

# ON THE INFLUENCE OF FOOD AND TEMPERATURE UPON THE DURATION OF LIFE.

BY JACQUES LOEB AND J. H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, September 4, 1917.)

## I. Introductory Remarks.

In order to find out the nature of the causes which determine the natural duration of life of metazoa a quantitative method is required, which permits us to represent the duration of life as the numerical function of one variable. Starting from the idea that chemical conditions in the organism are one of the main variables in this case, one of us raised the question whether there was a definite temperature coefficient for the duration of life and whether this temperature coefficient was of the order of magnitude of that of a chemical reaction.<sup>1</sup> The first experiments were made on the unfertilized and fertilized eggs of the sea urchin and could only be carried out at the upper temperature limits of the organism, since at ordinary temperatures this organism lives for years. In the upper temperature region the temperature coefficient for the duration of life was very high, probably on account of the fact that at this upper zone of temperature death is determined by a change of the nature of a coagulation or some other destructive process. Moore,<sup>2</sup> at the suggestion of Loeb, investigated the temperature coefficient for the duration of life for the hydranth of a tubularian at the upper temperature limit and found that it was of the same order of magnitude as that previously found for the sea urchin egg. In order to prove that there is a temperature coefficient for the duration of life throughout the whole scale of temperatures at which an organism can live experiments were required on a form whose duration of life was short enough to

<sup>1</sup> Loeb, J., *Arch. ges. Physiol.*, 1908, cxxiv, 411.

<sup>2</sup> Moore, A. R., *Arch. Entwicklungsmechn. Organ.*, 1910, xxix, 287.

## 104 Food, Temperature, and Duration of Life

measure the duration of life even at the lowest temperatures. Organisms especially fit for this purpose are insects.

We selected for this purpose the fruit fly (*Drosophila*) which can easily be raised in large numbers on a suitable culture medium in Erlenmeyer flasks. Since Metchnikoff pointed out that the poisons produced by bacteria in the intestine may shorten the duration of life it was necessary to work with flies whose whole body (the intestine included) was entirely free from microorganisms. We succeeded in producing such cultures of flies free from all microorganisms with the aid of a combination of methods introduced by Bogdanow<sup>3</sup> and by Delcourt and Guyénot.<sup>4</sup> Such flies will be designated as "aseptic." We have already published a preliminary report on some of our experiments<sup>5</sup> and intend to give in this paper the full results of our investigations.

The results published on aseptic flies, *i.e.*, flies free from microorganisms, in the preliminary paper, were as follows.

1. With a supply of proper and adequate food the duration of the larval stage is an unequivocal function of the temperature at which the larvæ are raised, and the temperature coefficient is of the order of magnitude of that of a chemical reaction, *i.e.*, about 2 or more for a difference of 10°C. It increases at the lower and is less at the higher temperatures.

2. The duration of the pupal stage of the fly is also an unequivocal function of the temperature and the temperature coefficient is for each temperature practically identical with that for the larval stage.

3. The duration of life of the imago is, with proper food, also an unequivocal function of the temperature and the temperature coefficient for the duration of life is within the normal temperature limits approximately identical with that for the duration of the life of the larva and pupa.

From this approximate identity of the temperature coefficients for the three stages of the life of the fly we drew the conclusion that the limiting factor for the duration of each of the three

<sup>3</sup> Bogdanow, A. E., *Arch. Physiol.*, 1908, Suppl., 173.

<sup>4</sup> Delcourt, A., and Guyénot, E., *Bull. Sc. France et Belg.*, 1911, xlv, 249. Guyénot, Recherches expérimentales sur la vie aseptique et le développement d'un organisme en fonction du milieu, Thèse de Paris, 1917.

<sup>5</sup> Loeb, J., and Northrop, J. H., *Proc. Nat. Acad. Sc.*, 1917, iii, 382.

stages is a process affected in the same way by the temperature. If it be true that this terminating factor for the larval or pupal stage is the production of a certain type of substance in sufficient quantity (as suggested by the influence of thyroid substance on the termination of the tadpole stage in the frog) or the removal of an inhibiting substance, it follows that a similar cause may be likely to exist for the termination of the last stage in life or for the duration of life.

In this paper we will discuss more fully the influence of the two main factors determining the duration of life, namely, food supply and temperature.

