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Effect of CO and Methylene Blue on Respiration of Embryos.*

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Barron *et al.*[1] have adduced evidence in support of the hypothesis that methylene blue and other dyes stimulate the oxygen consumption of certain cells by promoting the oxidation of carbohydrates or some of their degradation products. They suggest, moreover, that the catalytic process depends upon the reversible reduction and reoxidation of the dyestuff and that this is independent of the function of the respiration enzymes, for when these are poisoned by cyanide and carbon monoxide the addition of methylene blue or other dyes restores respiration to the normal levels.

Recently Reid[2] has shown that leuco-methylene blue is not autoxidizable, as is commonly supposed, but that the process involves a metal catalysis and is sensitive to CO poisoning. Cook, Haldane and Mapson[3] have demonstrated a CO sensitivity of methylene blue stimulated oxidations in *B. coli*. Likewise, Chang and Gerard[4] have shown that the stimulation of nerve respiration by cresyl blue is depressed by CO. We[5] have previously pointed out that the methylene blue stimulation of respiration in diapause (blocked) grasshopper embryos *(Melanoplus differentialis)* is sensitive to CO, whereas the normal respiration of such embryos is little if at all affected by this substance. In actively developing embryos the stimulation due to methylene blue as well as a large fraction of the normal respiration is depressed by CO. Our inability to obtain antagonism by methylene blue of cyanide inhibition of respiration except in cases where very low concentrations of cyanide were used and the results obtained by other workers as well as ourselves on the

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1 Barron, E. S. G., and Harrop, G. A., *J. Biol. Chem.*, 1928, **79**, 65; Barron, E. S. G., *J. Biol. Chem.*, 1928, **81**, 445; Barron, E. S. G., and Hoffman, L. A., *J. Gen. Physiol.*, 1930, **13**, 483; Barron, E. S. G., and Hamburger, M., Jr., *J. Biol. Chem.*, 1932, **96**, 299; DeMeio, R. H., Kissin, M., and Barron, E. S. G., *J. Biol. Chem.*, 1934, **107**, 579.

2 Reid, A., *Berichte*, 1930, **63**, 1920; *Bioch. Z.*, 1930, **228**, 487.

3 Cook, R. P., Haldane, J. B. S., and Mapson, L. W., *Bioch. J.*, 1931, **25**, 534; *Bioch. J.*, 1931, **25**, 880.

4 Chang, T. H., and Gerard, R. W., *Am. J. Physiol.*, 1931, **97**, 511.

5 Bodine, J. H., and Boell, E. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 629; *Physiol. Zool.*, 1936 (in press).

CO sensitivity of methylene blue stimulation strongly suggests that in some living systems methylene blue catalysis of respiration depends upon and functions through the normal respiratory mechanism of the cells.

The present communication is designed to furnish evidence that this suggestion is to some extent tenable. Techniques involved in respiration studies on embryos as well as the characteristics of the embryos themselves have been described previously.[5, 6]

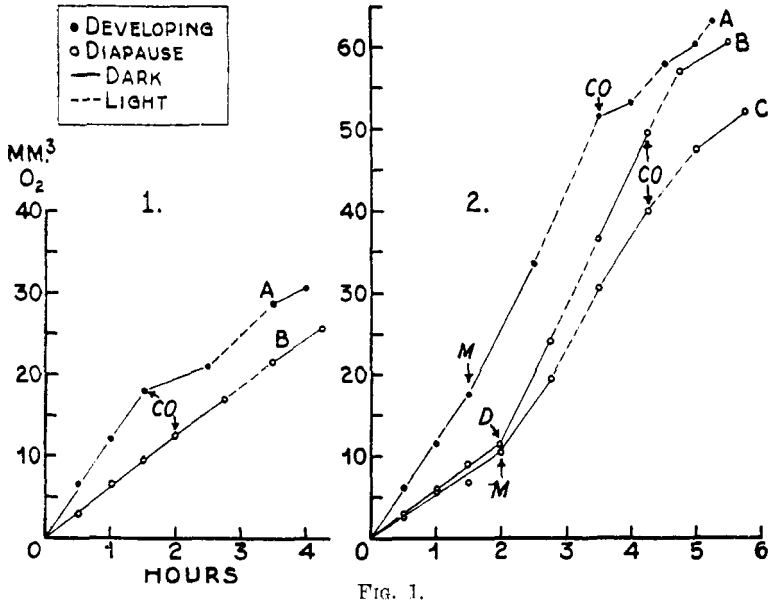

FIG. 1.

Comparison of the effect of CO on respiration of developing (A) and diapause (B) grasshopper embryos in dark and light. $CO/O_2 = 95/5$ added at arrows. Ordinate—O_2 uptake per 100 embryos; abscissa—time in hours.

FIG. 2.

Showing methylene blue stimulation of respiration and the reversible effect of CO in dark and light on developing (A) and diapause (C) embryos. Curve B shows similar effects of CO on stimulation of respiration by DNP. Concentration of methylene blue 12.6×10^{-5} molar, $CO/O_2 = 95/5$. Methylene blue added at arrow with M; DNP, at arrow with D. Ordinate and abscissa same as in Figure 1.

The essential features of the present work are depicted in the typical graphs shown in Figs. 1 and 2. The differential susceptibility of diapause and developing embryos to CO is shown graphically in Fig. 1. In developing embryos the CO inhibition is considerably diminished by light (450 W. tungsten filament lamp through glass-sided bath). Respiration in CO in the dark is 25% and in the light 75% of the normal. Curve A, Fig. 2, shows that the

[6] Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936, **8**, 357.

oxygen uptake of developing embryos in the presence of methylene blue is slightly greater in the light than in the dark. This increase is observed also in the case of diapause embryos (curve C) and may be attributed to the photodynamic action of methylene blue.[7] With dinitrophenol no such effect is noted. It is apparent from the curves of Fig. 2 that CO inhibits the methylene blue-stimulated respiration in diapause and developing embryos as well as the normal oxygen uptake of developing embryos (Fig. 1). Moreover, the inhibition is made reversible by light.

It has been pointed out by DeMeio and Barron[8] and confirmed by Krahl and Clowes,[9] and by Bodine and Boell[10] that DNP stimulates respiration by functioning through the normal oxidase-dehydrase systems of the cell. Thus any interference with the activity of the oxidase involved (as for example by KCN or CO) would restrict to a considerable extent the increased oxygen uptake induced by DNP. In grasshopper embryos methylene blue stimulation and DNP stimulation both appear to be similarly limited by CO. This fact, then, suggests that they both function through, although not necessarily in the same way, a CO sensitive mechanism whose affinity for CO is greatly reduced in the light.

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Analysis of the Acid-soluble Phosphates of Muscle Following the Injection of Glucose Plus Insulin.

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The following observations have been made in regard to changes in inorganic phosphate of plasma.[1] 1. Injection of insulin, while causing a lowering of plasma phosphate in normal rabbits, had little or no effect on plasma phosphate in adrenalectomized rabbits. 2. Injection of epinephrine was equally effective in normal and adrena-

7 Blum, H. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1935, **3**, 318.

8 DeMeio, R. H., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 36.

9 Krahl, M. E., and Clowes, G. H. A., *J. Biol. Chem.*, 1935, **111**, 355.

10 Bodine, J. H., and Boell, E. J., PROC. SOC. EXP. BIOL. AND MED., 1936 (in press).

1 Cori, C. F., and Cori, G. T., *Arch. Exp. Path. Pharm.*, 1933, **172**, 249.