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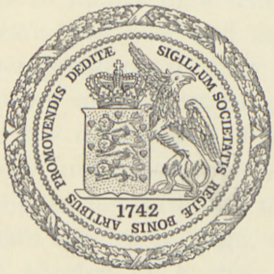
PROBLEMS OF HEAT DEATH AND HEAT INJURY

EXPERIMENTS ON SOME SPECIES OF *DIPTERA*

BY

ELLINOR BRO LARSEN



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I. Introduction.

The following work is published as a part of the investigations conducted by Professor MATHIAS THOMSEN on the biology of various species of flies, especially those associated with houses and domestic animals.

In a previous paper (E. BRO LARSEN & M. THOMSEN, 1940) the main subject was the duration of development of the species concerned at various constant temperatures within a temperature zone which permits the continuation of development. Those experiments showed that up to a certain point temperature has an accelerating effect on development, but if the temperature rises further the effect is injurious, development is retarded, various irregularities occur, and if still higher temperatures are applied, development is no longer completed and the insects die after a shorter or longer period.

For several reasons it was desirable, however, to study the reactions of the species to fatally high temperatures, partly because measurements show that now and again these insects are exposed to such fatal temperatures in their natural environment, partly because heat plays an important part in some of the methods advocated for the destruction of the eggs, larvae, and "pupae" of the house-fly and other species. So it is of interest to know the height of the temperature and the length of exposure necessary to kill a certain species or stage, and how great a percentage is likely to be affected by a given exposure.

Finally these investigations might perhaps claim some theoretical interest, as reports on the influence of fatal temperatures on insects are somewhat scanty and insufficient. In some of these previous experiments there is no statement of the duration

of the exposure; in others it was not possible to make sure if the insects actually were exposed to the temperature registered; moreover the exact determination of the age of the insects used was often difficult.

Most closely related to the subsequent investigations is OOSTHUIZEN's work 1935 on the influence of fatal temperatures on the confused flour beetle *Tribolium confusum*, in which eggs larvae, pupae and adults were exposed to high temperatures at varying intervals and at varying degrees of humidity; fertility under these influences was also examined, the results being discussed. In DARBY and KOPP's work (1933) on *Anastrepha ludens* (*Diptera, Trypetidae*) the age of the insects used in the experiments was considered, as well as the question whether they actually may be expected to have the temperature registered. MELLANBY (1932) examined the influence of the humidity of the air on the determination of the thermal death point, and like BUXTON (1931) found that at temperatures so high that a short exposure is fatal, humidity has no effect, provided that the test insects are so small that their temperature cannot be lowered essentially by evaporation, whereas with long periods of exposure low humidity is unfavourable owing to evaporation. However, most of the investigations on insects are confined to a bare determination of the thermal death point and often, employing the method given by BODENHEIMER (1929), to a determination of the phases of activity of insects under rising temperatures, a method which excludes the time factor in determining the fatal temperature. This factor, however, is of great importance, theoretically as well as practically; for instance it is often necessary, to use the lowest possible fatal temperature in order to avoid damage to the medium inhabited by the insects, e. g. flour, dung, bulbs, etc. For investigations dealing with heat injury as seen mainly from a theoretical point of view, organisms other than insects were generally employed, e. g. bacteria, spores of fungi, seeds and cells of plants, blood corpuscles etc., organisms readily supplying an abundance of uniform material. However, as there is a lack of such comprehensive investigations on insects, I have, in spite of the rather primitive experimental technique, attempted to procure a material as copious as possible, for the elucidation of the influence of the fatal temperatures.

My special thanks are due to Professor MATHIAS THOMSEN for his interest and suggestions during the years in which the investigations were carried out. I would also express my thanks to the Carlsberg Foundation which has given a grant for the work. Further, I am indebted to Dr. OLE HAMMER for collecting wild flies from farms and fields and to Mrs. RACHEL BAGER and Professor K. A. C. BONDORFF for valuable help in the statistical treatment of the results.

II. Technique and test animals.

The experiments were started during the winter of 1934 and continued till the winter of 1936 and thereafter at intervals, when suitable material was at hand, until 1938.

The following species were employed: *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans and Scatophaga stercoraria.*

Musca domestica is the species most thoroughly investigated, eggs, larvae and puparia at different stages of development have been examined. As to the four other species puparia and larvae have been examined.

If the purpose is to experiment with fatally high temperatures the technique must be another than that of the experiments, mentioned in the introduction, on the influence of temperature on the duration of development, since a very slight change of temperature causes a marked difference in the injurious effect to be examined, and it is difficult to keep a constant temperature in dung. Hence the experiments have been carried out by immersing the objects in a water-bath of the required temperature; here it is rather easy to keep the temperature very constant for a tolerably short experimental period.

The experiments have been carried out in two ways, adapted to the particular problems to be examined: I) What is the influence of a given exposure on the further growth of the individual? II) How long can an individual live at a certain temperature?

I. In the first experimental series the technique was very simple; at the beginning the objects were placed in small very thin-walled glass tubes which were immersed in a water-bath of the required temperature, later, however, they were immersed directly into the water in small gauze bags. Controls showed no difference between the results of the two methods, but the latter was by far the more convenient and provided the most uniform heating.

The experimental period having been expired, the bags were taken out, puparia and eggs were placed on moist sand at 25° C. to emerge larvae being confined in dishes containing dung. When emerging time arrived the culture glasses were watched and notes were made of the time of emergence and the final number of emerged insects; malformations during the pupation of the larvae and in the emerged insects were recorded, all puparia not broken were opened, and the moment when death had occurred was determined as exactly as possible by observing the stage of the pupa or larva.

All experiments were made at a relative humidity of 100 per cent which is very near to the optimum and corresponds to the normal humidity of the nutritive medium—dung—of the insects.

In all experiments the larvae and puparia used were taken from cultures kept at 25° C.

The experimental temperature has ranged from 40—56° C. adjusted according to previous experience of the temperature susceptibility of the individual species and stages; the experiments were made with intervals of 1° C. The experimental period generally was $^1/_4$, $^1/_2$, 1, 2, 4, 8, 16 and 32 minutes, so that the time of exposure for the same temperature increases on a logarithmic scale. In order to stabilise the established values, however, periods of exposure were often inserted between those given above.

It is to be noticed that no experiment lasted more than 32 minutes, partly because it was difficult to keep a constant temperature for a longer period, the heating of the water-bath not being automatically regulated, partly because it was to be feared that if longer experimental periods were used the effects of irrelevant factors might be felt, for instance hunger, deficiency of oxygen etc.

MELLANBY (1932) states that the death point of lice and fleas is influenced by starvation, but since both animals feed on highly watery food, it is probable that it is the large amount of liquid which makes the well-nourished insects more resistant.

II. As to the second experimental group, for which larvae only were used, a wide glass tube (fig. 1) was placed in the water-bath, one end of the tube being closed, while the other had a pierced rubber stopper, from which a short thermometer projected into the tube. Fused into the tube near its closed end

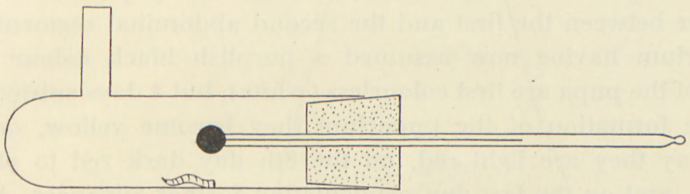


Fig. 1. Glass tube for temperature resistance experiments (see text).

was a branch which emerged from the surface of the water. When the air of the glass tube had reached the required temperature a larva was put through the branch tube down into the experimental tube, whereafter the reaction of the animal could be watched through the water from above. The time of occurrence of the various recognisable phases was noted, as also how long a time passed before death occurred (see later).

III. Experiments on *Musca domestica*.

1. Experiments of type I.

a. Experiments on puparia.

An accurate knowledge of the various easily recognisable phases of the development in the puparium, the time of their occurrence and their duration, is necessary, in order to know at which stage the insect is affected and how long after the exposure death occurred in the unbroken puparia.

At 25° C. the development of *Musca domestica* from the hardening of the larval cuticle—the formation of the puparium—until

the emergence of the fly, takes about 6 days. Hardening lasts from 2—3 hours, during which the colour changes from white to purplish red, this being followed by a final larval stage accompanied by a partial ecdysis (FRAENKEL 1938). This fourth larval stage lasts about 18 hours. Afterwards another and complete ecdysis takes place, whereby the larva becomes a pupa; at the beginning the head of the pupa is still invaginated (cryptocephalic stadium), but during the following day the head is everted (phanerocephalic stadium). After this the two minute tubular spiracles are protruded through the puparium on the border between the first and the second abdominal segment, the puparium having now assumed a purplish black colour. The eyes of the pupa are first colourless (white), but 4 days subsequent to the formation of the puparium they become yellow, on the 5th day they are light red, on the 6th day dark red to almost black, and on the last day pigmentation further advances. A few hours before emergence the movements of the frontal sac commence (E. BRO LARSEN and M. THOMSEN 1940).

For a preliminary examination of the susceptibility of the various stages experiments have been made on puparia at 8 different stages at identical temperatures. In fig. 2 they are named according to the time elapsing, counted by days, after the puparium has formed, as follows: 0: quite young, white puparіae; $^3/_4$: puparіae about 18 hours old containing larvae of the 4th larval stage; 1: puparіae 1 day old, containing pupae; 2: puparіae 2 days old etc., up to 6: puparіae 6 days old immediately before eclosion. The figure shows the result, the percentage of emergence of the 8 stages having been plotted, partly when the time of exposure lasted 1 minute (the full curve), partly after an exposure of 2 minutes (the stippled curve). It will be seen that the emergence percentage, i. e. the heat resistance, is the highest for 3 days old puparіae and the lowest for the stages: 0, $^3/_4$ and 6. The stage of the white puparium (0), however, does not last more than half an hour; hence it is difficult to procure sufficient material of this stage for a long experimental series. Stage 6 shows much irregularity and is difficult to determine with accuracy, since it is to symbolize the condition immediately prior to emergence. Thus there remains the "$^3/_4$ stage", lasting 18 hours; this stage has been chosen as a representative

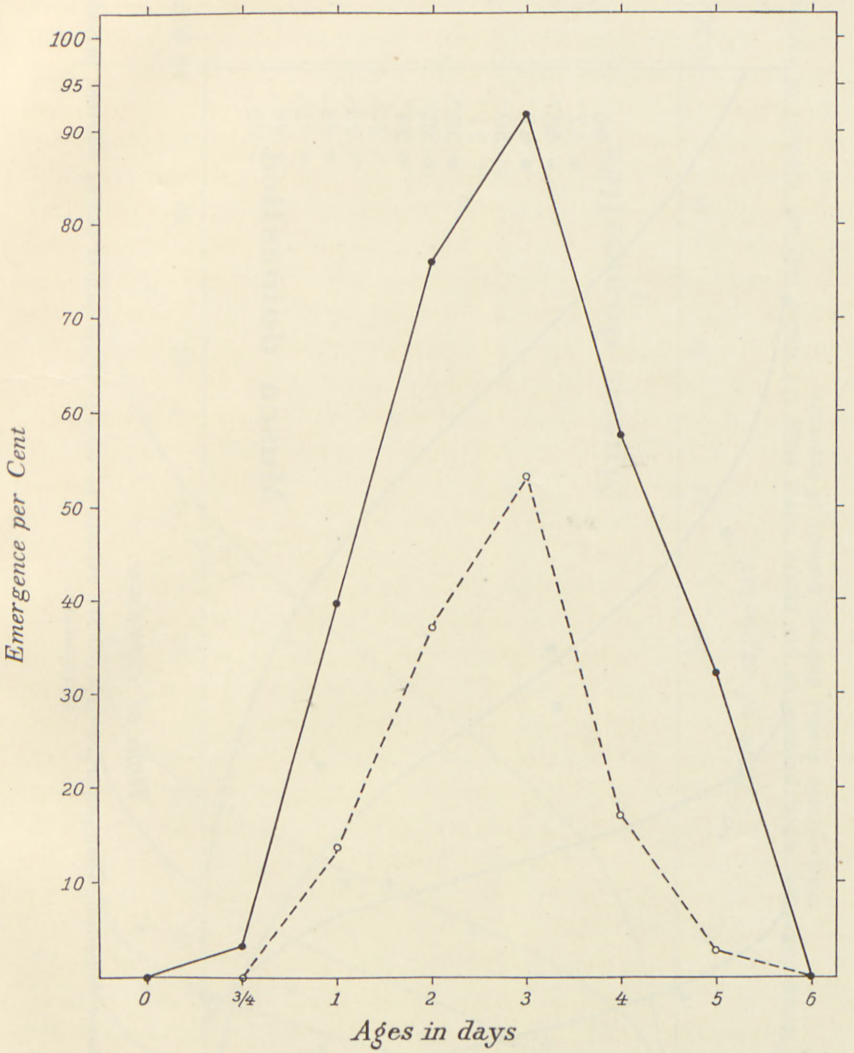


Fig. 2. Temperature resistance curves for eight age classes of puparia at the same temperature, ——— for 1 minute's exposure, ------ for two minute's exposure.

of the most sensitive stage or a very sensitive one at least, while 3 days old pupariae were chosen for experiments as the least sensitive.