## *II. Influence of Different Kinds of Food on the Duration of Life of the Imago.*

In some insects the imago takes up no food (as, *e.g.*, in the silk worm) but the duration of the life of the imago of the fruit fly depends on the nature of the food, though in an altogether different way from that of the larva. The growth of the insect takes place in the larval stage while neither the pupa nor the imago grows. It was found that while the larvæ cannot grow on "glucose-agar"<sup>6</sup> unless yeast is added, the imago can live as well on "glucose-agar" alone as when yeast is added. This difference need not surprise us since the larva needs food containing *all* the building stones required for the synthesis of the compounds of its body while the imago, which does not grow, can live on food which is lacking in certain ("accessory"?) substances found in the yeast, presumably because such accessory substances are no longer needed in the fully grown organism or are needed in such small quantities that they can be supplied by the hydrolytic processes going on in its own cells.

Larvæ were raised on yeast at room temperature and the newly hatched flies were then put immediately after hatching upon dif-

<sup>6</sup> "Glucose-agar," which proved an excellent culture medium for the flies in our experiments, had the following usual composition. 1 pound of beef was freed from fat, put into 1 liter of water, and placed in the refrigerator over night; boiled 30 minutes, filtered, 20 gm. of agar were added, and boiled till dissolved; 10 gm. of peptone added and boiled 20 minutes; neutralized to phenolphthalein, boiled 7 minutes, cooled to 60°; 2 eggs added, filtered, 20 gm. of glucose added.

## 106 Food, Temperature, and Duration of Life

ferent culture media and the duration of life was noticed. All experiments were made with aseptic flies under aseptic conditions in thermostats at 25° and 30° respectively.

It was found (Table I) that on agar alone or on agar with the necessary salts the imagos lived less than 2 days at 25° while if dextrose and salts were added to the agar they lived over 8 days at 25°, and on "glucose-agar" they lived 28.5 days at the same temperature. As the experiment for 30° shows, they lived as long on "glucose-agar" alone as when yeast was added.

TABLE I.

*Effect of Food on Duration of Life of the Imago (Both Sexes).*

Food.	1 gm. washed agar + 100 cc. H <sub>2</sub> O.	1 gm. agar 0.1 " K <sub>2</sub> HPO <sub>4</sub> 0.1 " MgSO <sub>4</sub> 100 cc. H <sub>2</sub> O.	1 gm. agar 0.1 " K <sub>2</sub> HPO <sub>4</sub> 0.1 " MgSO <sub>4</sub> 2.0 " dextrose 100 cc. H <sub>2</sub> O.	"Glucose-agar."		"Glucose-agar" + 5 gm. yeast per 100 cc.
				a.	b.	
Temperature . . . . .	25°	25°	25°	25°	30°	30°
Average duration of life, days . . . . .	1.92	1.75	8.25	28.5	13.7	13.1

TABLE II.

*Effect of Sex (30°, "Glucose-Agar" Food).*

Sex.	♂♂ ♀♀	♂♂	♀♀
Duration of life, days . . . . .	13.1	15.7	13.3

In these experiments both sexes were used. It was found that the isolated males lived a little longer than the isolated females or the males when mixed with females (Table II). "Glucose-agar" served as food.

### III. Influence of Temperature on the Duration of the Larval Period.

For these experiments the aseptic cultures were kept in water-jacketed incubators regulated to within  $\pm 0.1^\circ\text{C}$ ., and containing water so that the humidity was always about 100 per cent. The cultures of aseptic larvæ were made in 120 cc. Erlenmeyer flasks

containing 10 gm. of yeast, 15 cc. of water, and absorbent cotton. Slight variations in the amount of water, cotton, etc., do not affect the rate of growth. The flasks were sterilized in the autoclave for 30 minutes. Aseptic flies of both sexes were put in and allowed to remain 15 hours at room temperature, during which time a number of eggs were laid. The flies were then removed by the aseptic method devised by Delcourt and Guyénot and the flasks with the eggs put into an incubator. The larvæ hatch in a few hours after the eggs are laid, and at the time the flies were removed from the flask most of the larvæ had already hatched. The duration of the life of the larvæ was reckoned from the time the eggs were placed in the incubator to the time the pupæ were formed. Six to ten cultures were made for each temperature. The figures in Table III are the sums of the number of pupæ forming in the separate flasks on the day noted. In computing the averages for the time required to reach the pupal stage the middle of the interval at which the pupæ were formed was used; *i.e.*, if 48 pupæ formed between the 4th and 5th day they were all considered to have taken 4.5 days to form.

