relative humidity and saturation deficiency. There appears to be a slight

tendency for the larger values of b to be associated with low humidities at any one temperature. Many of the differences in the values of b for high and low humidities are statistically significant (Tables 14–19 and Fig. 13). There seems to be only a very weak relation between the values of b and saturation deficiency irrespective of temperature and none whatever with temperature when saturation deficiency is constant. It is probable that most of the variation of b is inherent in the material rather than due to the environment of the eggs.

(7) *The results of other workers*

Hase (1930) and Omori (1938) have worked with eggs at temperatures below 13° C. The percentage mortalities in my experiments agree well with those obtained by Hase at a temperature of 2° C. But Omori obtained much shorter survival times at 0° C. For example, he records 100 % mortality at 21 days' exposure; my observed exposures for complete mortality at 1° C. are 35 days for both humidities used. It is quite probable, however, that with a sufficient number of samples I also would have had some which gave 100 % mortality in 21 days, even at 1° C.

It has been stated (see Uvarov, 1931) that even short exposures of 3 days to 2° C. results in some mortality, for only 93 % of the eggs hatch. It is most probable, however, that this mortality was not due to the low temperature; for my controls which had not been subjected to a low temperature almost invariably showed such a slight mortality.

(8) *Tables and diagrams of experimental data*

The following Tables 14–19 (see Fig. 13) give data for percentage mortality of *C. lectularius* eggs after exposure to various temperatures between 1 and 13° C. and different humidities for varying periods in days. The exposures commenced within 24 hr. after oviposition. Eggs were laid and reincubated after exposure and controls were kept from oviposition at 23° C. and 90 % R.H.

The following data are given in the tables below:

I. Period of exposure in days.
II. Number of eggs used.
III. Percentage mortality corrected for control mortality.
b. The regression coefficient the probit-exposure relationship.
V(b). The variance of b.
χ^2. For the probit-exposure regression line.

Other necessary data are to be found in Table 12. These are: median exposure for death and variance in days and the exposure for 99·99 % mortality. Temperature variations, about the mean values which are given in the following tables, are also set out in Table 12.

Table 14. *Exposure temperature approx.* 1° C.

	1·0° C. 89 % R.H. 0·5 mm. S.D.		1·1° C. 5 % R.H. 4·7 mm. S.D.	
I	II	III	II	III
7	35	36·51	30	25·00
14	41	51·22	39	64·74
21	50	95·56	50	90·00
28	50	97·78	49	97·45
35	50	100·00	50	100·00
b	0·1347		0·1319	
V(b)	0·000386		0·000379	
χ^2	6·59		0·40	

Table 15. *Exposure temperature approx.* 4° C.

	4·1° C. 89 % R.H. 0·7 mm. S.D.		4·2° C. 70 % R.H. 1·8 mm. S.D.		4·0° C. 60 % R.H. 2·4 mm. S.D.		4·0° C. 15 % R.H. 5·1 mm. S.D.		4·3° C. 5 % R.H. 5·8 mm. S.D.	
I	II	III	II	III	II	III	II	III	II	III
9	—	—	24	4·20	—	—	—	—	59	10·57
10	30	11·35	—	—	25	4·00	25	17·53	—	—
16	30	18·44	29	16·86	25	12·00	—	—	80	46·16
17	—	—	—	—	—	—	25	29·90	—	—
23	28	62·01	30	52·52	27	37·04	—	—	50	72·34
24	—	—	—	—	—	—	60	62·20	—	—
28	—	—	—	—	—	—	—	—	50	87·23
30	40	65·43	32	86·30	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—	60	100·00
35	38	83·20	40	94·52	—	—	—	—	—	—
39	50	89·36	—	—	—	—	—	—	—	—
40	30	100·00	40	91·78	—	—	—	—	—	—
44	—	—	40	94·52	—	—	—	—	—	—
50	—	—	40	100·00	—	—	—	—	—	—
b	0·0886		0·1139		0·1124		0·0942		0·1259	
V(b)	0·000103		0·000151		0·001401		0·000557		0·000184	
χ^2	4·91		5·93		0·04		0·48		2·08	

Table 16. *Exposure temperature approx.* 7–8° C.