The experiments were carried out according to the following principle: In each series at identical temperatures, experiments

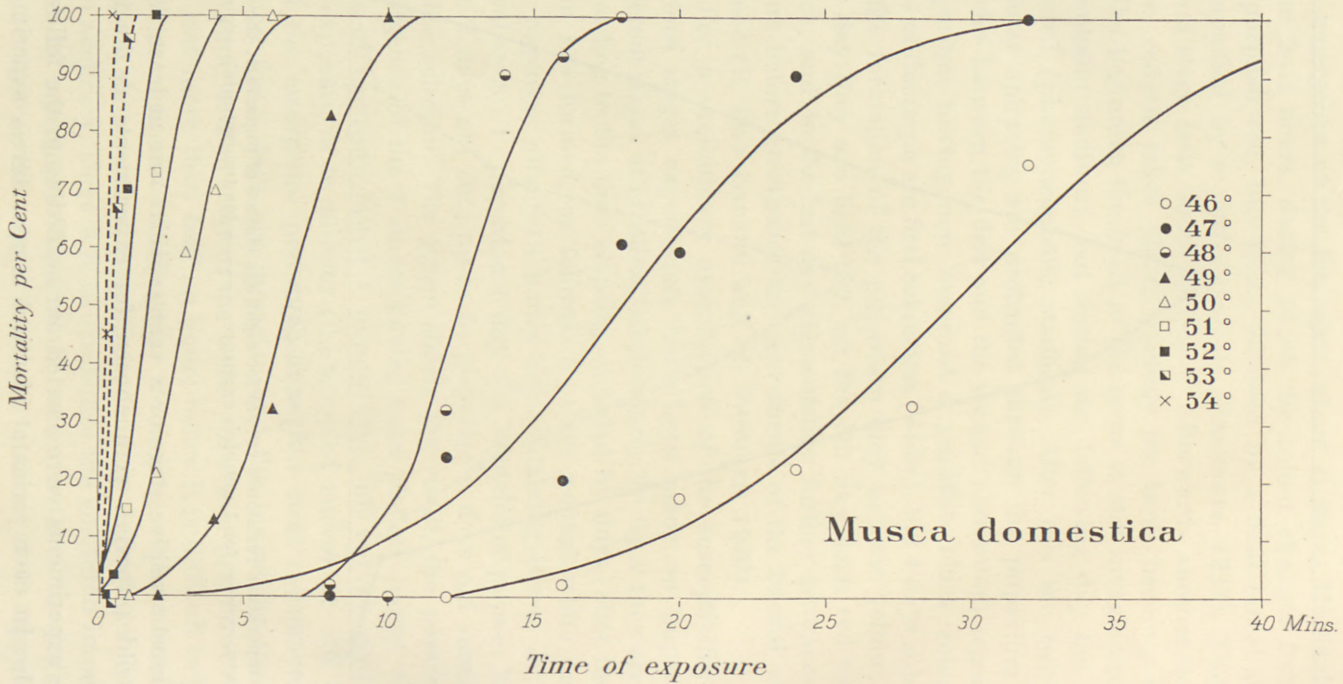


Fig. 3. The mortality in *Musca domestica*, when puparia 3 days old are exposed to various temperatures during different periods of exposure.

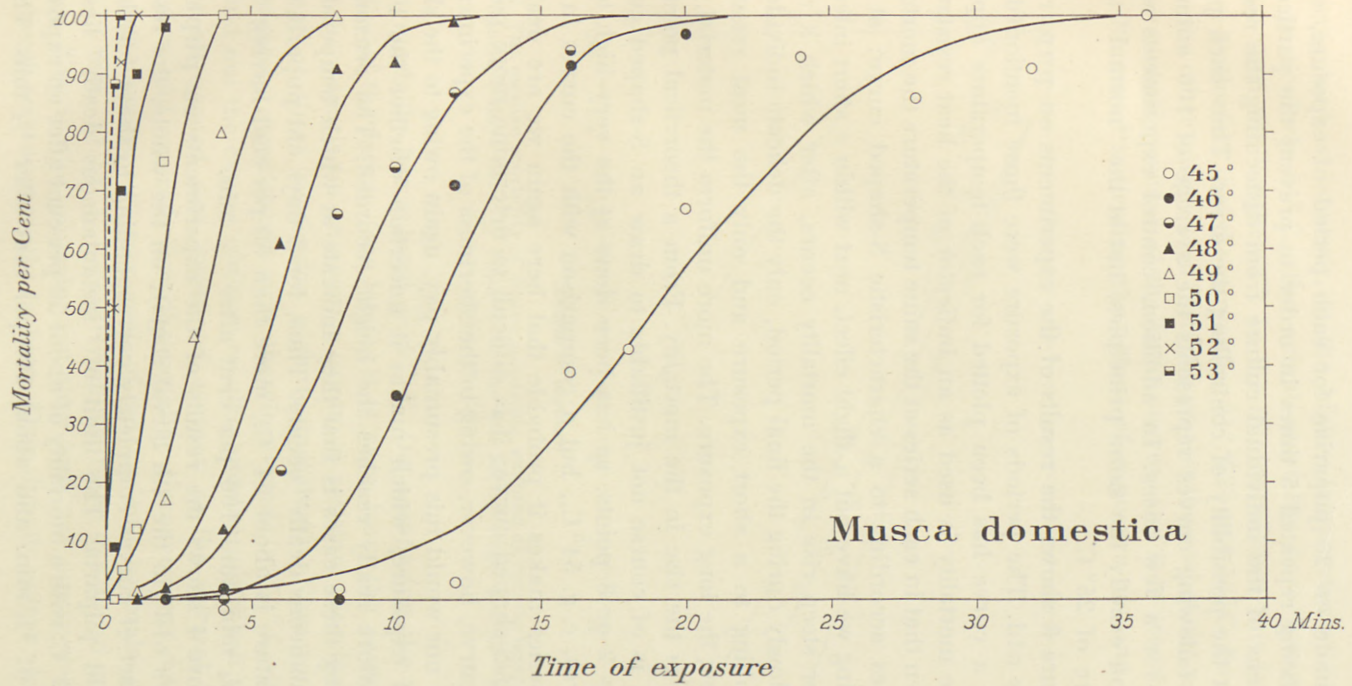


Fig. 4. The mortality in *Musca domestica*, when puparia in the fourth larval stage (18 hours old) are exposed to various temperatures during different periods of exposure.

were made on 20 puparіae for each period of exposure, each series being repeated 5 times in order to prevent the particular conditions of the individual culture from dislocating the results without the possibility of controlling the error. Thus each point of the following curves represents the average of 100 animals, except for a few cases. In addition, control experiments were made on exactly the same principles, but at the "normal" temperature of 25° C.

Figure 3 shows the results of the experiments on puparіae 3 days old. The periods of exposure were those mentioned on pag. 6, a curve has been plotted for each temperature applied and the mortality is used as an indicator of the heat resistance. It is seen that in each series at the same temperature the mortality increases according to a characteristic S-shaped curve: at the beginning we have but a slight effect, next within a short interval a rather steep rise in the mortality occurs, after which it rises more slowly during the final period, only the delicate individuals succumbing to a short exposure and only the most resistant surviving the long exposure. The more uniform the material, the steeper is the rise in the mortality. From a theoretical point of view it is of course not justifiable to draw an S-shaped curve through 2 or 3 points, as has been done at the very high temperatures, e. g. 54° C., but a comparison with the curves in the other series makes it probable that here again we are dealing with an S-shaped curve; it is difficult to determine more points of the curve, however, owing to the shortness of the experimental period, nor would this presumably pay, again owing to the short time of exposure, which makes it uncertain whether the body temperature really reaches the height recorded. The reason of including these cases is that they indicate at which temperature "instantaneous death" occurs. Thus, for 3 days old puparia this temperature is about 54° C., more than 50 per cent having died after $^1/_4$ min. and 100 per cent after $^1/_2$ min.

Figure 4 shows the results of the experiments on puparia $^3/_4$ days old (in the 4th larval stage); on the whole, the results give a set of curves completely corresponding to those for the 3 days old puparia. The limit of "instantaneous death" lies at 53°—52° C. with a mortality of about 50 per cent after an exposure period of $^1/_4$ min. and about 100 per cent after $^1/_2$ min. There

are irregularities, however, at the higher temperatures: 53°, 52° and particularly 51° C., the actual mortality here being much higher than the "expected". In the numerical data this is manifested by the fact that the curves for 51°, 52° and 53° C. lie remote from the other curves by a distance greater than what corresponds to the mutual position of the latter curves, and it is seen in fig. 5 that the curve indicating the extent of exposure causing 50 per

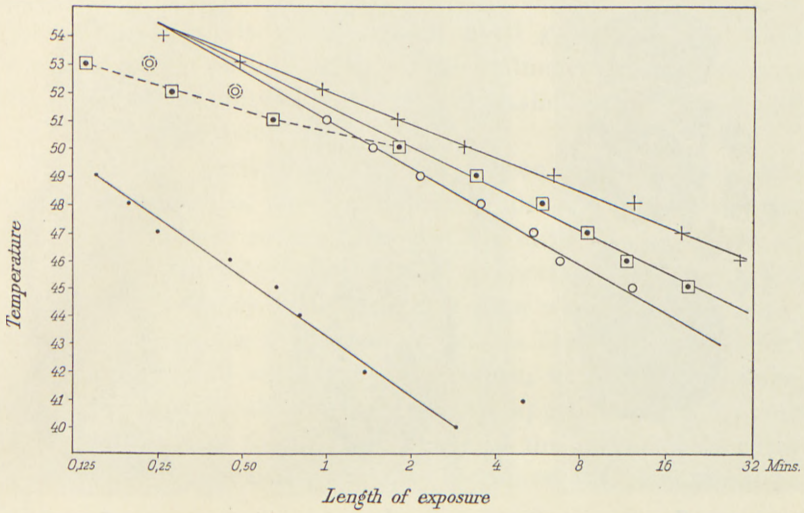
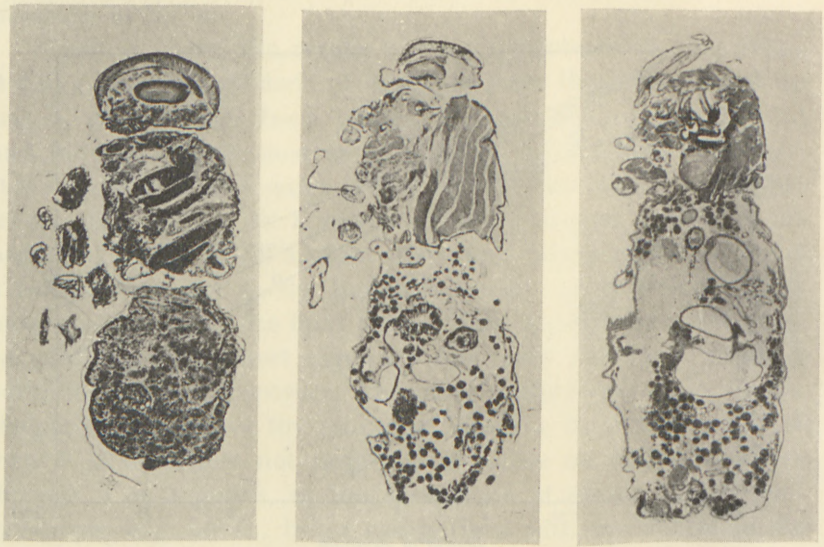