We could not well use temperatures lower than 10° since the pupæ did not hatch at that temperature and it was obvious that the growth of the larva was no longer normal. Temperatures above 27.5° no longer accelerated growth.

If we compute from these values the temperature coefficient  $Q_{10}$  for the larval period (Table IV,  $Q_{10}$ ) we find that it is of the order of magnitude of that of a chemical reaction, namely, 2 or more for a difference of 10°C. The temperature coefficients show, however, the irregularities characteristic of all the temperature coefficients for life phenomena, namely, increasing at the lower limit and diminishing at the higher limit. This peculiarity appears also in ordinary chemical reactions, but to a much less degree; but it appears more approximately to the same extent as in life phenomena in chemical reactions catalyzed by enzymes. It seems more natural to assume, as has been done by Arrhenius and others, that these deviations in the temperature coefficients are due to secondary effects of the temperature (*e.g.*, upon viscosity or the state of aggregation of catalyzer or the injury to the catalyzer or some other variable) than to conclude that the temperature coefficient does not indicate a chemical (enzyme) reaction.

# 108 Food, Temperature, and Duration of Life

It is well known that at the upper temperature limits the temperature coefficient of enzyme reactions falls off again when the

TABLE III.

*Influence of Temperature on Duration of Larval Period of Drosophila.*

Days elapsed after hatching of egg.		Number of pupæ formed at							
Day counted.	Average time.	10°*	15°*	20°*	25°		27.5°**	30.0°*	31.5°**
					a.*	b.**			
1-2	1.5								
2-3	2.5								
3-4	3.5					4	156	93	63
4-5	4.5				53	29	105	129	333
5-6	5.5				137	18	12	6	254
6-7	6.5			47	78				52
7-8	7.5			65	36				
8-9	8.5			68					
9-10	9.5			16					
13-14	13.5		2						
15-16	15.5		13						
17-18	17.5		27						
19-20	19.5		14						
21-22	21.5		6						
39-48	43.5	5							
49-58	53.5	20							
59-68	63.5	13							
69-78	73.5	3							
Total number of pupæ .....		41	62	196	304	51	273	228	702
Average duration of larval period in days (from egg to pupation).....		57.0	17.8	7.77	5.82	4.76	4.15	4.12	4.92

\* Flies used were of the 20th to 22nd aseptic generation.

\*\* Flies used were of the 29th to 31st aseptic generation.

temperature rises beyond a certain point. We were interested to know whether the same was true for the duration of the larval stage of the fruit fly, and found this to be the case (Table IV,

flies of the 30th to 32nd aseptic generation). On account of this probably secondary effect of the temperature the curve for the rate of larval development (the reciprocal value of the duration  $\frac{100}{\text{time}}$ ) becomes from 10 to 30° (Fig. 1) almost a straight line; and from

TABLE IV.

*Effect of Temperature on Rate of Growth of Aseptic Larvæ (Fed on Yeast).*

Temperature.	Days required to reach the pupal stage.	Rate ( $\frac{100}{\text{time}}$ )	Q <sub>10</sub>
	Flies of the 20th to 22nd aseptic generation.		
°C.			
10	57 (pupæ do not emerge).	1.75	} 10.0 } 4.0 } 1.78 } 1.99
15	17.8	5.62	
20	7.77	12.85	
25	5.82	17.2	
30	4.12	24.25	
	Flies of the 30th to 32nd aseptic generation.		
25	4.76	21.0	} 1.74 } -1.58
27.5	4.15	24.1	
31.5	4.92	20.3	

25 to 31.5° (Fig. 2) a curve concave on the lower side similar to that found for many simple enzyme reactions.

We noticed that the duration of the larval stage for the 20th to 22nd generation of aseptic flies was slightly longer than for the 30th aseptic generation and this was true for all temperatures and for all the stages. We cannot account for this difference at present.

