ON THE INFLUENCE OF INTERMITTENT STARVATION ON THE LONGEVITY OF THE IMAGINAL STAGE OF *DROSOPHILA MELANOGASTER*

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(*Received 2nd August* 1927.)

I. THE PROBLEM.

Many interesting results have been published concerning the relation of longevity in animals to particular experimental conditions such as temperature variations and other environmental factors, increased muscular activity, vasoligation, tethelin administration, different kinds of food, and continuous starvation with or without water (for references see Pearl, 1922; Pearl and Parker, 1924).

Independently of the above investigations, a totally different factor which appears to be of real significance in the study of experimental prolongation of life has been discussed in my previous papers. The opinion that death from starvation is caused by autointoxication of the organisms by certain products of "hunger metabolism" is becoming more and more firmly established. On the other hand, natural death in the light of modern research may be considered as due to an accumulation of detrimental products of normal metabolism which cannot satisfactorily be removed from the body of multicellular animals (for references see Lipschütz, 1915; Jackson, 1925; Morgulis, 1923; Pearl, 1922). If this is so, then the influence on longevity of intermittent feeding, *i.e.* intermittent starvation, may be expected to be favourable in its nature; for it may be supposed that in animals intermittently fed "the metabolism of the 'hunger days' would furnish antitoxins for the toxins accumulated in the organism during feeding, and on the contrary the metabolism of the 'feeding days' would give antitoxins against those toxins which were formed during the preceding hunger metabolism. This interchange would lead to the delay of natural death" (Kopeć, 1922 *a*).

By alternating the feeding days with days of complete inanition I succeeded in prolonging the larval life of the caterpillars of *Lymantria dispar* L. for a time, which varied from 16·2 to 89·5 per cent. (according to sex) of the average duration of the larval stage of control specimens, the prolongation being in direct proportion to the intensity of starvation. The duration of life of the imago (which never takes food) remained unchanged (Kopeć, 1921, 1924). Notwithstanding the fact that the pupal stage underwent, in my experiments, a distinct abbreviation (cf. here also Kopeć, 1927), the total duration of the whole period of development was increased, by feeding larvae only every second day, by 24·2 per cent. in males and 30·0 per cent. in females as compared with the duration of the analogous period in the controls. But, striking as are these results, they are not of general application, for such prolongation

of life by intermittent starvation only refers to the developmental stage of animals. The same is true in regard to my experiments on the retarding influence of intermittent starvation on the rate of metamorphosis in tadpoles (Kopeć, 1922, 1922 *a*), the more so as the latter observations were discontinued immediately after transformation had taken place[1]. At the same time the undoubted fact must be here emphasised that intermittent starvation had a beneficial influence on tadpoles reared under the unnatural conditions of the laboratory in that it retarded their death.

To decide whether and to what degree this factor may prolong the duration of life of fully developed animals, experiments on adult insects and on mammals were started. In the present paper a short report is given of the results of preliminary studies on the influence of intermittent starvation on the longevity of the imaginal stage of *Drosophila*.

I am greatly indebted to Dr A. W. Greenwood of the Animal Breeding Research Department of the University in Edinburgh for help and for most valuable advice during the course of this study.

II. MATERIAL AND METHODS.

Only males of a white-yellow mutant stock were used, and after long preliminary experimentation the following method was elaborated.

The insects were kept in ordinary test-tubes of approximately the same size and wall-thickness and plugged with cotton-wool. All tubes after adequate manipulations and observations had been made were put together in one large receptacle on the bottom of which was placed some moistened cotton-wool covered with iron gauze. This receptacle, after being tightly closed with a glass plate, was permanently kept in an electric thermostat maintained more or less exactly at 27° C. The artificial food medium used during the whole course of the experiments was prepared according to the formula received from Dr T. Marchlewski of the University of Cracow (Poland), and consisted of 21 gm. cornflour, 2 gm. agar-agar and 150 c.c. distilled water heated to 60° C., mixed with 20 gm. malt extract and boiled for ten minutes. A thin layer of the medium was placed on the side of the test-tubes, near the mouth. At the end of the feeding periods the flies were shaken (without etherisation) into other carefully cleaned and dried test-tubes where, during the starvation periods, were placed thin strips of chemical filter paper moistened with distilled water. In the control series the tubes and the food were changed every 12 hours. After some preliminary practice I succeeded in keeping the humidity in all the tubes, both during starvation and feeding periods, as well as in the control tubes, satisfactorily uniform by an adequate regulation of the quantity and of the humidity of the food, the quantity of the water administered and by the tightness of the plugging. Thus in all cases approximately the same quantity of water soon condensed on the walls of the tubes, the atmosphere in all tubes being therefore always saturated with water vapour. In a separate set of experiments the

