Effects of temperature on the metabolic rate of young and old *Drosophila*

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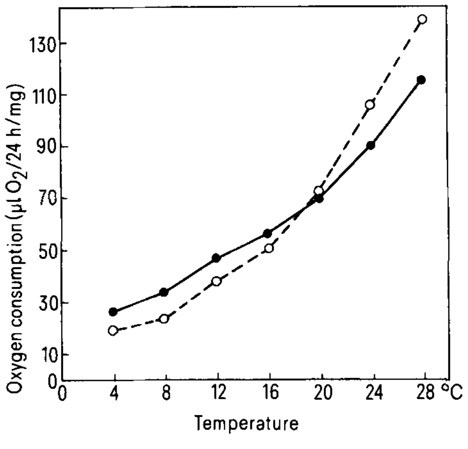
Summary. Respiratory measurements in young and old *Drosophila melanogaster* reveals significant differences between these two groups in response to temperature. The data suggest that there is some degree of temperature compensation of respiration in young insects but that this capacity is lost with age.

Considerable evidence has been assembled in recent years which suggests that an inverse relationship may exist between metabolic rate and lifespan in poikilotherms[2-6]. However, this concept remains controversial since several laboratories have reported findings to the contrary[7-9]. Nevertheless, studies from our laboratory do support the idea that lifespan correlates with metabolic rate, at least for *Drosophila*[2,3,10-12]. Moreover, we have found that the rate of age related physiological decline also correlates with the metabolic rate[2,11,12]. The mechanism of this metabolic 'wear and tear' that results in the loss of physiological function with age in insects is not well understood. We have previously suggested that this metabolic attrition is a direct consequence of respiratory damage to the mitochondria[13-15]. In apparent support of this idea, it has been noted in one insect species that resting metabolic rate actually increases with age[16]. This particular finding indicates that a decrease in the efficiency of the mitochondria to carry out oxidative phosphorylation (uncoupling) may be occurring during senescence. Although 1 study has shown that respiration rate decreases with age in both a wild and hybrid strain of *Drosophila* whereas in an inbred strain, respiration rate increases for the first 20 days of imago life and then decreases[7]. These same authors, in a later study, found that respiration rate decreases with age when larval population density is low but does not decline when the larval population density is high[8]. It is not clear how these findings relate to an understanding of senescence in *Drosophila*. Curiously, none of these previous studies measured metabolic rate as a function of both age and temperature. The measurement of respiration as a function of temperature is a sensitive indicator of respiratory efficiency in vivo. Therefore, we have measured oxygen consumption in young and old *Drosophila* at several temperatures. These studies show that the efficiency of respiration in *Drosophila* decreases with age.

Materials and methods. Drosophila melanogaster of the Oregon R strain used in these experiments have been inbred in this laboratory for over 17 years. Young flies were 14 days and old flies were 101 days post emergence and were raised at 21 °C under a 12:12 h light cycle. The lifespan and temperature dependent mortality kinetics for these flies have been reported[2]. The mean and maximum lifespans for flies raised in our laboratory at 21 °C are 86.3 ± 2.74 (SEM) and 116 days respectively. Oxygen consumption measurements were made using standard manometric techniques with a Gilson model IGA-14, direct reading differential respirometer. Groups of approximately 35-50 male flies were placed in 25-ml warburg flasks containing 250 μl of 20% KOH and fitted to the respirometer. Flasks were submerged in a circulating water bath adjusted to the desired temperature and flies were allowed to acclimate to each temperature for 2 h before measurements began. Three flasks of flies for each age group were used. After the equilibration period, oxygen consumption was determined by taking readings every 10 min for 1 h. Total weight of the flies in each flask was determined with a Cahn electrobalance model 7500. Oxygen consumption data is reported as ml oxygen consumed per mg fly per 24 h.

Results and discussion. It is clear from the figure that significant differences exist in the respiratory rate of young and old *Drosophila* at different temperatures. Of particular interest is the fact that the old flies exhibit a lower metabolic rate than young flies at low temperatures and a higher rate at high temperatures. Also, this effect does not seem to reflect differences in the levels of physical activity of these insects since at the low temperatures (4-12 °C) no activity was visible (flies were immobile) and no differences were observed at the higher temperatures where the flies were active. There is no difference in respiration rates at the temperature in which these flies were raised (21 °C). However, these data only reflect 2 points in the life cycle of an inbred strain maintained at one temperature (21 °C). Thus, it does not provide a comprehensive study of the relationship between temperature, respiration and age in *Drosophila*. Nevertheless, we interpret these data to mean that in young *Drosophila* there is some degree of temperature compensation of respiration but that this capacity is greatly diminished with age. The Q 10 over the entire temperature range is almost 1.5 times as great for the old flies as it is for the young flies. The Q 10 value is a temperature coefficient which represents the increase in the rate of a process (in this case respiration) produced by raising the temperature 10 °C. The Q 10 for young and old flies was 1.8 and 2.8 respectively. The mechanism for this greater sensitivity of respiration of old flies to temperature changes is presently not known. Newell[17] has noted that 'oxygen uptake of invertebrates at rest reflects mitochondrial oxygen requirements'. Therefore, the effect observed in this study may reflect significant alterations in the function and requirements of the mitochondria. This idea is supported by numerous studies which have shown that deteriorative changes in both the structure and function of the mitochon-