110 Food, Temperature, and Duration of Life

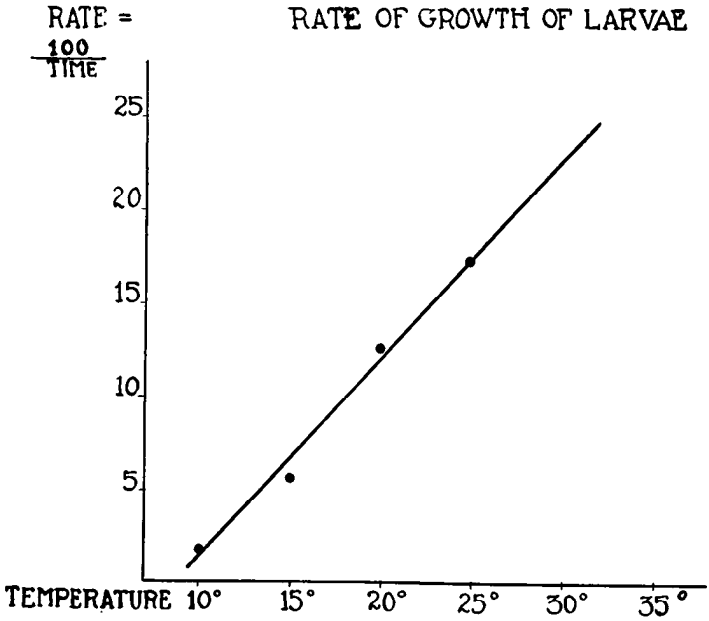


Fig. 1.

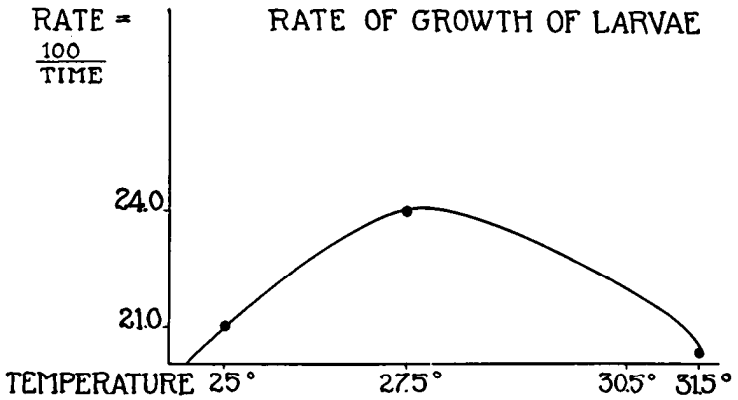


Fig. 2.



*IV. Influence of Temperature on the Duration of the Pupal Stage.*

Table V contains the data for determining the influence of temperature on the duration of the pupal stage. The stage terminates with the emergence of the imago from the cocoon. If we determine the time from the laying of the eggs to the emergence

TABLE V.  
*Egg-Imago and Pupal Period.*

Number of days after larvæ emerge from egg.	Number of imagos which emerge at				
	15°*	20°*	25°*	27.5°**	30°*
1.5					
2.5					
3.5					
4.5					
5.5					
6.5				58	30
7.5				205	114
8.5			6	17	39
9.5			151		
10.5			129		
11.5		4	23		
12.5		21			
13.5		36			
14.5		24			
15.5		21			
16.5		4			
17.5		5			
28.5	9				
30.5	25				
32.5	16				
34.5	10				
36.5	1				
Total number of imagos. . . . .	61	115	309	280	183
Number of days egg-imago. . . . .	31.5	14.10	10.05	7.35	7.55
Number of days egg-pupæ (Table III) . . . . .	17.8	7.77	5.82	4.15	4.12
Duration of pupa stage. . . . .	13.7	6.33	4.23	3.20	3.43

\* 20th to 22nd generation.

\*\* 30th to 32nd generation.

## 112 Food, Temperature, and Duration of Life

of the imago, and deduct from this the time from egg to beginning of pupation (Table III), we get the influence of temperature on the duration of the pupal stage. The temperature coefficient and the rate of development for the different temperatures are given in Table VI. In Fig. 3 the rate curve is plotted which is again between 15 and 30° approximately a straight line.

