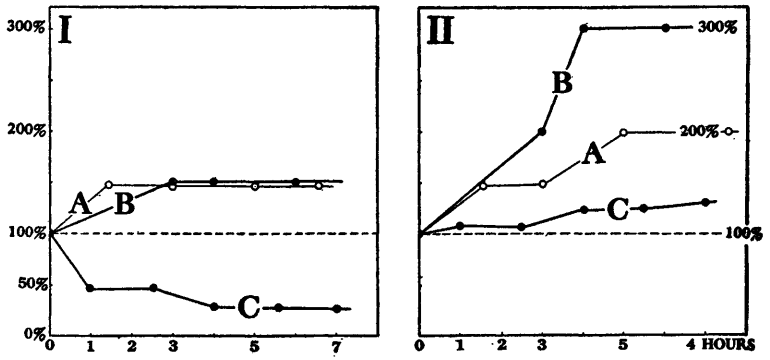

FIG. 3. *Effect of Refrigeration on Dilute Streptococcus Filtrate.*

Filtrates A and B were diluted with 4-volumes and filtrate C with 14-volumes of 0.8% NaCl-solution.

I. Change in lytic titer in dilute filtrates A, B and C as a result of storage at 37°C.

II. Parallel changes in titer of control samples stored at 4°C.

No theory is as yet suggested as to the probable mechanism of these unprecedented increases in lytic titer.

Whether or not similar augmentations of lytic activity take place in the animal body (*e. g.*, as a result of the administration of subtherapeutic doses of antistreptococcus serum), is a problem of practical clinical interest.

7956 C

Effects of 2-4 Dinitrophenol on Respiration of Commercial Cake Yeast.*

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De Meio and Barron[1] state that neither their findings nor those of Ehrenfest and Ronzoni[2] support "Field, Martin and Field's[3, 4]

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

1 De Meio, R. H., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 36.

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

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

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

contention that dinitrophenol is active toward yeast only in its undissociated form." Bearing in mind the fact that our "contention" relates only to studies on a pure culture of yeast, let us see to what extent the published data cited support the statement of De Meio and Barron.

Determination of the optimal concentration of 2-4 dinitrophenol (hereinafter DNP) at more than one pH level is essential for the decision of the point at issue. Ehrenfest and Ronzoni reported an optimal concentration of 0.36 mg. % DNP at pH 4.5 for their yeast. De Meio and Barron also reported work on yeast at one pH level only, viz: 6.64, and failed to state whether or not their dosage was optimal. Hence the published data cited afford no basis for the statement of De Meio and Barron, in fact the concentration of undissociated DNP corresponding to the optimum found by Ehrenfest and Ronzoni (assuming use of the sodium salt) is 0.00399 millimolar, which agrees very well with the values reported in the present paper for commercial yeast at other pH levels.

Moreover, De Meio and Barron used commercial yeast suspensions, which always contain several strains of yeast as well as numerous bacilli and cocci. Van Niel and Visser 'T Hooft[5] have pointed out that pure cultures are essential for reliable work in microbiology. Conclusions drawn from experiments on heterogeneous populations cannot safely be regarded as generalizations, although it may chance that a conclusion drawn from work on pure culture will also apply in the presence of other species. The experiments reported below are a case in point, and show conclusively that undissociated DNP is the active agent in the stimulation or inhibition of yeast respiration in commercial yeast suspensions as well as in suspensions of pure culture.[3, 4]

The experimental procedure was the same as in our previous work,[4] except that commercial cake yeast was used instead of pure culture. A small piece of yeast cake was fragmented, washed by centrifugation in glucose-phosphate, and finally suspended in glucose phosphate solution at the desired pH. Heavy bacterial contamination was always present in spite of repeated washing.

