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Observations on Cellular Oxidative Mechanisms Involved in Dinitrophenol Stimulation of Respiration.

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In an extensive investigation of the effect of dinitro compounds on tissue respiration and cell division, a point has been reached where it appears desirable to know more about the mechanism by which such compounds stimulate oxidative processes in the cell. We report here some experiments with 4,6 dinitro-o-cresol (DNC)* which indicate that dinitro compounds do not act on cell respiration either in the same way as methylene blue and other dyes which are reduced by the cell and re-oxidized by molecular oxygen,[1] or in the same way as dimethyl-p-phenylene diamine, which is reduced by the cell and reoxidized by the indophenol oxidase.[2]

When the oxygen consumption of sea urchin eggs is raised by DNC to 400% of the normal,[3] the R.Q. remains unchanged at the normal value of about 0.93. We have found that DNC is not an autoxidizable catalyst for the oxidation of glucose or cysteine, even in the presence of traces of metals or cytolyzed animal tissue. We have also found with rat tissues and eggs of invertebrate marine animals that the action of DNC as a respiratory stimulant can be completely and reversibly blocked by cyanide. Field, Martin and Field report similar results with cyanide on yeast.[4]

These facts indicate that DNC acts upon one or more of the cyanide sensitive oxidative chains in the cell. These may be roughly divided into 2 classes: (A) those depending for oxygen activation on Keilin's cyanide sensitive indophenol oxidase, and (B) those in which the substrate is activated by an autoxidizable cyanide sensitive dehydrase.[5] The mechanisms classifiable under (B) do not act through cytochrome, hence a distinction between the 2 classes can be made by studying the effect of DNC on the rate of oxidation or reduction of cytochrome in respiring cells.

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* This compound is 1-methyl 2-hydroxy 3,5 dinitro benzene.

[1] Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

[2] Keilin, D., *Proc. Roy. Soc.* (London), B, 1929, **104**, 206; Runnström, J., *Protoplasma*, 1932, **15**, 532.

[3] Krahl, M. E., and Clowes, G. H. A., *Biol. Bull.*, 1934, **67**, 332.

[4] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 997.

[5] Dixon, M., *Biol. Rev.*, 1929, **4**, 352.

A preliminary rough experiment, made August 20th, 1934, on a 25% suspension of normal yeast showed that the time of reduction of cytochrome was greatly accelerated by DNC, the time required, after aeration, for reappearance of the d band being reduced from 27 seconds to 5 seconds. After repeated confirmation of these results experiments were conducted on starved yeast with DNC in the presence of a variety of substrates.

Typical results of such experiments on starved yeast are presented in Tables I and II. The procedure in each case was as follows: A 3 cc. portion of a well aerated 17% suspension of Fleischmann's yeast in pH 6 McIlvaine phosphate-citrate buffer was mixed with 1 cc. portions of the designated substrate, DNC, and buffer solutions to give a constant volume of 5 cc. The final concentrations were: Yeast, 10%; substrate, 0.2%; DNC, 5×10^{-6}M for the experiments in Table I, and 6.25×10^{-5}M for the experiments in Table II. After 10 minutes reaeration, the effect was registered by noting the number of seconds which elapsed prior to the reappearance of the strong d band. Since the degree of acceleration effected by the DNC varies according to the length of time that the suspension has been allowed to stand, controls with yeast and buffer alone and yeast and DNC and buffer were run at frequent intervals in the course of the experiments.

TABLE I.

	Cysteine		Succinate		Glucose		Pyruvate		Lactate	
	None	0.2%	None	0.2%	None	0.2%	None	0.2%	None	0.2%
No DNC	292	272	285	243	223	63	164	65	106	93
5×10^{-6}M DNC	66	58	62	53	63	27	61	56	45	49

TABLE II.

	Cysteine		Succinate		Glucose		Pyruvate		Lactate	
	None	0.2%	None	0.2%	None	0.2%	None	0.2%	None	0.2%
No DNC	422	303	346	297	254	89	194	104	118	96
6.25×10^{-5}M DNC	90	85	89	100	91	50	88	94	86	83

The data show (a) that DNC greatly accelerates the reduction of cytochrome, and (b) that only with glucose is the reducing ability of the combination equal to the sum of those of the individual agents. Indeed, the pyruvate plus DNC and lactate plus DNC are no more efficient than DNC alone, although pyruvate and lactate both have a large reducing effect when used singly. It may be noted that this inability of lactate to increase the reduction of cytochrome in the

presence of DNC may account for the aerobic glycolysis which dinitro compounds produce in tissues.[6]

In subsequent experiments with iodoacetate poisoned yeast, carried out in the manner described above with M/1500 iodoacetate in the pH 6 buffer, it was found that DNC gave no acceleration of cytochrome reduction even when lactate or glucose was present. Since others[7] have observed that iodoacetate blocks the acceleration by 2,4 dinitro phenol of respiration in yeast, it is likely that some sulfhydryl containing enzyme system is essential for the DNC action.[8]

We have also found that such non-specific dehydrase poisons as sodium pyrophosphate and narcotics inhibit to a limited degree the reactivity of tissues to DNC stimulation.

From the evidence available we believe it likely that DNC stimulates cellular respiration by accelerating the oxidation by cytochrome of some substrate previously or simultaneously acted upon by the anaerobic dehydrases of the cell. It is too early to say whether DNC acts as a diffusible oxygen carrier between cytochrome and the substances normally oxidized or as an artificial substitute for a co-enzyme in the activation of substrates which do not normally play an important rôle in respiration. We are continuing these studies with cell-free cytochrome and individual dehydrases in an effort to demonstrate the relation between the individual components of such a system, eliminating at the same time the complicating factors connected with variations in the permeability of the cell.

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Studies on Acholic Cachexia: IV. Relation of Biliary Diversion to Duodenal Ulcer Formation.*

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The occurrence of peptic ulcer of the duodenum after the exclusion of bile has been noted with a wide variation in frequency and the factors concerned are not clearly understood. Kapsinow and

[6] Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966; Dodds, E. C., and Greville, G. D., *Lancet*, 1934, **1**, 398.

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

[8] Dickens, F., *Biochem. J.*, 1933, **29**, 1141; Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

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