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## EVIDENCE FOR AGING AS A CONSEQUENCE OF GROWTH CESSATION\*

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A previous investigation by Lansing<sup>1</sup> has indicated that a non-genic factor is active in aging of the rotifer. This factor has been shown to be transmissible, cumulative and reversible. The current report which is an extension of the initial experiments brings together new data which show that the aging factor becomes operative at a critical period in the life span. The period of growth cessation in the rotifer seems to coincide with expression of the aging factor as measured by accelerated aging.

The essential nature of the experimental method consisted of establishment by selection from isolation culture of series of generations of a parthenogenetic rotifer (*Philodina citrina*) with uniform parental age in all generations; such a series of generations was designated an "orthoclone." The isolation culture technique has been described previously.<sup>2</sup> It should be stressed that the experimental method involves no procedures which might alter the physiology of the rotifers. The longevity differences observed in various orthoclones are those apparently existing in nature and are only unravelled by the selection procedure.

The standardized environmental and genetic techniques yield longevity data for *Philodina citrina* which are remarkably consistent. Figure 1 shows diagrammatically the survival curves and egg production data for two closely related clones of rotifers. A wild stock group of 60 rotifers are contrasted with a similar group derived from eggs laid on the sixth day of life by the wild stock group. The curves for both longevity and egg production are virtually superimposed. The mean life span of the parent group was  $25.6 \pm 0.63$  days while that of the offspring (6F<sub>1</sub>) was  $25.7 \pm 0.55$ . The mean number of eggs laid per day during adult life is approximately two eggs per day. It is interesting to note that the adult rate of egg production is reached on the sixth day of life. Egg production falls

off rapidly to an insignificant level after the fifteenth day of life. As indicated previously,<sup>1</sup> *Philodina citrina* appears to produce a maximum of 32 eggs during the life span of an individual.

With the culture method employed *Philodina citrina* hatches from the egg in one day, initiates laying of eggs on the fourth and fifth days of life, reaches adult size on the sixth day and begins to show senile changes on approximately the fifteenth day of life.

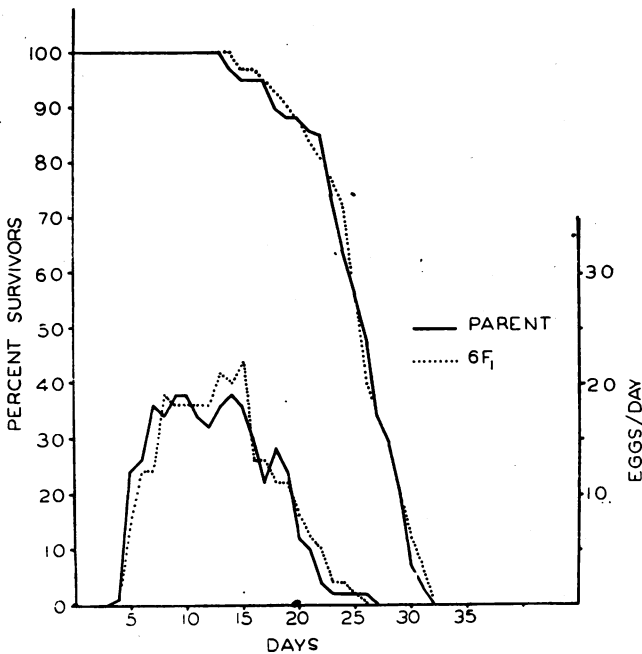


FIGURE 1

Survival and egg production curves for two related groups of *Philodina citrina*.

Measurements of body lengths were made with a micrometer ocular to determine the time at which maximum size is reached. It is difficult to obtain accurate data by this procedure since *Philodina* varies its length during movement. These measurements were made while the rotifers were fully extended in creeping along the bottom surface of the pyrex depression slides. The results of these measurements are graphically illustrated in figure 2 and represent the means of ten measurements for each day of life studied. *Philodina citrina* apparently reaches maximum length between the fifth and sixth days of life. This time coincides with that of initiation of the adult rate of egg production.

