essentially unchanged. The dehydrase activity, however, declined until none was detectable. (This decline commenced approximately 1 hr after maximal growth was attained.) At this latter point, the cells were viable when placed in minimal medium, but there was a 2-hr "lag" period before growth commenced. It is tempting to interpret this decline as an expression of enzyme repression, but the present in vitro evidence on the dehydrase supports the contention that the decline is due not to repression but, rather, to the instability of the enzyme within the cell. The labile nature of this enzyme is a critical feature when studies on cell-free extracts are carried out. For example, dehydrase activity in extracts was unstable not only upon storage (60% loss of activity after overnight freezing) but also during maintenance of freshly prepared extracts in ice. Various attempts to stabilize the activity have been unsuccessful to date. Thus, to obtain valid results, it was necessary to carry out the dehydrase assays within 3 hr after preparation of the cell-free extracts. Further evidence of the instability of the enzyme includes the observation of Armstrong et al. that high levels of activity are unstable in the in vitro assay when various amino acids are present and the observation that activity is lost during dialysis (unpublished data). The dehydrase is the only enzyme of the common pathway that displays this marked degree of instability and that requires careful and rapid handling.

The instability of the enzyme may account for the severe depression of the dehydrase activity that Armstrong et al. found to occur temporarily during the early period of growth of S. typhi-

![Graph](image)

**Fig. 1.** Specific activities of the four enzymes concerned with the biosynthesis of valine and isoleucine, as measured during the stationary phase of growth of Salmonella. See Armstrong et al. (Proc. Natl. Acad. Sci. U.S. 49:322, 1963) for details and references concerning the growth conditions and the enzymatic assays. The substrate utilized for the dehydrase assay was \( \alpha,\beta\)-dihydroxy-\( \beta\)-methylvalerate. The following abbreviations are used: Na Pfr = sodium pyruvate; AHB = \( \alpha\)-aceto-\( \alpha\)-hydroxybutyrate; KV = \( \alpha\)-ketoisovalerate (\( \alpha\)-ketovaline).

nutrum wild type in minimal medium containing valine. Thus, although published data clearly demonstrate the role of the dehydrase in the regulatory mechanisms of the pathway, it is prudent to examine closely any particular effect noted with this enzyme.

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**AEROBIOSES REQUIREMENT OF DIAGNOSTIC COLOR DIFFERENTIATION FOR RESPIRATION DEFICIENCY IN YEAST**

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Nutrient agar plates containing trypan blue mixed with Magdala red or eosin are convenient for diagnosing hereditary respirational deficiency in yeast as reported recently (Nagai, J. Bacteriol. **86**:289, 1963). The work was extended further to determine how such color differentiation between respirationally normal and deficient cultures of yeast is accomplished. Modified application of diagnostic color plates demonstrated that aerobiosis is a prime requirement for successful color differentiation.

Normal and deficient cultures of *Saccharomyces cerevisiae* IFO 0044, of *S. chevalieri* IFO 0210, and of *S. uvarum* IFO 0289 in heavy suspensions were...
streaked side by side on color plates arranged in two groups, and were allowed to grow at 30 C. One group was kept under ordinary aerobic conditions. The other group was forced to anaerobiosis in: (i) sealed glass jars where the air was deprived of oxygen by an alkali-pyrogallol mixture, (ii) liquid paraffin flooded 5 mm deep onto the plate surface, or (iii) the same nutrient color medium overlaid 5 mm thick. Normal streaks grown under aerobic conditions were close to white, whereas deficient streaks appeared in reddish-purple sheen as described before. In contrast, normal streaks under anaerobic condition appeared in heavy reddish-purple color the same as that of corresponding deficient streaks. Figure 1 shows typical pairs of such color plate cultures. The color of normal streaks grown under anaerobic conditions did not fade even when the plates were subsequently exposed to air, although some secondary outgrowths of much lighter color appeared. Also, the color did not develop any more in the aerobically grown normal streaks when the plates were later brought to anaerobiosis. Addition of $1.0 \times 10^{-2}$ to $1.5 \times 10^{-3}$ M potassium cyanide or $1.5 \times 10^{-4}$ to $2.5 \times 10^{-4}$ M copper sulfate to the color plates somewhat retarded the growth, and also made normal streaks heavily colored in aerobiosis as well as in anaerobiosis. Copper sulfate in that range of concentrations did not induce respiration deficiency, although it was known to do so at higher concentrations (Lindegren, Nagai, and Nagai, Nature 182:446, 1958). In addition to the diploid, wild-type yeasts and their "vegetative (cytoplasmic) petites" described above, several haploid cultures (derived from S. cerevisiae) including two strains of ultraviolet-induced "segregational (nuclear gene) petites" similarly showed color differentiation on the diagnostic plates under aerobic conditions. Reliability of Magdala red for these color plates was confirmed by using two recently manufactured preparations (from Bayer, Leverkusen, and Schuhardt, Munich, Germany) along with older ones used before.

These observations indicate that color differentiation is accounted for by a certain activity of normal yeasts that bleaches the absorbed dyes, but that does not occur in deficient cultures of either genic or cytoplasmic origin so far as phenotypic respiration deficiency is concerned. The bleaching appears to be closely linked with aerobiosis. Copper sulfate perhaps changes normal yeast to a quasi-anaerobic state, where it fails to reduce tetrazolium indicator, as reported elsewhere (Nagai and Nagai, Exptl. Cell. Res. 15:623, 1958), and also fails to bleach diagnostic dyes, as shown here.

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