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HARRY SOBEL
JESSIE MARMORSTON
HILDA ARZANGOOLIAN

Department of Biochemistry, Division of Laboratories; and *Institute for Medical Research, Cedars of Lebanon Hospital*; and *University of Southern California School of Medicine, Los Angeles*

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The Tetrazolium Reaction in Yeast

In studies dealing with phytotoxic effects of hydrocarbons, the use of yeast as test material was suggested by Collander and Äyräpää (*1*), who outlined a simple permeability experiment suitable for laboratory or for class demonstration. Neutral red was employed as a penetrating indicator.

Under the appropriate conditions, yeast absorbs the vital dye 2,3,5-triphenyltetrazolium chloride (TTC) and reduces it intracellularly to a red formazan (*2, 3*). Dead cells, following exposure to lethal concentrations of benzene, do not react. A simple demonstration of two factors related to vital TTC reduction is the following.

A quantity of wet compressed yeast cake is mixed with an equal weight of water, and 1 ml of the suspension is added to 100 ml each of the following four solutions. All contain 0.1 percent TTC and 0.01*M* phosphate buffer *p*H 7.6. In addition, the following are present: in 2, glucose 1 percent; in 3, benzene 0.1 percent by volume; in 4, glucose 1 percent and benzene 0.1 percent. The stoppered bottles are agitated from time to time.

Within about 5 min following addition of yeast, bottle 4 should be perceptibly pink, followed by 2, 3, and 1. In about 40 min, bottles 1–4 should show an increasing redness. Longer times will intensify the color but will decrease the differences. Strong illumination is undesirable, since it tends to reduce the dye photochemically outside the cells. A control bottle containing solution 4 but no yeast remains colorless.

The following interpretation of these results is suggested. Tetrazolium normally penetrates the yeast cell slowly. Benzene, through its lipid solvent action, increases permeability to the dye. This explanation finds support in the work of Hurst (*4*). Water that is 95-percent saturated with benzene is lethal to yeast in 8 hr at 25°C, but the approximately half-saturated solution used in these tests does not seem to be injurious. Sugar provides substrate for respiratory enzymes involved in the reduction of TTC. Yeast diaphorase is believed important in this regard (*5*).

That the cells were alive in all treatments after 1 hr was shown microscopically by a characteristic neutral red accumulation, by plasmolytic response to strongly hypertonic solutions, and by colony formation on grape-juice agar.

The question arises whether the action of benzene might not be one of increasing respiration. To obtain information on this point, oxygen uptake of yeast was determined by Warburg's direct method. At eight different concentrations of benzene, increasing from 7 to 83 percent of saturation, there was no significant effect on oxygen consumption within 1 hr. In saturated solution, however, the rate was 22 percent of the control. Further tests utilizing Dixon-Keilen flasks provided a measure of both O_2 and CO_2 exchange. A similar inhibition in saturated benzene solution was obtained, depressing CO_2 production more than O_2 absorption.

The foregoing considerations favor the interpretation that the benzene effect is an enhanced permeability toward TTC. Whether benzene increases permeability toward sugar at the same time, or whether sugar itself increases permeability, cannot be decided on the basis of these tests. The action of benzene is nonspecific, and the same effect is produced by other relative nonpolar substances, for example, ethyl ether, xylene, and 1-octanol.

In addition to laboratory use, these observations may have a bearing on the use of the tetrazolium reaction as a criterion of vitality. It has been noted that for certain cells (*6*) failure of TTC to penetrate constitutes a limitation.

H. B. CURRIER
B. E. DAY

Department of Botany
University of California, Davis; and
Department of Plant Physiology
University of California, Riverside

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