Decreased Oxygen Requirement for Growth of Fruit Fly Larvae After Continual Centrifugation.* (25974)

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Different laboratories reported that although increased gravity, as simulated by continual centrifugation, can reduce growth of plants and animals, these organisms can survive and actually demonstrate measurable growth(1-7). As expected, these organisms exhibit certain evidences of adaptation. One such evidence is that centrifugation can sometimes evoke a faster than normal growth. This occurs with very young fruit fly larvae during centrifugation at 500 to 700 times gravity(6), or after 24-hour centrifugation at 2000 to 3000 times gravity(5). It has been suggested that, since increased gravity could cause less food materials to be available for developmental processes the animal compensates in part by increasing the efficiency of processes necessary for development. This adaptation might over-compensate, permitting the faster than normal growth which occurs during moderate centrifugation. If such adaptation is permanent, it might well reflect itself after centrifugation by greater than normal growth rate. Under certain conditions this does occur even though there had been a slower growth in the centrifuge(6). The proposed increase in efficiency could have been masked by gravitational retardation of growth until centrifugation ceased. Should this be the case, experimental larvae, returned to control conditions, would consume less than normal amounts of oxygen to accomplish a given amount of growth. Our purpose is to report that the ratio of oxygen consumption to growth is indeed less for larvae returned to normal gravity after 24 hours continual centrifugation. Nyst studied oxygen consumption of Drosophila larvae(8). He found the rate at 25°C for resting larvae to be .04 mm^3/hour/mg of larvae during exponential growth. The rate was directly proportional to the animal's mass rather than surface area as is so often the case with larger animals. We confirmed that there is the same relation to mass for active larvae at 28°C(9). Oxygen consumption was approximately 0.10 mm^3/hour/mm^3 of larvae. Miller(10) has reported that wheat Coleoptiles exhibit altered oxygen consumption after prolonged centrifugation. Gray's group(11) also reported other changes which Coleoptiles undergo in adaptation to high gravity.

Materials and methods. The wild, red eyed strain of *Drosophila melanogaster* was employed. Newly hatched larvae, with volumes of 0.2 mm^3, were centrifuged 24 hours at 20 or 28°C. After removal from the centrifuge, oxygen consumption of active, growing larvae was measured at appropriate temperatures. They were then permitted to grow 24 hours under control conditions. Larvae were cultured and measured in essentially the same manner as previously described(3,5). They developed in plastic centrifuge tubes which contained a seed-free, banana-agar medium. The growth constant, k, is defined as rate of change in natural logarithm of volume (v). In other words, $k = \frac{\dot{v}}{v} = d(\ln v)/dt$. Volume is computed from measurements of larvae's length and width. Geometric means of volume are employed in computing k. Oxygen consumption measurements were performed in a simple respirometer (Fig. 1). It was prepared by inserting a 0.1 ml pipette into a small test tube. Petroleum jelly sealed the junction. Small volumes of ethyl alcohol served as manometer fluid. Rate of descent of this droplet in the pipette was measured. All but upper portions of pipettes were submerged in constant temperature baths. Values cited are corrected to standard temperature and pressure. Ratio of volume rate of oxygen consumption, $\dot{V}$, to absolute rate of growth, $\dot{v}$, can be employed as an index, I, of oxygen required for a given amount of growth. This

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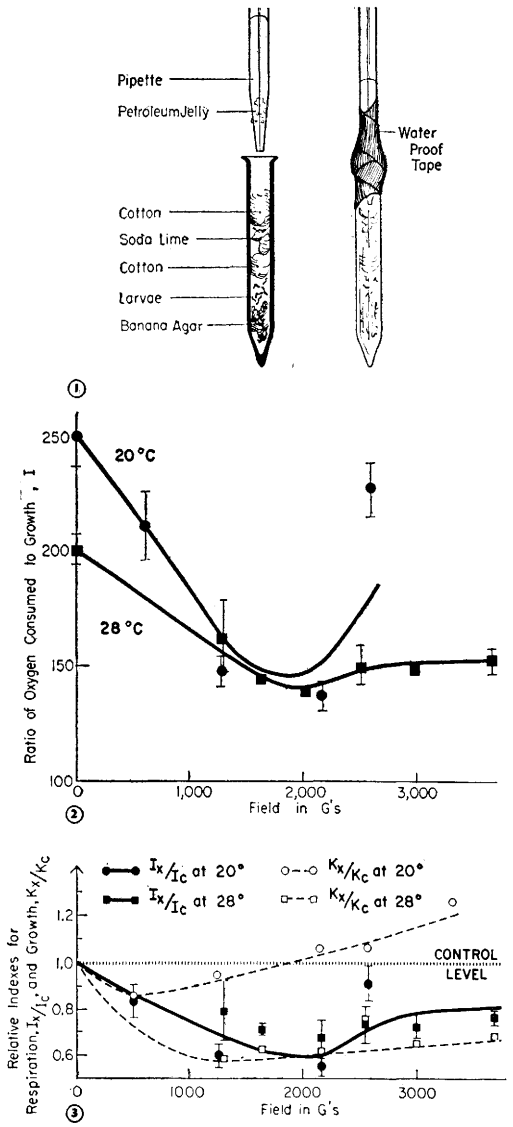

FIG. 1. Assembly of respirometer. The junction is first surrounded with masking tape to hold petroleum jelly. Approximately 100 one-day-old larvae were necessary to obtain reproducible readings. These respirometers were fashioned from medicine droppers and have a capacity of approximately 3 ml.