	6·9° C. 29 % R.H. 5·3 mm. S.D.		6·7° C. 65 % R.H. 2·6 mm. S.D.		7·8° C. 9 % R.H. 7·2 mm. S.D.		7·7° C. 90 % R.H. 0·8 mm. S.D.	
I	II	III	II	III	II	III	II	III
7	20	1·35	20	0	20	5·00	25	16·00
14	20	11·73	20	11·73	23	8·70	32	6·25
21	20	37·69	20	22·12	25	35·99	35	27·82
26	—	—	—	—	—	—	40	33·64
28	25	66·77	25	70·92	40	62·94	40	24·17
35	25	91·35	25	91·69	50	96·00	80	65·24
37	—	—	—	—	50	100·00	—	—
42	—	—	—	—	—	—	40	94·29
49	—	—	—	—	—	—	40	100·00
b	0·1219		0·1375		0·1419		0·0975	
V(b)	0·000369		0·000530		0·000299		0·000105	
χ^2	0·13		1·43		3·27		14·60	

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Table 17. *Exposure temperature approx.* 9–10° C.

	9·1° C. 72 % R.H. 2·4 mm. S.D.		9·3° C. 42 % R.H. 5·1 mm. S.D.		9·8° C. 89 % R.H. 1·0 mm. S.D.		9·8° C. 6 % R.H. 8·5 mm. S.D.	
I	II	III	II	III	II	III	II	III
7	30	4·70	20	0	19	10·11	30	6·67
14	30	4·70	—	—	30	7·47	40	10·59
21	30	22·35	20	20·00	40	19·94	40	32·94
28	30	36·47	25	72·00	50	65·84	40	72·06
35	30	83·96	20	85·00	39	86·31	33	100·00
42	—	—	—	—	50	100·00	—	—
b	0·1115		0·1448		0·1273		0·1280	
V(*b*)	0·000294		0·001093		0·000241		0·000310	
χ^2	3·81		1·88		3·43		2·31	

Table 18. *Exposure temperature approx.* 12° C.

	11·7° C. 89 % R.H. 1·1 mm. S.D.		12·1° C. 73 % R.H. 2·9 mm. S.D.		12·1° C. 46 % R.H. 5·7 mm. S.D.		12·1° C. 25 % R.H. 7·9 mm. S.D.		11·7° C. 5 % R.H. 9·8 mm. S.D.		12·1° C. 0–1 % R.H. 10·6 mm. S.D.	
I	II	III	II	III	II	III	II	III	II	III	II	III
7	32	9·38	—	—	—	—	—	—	29	6·90	—	—
14	31	9·68	19	1·68	20	6·59	20	29·29	20	31·58	20	4·21
21	30	36·66	25	25·27	25	16·48	30	15·82	30	57·89	30	32·66
27	—	—	—	—	—	—	—	—	20	48·86	—	—
28	—	—	30	23·08	30	45·05	40	72·22	40	92·11	40	82·32
29	40	59·16	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—	40	82·95	—	—
33	—	—	—	—	—	—	—	—	23	80·24	—	—
35	40	69·44	40	61·54	40	75·27	40	97·47	40	100·00	40	97·47
40	—	—	—	—	—	—	—	—	—	—	30	100·00
42	48	90·74	40	72·53	40	94·51	43	97·65	—	—	—	—
46	50	94·00	—	—	—	—	—	—	—	—	—	—
49	26	96·15	45	87·79	35	96·86	—	—	—	—	—	—
b	0·0800		0·0811		0·1110		0·1489		0·1034		0·1829	
V(*b*)	0·000058		0·000109		0·000186		0·000404		0·000162		0·000712	
χ^2	2·60		5·05		0·89		11·59		15·45		0·26	

Table 19. *Exposure temperature* 13° C.

	13·0° C. 0–1 % R.H. 11·1 mm. S.D.	
I	II	III
9	40	0
17	40	22·50
25	40	47·50
42	56	94·60
b	0·1015	
V(*b*)	0·000173	
χ^2	1·21	

