blood and oxygen supply to the bone marrow, thus tending to depress erythropoiesis.

It is concluded that choline aids in the production of this anemia, *not* by virtue of a lipotropic action, but rather by its vasodilator or pharmacological action.

References

1. Davis, J. E. *Amer. J. Physiol.*, 1944, **142**, 65, 213, 402.
2. Davis, J. E., and Gross, J. B. *Amer. J. Physiol.*, 1945, **144**, 444.
3. Dupee, C., Johnson, V., Marchello, A., Wilner, W., and Freeman, L. W. *Fed. Proc.*, 1944, **3**, 8.
4. Freeman, L. W., and Johnson, V. *Amer. J. Physiol.*, 1940, **130**, 723.
5. Johnson, V., Longini, J., and Freeman, L. W. *Science*, 1943, **97**, 400.
6. Loewy, A., Freeman, L. W., Marchello, A., and Johnson, V. *Amer. J. Physiol.*, 1943, **138**, 230.
7. Longini, J., and Johnson, V. *Amer. J. Physiol.*, 1943, **140**, 349.

A Nonrespiratory Variant of *Saccharomyces cerevisiae*[1]

Rita Whelton and H. J. Phaff

Division of Food Technology
University of California, Berkeley

In studies of the toxic effect of ethylene oxide on a strain of *Saccharomyces cerevisiae*, it was noted that, at a time when almost all of the yeast cells in a suspension have been killed by the poison, some of the surviving cells give rise to colonies unlike those of the original organism. These colonies are smaller and rougher than the normal type. The organisms in them are usually more spherical, somewhat smaller, and in some cases occur in clusters. When restreaked on agar plates, they gave rise to colonies showing much variation in size. The ethylene oxide-induced variants can be obtained repeatedly from the original strain after repurification of the latter and thus do not represent a constant degree of contamination.

The occurrence of mutated colonies has been described before (*2, 3*) and interpreted as being due to the development of haploid yeast cells. However, such variation is usually observed only after sporulation of the yeast is followed by isolation on special media or by dissection and cultivation of individual ascospores.

In a few instances we have observed what appeared to be fusion between cells of the variant colonies to form a diploid yeast. Haploid yeasts are often unstable, not only because of possible conjugation but also because of the high rate of mutation to which they are subject. Instability has been noted in the variants obtained in this work and has led to difficulties in studying them. However, certain strains of the apparently haploid type have been sufficiently stable to be examined in some detail, and one, which did not undergo further variation over a period of several months, has shown very interesting characteristics.

Unlike suspensions of the parent strain, resting suspensions of this variant show no ability to oxidize either glucose or alcohol under aerobic conditions. In any case, the respiration is so slight that it is not measurable in Warburg respirometers. This is true even when the organisms have been grown in a shaking liquid medium containing 0.5 per cent glucose and 5 per cent yeast autolysate and have been harvested during the period of exponential growth. As will be seen in Table 1, the rate of fermentation is high, and no Pasteur effect can be observed.

Suspensions of the parent yeast examined spectroscopically show absorption bands corresponding to reduced cytochromes A, B, and C at 605, 552, and 563 A. Similar suspensions of the variant show a strong band at 563 A. (cytochrome C), but lack the other two even when reducing agent is added in excess.

TABLE 1

Rates of Respiration and Fermentation of Parent and Variant Strains at 30° C. With 2 Per Cent Glucose and 2 Per Cent KH_2PO_4

	Parent	Variant
Q_{O_2}	42.5	0
$Q^{air}_{CO_2}$*	62	274
$Q^{CO_2}_{CO_2}$	145	268

* Only aerobic fermentative CO_2 production included in $Q^{air}_{CO_2}$.

In addition, the variant gives no test for indophenol oxidase with Nadi reagents according to the method of Keilin (*1*). The parent strain gives a strong positive reaction under identical conditions. Spectroscopic examination for cytochrome oxidase was not made.

The variant can ferment the same sugars as the parent strain and use them as substrates for growth. As might be expected, however, it does not grow with alcohol as carbon source, although the parent strain is able to do so. Morphologically, it differs in size and in the shape of the cells (parent—(4.2–7.2) × (5.5–10.8)μ; average: 5.5 × 7.2μ; variant—(4.2–6.6) × (4.8–8.4)μ; average: 5.3 × 6.2μ). While the thallus of the parent strain occurs as single cells and pairs, that of the variant consists of clusters of from 3 to 20 cells. In old liquid wort cultures, the variant shows no surface growth or ring formation. There are also noticeable differences in gross morphology of streak and giant colonies. The variant has lost the ability to form ascospores.

The question as to why these haploid variants, if such they are, appear after ethylene oxide treatment has not been satisfactorily answered. It might be the result of a specific metabolic effect of the poison on the yeast cells, but it seems more likely that haploid cells or spores may be present in the normal cultures and survive the treatment with ethylene oxide better than the diploid cells because of their slightly greater resistance to the poison. Since the particular variant discussed occurs in clusters of various sizes, it is difficult to compare its death rate in ethylene oxide with that of the original strain by the use of viable counts. Since any difference in resistance cannot be great (all of the yeast cells are eventually killed by ethylene oxide), they are difficult to establish by ordinary statistical methods. However, in studies with another variant, which had similar physiological characteristics (*i.e.* absence of oxida-

[1] A report on a joint research project of the Quartermaster General's Office, U. S. Army, and the University of California. The authors wish to express their appreciation to Prof. Gordon Mackinney for his aid with the spectroscopic work and to Miss Ruth Alleman for technical assistance.

tive metabolism) but which did not form clusters, an apparently slightly greater resistance to the poison was found than with the parent strain.