Fig. 5. The median mortality of *Musca domestica*, exposed at various temperatures. + Puparia 3 days old. ⊡ Puparia in the 4th larval stage. ○, ◎ Full-grown larvae. • Eggs.

cent mortality has a bend at 51° C. On comparing the susceptibility of the larvae (see later), it is seen from the same figure that the puparia on an average are more resistant than the larvae except at these particular high temperatures. Numerous supplementary experiments were therefore made at 51° C. in order to find out the causes of these results, and the unbroken puparia were all opened and examined as soon as the normal time of eclosion had expired. Now the peculiar discovery was made that a large number—47 per cent of all the test animals exposed for $^1/_2$ min. at 51° C.—were lying as fully developed and coloured flies in the puparia, but they had not emerged because the head was apparently lacking. Occasionally wings, legs and

setae were fully pigmented, the pupal skin ruptured etc., but the flies had not been able to break the puparium, because, among other reasons, the frontal sac was not capable of functioning. It is not correct, however, to say that there was no head, for various series of sections of "headless flies" (fig. 6 *c*) showed the head lying invaginated in the thorax of the fly. There were all



a *b* *c*

Fig. 6. Sections of *Musca* pupae *a:* Normal pupa, just before emergence. *b:* Heat-treated pupa with small head, just before emergence. *c:* Heat-treated "headless" pupa, just before emergence.

transitional stages from the completely undeveloped head to a fully developed head with pigmented eyes. A partly developed head may be everted; we then have flies with very small heads, fully pigmented but with undeveloped mouth-parts and small eyes (6 *b*). Thus the development of the head as well as the eversion itself may be disturbed or inhibited by heat effect, the development in the puparium continuing almost normally in other respects. Hence, several of the aforesaid 47 per cent would have emerged, since they were found alive and mobile in the puparia, if the lack of head had not prevented emergence, in which case the 51° C. curve would lie as do the other curves. In the tables these animals have been entered as dead in the stage "with red

eyes" or "with black eyes", judged according to the pigmentation of the body, since I thought it incorrect to place them under the stage "head absent", a stage characterising the transition of the normal puparium from the 1st to the 2nd day.

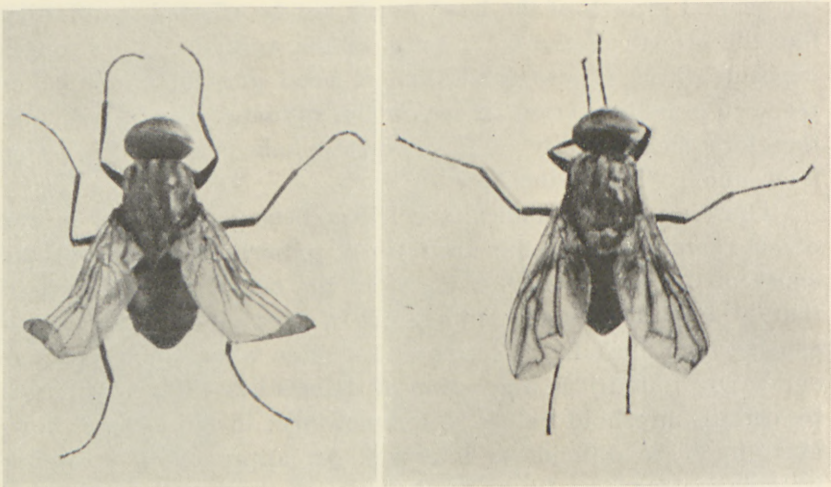
The explanation of this peculiar deformity is probably that special substances inducing the eversion of the head are destroyed by the influence of the heat, so that eversion is hindered, while the general growth of the head needs not be affected, continuing like the growth of the other parts of the animal. With stronger exposure there is also inhibition of head growth, which takes place just in the period in which the influence is applied, and therefore the mortality of the cryptocephalic stage is high (see later under "discussion").

Thus in this particular case it has been possible by means of an examination of the unruptured puparia to determine the cause of the change in the course of the curve, for it appeared that at a certain high temperature a special defect occurred causing high mortality. I suppose that often where such irregular curves are found it is a question of defects like this, conditioned by certain threshold values of temperature; in most cases, however, they are difficult to interpret. An approach to a similar irregular curve was found in the case of the larvae, and cases are mentioned repeatedly in the literature (cf. Bělehrádek 1935).

Apart from these instances deformities in the emerged flies are met with in the series of experiments on 3 days old puparia as well as in those aged 18 hours. Thus vesiculous wings filled with liquid are frequently found, and incomplete extension of the wings, which appear short, bent upwards (fig. 7a). A considerable deposit of pigment along the veins forming broad, brown or brownish black bars, is often seen (fig. 7 *b*). This very characteristic deformity appears mainly when third day puparia are exposed for a short time only at the highest temperatures: 54°, 53° and 52° C.

Günther Bodenstein (1940), in *Musca domestica*, by stimulating puparia at 42° C. at various intervals brought about a great number of various modifications of a kind similar to those mentioned above, e. g. "vesiculous wings", "drooping wings", "wings expanded", "wings crumpled"; in addition, however, his

material contains a number of other interesting modifications and mutations as to the form of the wing and the arrangement of the veins, modifications which resemble specific and racial characters of a number of other *Diptera*. As my examinations were brought to an end as early as 1938, I had not at that time become aware of modifications like the latter, for one thing



a *b*

Fig. 7. *a*: Heat-treated imago of *Musca domestica* with curled and expanded wings, *b*: *Musca domestica*, imago with pigment along the veins as a consequence of heat exposure during pupal life.

because the examinations in themselves were made for quite another purpose; hence only the most noticeable of them have been recorded. This is most unfortunate, for in a material so large and varying, numerous instances of the kind of modifications detected by BODENSTEIN were certainly present.

In order to compare three days old puparia with puparia of other stages, I have employed another method of representing the injury at high temperature: the exposure giving 50 per cent emergence or 50 per cent mortality being plotted in the coordinate system. This procedure is often used in medicine, f. inst. in analyses of the effect of poisons or in the standardisation of medicaments; it is stated what dose is necessary for a certain result: death, cramp, cure etc. in 50 per cent of the individuals used in the experiment.

This method of representation has likewise been used when comparing the resistance to heat of various species and various stages within the same species. If in the experiments on the puparia the temperature is plotted on the ordinate and the logarithm of the time on the abscissa, the result, as will be seen in fig. 5, will be nearly a straight line; the relation between the temperature and the logarithm of the time of exposure is consequently a linear function. (The temperature interval being so small, the picture of the curve will be almost the same if the logarithm of temperature is also used, as when calculating the temperature coefficient *b* (see p. 41)). In other words, the linear function means that the injury shows a linear proportionality to the percentage of increase of the exposure, not to the linear increase.

If on fig. 5 we compare the exposure necessary to bring about a mortality of 50 per cent, we see that everywhere it is less for puparia in 4th larval stage ("$^3/_4$") than for the 3 days old puparia. *A priori* it seems likely that this is due to the fact that the older pupariae have the shorter period left before emergence so that the active period of an advancing destruction caused by the heat will be shorter than in young puparia not due for emergence until several days later, however, the experiments mentioned on p. 8 on still older stages show that the whole explanation is not to be found here. In my opinion the essential point is that the influence on the young puparia takes place at a time when the latter are specially sensitive, because the metamorphosis is extremely active, numerous changes are in progress, for instance ecdysis, presumably hormonally conditioned, of the 4th larval stage to the pupa, the above mentioned eversion of the head and the formation of the limbs, the decomposition of the larval fat-body and the building up of the imaginal one, in short a period of profuse secretion of hormones and extensive formation of mitoses. It has been shown (see Bělehrádek 1935) that dividing cells are particularly susceptible to the exposure to heat compared with resting cells, so that the cause of the high mortality may be due to the large number of cells in mitosis.

The greater resistance of the 3 days old puparia suggests a quiet period in the pupal life and other circumstances point in the same direction. As is well known, measurement of the meta-

bolism in the course of the pupal life gives a U-shaped curve, the metabolism falling considerably during the middle part of the pupal life, to rise afresh towards the end of it. The phenomenon is known in a large number of insects, e. g. *Calliphora vomitoria* (WEINLAND 1906), *Tenebrio molitor* (KROGH 1914), *Phormia, Lucilia, Scatophaga* (TAYLOR 1927), *Lucilia sericata* (COUSIN 1932), and *Drosophila melanogaster* (POULSON 1935). KROGH advances the hypothesis that the metabolism is an expression of the quantity of organized tissue present, so that the drop in the beginning of the pupal life corresponds to histolysis, the rise to histogenesis. However, the explanation of metabolism as expressing the advance of the histolysis and the histogenesis is uncertain, since both processes in some cases may be completed before the drop in the metabolism occurs (WIGGLESWORTH 1939).

My experience seems to indicate that what we have to deal with is nothing but a resting period in the metamorphosis, the great resistance to heat and likewise to cold and to desiccation (E. Bro Larsen 1943) suggests that a quieter period of the pupal life has set in. The same explanation is suggested by the fact that hibernation in some closely allied species examined (there is no diapause in *Musca domestica*), e. g. *Lyperosia irritans, Haematobia stimulans* and *Scatophaga stercoraria*, takes place during this period. It is much more probable that hibernation takes place during a resting period than during a period with numerous katabolic and anabolic processes.

The hypothesis outlined above may also explain the fact that puparia 5 and 6 days old are more sensitive than those 3 days old; in the former the pigmentation of eyes, hairs, legs and wings as well as emergence coincides with or immediately follows exposure to heat, i. e. exposure is applied during a very active and critical period of the pupal life. It is difficult to make any definite pronouncement on the problem, of course, since the presence of essential processes evading observation may very well be imagined, including such as do not require an increased metabolism, e. g. possibly the differentiation of the brain or the like; nevertheless the great power of resistance to external influences coinciding with the period of low metabolism indicates a resting period.

b. Experiments on larvae.

For these experiments use was made of fully grown larvae with the formation of fat beginning, but still having food in the intestine; and as in the case of the puparia 5 groups of 20 larvae each were used for each temperature and each period of exposure. The result is as in the case of the puparia a series of S-shaped curves. One of the five series is pictured in Plate I. The values of the exposure causing 50 per cent mortality are seen in fig. 5; it is a straight line, when the logarithm of the period of exposure is used.

Larvae seem to be less resistant than puparia to high temperatures, this is shown by the position of the line of 50 per cent mortality below that for the puparia. This is apparently inconsistent with experiences made in nature, where puparia are always found in cooler places, just as laboratory experiments show that larvae prefer a higher temperature than that prevailing in the places in which the puparia are found (E. og M. Thomsen 1937); but in actual fact it is not possible to compare the circumstances. In the experiments with fatally high temperatures the question is not one of temperatures at which it is possible for the insects to live, but of an injury done by transitory exposure to a temperature otherwise fatal, hence it is comprehensible that, in a series of interdependent processes, a displacement will have greater consequences on the result of emergence for larvae than for pupae. In the first place larvae have to live for a longer time than pupae before emerging as flies, and in the second place the exposure is immediately followed by the formation of the puparium and its hardening, which is a very critical period. It has been demonstrated that these processes are induced by hormones secreted by the "ring gland" and if the activity of this hormone is inhibited the formation of the puparium is prevented (Fraenkel 1935, Hadorn 1937).

