used in discerning the essential components of this mixture.

Stock cultures of the organism were transferred twice weekly on Difco AC agar slants and incubated at 25 C. Broth cultures consisted of 5 ml of medium in 15 by 125 mm rimless tubes, calibrated for use on a Coleman Junior spectrophotometer. Broths were incubated at 25 C on a reciprocal shaker operating at 180 strokes per minute. Growth was determined turbidimetrically at 540 mμ in the calibrated tubes.

A 24 hour broth culture of the organism grown in Difco AC medium (8.5 mg per ml) provided inoculum. The cells were separated from the broth by centrifugation and washed three times with 3 to 5 ml of sterile saline. Finally a saline suspension was diluted to a turbidity of 0.1 optical density units.

The medium to be investigated was autoclaved for 10 minutes at 15 pounds in the calibrated tubes. One drop of inoculum was added to each tube by aseptic technique and the broths incubated 18 to 24 hours with shaking.

Table 1 outlines the composition of the media and some of the accessory factors tested. Although an acid hydrolyzate of casein (Difco casamino acids) generally was used as a source of amino acids, a proportional mixture of synthetic amino acids was equally active. The effect on growth of various combinations of accessory factors is shown in table 2. Guanine and thiamin were the most active, niacin was growth stimulatory, and adenine did not replace guanine. Earlier experiments indicated that no other common B vitamins influence growth under these conditions.

Although the basal medium plus thiamin, guanine, and niacin will not support growth of the original smooth colonies and results in limited growth of nonaerated rough cultures, it has repeatedly equalled Difco casitone as a medium for aerated cultures of the rough variant. Also, the half maximum inhibitory concentration of penicillin for the variant is approximately equal (0.001 to 0.003 IU penicillin per ml) in both media under these conditions. Thus, a chemically defined medium has been obtained for the study of penicillin inhibition of this sensitive organism.

A SIMPLE SCREENING TEST FOR GENETIC STUDIES OF RESPIRATION DEFICIENCY IN YEAST[1]

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Respiration deficiency in yeast has been correlated with a number of experimental observations other than failure to take up oxygen in the Warburg respirometer with glucose as substrate. Absence of certain lines in the cytochrome spectrum, and of a number of enzymes in the aerobic pathway, has been studied intensively for a few strains by Ephrussi and Slonimski (Compt. rend., **230,** 685–686). In most screening tests for genetical analysis, small colony size and the negative Nadi reaction appear to have been employed.

Certain ambiguities or difficulties in these tests have impelled us to seek a more convenient procedure for screening the thousands of strains in the Carbondale pedigree for respiration deficiency. Some preliminary observations on the utilization of organic acids as principal carbon sources for growth on agar medium indicated that whereas aerobic yeasts grew well on lactate agar, strains which failed to form colonies on lactate agar were respiration deficient with lactate, acetate, or glucose as substrate. On the suggestion of Dr. J. W. Foster we adapted an alkali production test, such as is often applied for the utilization of formate, and established the correlation between respiration deficiency and inability to produce excess alkali during growth on a medium containing sodium acetate.

The medium employed consists of glucose, 0.2 g; KH_2PO_4, 0.05 g; peptone, 0.2 g; sodium

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acetate, 0.4 g; yeast extract (Anheuser-Busch no. 3), 0.01 ml; trace metal stock solution, 0.1 ml; 0.1 per cent phenol red, 3.0 ml; and water to 100 ml. The medium is adjusted to pH 6.5, tubed in 2 ml aliquots in 10 by 1 cm tubes, plugged and sterilized at 15 lb for 15 minutes. The trace metal stock contained per 100 ml $MnSO_4 \cdot 4H_2O$, 2.27 mg; $ZnSO_4 \cdot 7H_2O$, 0.883 mg; $CuSO_4 \cdot 5H_2O$, 0.253 mg; and $FeSO_4 \cdot 7H_2O$, 1.99 mg. Tubes are stored at 5 C until needed.

Heavy loop inoculations are made directly from agar slants, and the tubes are shaken mechanically at the smallest possible angle to the horizontal at a temperature of 30 C.

Yeasts with normal respiration, Q_{O_2} (N) ca 1,000, produce the deep alkaline color of the indicator within 24 hours. Respiration deficient yeasts fail to change the indicator color even after 5 days.

Some ambiguities arise with inbred haploids from which representatives of an almost complete spectrum of respiratory weakness may be selected. Yeasts with $Q_{C_2} > 450$ yield the positive alkali production in peptone acetate in less than 48 hours, whereas yeasts with $Q_{O_2} < 200$ do not yield sufficient alkali in peptone acetate.

Based on this zone as an arbitrary boundary of respiration deficiency, qualitative correspondence between aerobic positive and alkali production in peptone acetate and between respiration deficiency and lack of alkali production in peptone acetate has been demonstrated in tests involving 42 respiration sufficient strains and 41 respiration deficient strains.