CARBON DIOXIDE PRODUCTION AND DURATION OF LIFE OF DROSOPHILA CULTURES.

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One of the most concrete and plausible mechanisms which have been proposed for the regulation of the duration of life is what may be termed the *energy limit*, elaborated by Rubner.[1] Rubner stated that the total energy transformed per kilo of body weight during the total life of the animal was approximately constant for a large number of animals. He suggested, therefore, that the duration of life of the individual was determined by the time required to transform this quantity of energy. Slonaker[2] found that 4 albino rats which were allowed to exercise freely died sooner than others which were allowed only limited exercise, and this has been considered by Pearl[3] as additional evidence in favor of Rubner's view. It was found by Loeb and the writer[4] that the duration of life of aseptic *Drosophila* cultures was a function of temperature, the insects living longer at a low temperature. Since they are also more sluggish at a lower temperature Pearl has suggested that this also is evidence in favor of the energy limit. There is further confirmation of the idea in the fact, as pointed out by Crozier,[5] that the temperature coefficients of the duration of life and of the rate of oxidation may be similar. No direct measurements comparing the total energy production with the duration of life have been made, and the present experiments with *Drosophila* were designed to test this point. It has been found that there is considerable variation in the total amount of CO_2 produced by cultures of *Drosophila*

[1] Rubner, M., Das Problem der Lebensdauer, München and Berlin, 1908.

[2] Slonaker, J. R., *J. Animal Behavior*, 1912, ii, 20.

[3] Pearl, R., The biology of death, Monographs on experimental biology, Philadelphia and London, 1922, 213.

[4] Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1917, xxxii, 103.

[5] Crozier, W. J., *J. Gen. Physiol.*, 1924–25, vii, 189.

during their entire life under different conditions, and that, therefore, the duration of life of the insect is not determined by the time required to transform a definite amount of energy.

Methods.

The flies used in these experiments were taken from the 195th to the 205th generation of aseptic cultures used by Loeb and the writer.[4] These flies have been inbred from the original pair of aseptic flies; the cultures have been kept in the dark at room temperature and raised on sterilized yeast. It will be noted that the duration of life differs markedly from that given previously. A progressive decrease

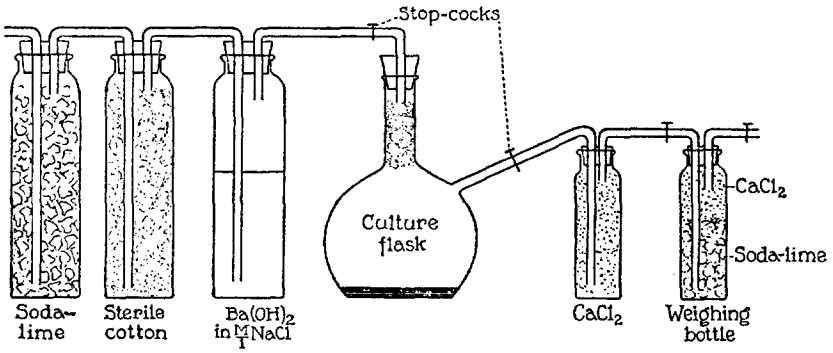

FIG. 1. Apparatus for CO_2 determination.

in the duration of life of these cultures, especially at low temperatures, had been noted for several years.

Determination of CO_2.

The arrangement of the apparatus for the determination of CO_2 is evident from Fig. 1. The cultures were connected to a soda-lime weighing tube as shown in the figure. A slow current of air was blown through the flask every 2 or 3 days for 3 hours and the CO_2 weighed. The number of larvæ, pupæ, or imagos was also counted, and the CO_2 produced per 100 individuals was calculated. A control flask containing no insects, run simultaneously, showed no increase in CO_2. The culture flasks themselves were also continued after all the insects were dead. After the death of the insects no CO_2 was found, showing

that the apparatus was air-tight and that the CO_2 in the air stream had been completely removed by the soda-lime. The larvæ were grown on sterilized yeast as already described and the imagos were kept on glucose agar. Any flasks showing contamination with micro-organisms were discarded.

The 15°, 26°, and 30°C. cultures were kept in the dark in water-jacketed incubators, and the 22–26°C. light culture was kept at the room temperature, in diffuse daylight, and in addition illuminated with a 40 watt bulb attached to a circuit breaker so that the light was turned on and off at irregular intervals. This culture was also disturbed more or less by persons working in the laboratory and the flies were much more active than those in the dark. Each culture contained from 300 to 400 flies.

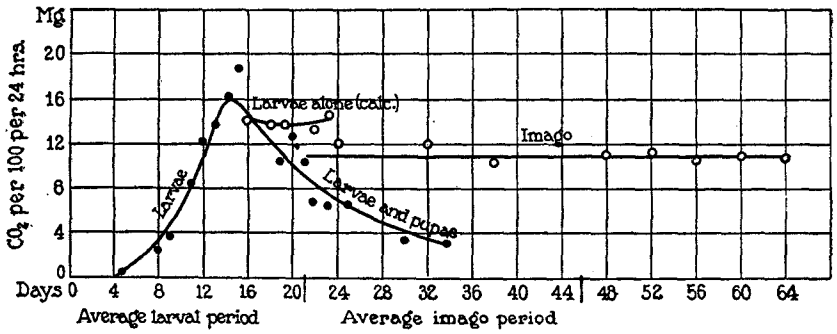

FIG. 2. Production of CO_2 per 100 individuals per day, in dark, at 16°C.