The greater sensibility of the larvae is also demonstrated in fig. 8, which shows the mortality rate at 49° C., the extraordinarily great susceptibility of the eggs being particularly noticeable. At 51° C., however, it is seen, as mentioned on pag. 13, that the young puparia are more susceptible than the larvae. In this connection it

may be mentioned that DARBY and KAPP (1933) in heat experiments on *Anastrepha ludens* find that the larvae are the less susceptible. But quite apart from it being a question of another species, and that quite different conditions may possibly play a part, it is to be noticed that the larvae are recorded as "living" if only they move after the heat application, while the pupae

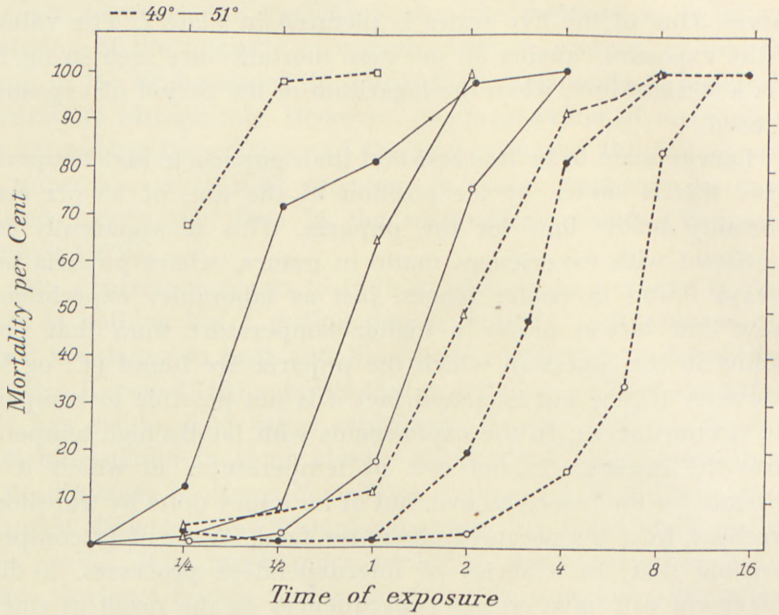


Fig. 8. Mortality of *Musca domestica* exposed to 49° ----- and 51° ———: 3 days old puparia ○, 18 hours old puparia ●, larvae △ and eggs □.

are not recorded as "living" till the moment of emergence, whereas larvae as well as puparia in my experiments are not entered as "living" until they have succeeded in carrying development through to emergence. On attempting a valuation similar to that for *Anastrepha ludens* we find that the larvae at any rate are less susceptible than the young puparia.

In the earlier experiments on the dependence of development on temperature (E. BRO LARSEN and M. THOMSEN 1940, pag. 22) larvae were kept at temperatures above the optimum, e. g. 39°, 40°, 41° C. In the behaviour of these larvae the effect of the high temperatures manifested itself in the form of increasing restless-

ness and loss of weight. The same effect is observed from exposure to fatally high temperatures if the exposure is slight only. The larvae move along quickly and restlessly as soon as the gauze bag is opened; but if the exposure is increased either by raising

Table 1.

	0 m.[1]	5 m.	10 m.	20 m.	30 m.	40 m.	24 hours
53° C.							
1/4 m.	5	50	45	70	..	85	90
1/2 m.	0	2	3	3	..	15	15
52° C.							
1/4 m.	5	40	55	70	..	95	95
1/2 m.	0	5	5	10	..	10	45
1 m.	0	0	0	0	..	0	30
51° C.							
1/4 m.	40	80	95	100	..	100	100
1/2 m.	15	55	90	100	..	100	100
1 m.	0	5	20	20	..	20	85
2 m.	0	0	0	0	..	0	5
50° C.							
1/4 m.	45	80	100	100	100	100	100
1/2 m.	20	95	65	100	100	100	100
1 m.	1	3	30	65	75	90	95
2 m.	0	0	0	0	0	0	40
4 m.	0	0	0	0	0	0	0

In the first column is given the time of exposure for each temperature. In the following columns is given the number of larvae capable of penetration into the dung at the given moment.

the temperature or by applying it for a longer period, the movements of the insects are paralysed. When the experiment is finished the paralysed animals lie motionless on the medium for a shorter or a longer time, and it is possible to obtain a measure of the heat injury in the first hand by recording the time of the return to mobility (table 1). If the paralysis ceases in the course of an hour, the insects are often able to penetrate into the dung and complete their development; but if they are completely insusceptible to excitation for more than about two hours, only

[1] m = time in minutes after end of exposure.

incomplete recovery takes place, and even if the insects after the course of 24 hours start moving a little, the injury sustained during the 24 hours will be so great that pupation and further development are inhibited.

In addition to paralysis other characteristic changes are often noticed in larvae affected by heat, for instance rhytmical, pulsating muscular movement, lack of capacity for orientation to the light, a capacity normally very well developed in *Musca* larvae. Similarly, miscolourings occur, brown and dark spots, particularly in the alimentary canal; and even if mobility is restored, the animals being able to penetrate into the dung, digestion seems to be difficult, possibly because certain enzymes have been destroyed by the heat or because the internal organs are still paralysed. The former view is held by Oosthuizen (1936) who has observed similar phenomena in larvae of *Tribolium confusum*.

Finally it may be mentioned that the ability of the full grown larvae to tolerate desiccation (see E. Bro Larsen 1943) is lost to a great extent; the skin becomes dull and flabby, and if the culture dishes are not covered, the larvae will dry up before the cessation of the paralysis. As has been stated, the formation of puparia is rendered difficult, this manifesting itself partly by the inhibition of the normal contraction, so that tapering, larva-like puparia appear (see Plate 1); if this abnormality is not too great, apparently normal flies may emerge. Further the hardening may be incomplete, which generally results in the loss of these puparia, because *e. g.*, like quite young puparia, they are very susceptible to desiccation.

In addition it is characteristic that the formation of the puparium is retarded, partly, as a matter of course, owing to the general paralysis, but partly also because dislocations in the interaction of the various processes seem to delay the formation of the puparia, and it has been noticed that the latter may be delayed up to 48 hours compared with control animals at the same temperature, a phenomenon also found by Oosthuizen in the various stages of *Tribolium confusum*.

A characteristic phenomenon, even if occurring rather sporadically, which likewise I consider related to abnormally high temperature, and which may therefore be mentioned in this

connection, is the occurrence of larvae that are unable to form a puparium. They are extraordinarily big, transparent-yellowish larvae, very glossy and distended; the normal white fat-body, seen towards the end of larval life through the cuticle, is not observable in these insects. These abnormal larvae wander restlessly about for several days after the time when pupation should normally have occurred, after which they either die or harden into a very incompletely developed puparium, characterized by its larval shape and incomplete pigmentation and hardening, and no flies emerge from these puparia. I have found these larvae in mass-cultures which have been exposed to too much heat, as well as in my experiments at fatally high temperatures; my conjecture is that the raised temperature has damaged the hormones which condition the physiological changes taking place as a preliminary to pupation.

In this connection it may be stated that Mellanby (1938) presumes that the puparium-forming hormones in *Lucilia sericata* are destroyed at 25°—37° C., because larvae at these temperatures do not develop further than the stage of prepupal diapause and fail to form puparia; if hereafter the temperature is lowered, puparia are formed after some time. Hadorn (1937) has described similar retardations and defects in the formation of puparia in a mutant of *Drosophila melanogaster* ("lethal giant"); it seems that these larvae secrete too small a quantity of the hormone of the ring gland, for it is known that the implantation of ring gland from normal larvae causes a formation of puparia. Thus it is also possible that it is a question of a spontaneous mutation without any relation to the influence of high temperature.

c. Experiments on eggs.

The incubation period of the eggs of *Musca domestica* is very short; as susceptible and non-susceptible periods alternate, it is difficult to procure material as uniform as desirable; hence a larger material has been used for these experiments than for those on the puparia and the larvae; the results nevertheless were not satisfactory.

Eggs quite newly laid were used for the experiments, and the temperatures employed were 41° (1061 eggs), 42° (1623 eggs), 44° (1276 eggs), 45° (1587 eggs), 46° (611 eggs), 47° (422 eggs),

48° (531 eggs) and 49° (339 eggs). For the control experiments 900 eggs were used, these control eggs on an average showing a mortality of 2.8 per cent.

A straight line through the exposures causing 50 per cent mortality is below the curves of the puparia and larvae (fig. 5). Consequently, eggs seem to be much more susceptible than larvae and puparia; however, a direct comparison between the physiological resistance of the different stages is actually not possible; thus it must be remembered that the units of time employed for exposure are comparatively much greater in the case of the eggs, the developing period of which is so very short (14 hours at 25° C.), while the puparia are exposed for a much smaller fraction of their total developing period.

Nothing can actually be concluded from these experiments but the fact is that, if the various stages mentioned above are given an exposure numerically identical, the mortality will be highest in the eggs, much lower in the larvae and young puparia and lowest in 3 days old puparia.

With the eggs the influence of the heat manifests itself also in a characteristic way apart from the rise of mortality. Hatching is retarded, as compared with the control eggs, and even if the development of the embryo proceeds so far that the larvae are seen within the eggs, with belts of spines, their movements are so slight that it is hard for the larvae to pierce the egg shell, whereby hatching is retarded or prevented for this reason alone. If hatching is successful the larvae often are so delicate that they die immediately after.

Here again characteristic brown spots appear during embryonic development after strong exposure.

2. Experiments of type II.

In this type of experiments the larvae were exposed to a definite temperature (see under technique) and a record was taken of the moment of death; still it must be observed that there is no guarantee that what is recorded is the actual death, since this cannot superficially be distinguished from heat rigidity. At intervals, however, a quivering of the skin is to be seen, even when movement has otherwise ceased, as long as the insect is exposed

to the heat. After the cessation of this reaction, I have not succeeded in restoring the insect to life by bringing it back to normal temperature; hence I have recorded this moment as the moment of death.

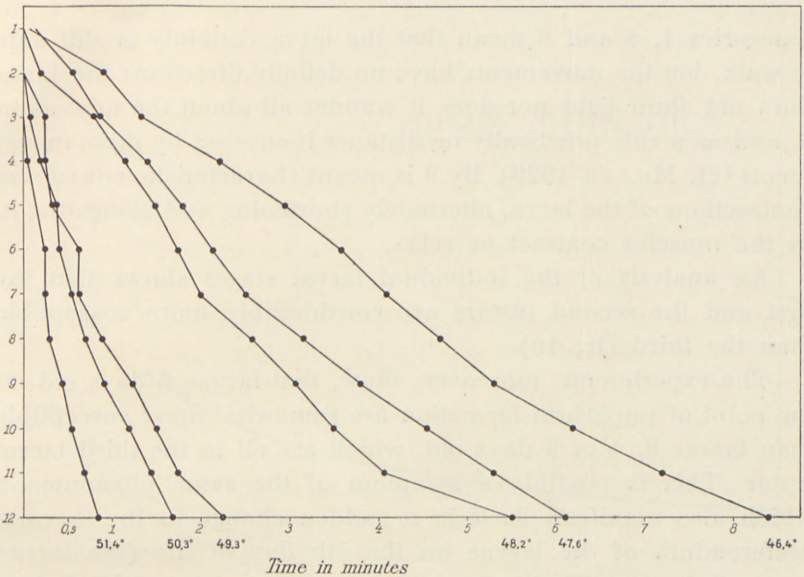


Fig. 9. The succession of the injury of larvae of *Musca domestica* when exposed to different temperatures (see text p. 25-26).

Gradually, as the changes caused by the heat exposure become greater, the individual insect undergoes a series of rather characteristic phases which can be watched through the wall of the glass tube. With rising temperature the stages are passed in quicker and quicker succession, as is seen from fig. 9 which shows the condition in full grown larvae.

A distinction is made between:

1. Normal movements.
2. Lively movements.
3. Violent movements.
4. Uncontrolled movements of gait.
5. Uncontrolled movements. a.
6. — — b.
7. Movements of front part and hind part.

8. Slight movements of front part and hind part.
9. Pulsating movements.
10. Slight pulsating movements.
11. Skin quivering and heat rigidity.
12. Heat rigidity and death.

Categories 4, 5 and 6 mean that the larva certainly is still able to walk, but the movements have no definite direction; the larva does not shun light nor does it wander all about the tube as in 3, and as a rule practically no distance is covered by these movements (cf. MILLER 1929). By 9 is meant characteristic convulsive contractions of the larva, alternately shortening and elongating it, as the muscles contract or relax.

An analysis of the individual larval stages shows that the first and the second instars are considerably more susceptible than the third (fig. 10).