[1] Only in those cases when starvation was practised on full-grown caterpillars, approximately from the 10th day after the last moult and on tadpoles during the period between the 50th and 65th day of life, could an unquestionable acceleration of metamorphosis be observed. The physiological explanation of this apparent discrepancy in the results may be found in my original papers.

insects were intermittently deprived of both food and water. In these cases the tubes were plugged very tightly. Although no condensation of water could take place, no especially unfavourable desiccation of the starved specimens was observed, for the atmosphere in the common receptacle in which all tubes were placed was always very humid.

Each series of experiments consisted of ten test-tubes each containing ten flies, The same number of males, viz. 100, was used as control. The whole material belonged to several bottles derived from a single stock bottle and thereafter interbred. Not all test-tubes were, however, started on the same day. The origin and distribution of the flies, obtained in this way, were as follows: Male flies hatching between May 4th and May 6th were on the morning of May 6th distributed at random into a series of test-tubes. One such tube was allotted to each series and was designated as tube *a*. Male flies hatching between May 6th and May 8th were similarly distributed to each series in tubes *b* and *c*. Male flies hatched between May 8th and May 10th formed the occupants of *d* and *e*, those hatched between May 10th and May 12th formed the population of *f*, *g* and *h*, whilst the last two tubes of each series, *i* and *k* contained male flies which hatched between May 14th and May 16th. Some of the flies were lost during the physical manipulations involved in the frequent changing of tubes. Others died an accidental death (*e.g.* by adhering to the food or to drops of water condensed on the sides of the test-tubes) and were therefore lost for the purpose of the experiment. The records of these specimens were omitted. Each series of experiments, however, was provided with one or two supplementary tubes, in which the flies from the hatch during the period from May 18th to May 20th were treated in a manner identical with their respective series. Thus, when the record of a fly in the experimental tube was lost in one of the above-mentioned ways the record of a fly which died a natural death in the corresponding supplementary tube was substituted *seriatim*. The number of such substitutions, however, amounted only to 52 in the whole material. The small number of losses was due partly to the fact that it was possible in many instances to rescue by means of a brush the flies which had become stuck and also to the fact that the flies in the narrow test-tubes very soon lost the habit of flying and merely crawled about.

All the data presented in this paper were calculated from the mornings of May 7th, 9th, 11th, 13th, 17th, and 21st respectively, for on the first day all flies were fed *ad libitum* as in the control tubes. The flies hatched in the later periods were distinctly smaller than those which emerged earlier, but as stated above, each series contained the same number of flies from each hatch. The tubes were examined for dead flies every twelve hours, the dead specimens being removed.

III. DESCRIPTION OF EXPERIMENTS.

The following series of experiments were performed:

A. Starvation without water, Series I–VII:

I. Continuous starvation, designated in the tables of data as *c.s.*

II. Starvation for three successive days out of every four, *i.e.* 72 hours out of every 96; designated in the tables as 72/96.

III. Starvation for two successive days out of every three; designated as 48/72.

IV. Starvation for 18 successive hours out of every 24, viz. from 9 p.m. till 3 p.m.; designated as 18/24.

V. Starvation for one day out of every two; designated as 24/48.

VI. Starvation for half day out of every day, viz. from 10 a.m. till 10 p.m.; designated as 12/24.

VII. Starvation for six hours out of every 24, viz. from 12 o'clock noon till 6 p.m.; designated as 6/24.

B. Starvation with water, Series VIII–XIV.

These series were analogously arranged and designated as the above.

C. Controls, fed every day *ad libitum*, designated in the tables of data as control.

As already stated, each series consisted of ten tubes containing ten flies each. The total number of specimens used for this study amounted therefore to 1500 (including the one supplementary experiment (see p. 208) to 1600).

In Tables I and II the mean duration of life is recorded for each of the ten test-tubes belonging to each series. The general means for each series are also expressed by calculating the averages of the above ten respective means[1].

A. Starvation Without Water.

In Table I it is seen that for insects which were starved for three days out of every four the mean longevity was, as a rule, less than 2·5 days and did not differ materially from that of specimens continuously deprived of food and water (cf. Series I and II). This result is in harmony with the fact that the maximal duration of life in both cases does not exceed 3·5 days. Pearl and Parker (1924), in their fundamental study on the longevity in *Drosophila* in complete absence of food give the mean duration of life of starved males as less than two days both in wild and vestigial stocks. The higher value of mean longevity of completely starved white-yellow males observed in the present study must be due to the different method of treatment. For although the fully fed wild flies live much longer than the vestigial mutants (Pearl and Parker, 1921), practically no difference between the two stocks was noticed by these authors when complete starvation was practised.

