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Action of 1-2-4 Dinitrophenol on Yeast Respiration and Fermentation.*

J. FIELD 2ND, A. W. MARTIN AND S. M. FIELD.

From the Department of Physiology, Stanford University.

Cutting and Tainter,[1] Magne, Mayer and Plantefol,[2] Hall, Field, Sahyun, Cutting and Tainter[3] and others have shown that 1-2-4 dinitrophenol (hereinafter called αDNP) increases the rate of oxygen consumption of animals by direct action on the tissue cells. Similar action on some plants has been demonstrated by Plantefol.[4] However, Genevois and Saric,[5] during the course of our experiments, reported that αDNP failed to increase yeast respiration, in fact that a concentration of 100 mg. per liter decreased respiration and the percentage of viable cells by 90%. These results will agree with ours as to respiration if the pH was below 6.0.

We have studied the action of αDNP on the respiratory and fermentative processes of pure cultures of *Saccharomyces cerevisiae,* race F, over a considerable concentration range and at various acidities, using Warburg[6] respirometers at 25°C. The respiration studies were carried out in an atmosphere of pure oxygen, those on fermentation (method of Negelein[7]) in 95% N_2 and 5% CO_2.

αDNP was used in the form of the sodium salt, which in no case shifted the pH from the level set by the buffers (glass electrode pH determinations were kindly made by Dr. F. DeEds). Nor did the pH level change during the course of the run.

αDNP is an acid with a pK of 4.02 at 25°C. The ions are yellow, the undissociated acid is colorless.[8] Our experiments indicate

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that only the undissociated form is biologically active, as the following data show.

RESPIRATION

pH	Total conc. DNP in mg. per l.	Conc. undissociated form in mg. per l.	Effect
5.2	400	25.08	Inhibition
5.9	10	0.13	Stimulation
	50	0.65	" (optimal)
	100	1.30	Inhibition
	400	5.20	"
6.8	Below 50	Below 0.08	No effect
	100	0.16	Stimulation
	400	0.64	" "
	800	1.28	Inhibition
8.03	400	Negligible	No effect

In all cases proper controls were run.

Similar findings were obtained in the studies on fermentation. Within certain limits the action is reversible. At pH 5.9, a total concentration of 400 mg. per liter inhibits strongly. If after one hour under these conditions enough alkali is added from the side-arm of the Warburg vessel to bring to pH 6.8, thus decreasing the concentration of the undissociated form, strong stimulation results. This holds for both respiration and fermentation.

The effect is independent of the presence of glucose, and the onset occurs within 5 minutes of addition of the αDNP.

Depression of respiration at pH 6.8 is not accompanied by death of cells (staining with Methylene-blue after Fink and Kühles[9] and plate counts).

The stimulation range of the undissociated form for yeast is near the same order of magnitude as that found for animals.[2, 10]

The contradictory results of other investigators may be ascribed to lack of appreciation of the activity of the undissociated form, and consequent use of unsuitable hydrogen ion concentrations. It may be that the biological inactivity of the ionic αDNP is associated with permeability relations. Experiments are now in progress on this and other points.

[9] Fink, H., and Kühles, R., *Z. physiol. Chem.*, 1933, **218**, 65.

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