FIG. 2. Oxygen consumption relative to growth, I, as a function of the previous centrifugal field intensity. These values of I are for animals after return to control conditions. Values next to the Y-intercept correspond to control values under control conditions, these larvae consume approximately 0.07 ml O_2/hr/ml of larval volume at 20°C and 0.10 at 28°. Vertical lines extending from the points indicate stand. errors. If errors are not shown their magnitude is less than 10.

FIG. 3. Relative oxygen to growth indexes $\frac{I_x}{I_c}$ as a function of gravitational field intensity of previous exposure. The subscript, x, refers to experimental and the subscript, c, to the control. These values were computed upon dividing values in Fig. 2 by control values. Vertical lines extending from points for these values indicate stand. errors. These are not indicated for magnitudes less than .05. Values for relative post-exposure growth constants $\frac{K_x}{K_c}$ are also shown. Their errors, although not shown, would be approximately 10% of the ratio's value. Values for growth constants during centrifugation at these temperatures have been published elsewhere(6).

oxygen to growth index would be equal to $\frac{V}{\dot{v}}$. Since k is defined as equal to $\frac{\dot{v}}{v}$, then the index would also be equal to $\dot{V}/kv$. That formula has been employed in this report. (For this computation, v is equated to arithmetic mean larval volume at time of oxygen measurements.) The index has the advantage of being essentially constant throughout exponential growth(9). It is a unitless quantity which indicates ratio of volume of oxygen consumed to volume of living material produced. Under control conditions, values for I are more reproducible than those for $\dot{V}$, $\dot{V}/v$, or k. Tubes containing experimental larvae were centrifuged in Servall angle centrifuges (Model XL). Centrifuges were enclosed in specially constructed constant-temperature chambers(6). Control tubes were placed in the same chamber. The symbol "G" is used to designate multiples of Earth's gravitational field. Centrifugal fields of from 500 to 3800 G's were employed.

Results. After centrifugation I, oxygen consumption/unit volume of larvae, was below control levels. The oxygen required during a given amount of growth was reduced (Fig. 2 and 3). For larvae centrifuged at 20°C, this was best demonstrated with those exposed to fields from 1200 to 2200 G's. At 20°C, there is an increased efficiency after centrifugation even though there is no marked effect upon growth rate either during(6) or after (Fig. 3) centrifugation. With animals exposed at 28° C, the most significant results were obtained after exposure to fields greater than 1500 G's. No marked difference between relative changes in experimental indexes at the 2 temperatures could be demonstrated for comparable fields

(see Fig. 3). During centrifugation at 28°C, the growth constant drops in a more or less linear manner as the field increases beyond 500 G's(6). Such linearity is not demonstrable for I. In all cases, there was little mortality after centrifugation. For both control and experimental cultures, 90% of animals surviving after centrifugation had ceased, were alive 24 hours later.

Discussion. Prolonged centrifugation causes fruit fly larvae to develop altered metabolism. This is evidenced by the fact that, upon return to control conditions, these animals can accomplish more than the normal amount of physical growth with a given quantity of oxygen. The mechanism of this alteration is unknown. It is tempting to interpret this as an increased efficiency of growth processes.

The validity of the preceding suggestion could well depend upon how broadly we wish to define "efficiency" and "energy for growth." Let us define efficiency in terms of the ratio of growth accomplished to food metabolized. Then, assuming a constant, caloric equivalent for oxygen, efficiency has increased.

One would not necessarily expect changes in chemical production of protoplasm to greatly alter the overall oxygen requirements. Processes other than production of protoplasm could be altered. These other processes might include various movements, digestion, and maintenance. Should any change cause a greater efficiency, then, once gravitational stress is removed, a larger fraction of food would be available for growth processes.

After centrifugation, larvae do develop into pupae. However, unlike controls, a sizable percentage of these individuals do not emerge as adults. Perhaps, food materials can be conserved by sacrificing structures.

Summary. 1. Prolonged centrifugation can alter an organism's growth. 2. After centrifugation at 20 and 28°C, fruit fly larvae do not require as much oxygen for growth. 3. It is suggested that the animal has adapted by developing a greater efficiency for growth.

1. Gray, S. W., Edwards, B. F., *J. Cell. Comp. Physiol.,* 1955, v46, 97.

2. Matthews, B. H. C., *J. Physiol.,* 1953, v122, 31P.

3. Wunder, C. C., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 544.

4. Smith, A. H., Winget, C. M., Kelly, C. F., *Growth,* 1959, v23, 97.

5. Wunder, C. C., Herrin, W. F., Cogswell, S. C., *Proc. First Nat. Biophysics Conf.,* 1959, Yale Univ. Press, Hartford, p639.

6. Wunder, C. C., Herrin, W. F., Crawford, C. R., *Growth,* 1959, v23, 349.

7. Walters, G. R., Wunder, C. C., Smith, L., *J. Appl. Physiol.,* 1960, v15, 307.

8. Nyst, R. H., *Ann. Soc. Roy. Zool., Belgique,* 1941, v72, 74.

9. Crawford, C. R., *Oxygen Consumption of Fruit Fly Larvae after Continual Centrifugation,* M. S. Thesis, Univ. of Iowa, 1959.

10. Miller, A. I., *Anat. Rec.,* 1950, v108, 619.

11. Edwards, B. F., Gray, W. S., *J. Cell. Comp. Physiol.,* 1956, v48, 405.

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Adaptation of Orbital Bleeding Technic to Rapid Serial Blood Studies. (25975)

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During investigations requiring serial blood samples on experimental animals our attention was drawn to the ingenious but apparently little known eye bleeding procedure (1-5). Tail bleeding is difficult, time consuming and unsatisfactory from the standpoint of contamination of the collected blood. Other methods have obvious drawbacks.

Methods. Since enzyme studies usually require plasma rather than whole blood it is desirable to centrifuge directly in the original blood collection tube. The technic requires a

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