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Effect of Repeated Washing on Stimulation of Yeast Respiration by 2-4 Dinitrophenol.*

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The stimulation of yeast respiration by 2-4 dinitrophenol (DNP) observed by Plantefol,[1] Field, Martin and Field,[2, 3] and others has been ascribed by Genevois and Creac'h[4] to the presence of extracellular catalysts carried over from the growth medium. The latter authors attribute the failure of Genevois and Saric[5] to note such stimulation to the fact that their yeast was always carefully washed before respiration was measured.

This suggestion seemed improbable to us, since it has been routine practice in our laboratory to wash yeast one or more times by centrifugation before measurement of respiration.[3] However, we have repeated the determination of the optimal concentration of DNP at pH 6.8, washing up to 20 times by centrifugation at 3000 r.p.m. for 5 minute periods either in distilled water or in 0.1 M phosphate buffer pH 6.8 before each experiment. The yeast was carefully resuspended between each washing. Two strains of yeast were used, our own pure culture of *Saccharomyces cerevisiae*,[2, 3] and a pure culture isolated from French baker's yeast supplied by Genevois, whose kindness we wish to acknowledge. The further experimental procedure has been described elsewhere.[3] Our results are presented in Table I.

It is clearly shown in the table that stimulation of yeast respiration by DNP is not abolished by washing either in distilled water or in phosphate buffer, once or 20 times. While the absolute values of the respiratory rate are low after 10 washings in distilled water, viability determinations by the method of Fink and Kühles[6] showed

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1 Plantefol, L., *Compt. Rend. Soc. de Biol.*, 1933, **113**, 147.

2 Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

3 Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

4 Genevois, L., and Creac'h, P., *Compt. Rend. Soc. de Biol.*, in press.

5 Genevois, L., and Saric, R., *Compt. Rend. Soc. de Biol.*, 1932, **111**, 181.

6 Fink, H., and Kühles, R., *Z. physiol. Chem.*, 1933, **218**, 65.

TABLE I.
The Effect of Washing on the Stimulation of Yeast Respiration by DNP.
Each series, 6 experiments, 24 readings.

Concentration sodium dinitrophenoxide (mg.%)	0	30	40	50
Concentration undissociated DNP, Millimols × 10^{-3}	0	2.22	2.96	3.70
Part 1. *Saccharomyces cerevisiae*				
Yeast washed 10 times in dist. water.				
†Suspension medium, glucose phosphate.				
Rate of oxygen consumption (cont. = 100)	100	171	194	150
Yeast washed 20 times in phosphate‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	103	122	81
Part 2. French baker's yeast (Genevois).				
Yeast washed once in phosphate.‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	161	165	156
Yeast washed 20 times in phosphate.‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	155	161	159
Yeast washed once in distilled water.				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	108	138	116
Yeast washed 10 times in dist. water.				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	118	143	127

*Suspension medium refers to the medium in which the yeast was suspended during the measurement of respiration in the Warburg apparatus. In every case this was 1% glucose, 0.1 M phosphate buffer, pH 6.8.

‡Phosphate = 0.1 M phosphate buffer, pH 6.8.

that this is not due to decrease in the number of living cells, and the values of percentage stimulation are in line with the rest. Accordingly we conclude that DNP acts directly on systems within the yeast cell, and that extracellular catalysts are not essential for such action. This is in accord with the view of Plantefol[7] and our own earlier opinion.[3]

7 Plantefol, L., *Compt. Rend. Soc. de Biol.*, 1934, **117**, 1167.