In the remaining series the longevity increased in proportion as starvation was less intense. But, even in Series VII, in which starvation lasted only six hours out of every 24, no prolongation of life in comparison with controls could be observed.

In Series V the insects were fed during 24 successive hours out of every 48. Notwithstanding a different length of the feeding and fasting periods, the quantitative ratio of these periods was the same here as in Series VI, in which starvation lasting 12 successive hours out of every 24 was practised. From the data it can be seen that the duration of life in the latter case was much greater than in the former. The same is true for Series II (starved 72 hours out of 96) when compared with Series IV (starved 18 hours out of 24). It follows therefore that the shortening of

[1] Data referring to the minimal and maximal duration of life in each test-tube will be given in my paper which will appear in *Acta Biologiae Experimentalis*, Warsaw, **1**, 1928.

life which is produced by intermittent starvation depends not only on the quantitative ratio of starving to feeding periods but also on the absolute length of the periods of starvation. The injurious influence of longer, though less frequent fasting intervals is more pronounced than that of shorter, though more frequent periods of inanition.

Examination of individual records of mortality in separate tubes, which are not given in the present paper, points on the whole to the conclusion that the injurious influence of complete starvation for six hours out of every 24 affected the flies most during the middle period of their life; on experiments being prolonged, the most resistant specimens can survive in unfavourable conditions and live almost as long as animals fed continuously.

The degree to which the resistance of *Drosophila* to the absence of food depends on the age of the flies becomes especially clear from the results of the following supplementary experiment. One hundred flies were continuously fed for 20 days and only from the twenty-first day of their life were they deprived of food and water one day out of every two. If resistance to starvation does not become lower with age it would be expected that these specimens would have lived under experimental conditions for at least two weeks longer, for the general mean duration of life in Series V (where feeding one day out of every two was practised from the very beginning) equalled 17·46, whilst the mean longevity in the control was not less than about 40 days (Table I). It was found, however, that not one of these flies survived 24 hours of starvation, all being dead before the end of the first period of starvation. There can therefore be scarcely any doubt that the resistance to inanition diminishes in older insects, although no conclusion as to the physiological nature of this phenomenon may be drawn from the experiments actually performed.

Table I. *Starvation without water.*

Mean duration of life (in days) in each test-tube.

Experiment started on	No. of test-tubes	Series of experiments							
		I *c.s.*	II 72/96	III 48/72	IV 18/24	V 24/48	VI 12/24	VII 6/24	XV Control
May 6th	*a*	2·60	2·40	1·90	15·10	22·40	29·35	41·90	36·65
May 8th	*b*	2·00	2·35	3·70	16·45	17·70	23·85	34·35	40·30
	c	2·10	2·85	4·80	17·05	17·85	32·20	36·35	42·45
May 10th	*d*	2·10	2·05	5·80	10·75	18·10	31·45	36·10	31·65
	e	2·50	2·10	7·90	12·25	20·50	34·00	38·75	41·75
May 12th	*f*	2·25	1·95	2·95	13·00	16·00	23·00	37·15	33·55
	g	2·70	2·20	3·10	13·95	17·85	31·35	39·60	36·20
	h	2·75	2·40	10·10	17·65	18·35	31·50	41·10	46·00
May 16th	*i*	2·00	2·10	2·95	11·65	11·00	26·80	34·55	37·60
	k	2·40	2·20	6·80	14·25	14·80	29·75	37·50	42·90
General mean		*2·34*	*2·26*	*5·00*	*14·21*	*17·46*	*29·33*	*37·74*	*38·91*

B. Starvation With Water.

From the data given by Lutz (1915) for wild *Drosophila* males which were continuously deprived of food but did not lack water, a mean longevity of 66·31 hours, *i.e.* of 2·76 days, was calculated by Pearl and Parker (1924). The average duration of life of analogously starved white-yellow males in the present study (Table II) equalled 2·74 days, which is in complete agreement with the above value.

Table II. *Starvation with water.*

Mean duration of life (in days) in each test-tube.