TABLE VI.  
*Temperature and Rate of Development of Pupæ. 20th to 22nd Aseptic Generation.*

Temperature.	Days as pupæ.	Rate ( $\frac{100}{\text{time}}$ )	Q <sub>10</sub>
°C.			
15	13.7	7.2	} 5.0 2.24 1.53
20	6.33	15.8	
25	4.23	23.7	
30	3.43	29.15	

RATE OF DEVELOPMENT OF PUPAE

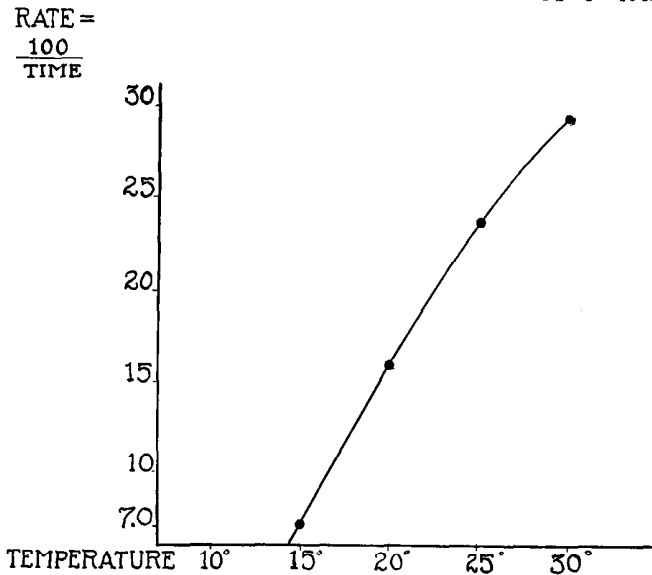


Fig. 3.

*V. Influence of Temperature on the Duration of Life of the Imago.*

Aseptic larvæ were raised at room temperature (18–20°) on yeast and the flies were removed aseptically from the culture flasks within 15 hours of the time they emerged. They were

TABLE VII.  
*Duration of Life of Imago on "Glucose-Agar."*

10°		15°		20°		25°		30°	
Days.	No. of dead.	Days.	No. of dead.	Days.	No. of dead.	Days.	No. of dead.	Days.	No. of dead.
17.5	1		1	14.5	1	12.5	6	3	31
24.5	2		4	24.5	5	22.5	30	8	54
31.5	1		0	34.5	14	32.5	20	13	66
52.5	1	38.5	1	44.5	23	42.5	14	18	20
59.5	5	45.5	2	54.5	6			23	43
66.5	1	52.5	3					28	14
73.5	3	59.5	3						
80.5	0	66.5	12						
87.5	2	73.5	18						
94.5	2	80.5	20						
101.5	5	87.5	12						
108.5	2	94.5	11						
115.5	5	101.5	3						
122.5	17	108.5	8						
129.5	14	115.5	4						
136.5	12	122.5	27						
143.5	19	129.5	7						
150.5	7	136.5	7						
157.5	5								
164.5	1								
Total number of flies..	105		143		49		70		228
Average duration of life, <i>days</i> ...	120.5		92.4		40.2		28.5		13.6

placed into sterile 500 cc. Erlenmeyer flasks containing 25 cc. "glucose-agar." This food is, as stated, perfectly adequate for the flies but is inadequate for the larvæ which cannot develop on it into flies. This was an important point in the method of our experiments. In order to determine the duration of the life of

## 114 Food, Temperature, and Duration of Life

the imago for a given temperature a definite number of flies were put into a flask, and the number of those which died was ascertained for each day. These flies laid eggs. If the larvæ hatching from these eggs had been able to develop into new flies it would have become impossible to find out the exact death rate of the old flies, since constantly new flies would have been added. This source of error was avoided by using "glucose-agar" as a culture medium for the old flies on which the eggs laid by the flies during

TABLE VIII.  
*Duration of Life of Imago on "Glucose-Agar."*

27.5°		31.5°		33.1°		35°		37.4°	
Days.	No. of dead.	Days.	No. of dead.	Days.	No. of dead.	Days.	No. of dead.	Minutes.	No. dead per 100.
2	10	3	21	1.5	48	0.5	61	10	9
4	13	5	22	2.5	40	1.16	27	30	37
6	14	7	43	3.5	17	1.66	27	50	25
8	10	9	40	4.5	9	2.16	2	70	26
10	14	11	7	5.5	1			90	4
12	9								
14	15								
16	28								
18	6								
20	1								
22	3								
24	3								
Total number of flies..	126		133		115		117		101
Average duration of life, days...	11.1		6.87		2.41		0.95		0.032

the experiment could not reach the imago stage. By special tests (smears and cultures from dead flies) it was ascertained that **all** the cultures used in the experiments remained sterile to the end of the experiment. The flasks containing the flies were plugged with cotton. Sufficient food and oxygen were present since cultures containing 30 to 40 flies had the same average duration of life as those containing 10 to 15.

