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]

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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.

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Reference

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