The most important link in the chain of evidence showing that undissociated DNP is the active agent in the stimulation of yeast respiration is the finding that in doses evoking optimal stimulation the concentration of the undissociated form is quite constant over a wide pH range, while the total concentration causing optimal stim-

[5] Van Niel, C. B., and Visser 'T Hooft, F., *Ber der Deutsch. Chem. Gesell.*, 1925, **58**, 1606.

ulation is a function of pH. A series of 14 experiments showed that this is true for contaminated commercial yeast suspensions within quite narrow limits. At pH 6.8 the total optimal concentration of sodium dinitrophenoxide was 60 mg. %, while at pH 5.2 it was 2.5 mg. %, a 24-fold difference. The corresponding concentrations of undissociated DNP are 0.00444 and 0.00692 millimolar respectively, which is good agreement in view of the unsatisfactory nature of the experimental material.

If undissociated DNP is the active agent, a given total concentration of the drug should cause stimulation at one pH level, inhibition at another, depending on the concentration of the undissociated form. It is shown in Table I that this is the case.

TABLE I.

Effect of change in pH on action of a given dose of DNP on respiration of commercial yeast. Yeast suspended in glucose phosphate of stated pH.

pH	Concentration of DNP mg. %	Concentration of undissociated DNP millimols X10^{-3}	Oxygen consumption cmm. per 10^8 yeast cells per hour
6.8	0	0.	173.2
6.8	40	2.96	242.5
6.0	0	0.	155.1
6.0	40	18.5	59.3

Finally, we have determined the effect of change in pH of the medium during a run on the physiological action of DNP. It is shown in Fig. 1 that a given total concentration which causes stimulation at pH 6.8 evokes marked inhibition when enough acid is added from the sidearm of the Warburg vessel to reduce the pH to 5.4. Here the same cells and the same concentration of DNP are

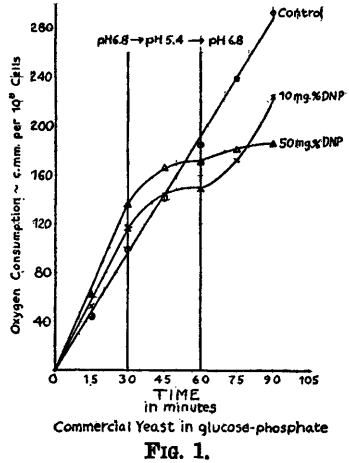

FIG. 1.

present throughout, but the increase in the concentration of the undissociated form consequent upon the decreased pH brings about prompt inhibition of respiration. Partial recovery occurs on restoration of the original pH level in the lower DNP concentration range. The control was not affected appreciably by the pH shift.

We have now shown on commercial yeast that a given total concentration of DNP will produce stimulation or inhibition of respiration depending upon the pH level, while the concentration of undissociated DNP is quite constant when optimal stimulation is evoked, and that change in acidity during a run will modify or reverse the effect of DNP on yeast respiration in a manner most directly explicable by changes in the concentration of undissociated DNP. We therefore conclude that in commercial yeast suspensions as in suspensions of pure yeast culture,[4] DNP stimulates or inhibits yeast respiration only in the undissociated form. We know of no evidence to the contrary.

7957 P

Production of Superovulation in Normal Immature Rats by Injection of the Principle in Menopause Urine.

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The study of the effects of urine collected from a considerable number of normal women in the menopause and after castration has led us to regard the presence of lutein tissue in the ovaries of normal test rats as a common finding. The 24-hour specimens from such women were precipitated by alcohol, extracted with water and centrifuged. One-third of the powder thus obtained from each case was injected over a period of 3 days into a group of three 24 to 25 day old rats. (The remaining two-thirds was combined with synergist or pregnancy prolan in further tests for the active components of menopause urine, as will be seen in the following communication.) Autopsy was performed 96 hours after onset of injection. The urine of some of the patients was examined repeatedly; of the 20 women examined, the urine of 14 stimulated corpora on at least one occasion. Of a total of 88 tests, 20 showed corpus production.

We have, furthermore, been surprised to encounter ovarian weights of from 70 to 140 mg. in cases in which corpora lutea were