Five to ten separate cultures were used at each temperature, each containing 5 to 20 flies. Several cultures with relatively few

flies in each were used since it was more convenient to count the number of dead flies under these conditions. The time was reckoned in the same way as for the larval and pupal periods.

Table VII gives the statistical results of the duration of life of the imago on "glucose-agar." The flies used were of the 20th to

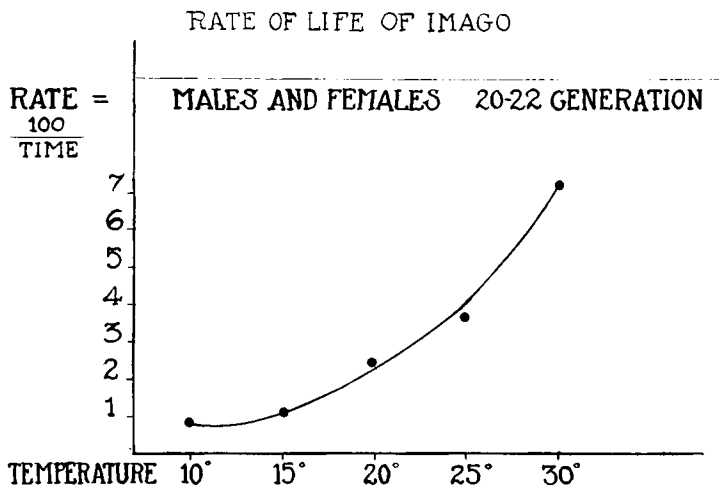


Fig. 4.

22nd aseptic generation. Table VIII gives the duration for the upper limit of temperatures, 27.5–37.4°. Both sexes were used indiscriminately in these experiments. In Table IX the average results are tabulated and the temperature coefficients for 10° are given. A temperature of 10° or lower is harmful for the organism, as are temperatures above 30°. In Table IX the temperature coefficients of the duration of life of the imago are computed and the reciprocal value of the duration of life—the rate at which an animal "gets through" with life—is calculated. If we plot this curve (Fig. 4) which corresponds to the rate curve of the larval and pupal stage we find that in the case of the imago it is no longer a straight line but more what we should expect for a chemical reaction curve. The reason that Curve 4 corresponds more to a chemical reaction curve than Curves 1 and 3 is that the rate does not decrease at the higher temperature limits. This decrease is then a merely secondary phenomenon but it is responsi-

## 116 Food, Temperature, and Duration of Life

ble for transforming the rate curve into a straight line; all of which corroborates our previous statement that the straight line character of the curve does not militate against the assumption that we are dealing in all three curves with a temperature coefficient of the order of that of a chemical reaction.

TABLE IX.

*Temperature Coefficient of Duration of Life of Imago. Males and Females Fed on "Glucose-Agar."*

Temperature.	Duration of life of imago.	Rate $\left(\frac{100}{\text{time}}\right)$	$Q_{10}$
	20th to 22nd generation.		
°C.	days		
10	120.5	0.83	} 1.70 5.25 1.99 4.4
15	92.4	1.08	
20	40.2	2.49	
25	28.5	3.51	
30	13.6	7.35	
	30th to 32nd generation.		
27.5	11.1	9.00	} 3.3 630 137 10 <sup>6</sup>
31.5	6.87	14.55	
33.1	2.41	41.50	
35	0.95	105.2	
37.5	0.032	3,125.0	

In conclusion we will give in Table X the total duration of life and its temperature coefficients for the temperatures at which the animal can complete its cycle.

### VI. The Mortality Curve.

When we plot the number of flies which die during successive days in terms of percentage of the original number of flies we get

TABLE X.  
Total Duration of Life.

Temperature.	Total duration of life.	Rate ( $\frac{100}{\text{time}}$ )	Q <sub>10</sub>
°C.	days		
15	123.9	0.81	} 5.0 } 3.0 } 3.0
20	54.3	1.84	
25	38.5	2.67	
30	21.15	4.65	

that curve of the death rate usually given in life insurance statistics, namely, a probability curve, the ascending branch of which is a little steeper than the descending branch. The death rate of a population of aseptic male flies on "glucose-agar" at 30° is thus given in Fig. 5 and Table XI.