The experiments, moreover, show, that larvae 6 days old on the point of puparium formation are somewhat more susceptible than larvae 3, 4 or 5 days old, which are all in the third larval instar. This is possibly a symptom of the same phenomenon which also manifests itself in a sudden change in the thermopreferendum of the larvae on the 5th day of life (the larvae were all kept at 25° C. at which temperature they take 6 days for their development), so that it is much lower during the last two days than on the previous days (E. THOMSEN and M. THOMSEN 1937). On the other hand the curve of heat resistance is highest for the big larvae 4 and 5 days old, and the drop on the 6th day is not so marked as might be expected, considering the evident lowering of the thermopreferendum of the larvae; so that in all probability the question is merely of a somewhat greater sensitivity owing to the changes which take place as a preliminary to the formation of the puparium.

If the temperatures used are plotted on the ordinate, and the logarithm of the time from, the start of the exposure until the occurrence of death, on the abscissa, the result will be little short of a straight line, expressing that the logarithm of the longevity—which here is an expression of the reversed dose of the exposure—is in inverse ratio to the temperature, or to get equal effect when the temperature de-

creases arithmetically the time must increase geometrically. Fig. 12 represents this phenomenon regarding *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans* and *Scatophaga stercoraria*. It should be noticed that at the very

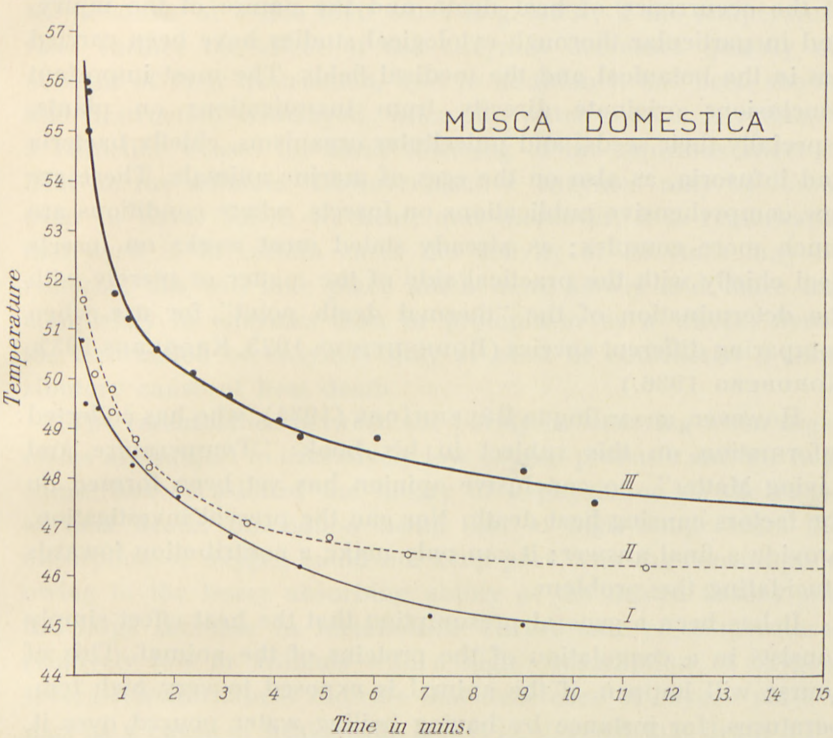


Fig. 10. Resistance curve for larvae of *Musca domestica* for the three instars.

high temperatures the points are above the straight line, i. e. a longevity is recorded greater than should be expected; such is the case with big larvae when the temperature of "instantaneous death" is approached. The explanation is presumably that during the short exposure the big larvae are not heated up to the temperature recorded; but there is also the possibility that, if the space is not completely saturated with vapour, they may, for a short time only, lower their temperature by evaporation (see Mellanby 1932).

3. Discussion.

On the causes of heat death and heat injury.

Numerous writers have worked on the problem of the causes of the occurrence of heat death and the nature of the injury, and in particular thorough cytological studies have been carried out in the botanical and the medical fields. The most important conclusions originate directly from investigations on plants, especially their seeds, and unicellular organisms, chiefly bacteria and infusoria, as also on the eggs of marine animals. There are few comprehensive publications on insects, where conditions are much more complex; as already stated most works on insects deal chiefly with the practical side of the matter or merely with the determination of the "thermal death point" for use when comparing different species (Bodenheimer 1925, Krogerus 1932, Nordberg 1936.)

However, according to Bělehrádek (1935), who has collected information on this subject in his book: "Temperature and Living Matter", no conclusive opinion has yet been formed on the factors causing heat death. Nor can the present investigation, provide a final answer; it can only make a contribution towards elucidating the problem.

It has been a general presumption that the heat effect simply consists in a coagulation of the proteins of the animal. This of course will happen, if the animal is exposed to very high temperatures, for instance by having boiling water poured over it, and it is easily understood that the heat coma, in which the animals lie extended, stiff and tense, was a temptation to biologists to assume a coagulation or fixation of a nature similar to that mentioned above. However, the fact that the heat coma may be transient, the animal once more becoming active, has been difficult to explain, since coagulation of protein only to a slight degree is a reversible process; and when moreover it was maintained that heat coma may occur and vanish an indefinite number of times, this being a normal link of the diurnal activity of the animal, there was considerable disagrement between the observations of chemists as to protein coagulation and the observations made of what actually takes place in nature. Similarly, it is

remarkable that heat injury occurs at a temperature much lower than that causing a coagulation of the proteins in question.

Among the other theories advanced to explain heat death is that of the destruction of the enzymes of the organism; this theory is supported by the fact that enzymes have an optimum temperature, at which their effect is greatest; if the temperature rises further the effect of the enzymes decreases quickly on account of their destruction, and in addition it has been shown that destruction is delayed, when the water content is reduced, a reduction eauses the same lowering of the injurious effect of heat on the animals. However, active enzymes may be found even in tissue killed by heat; and moreover, it is remarkable that even if in certain cases the activity of enzymes may be restored, this will take place much more slowly and more imcompletely in enzymes than in protoplasm as a whole; hence the destruction of enzymes may at most be considered a contributory cause of heat death.

The resemblance between the paralysis occurring when organisms are subject to excessively low oxygen pressure and the heat coma, has occasioned the theory of asphyxiation as the cause of heat death, the notion being that at high temperature the absorption of oxygen could not keep pace with the consumption owing to the lesser absorbing ability of the heated tissues and the large increase in metabolism. In the case of temperatures relatively low in animals with a high consumption of oxygen, several circumstances indicate that deficiency of oxygen plays a part as a cause of heat death, but it is objected that death often occurs so early that oxygen deficiency cannot possibly have set in.

The same is true of an accumulation of toxins in the organism as a cause of heat death. As a result of increased metabolism owing to the heat, poisonous metabolites have been supposed to accumulate in the organisms; in certain cases this, no doubt, is so, but it may be stated here, as with the theory mentioned above, that the injury often sets in so rapidly that there can be no question of an accumulation of metabolites.

Finally a theory much favoured is that the influence of heat causes a change in the fats of the protoplasm, the lipoids, so that at the critically high temperatures the injury consisted of the melting of the cell fats. It is taken for granted that the melting

points of the lipoids of animals have a relation to the temperature at which they are formed so that heat-adapted animals have fats with a high melting-point, whereas the lipoid melting-point of cold-adapted animals would be lower. This theory of the melting of the lipoids as the cause of heat death is supported by the observation that animals adapted to heat often have a greater resistance to fatally high temperatures than those adapted to cold. Various investigations of the chemical and physical changes of the cells point in the same direction, whereas others weaken the theory (Bělehrádek 1935, p. 214).

All in all it may be said that none of the above theories alone covers the cause of heat death and heat injury.

If we ask what contribution the present investigation makes to the elucidation of the problem, it must be said at once that it does not support the theory of the general coagulation of proteins; heat death does not occur at a definite temperature, there must always be a combination of temperature and time so that the "thermal death point" as expressing the coagulation point of the proteins of the species concerned must be abandoned.

However, in the present investigations special stress is laid upon the determination of the time at which the heat death occurs, and by opening unbroken puparia and by the analysis of dead eggs we find that the characteristic feature is that development continues for a shorter or a longer time after the heat exposure. Under severe exposure the effect of the injury advances rapidly, death occurring early; under less severe exposure there is considerable development before death occurs. An attempt is made to illustrate this in table 2, where the results of all the experiments on 3 days old puparia are grouped, arranged to temperature and duration of exposure. It will be seen that with the weak stimulations, for instance with short periods of exposure, development continues for a long time after the cessation of the exposure, death occurring only late; with stronger stimulations death interrupts development at an earlier stage.

In addition, we see from table 3 that the mortality rate is not evenly distributed over all stages of development but is more frequent at certain stages. The table gives the course of the experiments on $^3/_4$ day old, and 3 days old puparia.

All experiments with periods of exposure of $^1/_4$, $^1/_2$, 1, 2, 4,

Table 2. Puparia 3 days old. Mortality and Emergence of Flies.

Temperature	Time of exposure	Stages of development					
		0	1 Yellow eyes	2 Red eyes	3 Black eyes	4 Half emerged	5 Emerged
54° C.	1/4 m.	6	..	6	21	14	92
	1/2 m.	40	17	45	32	..	..
53° C.	1/4 m.	..	..	2	1	2	97
	1/2 m.	..	..	23	29	8	43
	1 m.	45	14	16	21	1	4
52° C.	1/4 m.	..	..	..	..	..	101
	1/2 m.	..	..	3	2	1	96
	1 m.	15	..	19	28	9	29
	2 m.	34	22	26	18	..	..
	4 m.	95	5	..	..	..	..
51° C.	1/4 m.	1	..	..	..	..	99
	1/2 m.	..	..	..	3	..	97
	1 m.	..	..	9	6	3	82
	2 m.	..	..	27	43	5	25
	4 m.	2	41	51	6	..	..
	8 m.	50	44	6	..	..	..
50° C.	1/4 m.	..	..	..	..	..	40
	1/2 m.	2	..	..	..	..	38
	1 m.	1	..	..	..	..	99
	2 m.	..	..	9	10	4	77
	4 m.	11	..	20	34	13	24
	8 m.	2	21	52	5	..	..
	16 m.	19	14	3	..	..	..
	32 m.	100	..	..	..	..	..

Column 0: Number of individuals died during or immediately after exposure.

Column 1: Number of individuals died in pupal stage with yellow eyes (4 days old).

Column 2: Number of individuals died in pupal stage with red eyes (5 days old).

Column 3 and 4: Number of individuals died in pupal stage with black eyes or half emerged (6 days old).

Column 5: Number of individuals emerged.

8, 16 and 32 minutes are here summarized, including the supplementary experiments with other periods of exposure, than those originally planned. For 3/4 day old puparia there are the

following phases: 0: death occurs during exposure or immediately afterwards, viz. in the 4th larval stage; 1 and 2: death occurs after the formation of the pupa, but prior to the eversion of the head (1), or during eversion, so that a very small head results (2); 3: death occurs during the period of white eyes; 4: during the period of yellow eyes; 5: during the period of red eyes; 6: during the period of black eyes; 7: indicates that emergence has taken place. For 3 day old puparia the following phases are distinguished: 0: death occurs during exposure or immediately afterwards, viz. during the stage of white eyes; 1: death occurs during the stage of yellow eyes; 2: death occurs during the stage of red eyes; 3: death occurs during the stage of black eyes; 4: death occurs during emergence so that the puparium is broken, but the fly too much weakened to complete the process; 5: emergence apparently normal.

It is seen from tables 2 and 3 that when the exposure is increased, either by raising the temperature or prolonging the period, the mortality rises; but there is a remarkable difference between the two groups in table 3; where young puparia in the 4th larval stage are exposed, death chiefly occurs during or immediately after exposure, but when older puparia are subjected to the same doses, death occurs more frequently later, i. e. when development has passed the resting stage and reached the sensitive stages in which the formation of the imago is completed.

Table 3. Percentage of Mortality and Emergence of Flies.