Relationship between oxygen consumption and temperature for 2 different age groups of *Drosophila melanogaster*. —●—, 14-day-old flies and ---○---, 101-day-old flies. Each point represents the mean of 3 flasks of flies with 35-50 flies per flask. Repeated measures ANOVA were performed to test for parallelism of the graphs. The graphs are significantly non-parallel (p = 0.0002).

dria do accompany aging in invertebrates[15]. In this context, it has been recently noted by Driver and Lamb that old *Drosophila* have impaired energy metabolism and are less energy efficient than young flies[18]. Moreover, since respiratory activity is directly related to the activity of cytochrome oxidase, changes in the activity of this enzyme may stem from changes in the inner mitochondrial membrane. It is thought for example, that temperature plots of membrane bound enzyme activities are reflected by changes in the lipid components of the membrane[19,20]. Possibly, this effect is the result of membrane lipid changes induced by peroxidation of mitochondrial lipids. The fact that lipid peroxidation is related to aging rates is well established[21]. Furthermore, one of the well characterized effects of lipid peroxidation on the mitochondria is an alteration in the physical properties of the phospholipids in the inner membrane bilayer[22]. Thus, age induced lipid peroxidation would be expected to affect mitochondrial enzyme activity and this would be manifested in in vivo respiratory activity. This could explain the altered respiratory rates observed as a function of temperature in this study.

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Heat shock response in the Atlantic sea urchin, *Arbacia punctulata*[1]

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Summary. Synthesis of heat shock proteins of blastulae and gastrulae of the sea urchin, *Arbacia punctulata*, was characterized by analyzing proteins labeled in vivo with [35S]methionine on 2-dimensional polyacrylamide gels. Trypsin was also shown to stimulate synthesis of heat shock proteins.

Giudice and coworkers[2,3] have demonstrated that sea urchin embryos resemble other organisms[4] in responding to heat shock by altering the relative synthesis of certain proteins, with the major such heat shock protein (HSP) having a mol.wt of 70 kdal (HSP70). This paper defines this response further by demonstrating isoelectric variants of HSP70 and by quantitating the synthesis of selected polypeptides in response to heat and trypsin.

Materials and methods. Adults and embryos of *Arbacia punctulata* (fertilization ≥ 95%) were maintained at 21 °C. Trypsin (150 µg/ml) was added to some embryos after fertilization. Hatched blastulae (8 h after fertilization) and early gastrulae (13 h) were cultured as follows. After 70 min at either 21 °C (control) or 31 °C (heated), control and heated embryos were labeled 20 min at their respective temperatures in artificial sea water (1 ml) containing streptomycin (150 µg) and [35S]methionine ([35S]met, 45 µCi). Non-labeled, heated embryos were quickly cooled to 21 °C after 90 min at 31 °C. Samples of control and heated cultures, taken 1 and 4 h after the heating period, were also labeled 20 min at 21 °C.

Labeled embryos were prepared for electrophoresis[5] with the total hot trichloroacetic acid (TCA)-precipitable cpm and total protein[6] being determined for each sample. Isoelectric focusing (IEF) gels were prepared according to O'Farrell[7] with electrophoresis in the 2nd dimension through 9% separating and 3% stacking gels[8]. The gel slabs were stained, dried, and autoradiographed (Kodak XAR-5) for 10–14 days. Selected spots were cut from dried gels, solubilized, and counted by liquid scintillation spectrometry. [35S]met incorporation into each polypeptide was recorded as the percentage of total hot TCA-precipitable cpm loaded in the IEF dimension. Because this computation assumes 100% recovery in both electrophoretic dimensions, the values obtained probably underestimate the actual percentages.

Results. Heating blastulae and early gastrulae stimulated the synthesis of 5–6 polypeptides (HSP70) with a mol.wt of approximately 70 kdal and isoelectric points (pI) between 5.8 and 6.5. The stimulated synthesis was 6.7 and 9.0 times that of control blastulae and gastrulae, respectively (table). The HSP70 were synthesized slowly, if at all, at 21 °C unless the embryos had been exposed to trypsin (fig., table). Incubation at 31 °C reduced overall protein synthesis about 50%, but not uniformly. Actin and tropomyosin synthesis was much less sensitive to heat than that of tubulins and polypeptide 2 (fig., table).

Heated gastrulae, but not blastulae, showed increased rela-