The result of a complete experiment is shown in Fig. 2, in which the CO_2 per 24 hours per 100 individuals has been plotted against the time in days. The curve confirms the results of Fink,[6] in that it shows a rapid increase during the larval period followed by a decrease in the pupal period. Since the curve is per individual and not per unit of weight, the rise is sharper than in the CO_2 per unit weight curve as it includes the increase in size of the larvæ. It will be noted that the curve of CO_2 production of the imagos is practically constant throughout life. This result was obtained in every experiment, the only decrease noted being at the end of the experiment when the number of

[6] Fink, D. E., *J. Gen. Physiol.*, 1924–25, vii, 527.

TABLE I.

Duration of Life and CO_2 Production of Drosophila Cultures under Various Conditions.

Larvæ.						Imagos.						Total CO_2 for entire duration of life.
Experiment.	Temperature, etc.	Average duration of larval period.	Average CO_2 per 24 hrs. per 100.	Total CO_2 per 100 larvæ.		Experiment.	Temperature, etc.	Average duration of life.	Average CO_2 per 100 imagos per 24 hrs.	Total CO_2 per 100 imagos.		
	°C.	*days*	*mg.*	*mg.*			°C.	*days*	*mg.*	*mg.*		*mg.*
1/8	15 Dark.	21	8.0	170		2/10	16 Dark.	24	11.4	275		445
3/17	15 "	20	7.5	150		4/15	16 "	26	11.1	290		440
2/19	26 "	6.3	13.0	83		4/29	26 "	13	14.5	189		
2/19*a*	26 "	6.1	13.4	82		5/17	26 "	9.3	12.0	110	192	272
1/9	26 "	6.2	12.0	73		4/15	26 "	9.0	29.0	260		
						5/17	26 "	9.2	23.0	210		
1/9	22–26 Light.	6.5	10.8	70		3/2*a*	22–26 Light.	15.0	22.0	330		
2/11	22–26 "	7.2	12.0	87		3/2*b*	22–26 "	16.0	21.0	331	317	411
2/19	22–26 "	6.6	17.0	112	94	3/31	22–26 "	15.2	18.0	275		
2/19	22–26 "	7.3	15.0	108		3/31*b*	22–26 "	15.0	22.0	330		
3/16	30 Dark.	4.6	14.0	66		3/30	30 Dark.	7.6	19.6	149		
2/19	30 "	6.1	15.4	94		2/26	30 "	8.1	17.5	141	166	246
						3/9	30 "	11.9	17.6	210		

flies was very small and the error proportionately large. There is, therefore, no evidence of any "running down" as might have been expected were the duration of life determined by the transformation of a limiting amount of energy.

A summary of all the experiments is given in Table I. The experiments show that the total amount of CO_2 produced by the insects during the larval, the imaginal, or during the entire duration of life varies considerably with the conditions.[7] More CO_2 is produced at 15° than at 26° when both cultures are in the dark; *i.e.*, the temperature coefficient of CO_2 production is smaller than that for the duration of life or the duration of the larval period. The cultures which were exposed to the light, however, produced much more CO_2 than those in the dark.[8] This effect of light on CO_2 production is well known, and was shown by Loeb[9] to be due to an increase in muscular activity, since insect pupæ are not affected. At 30°C., in the dark, there is a still further decrease in the total CO_2, owing to the fact that the CO_2 per day remains nearly constant while the rate of growth and duration of life is shortened. 30°C. is above the normal temperature range of the insect since successive generations cannot be reared at this temperature, and it is possible that the results at this temperature are not significant on this account, since it is evident that death due to injury cannot be determined by energy limitations.

The results are corroborated by the fact that quite high light intensities have no effect on the duration of life of these insects,[10] whereas numerous investigators have shown that illumination markedly increases the CO_2 production.

SUMMARY.

The total CO_2 produced by aseptic *Drosophila* cultures during the entire duration of life has been determined at 15°, 26°, and 30°C. in the dark and at 22–26°C. in the light.

[7] Owing to experimental difficulties the CO_2 production of the pupæ has been omitted. It is very small compared to that of either larvæ or imagos.

[8] The longer duration of life of the light cultures is due to the slightly lower temperature.

[9] Loeb, J., *Arch. ges. Physiol.*, 1888, xlii, 393.

[10] Northrop, J. H., *J. Gen. Physiol.*, 1925–26, ix, 81.

The total amount of CO_2 produced is not constant but is greater at 15° than at 26° or 30°, and is much greater in the light than in the dark.

The total duration of life, therefore, is not determined by the time required to produce a limiting amount of CO_2.