Goldin, Herbert. University of Oregon, Eugene. Oxygen uptake in two Minutes of D. melanogaster.

Since all heterozygous Minute larvae, regardless of their locus, exhibit delayed growth and development, as compared to normal sibs, and since investigations by Farnsworth (J. Expt. Zool. 157:345-352, 1964) have indicated abnormalities in cellular respiration and protein synthesis of $M(2)1^2$, it was felt that respiratory studies of $M(2)1^2$ and M(3)w heterozygous as well as homozygous larvae might prove fruitful in further elucidating the Minute effect.

Larvae were collected according to the method of Farnsworth (DIS 37:139, 1963). Controls (+/ltd and +/ca) were tested for O_2 consumption at 2, 24, 48, 72 and 90 hours of development. Since Minute homozygotes do not grow appreciably after hatching, a control of approximately the same size was considered more appropriate than one of similar age. Therefore, +/+ larvae, two hours post hatching were used as controls for the Minute homozygotes.

Heterozygote Minutes ($M(2)1^2$/ltd and M(3)w/ca) were obtained by mating ltd/ltd (ca/ca) virgin females with $M(2)1^2$/ltd (M(3)w/ca) males. The resultant larvae could be readily distinguished at 48 hours of development, on the basis of Malphigian tubule color, the Minute heterozygotes having the characteristic yellow pigmentation and ltd/ltd or ca/ca an absence of pigmentation. The heterozygotes were tested for O_2 consumption at 48, 72, and 96 hours of development.

Homozygous Minutes live for about 50-60 hours and can be readily distinguished from their wild type sibs in culture at 24 hours of development on a size basis. These were tested for respiration at 24 and 48 hours of development.

The larvae were removed from a yeastless culture medium directly to a micro-respirometer. The apparatus was a modification of that described by Thimann, Yocum and Hackett (BBA 53:239-257, 1954) and consisted of a 5 ml vial with a moistened piece of filter paper on the bottom. In the mouth of the vial was inserted a one-hole rubber stopper, through which was placed a 0.2 ml pipette, calibrated to 0.001 ml (one microliter). A small piece of cotton wick saturated with 10 N KOH served to absorb CO_2 and was suspended in the chamber on an insect pin inserted in the rubber stopper. The entire unit was immersed in a water bath, the temperature of which was effectively regulated at 25°C by means of a heating unit, a stirring attachment, and a copper coil through which cold water could be run for cooling. As noted above, the KOH filter served as a trap for CO_2 given off by the larvae during respiration, and thus when the oxygen in the vial was used, water was drawn into the pipette. Oxygen uptake readings, in microliters, could thus be taken directly by noting the initial and final levels of water in the pipette.

After the larvae had been transfered to the vial, the whole unit was assembled and placed in a water bath for a 15 minute equilibration period, after which readings were taken at 10 minute intervals for 30 minutes. A blank respirometer was always inserted in the bath to serve as a thermobarometer to compensate for any changes in barometric pressure.

The results were recorded as the mean value of oxygen uptake in microliters of oxygen per larva per hour, as shown in Table I, and represent a minimum of five experiments from two different cultures on different experimental days for each genotype and age.

The data show that the control respiratory rate steadily increases to 48 hours post hatching. At 72 hours of development, the rate has quadrupled that of the 48 hour stage. At 90 hours, just prior to pupation, there is only a slight increase in respiration compared to 72 hour larvae.

In the case of the Minute heterozygotes, there is no significant difference in O_2 consumption, when compared to controls, at 48 hours after hatching. At 72 hours, however, the $M(2)1^2$/ltd respiratory rate is only half that of its +/ltd control and O_2 consumption is even further depressed in M(3)w/ca, when compared to its +/ca control. At 96 hours of development, the Minute heterozygotes show a slight increase in respiration compared to 72 hour larvae but, still do not approach the value obtained for 90 hour controls.

In the Minute homozygotes, O_2 consumption of the 24 hour larva was slightly lower than that of the two hour wild type control. $Minute(2)1^2/M(2)1^2$ larvae had somewhat increased their respiratory rate at 48 hours but, M(3)w homozygotes, although still apparently living, had a respiratory rate which was undetectable by the apparatus.

The data thus presented, have indicated another facet of the $M(2)1^2$ and $M(2)1^2$ and M(3)w phenotype, i.e., a lowered respiratory rate, which is time specific during larval development, occuring some time between 48 and 72 hours after hatching.

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Table 1: Effect of genotype on O_2 uptake in ul O_2/larva/hour ± SD

genotype	age (hrs., post hatching)					
	2	24	48	72	90	96
+/+	.265±.05					
+/ltd$_2$	.29 ±.15	.926±.37	1.82± .74	7.96±1.4	8.75±3.03	
M(2)l^2/ltd			1.77±1.07	3.99±1.8		5.83±1.79
M(2)l^2/M(2)l^2		.195±.048	.25± .09			
+/ca		.603±.37	2.16± .88	8.32±1.91	9.71±1.71	
M(3)w/ca			2.16± .5	3.24±1.1		4.3 ±1.35
M(3)w/M(3)w		.132±.12	~0			

Sharma, R. P. Indian Agricultural Research Institute, New Delhi, India. Radio-sensitization of Drosophila melanogaster by N-Ethylmalemide.

It has been demonstrated by Bridges (1960) in E. coli and Sharma (1965) in Vicia faba, that N-Ethylmalemide possesses radiosensitizing ability. A preliminary report on the radiosensitizing effect of this chemical in Drosophila is presented here.

2.5 ml of 100 uM solution (pH7) of N-Ethylmalemide was mixed with 2.5 ml of basic medium, comprised of agar (3%), yeast (10%), glucose (10%), propionic acid (0.4%) and water (100 ml), to get 50 uM concentration of the chemical. Freshly laid Drosophila eggs (Oregon-K) were transferred to this medium and allowed to develop up to adult stage. The newly emerged males were collected and kept for two days. One batch was kept as such, whereas the other batch was irradiated with 2400 r of X-rays. The males collected from the normal medium were irradiated with the same radiation dose to serve as control for the chemical-radiation combination treatment. The males were crossed with M-5 virgin females at the rate of one male and three females. The sex-linked recessive lethals were scored in F_2.

From the data (Table 1) it is seen that the combination treatment of chemical and radiation shows about 2-fold increase (5.4%) in the frequency of sex-linked recessive lethals over radiation (2.8%). The chemical alone is not able to produce any mutation. The possible explanation for such radiosensitizing effect produced by X-Ethylmalemide may be due to its ability to combine and inhibit the sulphydryl groups.

Table 1

Treatments	Chemical dose	Radiation dose	No. of Chromosomes tested	% of sex-linked recessive lethal
N-Ethylmalemide	50 uM	-	695	-
X-rays	-	2400 r	634	2.8
N-Ethylmalemide + X-rays	50 uM	2400 r	646	5.4

References: Bridges, C. B. (1960). Sensitization of E. coli to radiation by N-Ethylmalemide. Nature 188:415.
Sharma, R. P. (1965). The radiosensitizing effect of N-Ethylmalemide on Vicia faba. Curr. Sci. (In press).