Stage of development reached	Puparia 3/4 days old	Puparia 3 days old	Stage of development reached
0.* 4th larval stage ...	32 per cent		
1. headless	6 —		
2. small head			
3. eyes white	1 —	8 per cent	0.* eyes white
4. — yellow	1 —	6 —	1. — yellow
5. — red	4 —	12 —	2. — red
6. — black	4 —	12 —	3. — black 4. head everted
7. emergence.....	53 —	61 —	5. emergence
	101 per cent (4668 Ind.)	99 per cent (5126 Ind.)	

* 0 = stage at exposure.

It is also seen from this table that no matter what the exposure the mortality is the lowest during the stages of white and yellow eyes, whereafter it rises. Similarly it is seen that mortality during the exposure or immediately after is remarkably high, if the exposure takes place in the critical 4th larval stage, whereas it is but one fourth if the exposure takes place during the stage with white eyes.

The same is true of the experiments on the full grown larvae. If the results of all experiments are summarized, it is seen that the mortality within the period from exposed larva to emerged fly is not evenly distributed over the different stages, a calculation of the mortality showing the following figures:

Table 4. Percentage of Mortality and Emergence of Flies.

Died during or immediately after exposure	Died during formation of puparium	Died during the rest of pupal life	Emerged from larva-like puparia	Emerged from normal puparia
	a b c			
39 per cent	3—8—1 per cent	3 per cent	1 per cent	45 per cent

The columns *a*, *b* and *c* are stages of abortive formation of puparium, so-called "Larva-like puparia" or larval puparia (see Plate I) being formed. If the formation of the puparium is successful, no more than 3 per cent die during the whole pupal life, while 45 per cent develops to flies. Of the "Larva-like puparia" only 1 per cent develops, and here the formation of puparia proved to be a critical stage with high mortality (see also Plate I).

These observations, which show the successive advance of injury after exposure, and the higher frequency of death in some stages than in others, first and foremost are inconsistent with the theory of a general coagulation of proteins as the cause of heat death; nor do these observations support the theory of death owing to the melting effect of the temperature on the fats of the protoplasm.

The experience that with an exposure at one time the result appears at a later stage and that some stages are more suscep-

tible than others suggests a relation to the phenomenon that in development there is an alternation between labile and stable periods—active periods and resting periods. During the labile periods are induced the processes which later on will make their mark upon developments. We know only incompletely how this induction is carried out, but very likely certain determinator or induction substances are secreted during the labile periods, their task being to predestine, to start and regulate processes during embryonic as well as the post-embryonic development. Having the above phenomena in view I think it most likely that the injurious effect of temperature consists in a destruction or weakening of such determinator substances; for the consequence involved will be that the various processes are relatively displaced, i. e. that the equilibrium of development is disturbed.

If the destruction is only slight, the displacements will be so small that development can be completed, with or without defects as the result. If the displacements of the processes necessary to development are too great, development comes to a standstill and the animal will die, not so often in the resting periods as in the active periods, which require very intimate interaction between the various processes. If exposure takes place immediately prior to or during an active period, death or the defects will occur in this; if it takes place during a resting period, the consequences will mainly be seen when the effect of the determinator substances acting during the resting period is on the point of manifesting itself. This conforms with experiments made for instance on *Lepidoptera* (Prochnow 1914) in which the heat influence takes place in a critical period shortly after pupation, whereas the result does not appear until the pigmentation of the wings takes place during the last days of pupal life.

I am not of the opinion that there is any special cause of heat death, as sought by several investigators; there is no fundamental distinction between the injurious effect of heat from heat defect to heat death.

The nature of thermal injury to the living organism is, however, so very complex (cp. Bělehrádek 1935) that one cannot imagine it to be due to one single cause. The very characteristic general paralysis due to the influence of fatally high temperatures suggests, first and foremost, that the nervous system is

attacked; this is not to be wondered at, since in numerous other fields it has been found that the cells of the nervous system are more sensitive than the other cells of the organism. Here, as little as in the case of a destruction of determinator substances, the direct cause of the injury need be the high temperature itself. I think it most likely, that the injury in itself is due to the formation of toxic metabolites arising in consequence of the high temperature, perhaps poisonous substances of a kind similar to the histamines appearing if mammalian tissue is heated. The linear relation of the injurious effect to the logarithm of the dose recalls phenomena known in medical science in case of poisoning of the organism.

An accumulation of toxic metabolites in the cells is due probably to a defective oxygenation during cellular metabolism, the absorption of oxygen being deficient in heated tissues, i. e. a kind of asphyxiation within the individual cells. On the basis of my experiments I consider that the objection made that the heat exposure is often so short that there can be no question of oxygen deficiency or of accumulation of metabolites, is not well supported; the injury seems to be of the same nature with the shortest periods of exposure—$^1/_4$ min.—as well as with the longest. Moreover, it is known in medicine that toxic metabolites (histamines) may appear almost instantaneously. It is likewise known that a very brief reduction (a few seconds) of the supply of oxygen may paralyse, wholly or in part, the activity of brain cells. For instance, transitory pressure on the carotid arteries, about 15—30 seconds, will cause disturbances of vision and uncontrolled movements of the musculature of the limbs and the face; additional pressure causes paralysis (cp. the uncontrolled movements of *Musca* larvae prior to the occurrence of the paralysis). Thus it may very well be imagined that a brief oxygen deficiency may cause the formation of toxins, which take a long time to wash out of the organism (cp. the duration of paralysis in fly larvae) and which may destroy substances essential to development, so that the processes of development will be displaced and the animal at last will die.

Presumably it is incorrect to resort to such phenomena as the reduced capacity of absorption of heated tissues, etc. for there is no specific effect of fatal temperatures; exposing the

insects to lower temperatures results in similar symptoms of a poisoning of the organism, paralyses, defects etc. The more general explanation no doubt is that the high as well as the low temperatures are outside the temperature range to which the species is adapted and inside which the processes of the animal proceed harmoniously, without poisoning the animal.

IV. Comparison between various species of flies.

In addition to *Musca domestica* four other species have been examined, viz. *Stomoxys calcitrans*, *Lyperosia irritans*, *Haematobia stimulans* and *Scatophaga stercoraria*. These five species are characterized by being associated with the domestic animals, the nutrition of their larvae is almost identical, they all live in dung, and they are all capable of existing in cow dung, though *Musca domestica* prefers pig or horse dung. Moreover, it is common to them that they produce several generations throughout a year, and that the duration of development from egg to imago is short—shortest for *Musca domestica:* 11 days at 25° C. However, there are characteristic differences in their reactions to temperature. In a previous publication the duration of development of these species in relation to temperature has been studied (E. Bro Larsen and M. Thomsen 1940), and in another paper a comparison of the thermopreferenda of the larvae has been carried out on the basis of laboratory experiment, the results having been placed in relation to existing experience of the behaviour of the species in nature (E. Thomsen and M. Thomsen 1937).

The biology of the species examined according to observations in this country may briefly be characterized as follows: *Musca domestica* and *Stomoxys calcitrans* are both indoor forms. *Musca domestica* is particularly thermophilous, the larvae live in the strongly fermenting pig dung, and the species has a marked summer maximum. *Stomoxys calcitrans* is less thermophilous, the larvae live in the less fermentive cow and calf dung; the occurrence of the maximum depends on the temperature of the cow byre and the available quantity of nourishment (blood),

i. e. the number of cattle present, generally it occurs in the autumn when the cows are taken to the byre.

The other three species breed in the open. *Lyperosia irritans* is a marked summer form with a maximum in July-August, *Haematobia stimulans* is a spring and autumn form with a pronounced summer depression, and the same is true to a still

Table 5. Ecological data of species studied.

	Thermal constant	Threshold of development	Optimum temperature	Shortest duration	Upper temp. limit	Phenology	
						Summer	Winter
Musca domestica	146	12.2	33.2	6.92 d.	40	Several generations throughout the summer.	Slow development indoors.
Stomoxys calcitrans	193	12.3	31.4	10.75 d.	35	do.	do.
Lyperosia irritans	141	12.9	32.3	7.54 d.	36	Midsummer maximum.	pupal diapause.
Haematobia stimulans	189	10.7	28.3	10.96 d.	31	Spring and autumn maxima; summer depression.	do.
Scatophaga stercoraria	372	2.1	25.3	15.58 d.	27	do.	do.

greater extent of *Scatophaga stercoraria*, for it appears earlier in the spring and disappears later in the autumn, so that the summer depression becomes more pronounced (O. Hammer 1941). The table above gives information of the data obtained so far regarding the ecological constants of the species and their behaviour in nature. (For the explanation of the termini see E. Bro Larsen and M. Thomsen 1940, p. 16).

1. Experiments on puparia.

Owing to the difficulty of procuring uniform material the experiments have been carried out only on puparia of *Stomoxys* and *Scatophaga* of an age corresponding to the most resistent stage in *Musca domestica* and only for every second degree: 40°,

42°, 44°, 46° C. etc. The results are series of S-shaped curves as an expression of the mortality at the different degrees of exposure, and a curve through the points of 50 per cent of mortality is little short of a straight line (see fig. 11). If the curves of 50 per cent mortality for *Musca domestica* and the two other species are compared, it is seen that the puparia of *Scatophaga* are the least resistant, those of *S. calcitrans* somewhat more resistant, while

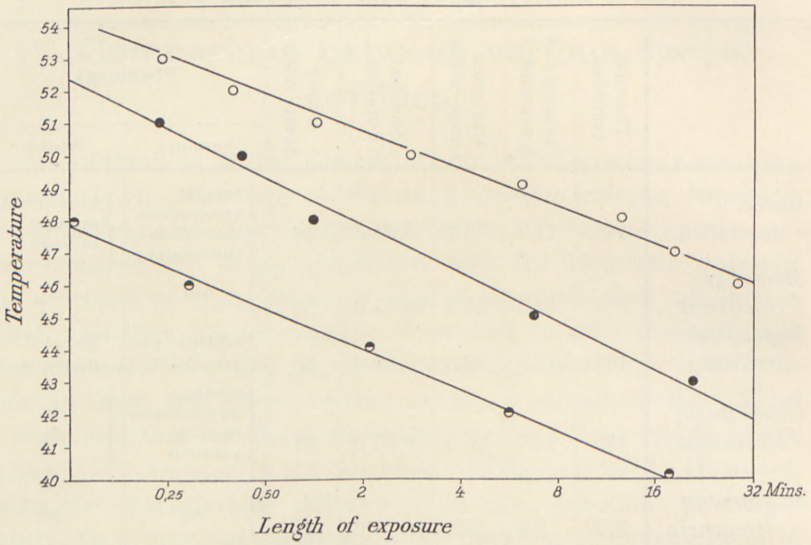


Fig. 11. The median mortality of *Musca domestica*, *Stomoxys calcitrans*, and *Scatophaga stercoraria*, exposed as puparia with white eyes to different temperatures and for various periods of exposure. ○ *Musca domestica*, ● *Stomoxys calcitrans*, ◓ *Scatophaga stercoraria*.

the resistance to high temperature is greatest in *Musca domestica*, which corresponds exactly to the information provided by table 5. On opening dead puparia we find the same phenomena as those seen in the puparia of *M. domestica:* that the pupae exposed to the smallest dose of heat have been able to continue development for several days after the exposure, while those exposed to larger doses die shortly after or during the exposure.

2. Experiments on larvae.

All experiments on larvae were carried out according to experimental type II in 1935.