The preceding investigation<sup>1</sup> showed that the longevity of successive

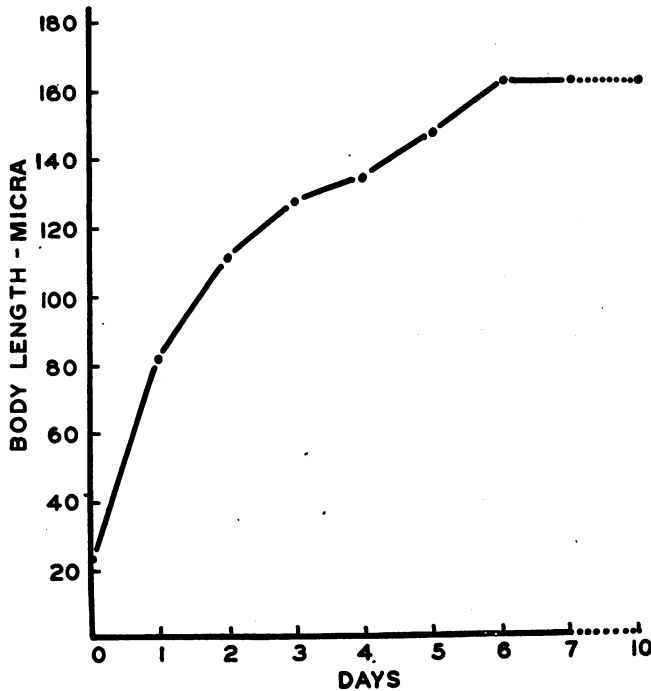


FIGURE 2

Graph showing changes in length of the rotifer in early life. Maximum length appears to be reached between the fifth and sixth days of life.

generations in old (16- and 17-day) orthoclones decreased progressively and that such old orthoclones invariably died out. Moreover, a similar pattern of events held true for eleven- and eight-day orthoclones. The latter result, illustrated graphically in figure 3, was particularly striking, for an eight-day old rotifer is a vigorous young adult animal. Since it was shown that orthoclones derived from adolescent (5-day old) rotifers manifest a progressive increase in longevity rather than a decrease, it seemed apparent that the aging factor must make its appearance or express itself early in life at some time between adolescence and early maturity. Six- and seven-day orthoclones were traced in detail in order to determine whether the factor appears abruptly or gradually between adolescence and early adult life.

Figure 4 contains the essential data of the 7-day orthoclone experiment. Survival curves for each generation in the orthoclone are shown in relation to one another. The pattern of events in the seven-day orthoclone parallels that of older orthoclones. The middle portion of the rectangular survival curve found in the first few generations tends to collapse as more animals

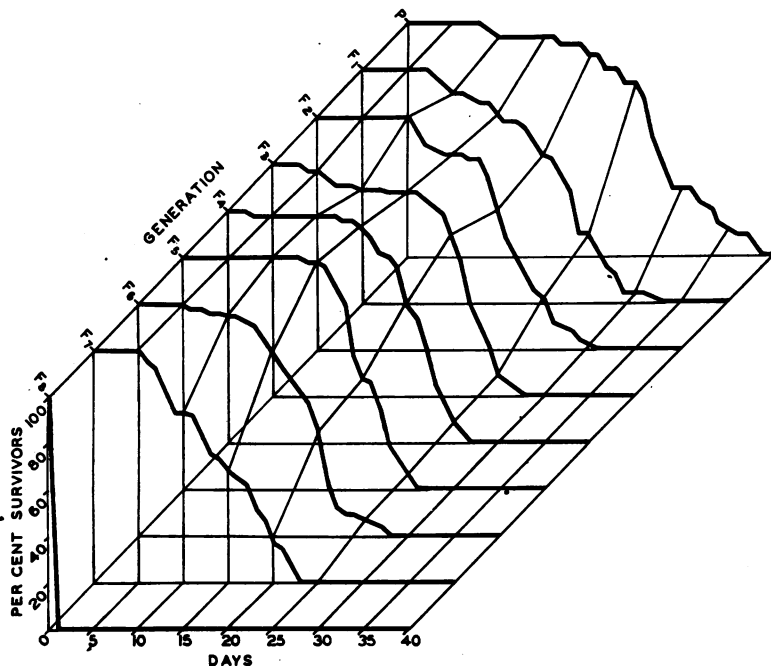


FIGURE 3

Three-dimensional graph of survival curves in successive generations of an 8-day orthoclone.

die at an early age. The  $7F_6$  and succeeding generations show a fairly uniform mortality rate with little change in maximum life span. The  $7F_{12}$  exhibits a distinct drop in mean life span and this is furthered through the  $7F_{15}$  which yielded a mean life span of  $6.5 \pm 0.19$  days. The  $7F_{15}$  was the last generation that could be obtained since no eggs were laid by these animals at any time during their short life spans.

The six-day orthoclone (Fig. 5) exhibits a pattern of change essentially like that of the 7-day orthoclone. Here, again, the middle portion of the survival curve collapses, no further marked changes occur until the fourteenth generation. The orthoclone died out for lack of eggs in the seventeenth generation which yielded a mean life span of  $7.7 \pm 0.27$  days.

