increasing the acidity of the milk shortened the coagulation time for both preparations, or rendering the milk alkaline prevented clotting, with the amount of extract and rennin regularly employed; adding ammonium oxalate, with resulting precipitation of the calcium together with a slight increase in pH, had an unfavorable effect for both products; and finally, a boiling temperature for 5 minutes rendered each entirely incapable of causing coagulation.

The effect of environmental conditions on the ability of growing organisms to produce clotting was studied. In each case in which an alteration in the milk was made, a decided effect was exerted on the *M. albicans'* ability or time to produce coagulation. Moreover, if the change was such that either rennin or the Monilia extract would be inhibited in its clotting action, then the live inoculum would likewise be affected. Similarly, changes more favorable for rennin action also rendered the milk more coagulable by growing organisms.

pH determinations, made at daily intervals, showed little change in reaction with the *M. albicans* cultures at the time of coagulation. However, at this time the *M. candida* displayed decided increase, a rise from pH 6.05 to 6.50 in 3.5 days, and a pH 7.4 in 8 days. The combination of reaction change together with *candida's* marked deficiency of the coagulating enzyme is considered responsible for its failure to exhibit coagulation. With the *M. albicans* a pH rise begins to occur after coagulation doubtless due to deaminization of protein material. However, even at the tenth day pronounced pH differences still obtain between the two species. That there is a definite biological difference between *Monilia albicans* and *Monilia candida* is shown on the basis of milk coagulation.

8897 P

Effect of 4-6 Dinitro-O-Cresol on Oxidation of d'- and l'-Arabinose by Previously Starved Yeast.*

J. FIELD, 2ND AND E. G. TAINTER.

From the Department of Physiology, Stanford University.

It has been shown in previous papers from this laboratory that the respiratory rate of yeast suspended in non-nutrient phosphate

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine and in part by Grant 358 of the Committee on Scientific Research of the American Medical Association.

buffer solutions or in glucose phosphate can be markedly increased on treatment with proper concentrations of 2-4 dinitrophenol (DNP), 4-6 dinitro-o-cresol (DOC) and some related compounds.[1-5] We report herewith the results of experiments in which it is shown that the effects of such metabolic stimulants upon the respiration of yeast is profoundly affected by the type of "exogenous"[6] carbohydrate fuel available.

A pure culture of *Saccharomyces cerevisiae* and pure culture methods were used throughout. The experimental procedures were the same as described previously[2, 8] except that yeast was first grown 48 hours on Orla-Jensen agar medium, then taken up in 0.2M phosphate buffer, pH 6.8, and starved 24 hours under aerobic conditions in Fraser tubes[7] at 25° ± 0.02°C. before the beginning of each ex-

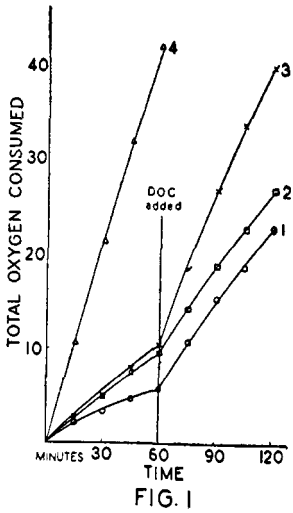

FIG. 1

Graph showing total amount of oxygen consumed in cmm. at N.P.T. per 10^8 cells as a function of time. Yeast suspended in 0.1M phosphate buffer, pH 6.8 (1), and in the same buffer with *d'*-arabinose (2), *l'*-arabinose (3) and glucose (4).

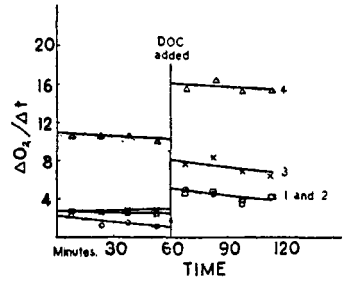

FIG. 2.

Graph showing oxygen consumption in cmm. at N.P.T. per 10^8 cells as a function of time. Numbers of curves have same significance as in Fig. 1. Values of $\Delta O_2/\Delta t$ are for 15-minute intervals.

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

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

3 Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Pharm. and Exp. Therap.*, 1935, **53**, 314.

4 Field, J., 2nd, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1342.

5 Field, J., 2nd, and Martin, A. W., *Compt. rend. Soc. de Biol.*, 1935, **119**, 458.

6 Stier, T. J. B., and Stannard, J. N., *J. Gen. Physiol.*, 1936, **19**, 461.

7 Fraser, C. G., *J. Physical Chem.*, 1921, **25**, 1.

periment. Respiration was measured in the conventional form of Warburg apparatus at 25° ± 0.02°C. Each set of quantitative comparisons was made on yeast from a single subculture. The observations reported represent typical findings in a series of 8 experiments involving 7 Warburg vessels each.

Fig. 1 illustrates integral curves showing the total amount of oxygen consumed in cmm. at N.P.T. as a function of time when previously starved yeast is suspended in 0.1M phosphate buffer, pH 6.8, or in the same buffer containing 1% glucose (for comparison), 1% d'-arabinose (levo-rotatory) or 1% l'-arabinose (dextro-rotatory).

In this experiment DOC was added from the sidearms of the Warburg vessels after a 60-minute control period. The strength of the DOC was such as to furnish a concentration in the Warburg vessel of 5.05×10^{-4}M (giving 7.96×10^{-6}M free acid at pH 6.8), which was found optimal in a series of preliminary experiments. After addition of DOC, the slope of the integral curves in the presence of glucose (not shown in Fig. 1, but can be inferred from Fig. 2) and of l'-arabinose is greater, in the presence of d'-arabinose the same, as in the non-nutrient control. Since the integral curves for the two pentoses practically coincide before addition of DOC and draw apart afterward (Fig. 1), this finding constitutes an interesting case of stereoisomeric preference on the part of the yeast cell.

Fig. 2 illustrates differential curves showing oxygen consumption as a function of time in the same experiment. It is shown that the increase in $\Delta O_2/\Delta t$ consequent upon addition of DOC in optimal concentration is definitely greater in glucose, slightly greater in l'-arabinose and definitely less in d'-arabinose than in the non-nutrient control. These results suggest that under the conditions of these experiments there is a respiratory "ceiling" for any given fuel. The action of metabolic stimulants, which increases $\Delta O_2/\Delta t$ is limited by the position of this ceiling as well as by the properties of the stimulant.[8] Further work on this point is in progress.

[8] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 388.