Miss Chick<sup>7</sup> has stated that bacteria are killed by disinfectants at a rate corresponding to that of a monomolecular chemical reaction, *i.e.*, that in each interval of time the same percentage of individuals alive at this time is killed. She was probably led to such an assumption by the fact that the ascending branch of the mortality curve in her experiments was generally very steep. The agencies used by her for killing the bacteria were so powerful

TABLE XI.  
Rate of Death at 30°. Males.

Time.	No. of dead.	No. of dead in interval.	Percentage of original number dying in interval.
days			
3.5	5	5	4.8
7.5	19	14	13.5
13.0	60	41	39.4
18	78	18	17.2
23	93	15	14.4
28	104	11	10.6

<sup>7</sup> Chick, H., *J. Hyg.*, 1910, x, 237.

## 118 Food, Temperature, and Duration of Life

that the ascending branch became almost a vertical line, thus escaping detection. Hence she noticed usually only the less steep descending branch which could be interpreted as a monomolecular curve for the reason that her experiments lasted only a short time. In Fig. 6 we give the frequency curve of deaths of a culture of males for a very high temperature, namely,  $39.45^{\circ}$ . The ascending branch of the curve is steeper than that for the lower

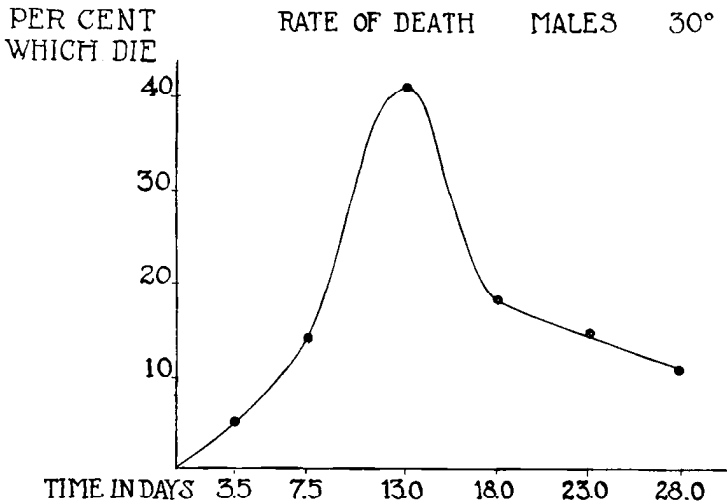


Fig. 5.

temperature in Fig. 5, but the fact that we are dealing with a probability curve is still very clear. Table XII gives the observations on which this curve is based.

The fact that the frequency curve of deaths is that of a probability curve shows that the difference in the duration of life of different individuals for the same temperature is due to individual variation. Incidentally it may be stated that observations on the rate of death of *Fundulus* embryos under the influence of acids, alkalis, and potassium salts show that the mortality curve in these cases is also a probability curve, the descending branch of which is less steep than the ascending branch. This difference may possibly be ascribed to a slight adaptational effect of the destructive agency.



30 to 40 flies were put in test-tubes and placed in a water bath kept at  $39.45^\circ \pm 0.02^\circ$ , then taken out at time stated and left at room temperature over night. The number of live and dead males was counted after 14 hours.

TABLE XII.  
*Rate of Death at 39.45°. Males.*

Time.	No. of dead.	Total per cent dead.	Per cent dying in interval.
<i>min.</i>			
25	30	4.5	4.5
30	130	17.0	12.5
35	177	34.6	17.6
40	318	54.6	20.0
45	489	72.0	17.4
50	550	82.9	10.9
55	440	88.2	5.3
60	364	91.2	3.0
65	532	97.5	(5.3)
70	350	98.9	1.4
75	609	99.7	0.8

