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Effect of Methylene Blue on Respiration of Blocked and Developing Embryonic Cells.*

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We[1] have recently shown that actively developing embryos of the grasshopper, *Melanoplus differentialis,* have a respiratory rate 2 to 3 times higher than do embryos whose activity is to a large extent blocked as the result of diapause. The average respiratory rate for embryos immediately before and after diapause ranges between 0.1 and 0.15 mm.^{3} per embryo per hour; the respiratory rate of blocked embryos averages approximately 0.05 mm.^{3} per embryo per hour. In these 3 states of development the embryos are morphologically identical—moreover, the dry weight of the embryos is the same in each case, *viz.,* ca. 0.07 mg.

In an attempt to gain some information regarding the nature of the block to respiration which occurs during diapause, the effect of methylene blue on the respiratory activity of blocked and developing embryos has been studied.

The embryos were freed from egg membranes and extra-embryonic materials and were suspended in a medium consisting of 0.9% NaCl, 0.02% each of KCl and $CaCl_2$ and 0.5% dextrose. The solution was buffered with phosphate (final concentration 0.01 molar) to pH 6.3—the pH of the perivitelline fluid of the egg.

Oxygen consumption of the embryos was determined by means of sensitive microdifferential manometers at 25° ± 0.02°C. Methylene blue solutions were prepared fresh daily in the saline-dextrose solution described above. In the use of cyanide the technique outlined by Huff and Boell[2] was followed.

The data in Table I show that methylene blue has only a slightly stimulating effect on the oxygen consumption of developing embryos, the average increase in respiration approximating 25%. On the other hand, the addition of methylene blue to blocked embryos practically doubles the rate of oxygen uptake. Nearly maximal stimulation is obtained with 3×10^{-5} molar methylene blue. When the concentration of the dye is increased beyond 15×10^{-5} molar,

* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

1 Bodine, J. H., and Boell, E. J., 1936 (in press).

2 Huff, G. H., and Boell, E. J., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 626.

TABLE I.

Type of Embryo	O_2 uptake per hour: Control mm.³	O_2 uptake per hour: M.B. added mm.³	% increase in M.B.	Molar conc. of M.B.
Pre-diapause	.100	.102	2	3×10^{-5}
Diapause	.050	.096	93	3×10^{-5}
"	.057	.117	105	15×10^{-5}
Post-diapause	.106	.131	24	3×10^{-5}
" "	.102	.132	29	6×10^{-5}

TABLE II.

Experimental Condition	mm³ O_2 uptake per hour: Control	M.B. added	KCN added	KCN + M.B. added
10^{-4} M KCN 6×10^{-5} M M. Blue	.102 (100%)	.132 (129%)	.046 (45%)	.132 (129%)

respiration is usually stimulated to a lesser extent. It is noteworthy that methylene blue raises the respiration of blocked embryos to the level which is characteristic for normally developing embryos. (Runnström.[3])

When the respiration of developing embryos is inhibited by cyanide the addition of methylene blue restores respiration to the normal level. As is indicated in Table II 6×10^{-5} molar methylene blue can completely annul the inhibiting effect of 10^{-4} molar cyanide.†

The above facts suggest that methylene blue can substitute for that portion of the respiratory mechanism which is inactivated either naturally as the result of the diapause block or artificially through the action of cyanide.

[3] Runnström, J., *Protoplasma*, 1930, **10**, 106.

† No antagonism between carbon monoxide and methylene blue has been observed.