The results are pictured in fig. 12 indicating the time elapsing from the beginning of the fatal exposure until the occurrence of death. The points represent mean values of individual experiments summed up for each half degree; generally each point is the average of about ten individual experiments, only in the case of the lower temperatures the average is of less than ten. For the points representing very high temperatures the same holds good

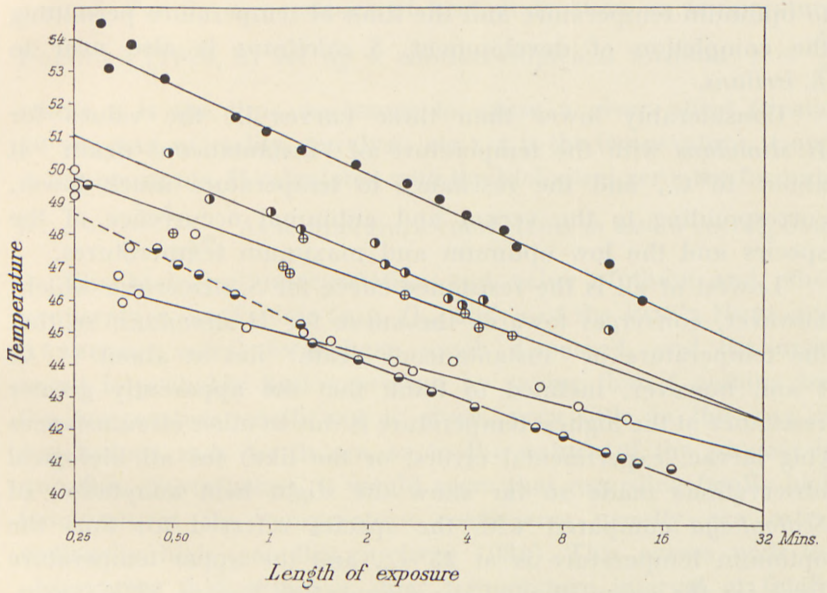


Fig. 12. Temperature resistance curves for larvae of ● *Musca domestica*, ◑ *Lyperosia irritans*, ⊕ *Stomoxys calcitrans*, ○ *Haematobia stimulans*, ◒ *Scatophaga stercoraria*.

as in the case of *M. domestica:* the larvae have a greater viability than should be expected in view of the temperature recorded; this is true particularly of *Scatophaga stercoraria* having the biggest larvae.

The results are seen to be in close accordance with the ecological facts given in table 5 on p. 37. At the top is *M. domestica*, the species on the whole showing the greatest adaptation to high temperatures, with the temperature for "instantaneous death" lying at about 53° C. (death occurring within the first half minute).

Next below is *L. irritans* with the temperature for "instanta-

neous death" at 50—51° C.; evidently it has a somewhat lower resistance to temperature corresponding to its outdoor life in the only slightly fermentative cow dung, but with a marked summer maximum revealing adaptation to high temperature. Table 5 shows that even regarding "optimum temperature" and "upper temperature limit of development" it comes next to *M. domestica*.

The curve of *S. calcitrans* is very near to this curve, with the temperature of "instantaneous death" at 48—49° C. In respect to optimum temperature and the limit of temperature permitting the completion of development, *S. calcitrans* is also near to *L. irritans*.

Considerably lower than these curves lie the values for *H. stimulans* with the temperature of "instantaneous death" at about 46° C., and the resistance to temperature much lower, corresponding to the vernal and autumnal occurrence of the species and the low optimum and maximum temperatures.

Lowest of all is the resistance curve for *S. stercoraria* which, however, converges towards the curve for *H. stimulans*, so that the temperature of "instantaneous death" lies at about 47° C. I am, however, inclined to think that the apparently greater resistance at the highest temperature is due to other circumstances (big larvae, experimental errors, or the like) for all biological observations made so far show the slight heat adaptation of *Scatophaga* compared with the species referred to; thus the optimum temperature is at 25° C., and the upper temperature limit for the completion of development as low as 27° C.

Hence it is seen that the results obtained from the application of fatally high temperatures show conformity with the habits of the animals in nature and their relation to temperature. Nieschulz (1933) on the basis of another experimental principle (see Bodenheimer 1925) found a similar agreement when determining the fatal heat maximum for imagines of *Musca domestica* (46.5°), *Stomoxys calcitrans* (43.8°), and *Fannia canicularis* (40.9°) corresponding to the thermopreferendum of the three same species, determined as 33.1°, 27° and 20.5°.

For the dependence of biological processes on temperature Bělehrádek (1926) drew up an empirical formula: $y = \frac{a}{t^b}$, in which y is the time, t the temperature and a and b are constants.

If the temperature is calculated from the biological zero (comp. E. Bro Larsen and M. Thomsen 1940, p. 16), the formula will be: $y = \frac{a}{(t-\alpha)^b}$ in which α is the biological zero, and if we write $b = 1$ the formula is: $y\ (t-\alpha) = a$. This is the formula of thermal summation which consequently is a particular case of Bělehrádek's formula. b is called the temperature coefficient and is considered a characteristic of the course of the process concerned. For the dependence of heat destruction on temperature Porodko (1926, b) set up a similar empirical formula: $z = \frac{A}{t^m}$ where z is the time necessary to cause a given effect (death, 50 per cent mortality, paralysis etc.), t is the temperature, A and m are constants. If calculated with the biological zero, the formula is: $z = \frac{A}{(t-\alpha)^m}$. As temperature coefficients m and b correspond.

The biological interpretation and value of these and other temperature coefficients (e. g. Q_{10} and μ of the van 't Hoff and Arrhenius rules) have been much discussed, and the main result is probably best expressed by saying that if nothing but the temperature coefficient is given, very little in the way of conclusions may be drawn as to the nature of the process in question. Nevertheless, it would seem that regarding fatally high temperatures the temperature coefficients usually are extraordinarily high (see Bělehrádek 1931). This agrees with the observation that within a short temperature interval at fatally high temperatures the effect of the injurious processes is doubled, while the velocity of the process is doubled within a much longer interval with moderately high or low temperatures. Thus the duration of development of *Musca domestica* at 21.5° C. is 15.67 days, at 30.3° C. 8.04 days, the velocity is doubled within an interval of 8.8° C. On the other hand at 48.6° C. *Musca* larvae will be killed in 4 minutes and at 49.2° C. in 1.8 minutes, a doubling of the intensity within less than 2° C. In accordance with this we find the temperature coefficient of the first mentioned process to be: $b = 1.002$, if $\alpha = 12°$ C., while in the latter case b is found to be 7.8 for $\alpha = 33.2°$ C. (the optimum temperature). This simply means that the supraoptimal temperature interval up to instantaneous destruction is only small.

An attempt of characterising the five species examined by means of *b* will give:

	α	*b*	α	*b*
Musca domestica	0	24.8	33.2	7.8
Lyperosia irritans	0	26.2	32.3	8.4
Stomoxys calcitrans	0	35.5	31.4	11.5
Haematobia stimulans	0	38.7	28.3	13.8
Scatophaga stercoraria	0	26.1	25.3	10.7

For the biological zero the optimum temperature was chosen from the argument that at the point at which mortality is the lowest and the completion of development most rapid, the heat injury will be least. This point therefore may be regarded as the biological zero for the special injurious processes of temperature.

It is seen from the table that for the four related *Muscidae b* rises with the increasing susceptibility to temperature; however, for *Scatophaga b* is 26.1 and 10.7 respectively and the course of the curve is another (fig. 12), so that *b* in no simple manner expresses the relation between the susceptibility of the five species to heat influence. Hence, we must be content to give a numerical expression of the empirical facts, as for instance:

The longevity is 4 min. for:

M. domestica at	48.5° C.
L. irritans -	46.0° C.
S. calcitrans -	45.5° C.
H. stimulans -	43.5° C.
S. stercoraria -	42.9° C.

In these comparative experiments the experimental animals are stimulated until they die; hence there is a question of an instantaneous destruction of vital substances and not, as in the time-limited exposure, of a chock resulting in a displacement of the equilibrium of processes and leading to the death of the animal.

If then the cause of death is to be sought in a destruction or melting of the lipoids of the cells (see p. 29) the succession of the melting points of the various species should be as given on p. 37: *Musca domestica, L. irritans, L. calcitrans, H. stimulans, S. stercoraria.* I have not had the opportunity to examine whether

this is the case, but even if it were, one must be cautious of drawing conclusions that are too general and believing in a simple relation between the melting point of the lipoids and the resistance to fatally high temperatures. If we take FRAENKEL's experiments (1938, b) on the thermal adaptation of *Calliphora* larvae, we find that if the larvae are kept at 12° C. and 31° C., the power of resistance of the latter to fatally high temperatures is about twice the resistance of the former, and equivalent injury at 39° being reached in 5 hours in the latter, but in 3 hours in the former, and a rise in the melting point of the lipoids is actually demonstrated. But if we take the two species *Musca domestica* and *Haematobia stimulans*, the resistance of *Musca* at 46° C. is 28 times greater than that of *Haematobia*, an equivalent effect being obtained in half a minute in *Haematobia* and in 14 minutes in *Musca;* and even if my experiments have been merely preliminary, I have found that if *Musca* larvae are kept at about 18°—20° C. their thermal resistance nevertheless is much greater than that of *Haematobia* larvae kept at 25° C., just as the difference of the resistance of *Musca* larvae kept at 25° C., and of those kept at 33° C. is so small that I am not even sure that I have demonstrated it. The very great difference found between the resistance of different species is of an extent much wider than what can be reached through experimental adaptation, and it cannot be directly due to differences in the melting points of the lipoids.

Statistical Treatment.

It appears from the foregoing that there is an individual variation in reaction, even if the exposure is the same, for we get a certain percentage of mortality and a certain percentage of emergence, not an all or none reaction, which result is due to the fact that all individuals were not equally resistant to the heat. Among the dead unbroken puparia is seen a marked variation of the degree of the fatal injury—some have died immediately after the exposure, others not until immediately before emergence (compare table 2 as also the individual experiments of the figs. 3 and 4). I have attempted to illustrate the results diagrammatic-

ally in fig. 13. The distance between the two parallel lines x and y indicates the variation of the test animals, the weakest specimens being found along the line x, those of medium resistance in the middle, and the most resistant along the line y. Along the horizontal axis the intensity of the exposure is plotted, the lowest

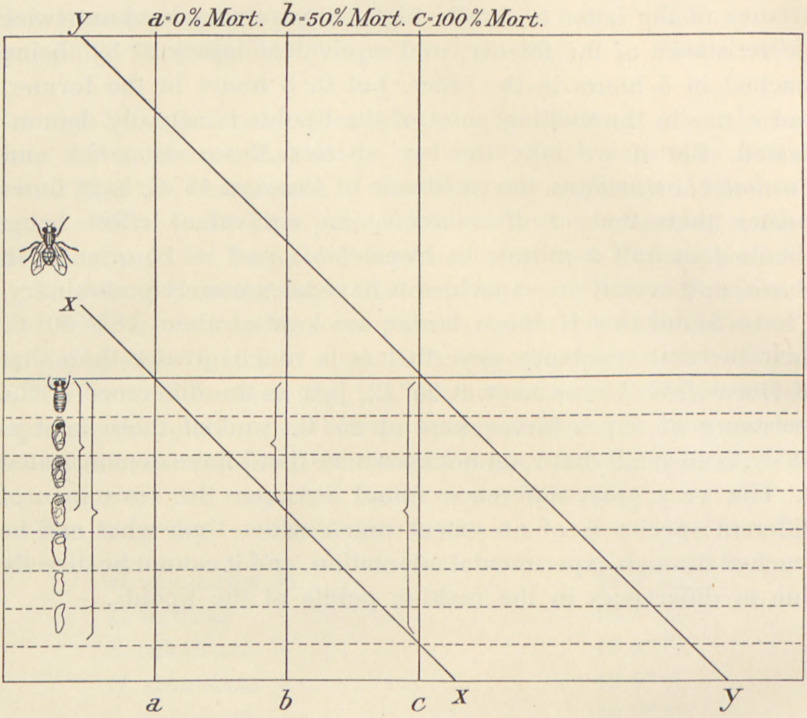


Fig. 13. The influence of variability in a given population of puparia exposed to heat (see text).

exposures being found on the left, the most severe on the right. Along the vertical axis diagrammatical drawings show the stage of development at which death occurred (cp. the classification on p. 32). The upper horisontal line represents the boundary between emergence and death, beyond this are the emerged flies[1].

If a definite heat dose b is given, both weak and resistant individuals are damaged, as the vertical line from b is meant

[1] In the diagrams no regard has been taken to the varying sensibility of the individual stages.

to illustrate, the consequence being that a percentage of 50 will emerge and a percentage of 50 will die, viz. the 50 per cent nearest to the x-line. Within the latter, however, some died in the stage with yellow eyes, some with red eyes and some with black eyes, and still others died immediately before emergence should have taken place. In the same way a continuity of the injury must be imagined among the emerged animals, only it evades analysis to a greater extent; an examination of fertility and viability in the survivors, would, however, probably bring it to light.