It appears, then, that the aging factor expresses itself at some time between the fifth and sixth day of life. The time cannot be defined more clearly with the present technique since observations are made at 24-hour intervals.

The fact that the period between the fifth and sixth days of life in *Phlo-dina citrina* marks the transition is significant when related to appearance

of the aging factor. Certainly the view that aging begins in the fertilized ovum becomes untenable. These experiments to the contrary suggest that aging is a direct sequel to growth cessation. Apparently, at any time during active growth the organism is at least potentially immortal. It is only with growth cessation that the capacity for aging develops.

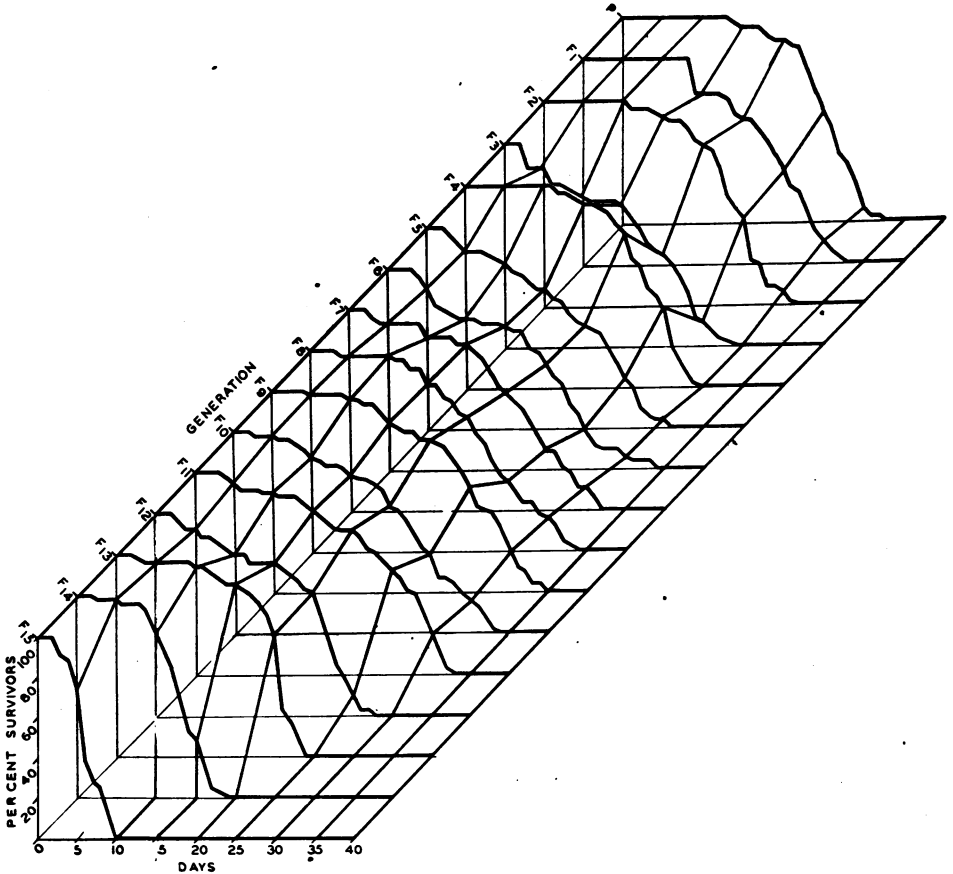


FIGURE 4

Three-dimensional graph showing relation between successive generations of a 7-day orthoclone.

Examination of figure 6 indicates that there is an orderly relation between longevity in generations of an orthoclone and age of the orthoclone. The curve that can be derived from the block diagram is essentially asymptotic. It seems, therefore, that the aging factor not only appears abruptly during the period of growth cessation but also increases with age after maturity.

Some critical observations remain to be made. The vertical limb of the asymptotic curve in figure 6 suggests that the longevity of a five-day orthoclone should be essentially infinite. This possibility is in accord with the observation that longevity of five-day orthoclones tends to increase. It remains to be demonstrated that the five-day orthoclone can