Castor (*4*) has described a nonrespiratory yeast variant resulting from treatment with HCN. In contrast to our variant, his organisms lacked cytochrome C. Ethylene oxide does not appear to act as a specific respiratory poison. However, although its effect on the metabolism of yeast, unlike the effect of HCN, appears to be general, it does inhibit fermentation more rapidly in the presence of air than in its absence. Not all of the morphological variants appearing after ethylene oxide treatment lack oxidative metabolism. Several of these showed Q_{O_2} values comparable to that of the original strain.

If greater resistance to the poison explains the appearance of such variants, it may be that only certain types of haploids will be found by this method. To study all of the types found, particularly in view of their further mutation, would be out of the scope of our work. However, the strain discussed, providing its physiological characters remain stable, offers many interesting possibilities for investigation.

References

1. KEILIN, D. *Proc. roy. Soc.*, 1929, **104B**, 206–252.
2. LINDEGREN, CARL C. *Bact. Rev.*, 1945, **9**, 111–170.
3. NYBERG, CARL. *Z. Bakt.* (Abt. 2), 1941, **103**, 272–276.
4. STIER, T. J. B., and CASTOR, J. G. B. *J. gen. Physiol.*, 1941, **25**, 229–233.

The Deposition of C^{14} in Bone[1]

WILLIAM BLOOM[2]

Metallurgical Laboratory, University of Chicago

HOWARD J. CURTIS[3]

Clinton Laboratories, Monsanto Chemical Company

FRANKLIN C. MCLEAN[2]

Metallurgical Laboratory, University of Chicago

In the course of experiments on the mineral metabolism of bone salt in rats, we made some observations on the deposition of C^{14} in bone which are worth recording now, although our studies are incomplete.

Beginning in July 1945, we injected a number of rats intraperitoneally with $BaCO_3$ or $NaHCO_3$ in which 75–150 μc. of C^{14} were present. Some of the rats (weighing 52–75 grams) had been on a normal diet; others, on a low-phosphorus, vitamin-free diet before the injection. All animals were on a normal diet thereafter. The rats were killed at 3 days and 2, 4, 8, and 16 weeks after injection. Among the controls were rats injected with P^{32} or Sr^{89}. The bones, after fixation in absolute alcohol and embedding in nitrocellulose, were sectioned *without decalcification* by the routine of McLean and Bloom (*1*). Autoradiographs of these sections as well as of the alcohol-fixed soft tissues were prepared for us by Mr. George Svihla. We found no differences in the results dependent on the normal versus the low P diets.

As was expected from previous studies on the deposition of bone salt as shown by staining with $AgNO_3$ and by the use of Sr^{89} and other calcium substituting isotopes, we found in the autoradiographs of the long bones of rats killed after 3 days that Sr^{89} deposits most heavily in the metaphysis (extending through the calcified cartilage and all of the spongiosa) and is present in smaller amounts in the bone structures of diaphysis and epiphysis. These differences in the amount of Sr^{89} in the several parts of the bone can be accounted for by the occurrence of two processes: (1) a fresh deposit of bone salt in the zone of new growth of bone in which Sr^{89} substitutes for some of the Ca atoms, and (2) an interchange between Sr^{89} and Ca of the bone salt previously deposited in the bone.

Autoradiographs made from rats injected with P^{32} show that P^{32} is deposited in much the same situations in bone as Sr^{89}.

The autoradiographs of the bones of rats injected with C^{14} show a markedly different picture from those of the Sr^{89} rats. Those from rats killed 3 days after injection of C^{14} show the shaft of the bones as black lines. The bone in the epiphysis is a faint gray, while the metaphysis of the growing end of the bone is negative, or practically so, leaving a gap of about 2 mm. between epiphysis and diaphysis. The nongrowing end of the bone is completely outlined in gray. The marrow cavity is so pale that it is probably negative. The autoradiographs of the 2-, 4-, 8-, and 16-week specimens show essentially the same picture as those after 3 days, except that the bones have grown in length and width. With the growth in length, the unblackened zone at the metaphysis increased to about 3 mm. at 2 weeks, 4 mm. at 4 weeks, 10 mm. at 8 weeks, and 12–17 mm. at 16 weeks. Since the blackened lines representing the lateral extent of the diaphysis at the time of injection are still present after 16 weeks, it would seem that the marrow cavity did not increase much in diameter at the site of deposition of radioactive carbon. However, new bone was deposited externally so that the bone as a whole increased in thickness.

Sections of the liver and kidney gave fairly intense autoradiographs at the 3-day and 2-week stages, but were negative after the longer intervals. Since the films were exposed for the same length of time, it would appear that there was approximately as much C^{14} in the bones after 16 weeks as after 3 days, while there was a great decrease in the C^{14} content of the soft tissues. Organ analyses are now being made.

From the autoradiographs of bones of these few rats we would conclude that C^{14} injected as carbonate appears primarily in those areas occupied by pre-existing bone. It does not appear in appreciable quantities in the areas of most recently deposited bone salt. This observation demands further study on the carbon metabolism of bone.

Since the C^{14} content of the bones did not decrease appreciably in 4 months, we believe that the health hazards involved in working with this isotope must be studied, particular attention being paid to the possible development of bone tumors.

[1] This work was carried out under contract between the University of Chicago and the Manhattan District, Corps of Engineers, War Department.

[2] Now with the Argonne National Laboratory and the University of Chicago.

[3] Now at the College of Physicians and Surgeons, Columbia University.

Reference

1. MCLEAN, F. C., and BLOOM, W. *Anat. Rec.*, 1940, **78**, 333.