If we take another exposure, a, a percentage of 100 flies will emerge, while the exposure c results in a mortality of 100 per cent, and these puparia are more severely injured ("more dead") than in case of exposure b, so that some will be dead immediately after the exposure, some in the 4th larval stage etc.

In order to obtain a truer picture of the extent of injury, these circumstances ought to be taken into consideration and corrections made in the curves of mortality for the extent of the injury to the dead puparia. This was done, the result being that the course of the S-shaped mortality curves become more clearly and regularly S-shaped. This suggests that the S-shaped curve is an expression of properties in the experimental material.

As mentioned it is characteristic of the curves that at the beginning mortality increases at an ever accelerating rate, afterwards increasing at an ever falling rate; the turning point is at about 50 per cent mortality, at which figure the rate is the highest, the curve being approximately symmetrical about this point. Moreover all curves are identical, the mortality rate alone being different. The most likely explanation of this shape of curve is that it is an expression of the individual variation among the experimental animals regarding the quality: resistance to heat. Thus the S-shaped curve is the summation curve deduced from the probability curve concerned, expressing for instance for the curve fig. 3 at 49° C. that 25 per cent of the specimens are just incapable of surviving an exposure of 1.4 minutes, that 50 per cent of the specimens are just incapable of surviving an exposure of 2.1 minutes and so on.

In order to test whether the distribution in question is a normal distribution, corrections were first made in respect of the

natural mortality, i. e. the mortality always found within the experimental cultures, independent of the intensity of the exposure. This was determined by control experiments at 25° C. Afterwards a summation curve of a normal distribution was plotted to fit as close as possible the points of the experimental curve, and the deviation of the points from the normal curve was tested by their standard error, and the standard error of the whole curve was determined. Thus the criterion of whether or not the experimental distribution is normal, is the size of these deviations measured by the standard error of the adjustment curve. If the deviations are distributed according to the probability curve the adjustment has succeeded. In table 6 is shown the figures according to the normal distribution and their actual positions.

Table 6.

3-days old puparia			¾-days old puparia			Full-grown larvae		
Within	Was observed	Normal	Within	Was observed	Normal	Within	Was observed	Normal
0.10 μ	0	3	0.10 μ	5	3	0.10 μ	2	3
0.20 μ	1	6	0.20 μ	11	7	0.20 μ	6	7
0.30 μ	5	8	0.30 μ	12	10	0.30 μ	9	10
0.40 μ	8	11	0.40 μ	14	13	0.40 μ	12	13
0.50 μ	10	13	0.50 μ	15	16	0.50 μ	16	16
0.70 μ	15	18	0.70 μ	19	22	0.70 μ	20	22
0.90 μ	20	22	0.90 μ	22	27	0.90 μ	25	27
1.10 μ	27	26	1.10 μ	30	31	1.10 μ	32	31
1.30 μ	31	28	1.30 μ	30	34	1.30 μ	36	35
1.50 μ	33	30	1.50 μ	40	36	1.50 μ	39	37
2.00 μ	35	33	2.00 μ	42	40	2.00 μ	43	41
2.50 μ	..	35	3.00 μ	42	42	3.00 μ	43	43

The differences are not greater than that they may be due to chance, and it is to be noticed that in all cases the curve seems to be a little more acute than the normal curve, the low values being slightly lower, the high values slightly higher than those of the distribution curve, a feature that is known from other fields of biology.

Thus it has been made probable that the S-shaped curves are an expression of a normal distribution with respect to the

property of resistance to heat. Bělehrádek in "Temperature and Living Matter" writes that the S-shaped curves frequently met with in biology, often quite simply may be transposed into curves of distribution; an attempt at this was indeed made by e. g. Henderson Smith (1923) with the mortality curves of the fungus *Botrytis cinerea*, in experiments with killing by means of phenol and heat, and in the latter case he finds that the experiments are covered by Student's distribution curve. Among insects, the material used for the experiments is often so difficult to deal with that the results only rarely can be treated statistically, the experimental data are too scarce and heterogeneous, whereas in experiments on bacteria and spores of fungi an almost unlimited abundance of data may be obtained, and the simplified conditions of life mean that the material is more homogeneous.

The statistical material has been deposed in the library of the Royal Veterinary and Agricultural College, Bülowsvej, Copenhagen, from where it may be had on loan on application.

Summary.

1. The influence of fatally high temperatures on various stages of development has been examined in the following species of *Diptera*: *Musca domestica*, *Lyperosia irritans*, *Stomoxys calcitrans*, *Haematobia stimulans*, *Scatophaga stercoraria*. The animals used for the experiments have been exposed to the effects of high temperatures during a definite time interval ($^1/_4$, $^1/_2$, 1, 2, 4, 8, 16 and 32 min.) and the extent of the injury has been examined. Full-grown larvae were exposed to high temperatures, and a record was taken of the time elapsing before death occurred.

2. *Musca domestica*. Puparia, 3 days old, $^3/_4$ day old, full-grown larvae in the 3rd larval stage, and eggs, were tested. A linear relation was found between the logarithm of the period of exposure and the temperature, when 50 per cent of mortality was taken as a measure of the injury.

3. It is shown that susceptibility to fatally high temperatures increases in succession as follows: puparia 3 days old, puparia $^3/_4$ day old, larvae, eggs.

4. Larvae of *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans* and *Scatophaga stercoraria* were exposed to fatally high temperatures, and it was found that if the temperature rises in arithmetical progression the effect increases geometrically, the relation between the temperature and the logarithm of the time taken to kill the insect at the temperature in question being a straight line.

5. It is shown that the susceptibility to heat in the five species of *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans, Scatophaga stercoraria* increases in the succession given above, whereby conformity is created between that succession and the other biological constants of the species, as well as previous experience on their behaviour in nature.

6. It is shown: a) that death from heat often does not occur during or immediately after exposure, but later in the development; b) that mortality mainly occurs soon after the close of the exposure if the latter is strong, but that it occurs only later in life if it is slight; c) that mortality, regardless of the stage in which exposure takes place, is not evenly distributed in the period after the exposure, but is found in those developmental periods in which activity is greatest, and in which the most intimate interaction between the various processes is demanded (hatching, development and pigmentation of the organs); d) the hypothesis is advanced that the injury partly consists in a total or partial destruction of induction substances in the labile periods, so that the consequences of the heat exposure do not appear until the processes normally induced by the induction substances were about to co-operate. If then the equilibrium is seriously disturbed, development will stop and the animal will die; if the character of the disturbance is less serious, development continues in a more or less defective way.

7. If the exposure takes place in periods with an intense activity (formation of mitoses), mortality during or immediately after the exposure is greater than if the exposure takes place during a resting period.

8. It is made probable that the S-shaped curves of mortality are simply an expression of the variation in the experimental material, and the curves are compared with the curve of normal distribution.

9. At determination of the temperature coefficient *b* (or *m*) (according to BĚLEHRÁDEK's formula) for the various species named under 5, the following figures are obtained: 24.8, 26.2, 35.5, 38.7, 26.1 respectively, i. e. very high temperature coefficients, which agrees with previous experience of the great acceleration of the processes exposed to fatally high temperatures.

(From the Zoological Laboratory of the Royal Veterinary and Agricultural College, Copenhagen.)

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Færdig fra Trykkeriet den 31. December 1943.

PLATE I.

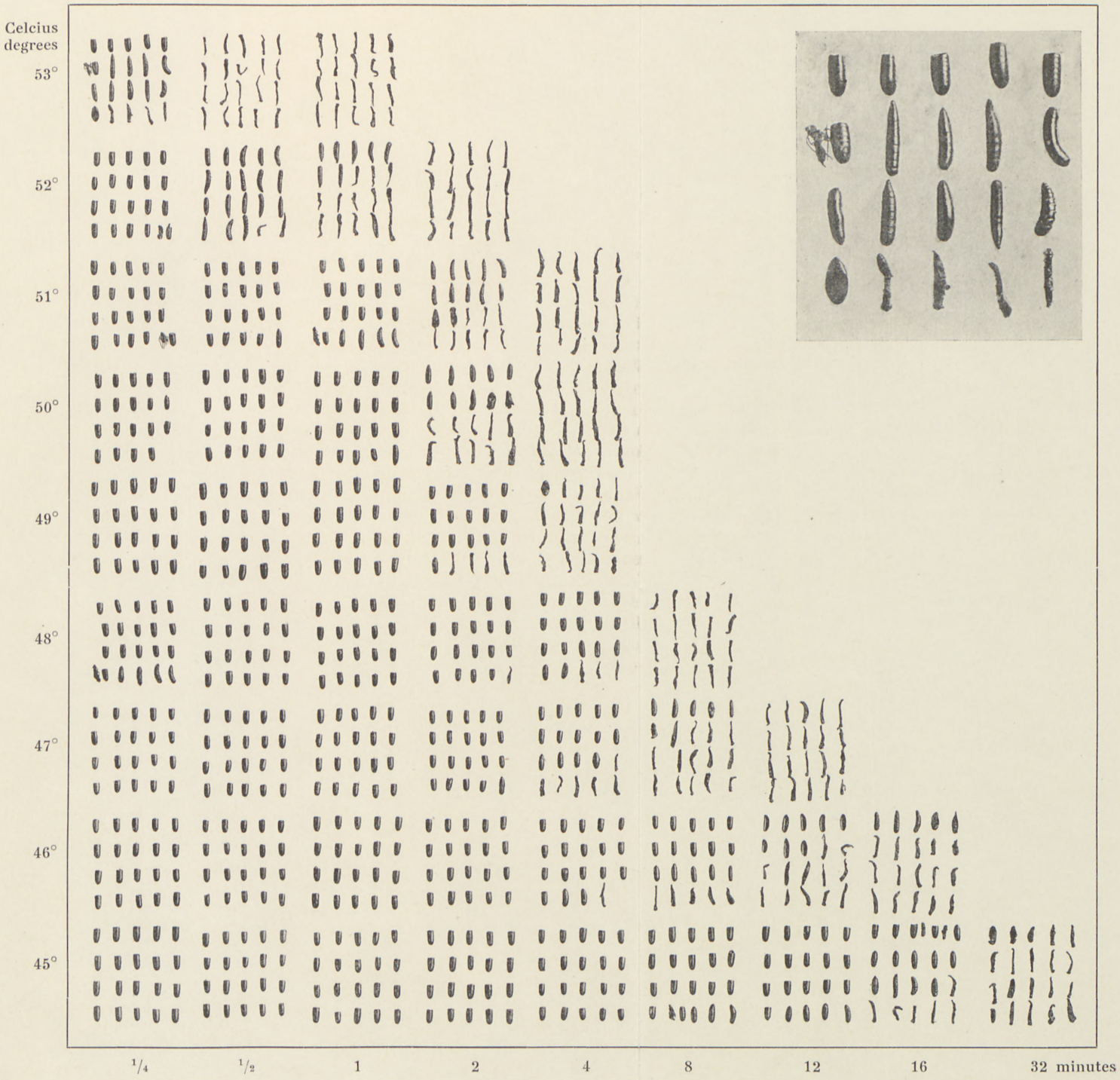
Photographic reproduction of the result of an experimental series with fully grown larvae, each group comprising 20 individuals in a single experiment.

The horizontal rows of groups show experiments at the same temperature, the vertical rows with the same period of exposure.

Within the single experimental groups the individuals have been arranged so as to show uppermost the larvae which have been able to carry through the development until the emergence of the flies, below them the individuals for which the formation of the puparia failed as a consequence of the exposure, and lowermost and last larvae which died immediately after cessation of exposure.

From the magnified detail picture, right (53°, 1/4 min.) it is seen that 5 larvae carried through the development until the flies emerged, 11 larvae made incomplete puparia, "larvaepupae", of which the first, however, contains a fully developed fly, while the eleventh is not hardened at all, for which reason it died soon. 4 larvae died immediately after the exposure.

If the exposure is weak, e. g. 46° and 1/4 min., all the larvae succeed in carrying through the development to flies; if the exposure is strong, e. g. 47° and 12 min., all the individuals die as larvae.


Celcius degrees
53°
52°
51°
50°
49°
48°
47°
46°
45°
¼
½
1
2
4
8
12
16
32 minutes