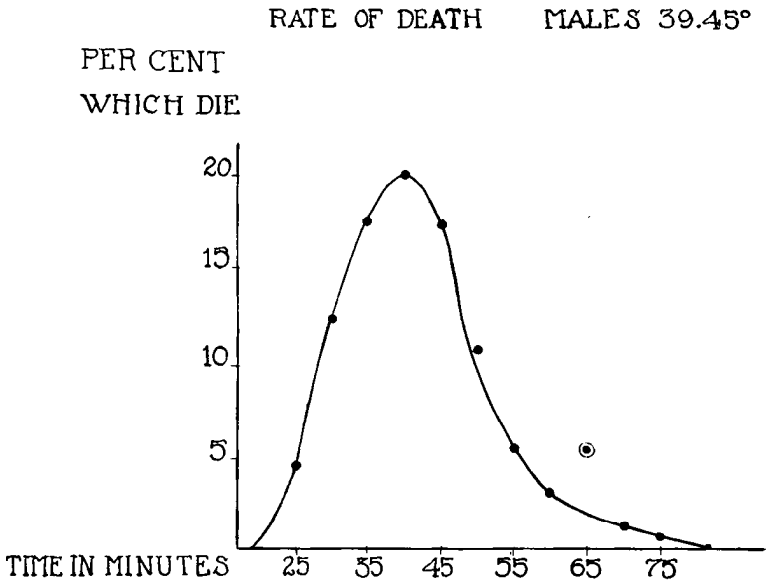


Fig. 6.

## 120 Food, Temperature, and Duration of Life

### VII. Comparison of the Temperature Coefficients for the Larval and Pupal Stage with That for the Duration of Life.

The life of *Drosophila* is normal only within temperatures above 10° and below 30° or roughly between 15° and 25°C. At 10° the larvæ reach the pupal stage but the animals then die without ever emerging from the cocoon. The imago, however, lives at 10°C. At 5° or less the duration of life of the imago is less than a week while at 15° it is 92.4 days. The fact that at 5° the duration of life is less than at 15°C. shows that the former temperature is incompatible with the life of the organism. At temperatures between 27.5° and 31.5°, at which temperature the coefficient for the rate of growth of the larvæ becomes negative, life is also no longer normal. The life of the fly is normal between 15° and

TABLE XIII.  
*Temperature Coefficients of Various Stages of Development.*

Temperature. °C.	Q <sub>10</sub> for rate of		
	Larvæ.	Pupæ.	Imagos.
15-20	4.0	5.0	5.25
20-25	1.78	2.24	1.99

25°C., and it is, therefore, for this range that a comparison of the temperature coefficients for the three stages becomes permissible. Table XIII shows the temperature coefficients for 15-20° and 20-25° and it is obvious that they are approximately the same for all three stages.

As we have already stated in a previous paper, this proximity of the three values suggests a proximity of the cause limiting the three stages. If the limiting factor for the larval and pupal stages be the production or destruction of a substance ("hormone") the same limiting factor may be suspected for the duration of life. Experiments made by Northrop<sup>8</sup> show that thyroid has no influence on metamorphosis in the fly.

<sup>8</sup> Northrop, J. H., *J. Biol. Chem.*, 1917, xxx, 181.

## SUMMARY.

1. The paper proves the existence of a definite temperature coefficient for the duration of life of the fruit fly (*Drosophila*).

2. Since the experiments were made with fruit flies free from microorganisms death cannot be ascribed to bacterial poisoning.

3. The temperature coefficient for the duration of life of the fruit fly is approximately identical with the temperature coefficients for the duration of the larval and pupal stage between 15° and 25°C., *i.e.*, within the limits where development is normal.

4. The duration of the three stages in the life of aseptic *Drosophila*, and the total duration of life is, for temperatures between 10° and 30°, as follows.

Temperature.	Duration (in days) of			
	Larval stage.	Pupal stage.	Life of imago.	Total duration of life from egg to death.
°C.				
10	57	Pupæ die.	120.5	177.5 + X
15	17.8	13.7	92.4	123.9
20	7.77	6.33	40.2	54.3
25	5.82	4.23	28.5	38.5
27.5	(4.15)	3.20		
30	4.12	3.43	13.6	21.15

5. Small variations in the duration of life were noticed in different aseptic generations of the flies; in the 32nd generation the rates were all slightly quicker than in the 20th to 22nd generation.

6. Aside from the temperature the nature of food influences the duration of life and an "adequate" food supply is presupposed for work on the influence of temperature as stated in the previous paragraphs. An adequate food supply for the growing larva includes yeast, while for the adult fly which no longer grows "glucose-agar" (with or without yeast) is sufficient.

7. The observations on the temperature coefficient for the duration of life suggest that this duration is determined by the production of a substance leading to old age and natural death or by the destruction of a substance or substances which normally prevent old age and natural death.