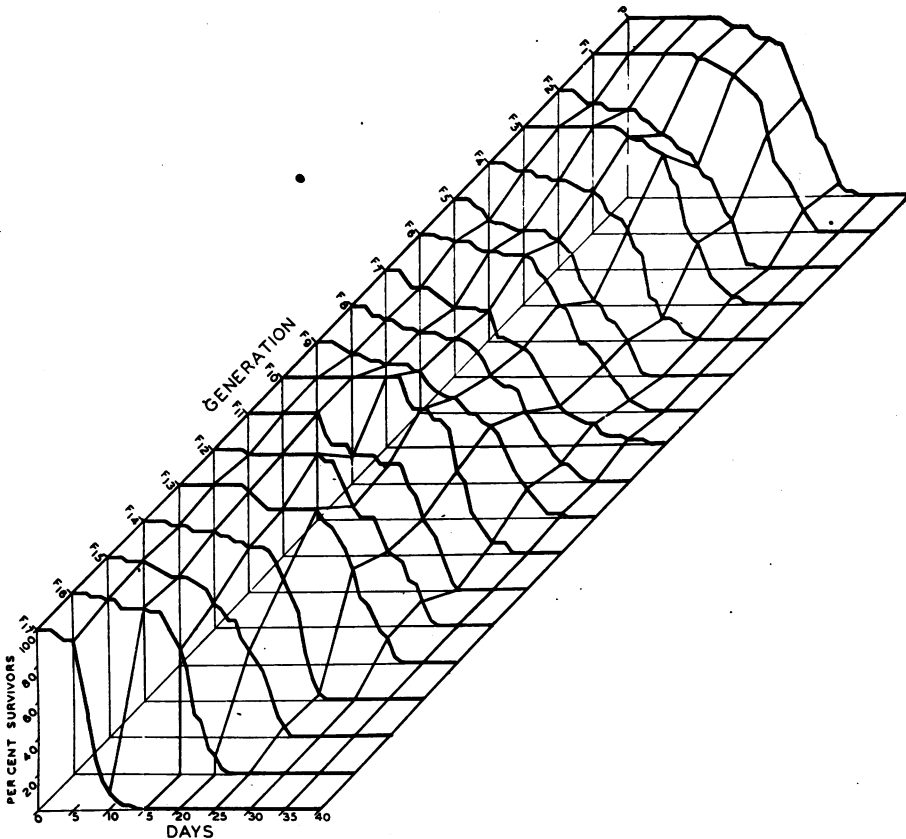


FIGURE 5

Three-dimensional graph showing relation between successive generations of a 6-day orthoclone.

be maintained indefinitely. Further, there as yet is no indication as to the maximum life span of rotifers in a five-day orthoclone.

The behavior of the aging factor and its relation to growth assumes additional significance when one examines in retrospect the meager literature on aging. Various experiments<sup>3, 4</sup> on the effect of starvation on longevity contain the basic pattern that extension of the period of growth results in extension of the total life span. Sonneborn's<sup>5</sup> experiments on

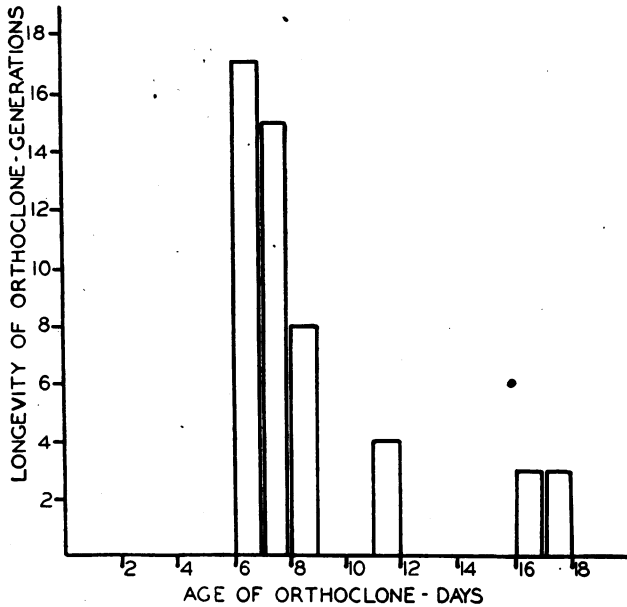


FIGURE 6

Recapitulation of relation between longevity of orthoclones and age of various orthoclones.

*Stenostomum* suggest that growing tissues do not age while non-growing tissues do age. In general it seems true that post-mitotic cells are the only ones which age, growing intermitotic cells do not show age changes.<sup>5</sup>

*Summary.*—It has been shown that aging of the rotifer involves a non-genetic factor which is transmissible, cumulative and reversible. New data have been obtained which indicate that the time of appearance of this aging factor coincides, within the limits of the experimental technique, with the time of cessation of growth.

There appears to be an inverse relation between the age of an adult orthoclone and longevity of such orthoclones.

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<sup>5</sup> Sonneborn, T. M., *J. Exp. Zool.*, 57, 57-108 (1930).

<sup>6</sup> Cowdry, E. V., *Problems of Ageing*, Williams & Wilkins, Baltimore (1942).