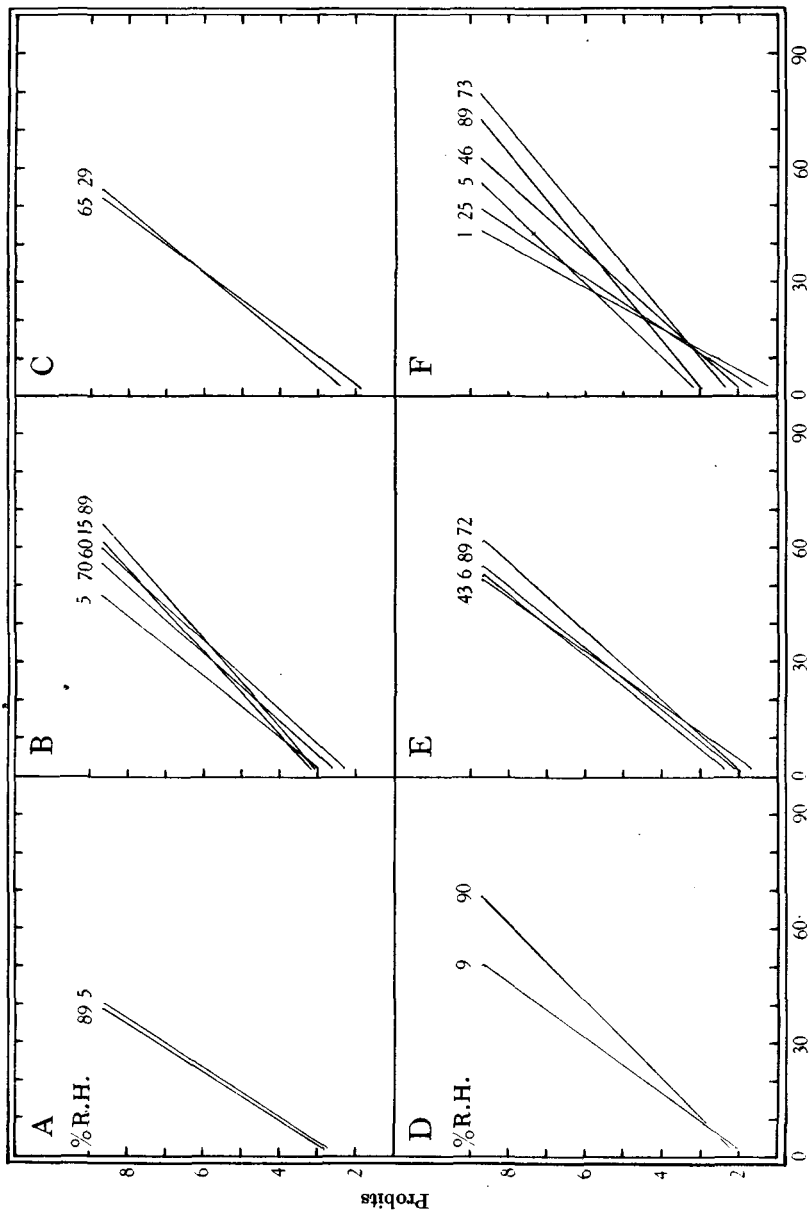

Fig. 13. Regression lines for probits on exposure for *C. lectularius* eggs exposed to temperatures between 1 and 12° C. and various humidities. Data in Tables 14–19. A, 1·0–1·1° C. B, 4·0–4·3° C. C, 6·7–6·9° C. D, 7·7– 7·8° C. E, 9·1– 9·8° C. F, 11·7–12·1° C.

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VII. Discussion and future work

The investigations described in this paper were undertaken with the ecology of the bed-bug, rather than its physiology, in mind; and no attempt has been made to discover the factors intimately associated with the causes of mortality, preconditioning effects or the different rates of development under the various climatic conditions. But from the ecological point of view some obvious problems still remain to be investigated.

In all this work, no serious effort was made to control the intensity or the duration of illumination. The eggs were kept in incubators or in constant temperature rooms, for most of the time in darkness but with frequent short exposures to daylight and to electric light of low intensity. Consequently the effects of light on the eggs are unknown.

The transference of eggs to various temperatures was made suddenly in the experiments which have been described. But in nature seasonal and diurnal temperature changes take place gradually. The effects of slow temperature changes should be studied and compared with the effects of sudden ones, for Robinson (1926) states that probably with non-hibernating insects a gradual approach to low temperatures is associated with less resistance than a more sudden change. It has been suggested above (p. 142) that fluctuations in temperature likely to occur in English houses are probably without much effect on the position of the developmental-hatching threshold; but work on the rate of development at temperatures fluctuating above and below 13° C. still has to be done. With fluctuating temperatures below the developmental-hatching threshold, condensation of dew on eggs may have marked consequences on their survival times; for I have noticed that the dents in the chorions of old *Cimex* eggs disappear if the eggs are placed in a film of water.

Since the rate of change of temperature in houses is gradual, and it is improbable that eggs laid at 23° C. would ever be suddenly subjected to 7° C. or lower and then later be raised quickly back to 23° C., perhaps it would have been better to have kept controls and to have reincubated eggs at a lower temperature than 23° C. Table 13 shows, however, that in the early stages of development at least, the temperature from which the eggs are transferred has no effect on the mortality at 10° C. Whether the magnitude of the temperature change has any effect on older eggs as they are removed back to a higher temperature still remains to be discovered. It must be remembered too, that in the exposure experiments the actual length of life of the eggs is the length of exposure plus the subsequent incubation time at 23° C.; in nature, therefore, the length of life at low temperatures may be longer than my experiments suggest, although of course they would not hatch if the temperature was below the threshold for hatching. Thus although the median exposure for death in my experiments is probably not the true value if the particular low temperature was held constant until half the eggs died, its ecological significance is unimpaired.