Experiment started on	No. of test-tubes	Series of experiments							
		VIII *c.s.*	IX 72/96	X 48/72	XI 18/24	XII 24/48	XIII 12/24	XIV 6/24	XV Control
May 6th	*a*	2·65	3·05	12·80	24·75	28·30	48·05	42·35	36·65
May 8th	*b*	2·50	3·35	11·85	22·35	33·95	34·80	42·40	40·30
	c	2·50	4·20	17·85	25·75	35·05	44·00	43·25	42·45
May 10th	*d*	2·90	3·65	8·65	22·65	29·05	36·65	36·50	31·65
	e	3·00	4·30	14·70	30·10	33·65	36·95	36·85	41·75
May 12th	*f*	2·65	2·75	5·05	21·50	23·35	35·60	36·60	33·55
	g	2·75	2·85	5·85	23·20	29·25	39·30	42·25	36·20
	h	2·90	3·90	11·30	26·50	32·00	40·00	46·10	46·00
May 16th	*i*	2·60	2·75	6·75	24·90	22·05	26·15	35·00	37·60
	k	2·90	3·35	7·40	25·65	28·90	34·55	35·90	42·90
General mean		*2·74*	*3·42*	*10·22*	*24·74*	*29·56*	*37·61*	*39·72*	*38·91*

In each series the mean duration of life of insects intermittently starved but continuously supplied with drinking water was distinctly greater than when the flies were deprived both of food and water (cf. Tables II and I). No exact inferences can, however, be drawn from these differences concerning the effect of water on the duration of life of intermittently starved insects, for in Series I–VII the humidity was not the same as in Series VIII–XIV (cf. Section II). The results obtained in both sets of experiments may only roughly be compared with each other.

By comparing *seriatim* Series IX–XIV in the present set of experiments a distinct increase of longevity can also be observed in inverse proportion to the intensity of starvation. In Series XIV the mean longevity in separate test-tubes was as a rule even greater than in controls; tubes *i* and *k* (both from the last hatch) together with tube *e* being the only three exceptions out of ten cases examined (Table II).

Only in the last test-tube was the maximal longevity higher in the continuously fed insects than in Series XIV, whereas in the remaining ones only in the test-tube *c* did the maximal duration of life of experimental flies not surpass the value for controls, being the same in both materials (see foot-note p. 207).

Stress should be laid on the fact that under normal conditions *Drosophila* feeds through the whole day, consequently the 6-hour periods of starvation practised in Series XIV cannot be considered as indifferent periods: at the end of each period

of starvation the flies at once began to eat gluttonously, their empty abdomens becoming immediately and abnormally distended with food.

These results undoubtedly point to the conclusion that periods of intermittent starvation for six hours out of every 24 increases the longevity of flies which were continuously provided with water. Further experiments with a larger number of specimens hatched on one and the same day and therefore suitable for biometrical examination would provide really satisfactory data for a complete analysis.

From the behaviour of animals in Series XII and XIII as well as in Series IX and XI the same inferences can be drawn in regard to the importance of the length of the fasting intervals (independently of the mutual ratio of the starving and feeding periods) as in the previous set of experiments.

Individual records indicate that starvation for 12 successive hours out of every 24 has a particularly injurious effect upon middle-aged flies.

C. CONTROLS.

The only fact which should be emphasised concerning the controls is that longevity was here great enough to show that the methods used in the present investigations provided a satisfactory index of the length of life in *Drosophila* (see the valuable data concerning longevity of several mutant stocks given by Gonzalez, 1923).

IV. SUMMARY.

1. Under all the intensities of starvation investigated, the longevity of the imago stage of *Drosophila* was as a rule diminished. At the same time, flies which were starved for six hours out of every 24 and which were not deprived of water, lived at least as long as the controls.

2. The longevity of flies increased in inverse proportion to the intensity of starvation.

3. The longevity was greater in series in which the flies were never subjected to lack of water than in those in which intermittent starvation without water was practised.

4. The injurious influence of longer, though less frequent fasting intervals was more pronounced than that of shorter though more frequent periods of inanition, both with or without administration of water.

5. Starvation for six successive hours out of every 24 without water affected the flies most during the middle period of their life. The same effect was found during starvation for 12 successive hours out of every 24 when the flies were supplied with water.

6. With lapse of time the flies became less resistant to the temporary absence of food.

Though the experiments performed did not give any clear results as to the supposed prolongation of life under the influence of intermittent starvation, some valuable hints for further study may be obtained from the observations recorded. The experiments on the influence of starvation for six hours out of every 24 with

administration of drinking water must be repeated on a larger scale and it is intended to perform further experiments using a less intense degree of inanition. As with lapse of time the animals become less resistant to starvation, special experiments are also planned in which the flies will be intermittently starved until middle age and thereafter will be fed continuously.

This work was performed whilst holding a fellowship of the International Education Board (Rockefeller Foundation).

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