9031 C

Effect of Dinitrophenol and Dinitrocresol on Oxygen Consumption of Diapause and Developing Embryos.*

J. H. BODINE AND E. J. BOELL.

From the Zoological Laboratory, State University of Iowa.

The stimulating action of the dinitrophenols on the oxygen consumption of cells and tissues is generally thought to reside in an acceleration of metabolic processes involving primarily the oxidation of carbohydrate. Dodds and Greville[1] showed that the extra oxygen uptake induced by 4,6-dinitro-o-cresol (DNC) in kidney slices had a R.Q. of 1. Ehrenfest and Ronzoni[2] demonstrated an increased oxidation of carbohydrate in yeast treated with dinitrophenol (DNP). Later[3] they demonstrated that DNP was without effect in iodoacetate poisoned muscle. Clowes and Krahl[4] showed that the R.Q. of sea urchin eggs in the presence of DNC remained at the level characteristic of untreated eggs, *viz.*, 0.93 to 0.95. Field and Tainter[5] present data to show that the stimulation of respiration in yeast by DNC is maximal in the presence of glucose.

In view of the fact that most of the work dealing with the effects of dinitrophenols has been done on biological systems whose normal fuel is in the main carbohydrate, it seems advisable to investigate the effects of DNP and DNC† on the oxidative metabolism of the grasshopper embryo *(Melanoplus differentialis)* where metabolism is predominantly at the fat level.[6] This paper will present preliminary facts relating to the stimulating effects of DNP and DNC on respiration together with a description of the influence of carbon monoxide and cyanide on the increased oxygen uptake.

The method of preparing the embryos for experiments has been described previously.[7] O_2 uptake was measured in the Barcroft-

* Aided by a grant from the Rockefeller Foundation for Research in Cellular Physiology.

1 Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966.

2 Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

3 Ronzoni, E., and Ehrenfest, E., *J. Biol. Chem.*, 1936, **115**, 749.

4 Clowes, G. H. A., and Krahl, M. E., *Publ. Eli Lilly Research Lab.*, 1934.

5 Field, J., 2nd, and Tainter, E. G., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 168.

† The compounds used were 2,4-dinitrophenol and 3,5 dinitro-o-cresol (Eastman Kodak Company).

6 Boell, E. J., *J. Cell and Comp. Physiol.*, 1935, **6**, 369; Bodine, J. H., and Boell, E. J., unpublished data, 1936.

7 Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936, **8**, 357.

Warburg apparatus for a period of from 1 to 3 hours in order to obtain a normal rate of oxygen consumption. DNP was then added to the manometer contents from the side bulb and the effect of the addition noted for a second period of from 1 to 3 hours. In the experiments involving the use of cyanide the technique of van Heyningen[8] was used.

The comparative stimulating effects of various concentrations of DNP on the oxygen uptake of diapause (blocked) and developing embryos is indicated in Table I. The QO_2 values represented

TABLE I.

Oxygen Uptake of Diapause and Developing Embryos in Presence of Dinitrophenol. The embryos were suspended in a medium consisting of 0.9% NaCl, 0.02% KCl, 0.02% $CaCl_2$, M/100 phosphate buffer to give pH 6.2, and dinitrophenol to make concentration indicated.

Qo_2 = mm.^{3} O_2/hour/mg. (dry weight) of embryo.

Molar conc. DNP × 10^{-5}	Diapause Qo_2	%	Developing Qo_2	%
0 (control)	.75	100	1.55	100
.125	.90	120	1.71	110
.25	1.46	195	2.23	144
.5	1.88	250	3.43	221
1.	1.93	258	3.67	237
2.5	2.55	340	4.28	276
3.	2.56	342	—	—
5.	2.16	288	3.87	250
25.	.85	113	—	—

for each type of embryo are averages of from 5 to 25 experiments in each concentration group. Maximum stimulation both in diapause and developing embryos is achieved with a concentration of DNP of 2.5×10^{-5} molar. Although the percentage increase in respiration is higher in diapause than in developing embryos, the absolute value of oxygen consumption is greater in embryos of the latter type since normally they respire at a rate 2 to 3 times that of blocked embryos.

DNC is more than twice as active physiologically as DNP for the concentration of this substance which produces maximal stimu-

TABLE II.

Effect of Carbon Monoxide on Stimulation of Respiration Produced by Dinitrophenol.

Concentration DNP = 2.5×10^{-5} molar; CO/O_2 = 95/5.

	Diapause embryos Qo_2	%	Developing embryos Qo_2	%
Control	.73	100	1.48	100
CO	.70	96	.55	37
DNP	2.01	276	3.39	229
DNP + CO	1.00	137	.42	28

[8] Heyningen, W. E. van, *Bioch. J.*, 1935, **29**, 2036.

lation is $1x10^{-5}$ molar. With both DNP and DNC the increased rate of respiration is maintained for several hours without undergoing appreciable change.

Table II shows that the stimulating effect of DNP is completely restricted by carbon monoxide in the case of developing embryos. With diapause embryos CO normally has only a slightly depressing effect; moreover, it does not suppress completely the extra oxygen uptake induced by DNP (compare DeMeio and Barron[9]).

Cyanide (Table III) when added to embryos, either before, after or simultaneously with the DNP, can reduce stimulation, prevent it entirely or depress respiration below the normal level. With developing embryos there seems to be no antagonism between DNP and the stronger concentrations of cyanide.

TABLE III
Stimulation of Respiration by Dinitrophenol in Presence of Cyanide.
Concentration of DNP = 2.5 × 10–5 molar.

Molar KCN × 10–5	Q_{O_2} Diapause embryos				Q_{O_2} Developing embryos			
	KCN	%	KCN + DNP	%	KCN	%	KCN + DNP	%
0 (Control)	.66	100	2.35	356	1.47	100	3.02	206
5	.70	106	1.56	236	1.46	99	2.62	178
50	.56	85	1.16	176	1.05	71	.96	65
100	.29	44	.60	91	.48	33	.55	37

A slight antagonism between DNP and KCN seems to be apparent with diapause embryos.

That the stimulation of respiration in both types of embryos by DNP or DNC involves the functioning of the normal oxidative mechanism of the cell is suggested by the sensitivity of the stimulation to carbon monoxide and cyanide, and also, by the fact that the temperature coefficients of the normal and DNP stimulated respiration are approximately the same. The data suggest, moreover, that the dinitrophenols can increase respiration in biological systems where carbohydrate metabolism does not predominate.

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