In English houses temperatures approaching the upper thermal death point of *C. lectularius* eggs (45° C. or 113° F. for 1 hr.) rarely occur except perhaps in roof-spaces or on dark surfaces exposed to direct sunlight. I have, therefore, considered that the upper fatal limits are of secondary importance in the ecology of *Cimex* in this country. Mellanby (1935) has investigated these limits, but we still know nothing about the effects of age or of preconditioning on the upper death point.

The whole subject of preconditioning or acclimatization needs systematic study. We do not know the minimum times necessary for a preconditioning temperature to produce an effect; and in the experiments with eggs laid at different temperatures it is not certain whether the eggs must be developed from the ovarioles or if merely oviposition at that temperature is sufficient for the effect to occur. But from Table 7 it seems that either the period of early development while the eggs are still inside the female, or the period of the first 24 hr. after oviposition, are critical times when preconditioning is brought about.

The mortality of eggs at low temperatures appears to be little influenced by the atmospheric humidity. Mellanby (1939*a*) thinks that it is probably the serosa and not the chorion of fertilized eggs which is responsible for the retention of water in the egg. I have sectioned eggs and have found an apparently chitinized membrane identical in appearance to that described for *Notostira* (Capsidae) (Johnson, 1934). Slifer (1938) has shown that a similar membrane in *Melanoplus* is permeable to water only at one part, the hydropyle; it would be interesting to discover if a hydropyle area is absent in *Cimex* eggs, thus rendering them relatively impermeable to water. For they lose water very slowly indeed and do not absorb it during development as with *Melanoplus* and *Notostira*. Clark (1935) states that with *Rhodnius* eggs the humidity probably has little effect on the embryo itself and only becomes important immediately before hatching. In *Cimex* humidity is not important even at hatching, but the similar lack of effects of humidity on the embryo may be due to a chitinous membrane in *Rhodnius*. The work of H. Mellanby (1936) suggests (see her Text-fig. 4A) that such a membrane is present although she does not figure an anterior thickening of it as exists in *Cimex* and in *Notostira*.

This work on the relations of *Cimex* eggs to climate is significant in the ecology of the bug only in conjunction with other aspects of its life—particularly with the factors influencing oviposition. The threshold for oviposition is at approximately 13° C., but if eggs were laid at this temperature it is doubtful if they would develop even if the temperature was kept two or three degrees higher than 13° C. (see Table 7). But the mortality among eggs and the lowest temperatures at which they will hatch in nature will depend a great deal not only on the temperature of oviposition but on the rate at which the temperature subsequently falls. If the eggs are laid at a fairly high temperature and the rate of fall is sufficiently gradual, considerable numbers might be expected to hatch at temperatures as low as 8° C. So far only macroclimatic

temperature changes in rooms have been studied and these have served as a guide for the planning of experiments; microclimates and their rate of change still remain to be worked out in conjunction with our knowledge of the temperature and humidity relations of eggs. But even if eggs hatch after exposures to low temperatures in nature the larvae may be weak in spite of their normal appearance. It is important, however, that collections of eggs should be made from natural infestations at different times of the year and tests made to reveal the proportion of viable eggs present amongst them.

The results on the survival of eggs at winter temperatures may ultimately be of use to workers interested in bed-bug control. It would perhaps be worth ascertaining if eggs which had suffered winter temperatures for some time were more easily killed by fumigants than the newly laid eggs used in laboratory tests. It is probable that in some rooms, where the winter temperatures remain below 10° C. for many weeks at a time, the concentrations which have been found necessary to kill eggs in the laboratory need not be employed since the eggs, if not already dead from exposure to low temperatures, may be easily killed by concentrations lethal to adults and nymphs.

VIII. Summary

1. The period which elapses between a blood-meal and oviposition on the part of the female bed-bug affects the duration of the egg stage. Eggs laid soon after the meal take longer to hatch than those laid later. It is supposed that embryonic development of eggs inside the female occurs at a rate relatively greater than that of oviposition.

2. Atmospheric humidity is without effect on the duration of the egg stage. Although the temperature-velocity graph appears to be fairly linear between 18 and 30° C. the thermal constants show considerable variation.

