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Effect of Respiratory Stimulating Factors on Endogenous Respiration of Yeast.

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Previous papers from this laboratory have reported the preparation from yeast[1, 2, 3] and malt-combings,[4] of factors which stimulate the respiration of yeast. In the above studies the stimulating substances were tested by the Warburg manometric technique using Ringer-phosphate-glucose as the suspending medium for the yeast. Under these conditions the respiratory stimulating factor (R.S.F.) could act by increasing the utilization of exogenous glucose. Hence, it seemed desirable to determine the activity of the R.S.F. on yeast suspended in sugar-free media. It has been shown by Stier and Stannard[5] that in the absence of exogenous glucose the oxygen uptake of a yeast suspension represents true respiration uncomplicated by fermentation and is properly called endogenous respiration rather than autofermentation. Under these conditions the carbohydrate reserves (glycogen) of the cell are utilized.

In the present experiments Fleischmann's bakers' yeast *(Saccharomyces cerevisiæ)* from the center of a cake was suspended in Ringer-phosphate and Ringer-phosphate-glucose (0.02% glucose) at a pH of 7.3. Potassium phosphate at the same pH was also used as a suspending medium with similar results, but lowering the pH to 4.3 somewhat decreased the respiration. A preliminary centrifugal washing with the suspending medium was carried out in some experiments but seemed to be unnecessary. A final yeast count of 250 (count x 250,000 = cells/cc.), determined with Beck's photodensitometer,[6] was used in all experiments. At a temperature of 37.5°C. and after a 15-minute equilibrium period in air, the oxygen

1 Norris, R. J., and Ruddy, Sr. M. Veronita, *Studies Inst. Divi Thomae*, 1937, **1**, 53.

2 Norris, R. J., and Hart, Sr. M. Jane, *Ibid.*, 1937, **1**, 65.

3 Cook, E. S., and Kreke, C. W., to be published.

4 Norris, R. J., and Kreke, C. W., *Studies Inst. Divi Thomae*, 1937, **1**, 137.

5 Stier, T. J. B., and Stannard, J. N., *J. Gen. Physiol.*, 1936, **19**, 461, 479; *J. Cell. and Comp. Physiol.*, 1937, **10**, 79.

6 Beck, W. A., *Science*, 1937, **85**, 2206.

uptake was measured for a 25-minute period. As is well known,[7] the oxygen uptake in the presence of glucose is much higher than in glucose-free media, and we found that an increase in the glucose concentration above 0.02% caused too great an oxygen consumption to be read under the conditions of the experiments.

R.S.F. preparations from yeast[3] and malt-combings[4] were added in concentrations of 0.5 mg. to 1.0 mg. per cc. The results of 36 determinations indicated that the respiration was increased both in the presence and absence of glucose and to the same extent in both cases. In these experiments the maximum variation was 18% and the average variation 6%, which is within experimental error. Data from a typical experiment are given in Table I. Stimulation of 150% to 250% has been obtained with yeast of varying age and with varying R.S.F. preparations. Similar results were obtained when a pure 48-hour culture of yeast was used but the degree of stimulation was lower. This was not wholly unexpected and is receiving further investigation.

TABLE I.
Respiration Stimulation by 1 mg./cc. of R.S.F. Sample 15.

	Ringer	Ringer+R.S.F.	Ringer+Glucose	Ringer+Glucose+R.S.F.
Cmm. O_2 absorbed	79.1	149.26	111.65	207.41
% stimulation		85.03		85.7

The R.S.F. preparations were also examined in Ringer-phosphate solutions with and without glucose but in the absence of yeast. No oxygen uptake was observed even when the respiration period was extended to 2 hours. Furthermore, no oxygen consumption occurred when R.S.F. preparations were added to suspensions of yeast killed by autoclaving. Thus, it is seen that the R.S.F. preparations do not, of themselves, "respire", nor are they capable of oxidizing glucose in the absence of the living cell.

Summary. Respiratory stimulating factors, obtained from yeast and malt-combings, stimulate the respiration of yeast to the same degree in the absence of exogenous glucose as in its presence. These factors under the same conditions but in the absence of living yeast, cause no oxygen uptake. Hence, the factors stimulate the true respiration of yeast. These studies are being extended.

[7] *Cf.* Warburg, O., *Biochem. Z.*, 1927, **189**, 354; Meyerhof, O., and Iwasaki, K., *Ibid.*, 1930, **226**, 16.