3. A daily alternation of temperature with a range of 10° C., between the threshold and optimum temperatures results in an acceleration of development; this can, however, be accounted for by the non-linearity of the temperature-velocity relationship, if the usual methods of thermal summation are used.

4. Eggs have been exposed to temperatures between 1 and 12° C. and the time taken to hatch on subsequent incubation at 23° C. has been ascertained. When these times are compared with times for hatching of control eggs kept at 23° C. from oviposition there is some evidence that a slight amount of development may occur at as low a temperature as 4° C.; i.e. 9° below the developmental-hatching threshold. Times after exposure are shorter than times for control eggs.

5. Thermal summation, however, suggests that temperatures between 13 and 11·7° C. result in a retardation of development since the times after exposure at which hatching occurs at 23° C. are longer than would be expected.

6. The method of thermal summation is criticized mainly on the grounds

that it assumes that a temperature has the same accelerating effect on all stages of embryonic development. A retardation of development such as that mentioned in the preceding paragraph may be either a true retardation or due to errors resulting from the assumption that the reciprocal of the time for complete development represents the true amount of daily development at all stages of embryonic growth.

7. The lowest constant temperature at which complete development with hatching, of eggs laid at 23° C., can occur is 13° C. I have called this temperature the *developmental-hatching threshold*. The *developmental threshold* may be as low as 4° C., while the *hatching threshold* is at approximately 8° C.

8. Alternating temperatures such as occur in English houses are unlikely to affect the position of the developmental-hatching threshold. Atmospheric humidity does, however, affect it and 75–90 % R.H. appear to be the only humidities at which development with hatching can take place at a constant temperature of 13° C.

9. Mortalities near the developmental-hatching threshold appear to depend also on the temperature at which eggs are laid (or perhaps the temperatures at which they develop within the female). Eggs laid at 15° C. and incubated at 15° C. and 7 % R.H. suffer 97·1 % mortality while those laid at 23° C. and incubated under identical conditions have 32·9 % mortality.

10. If eggs are laid at 23° C. and are incubated at 15, 18 and 23° C. until nearly ready to hatch, the percentage hatch at temperatures near the hatching threshold is higher with those eggs previously kept at 18° C. than with those from 15 or from 23° C. At 8° C., the lowest observed temperature for hatching, preliminary incubation at 15° C. is probably more favourable than one at 23° C.

11. Mortality of eggs above 13° C. is only slightly affected by atmospheric humidity over the optimal range. But the effects are more noticeable near the upper and lower temperature limits. 99–100 % R.H. appears to be associated with a higher mortality than 90 % R.H. The extreme temperature limits for eggs laid at 23° C. are 13 and 37° C. With eggs laid at 15° C. the range is restricted at both upper and lower temperature limits compared with the range for eggs laid at 23° C. The temperature of oviposition, whether it is 15 or 25° C., seems to make no difference to mortalities between 18 and 28° C.

12. The mortalities of eggs exposed for varying periods to temperatures between 1 and 13° C. and various humidities are discussed. By means of probits estimates of the times for 50 and 99·99 % mortalities have been made for each of the temperature and humidity combinations.

13. Exposure for 50 % mortality is affected slightly by humidity below 13° C., but no simple law relating survival to humidity has been found either above or below 13° C. Variations in temperature within the range 0–34° C. are likely to influence survival more than the humidity variations possible within this temperature range.

14. At constant saturation deficiencies the eggs survive longer at the higher temperatures between 1 and 13° C. The median exposure for death

(i.e. exposure for 50 % mortality) increases by 1·5–1·8 days per 1° C. rise of temperature.

15. The scatter of mortalities about the median exposure for death as measured by the regression coefficient of probits on exposure times is slightly influenced by humidity, but is not affected by temperatures between 1 and 13° C. Much of the variation in the slopes of the mortality curves is thought to be due to inherent variation in the eggs themselves.

16. With eggs laid at 23° C. and exposed to temperatures below 13° C. the longest exposure necessary to produce 99·99 % mortality (as judged by subsequent incubation at 23° C.) was estimated at 79·8 days. This occurred at 12·1° C. and 73 % R.H. The actual observed times for exposure for 100 % mortality are somewhat shorter than the estimated times.

17. Eggs with embryos in an advanced state of development are more quickly killed by exposure to 7·7° C. and 90 % R.H. than are newly laid eggs.

18. Ovipositional temperatures of 15, 18 and 23° C. produced no different effects on the mortality rates of eggs exposed to 10° C. and 90 % R.H.

19. Future problems and the ecological significance of the experimental results are discussed.

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