THE EFFECT OF DEUTERIUM ON THE GROWTH OF YEAST

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Water composed chiefly of deuterium, the heavier isotope of hydrogen, has been found unsuitable for living organisms when they are placed in it without gradual adaptation. As with most poisonous substances, less injury, some stimulation, and finally no effect should be obtained with increasing dilution of the heavy water. Barnes (1933) gives references to earlier investigations on this subject.

A pure strain of yeast, *Saccharomyces cerevisiae* Hansen, was grown in Williams' medium[1] made with deuterium containing water[2] having a specific gravity of 1.000061 (1 part in 2000) and in the same medium made with distilled water using the controlled methods described elsewhere (Richards, 1932a; Richards and Jahn, 1933).

No significant difference was found in the number of cells per unit volume during the growth in the two waters as the differences noted were only about one-fourth the standard error of these differences. Nor were significant differences found in the percentage of buds and of injured cells, or in the mean cell size of the two populations of yeast grown under these conditions. The cells grown in the heavier water were slightly more uniform

[1] Williams' medium contains sugar 20 grams; $(NH_4)_2SO_4$, 3 grams; KH_2PO_4, 2 grams; Asparagin, 1.5 grams; $CaCl_2$, 0.25 gram; $MgSO_4$, 0.25 gram; and distilled water 1000 ml.

[2] The heavy water was furnished to me through the kindness of Dr. T. C. Barnes and for his description of it *cf. Jour. Am. Chem. Soc.*, 1933, **55**, 4332. The specific gravity was measured to $\pm 5 \times 10^{-6}$. This is a greater uncertainty than advised by Washburn, but the difference found does indicate a significant concentration of heavy water.

in size than those grown in distilled water as the coefficient of variation of the former was about 8 per cent less than that of the latter.

Measuring the growth of the populations with a photoelectric nephelometer gives a criterion of growth depending on the number of cells present, the distribution of the sizes of the cells and the optical density of the cells. Table 1 shows an increased growth of 11 per cent (standard error 1.5 per cent) for all of the determinations of three series of the yeast grown in the heavier water above that of the yeast grown in distilled water. With no

TABLE 1

Growth of yeast in heavy water in per cent of the growth of the control yeast in distilled water

SERIES	NEPHELOMETER		AGE	VOLUME	DRY WEIGHT
	N*	Per cent			
			hours	*per cent*	*per cent*
130	24	108			
139A'†	16	103	106	114	111
134	30	117	114	117	120
134		Av. 111*	143	159	135
139B'†			106		86
139C'†			106		92

* Average weighted in terms of the number of measurements (N).

† Series 130, 134 and 139A' in heavy water of specific gravity 1.000061; Series 139B' one-fourth heavy water and three-fourths distilled water; Series 139C' one-half of the above heavy water and one-half distilled water.

significant differences in the number of cells in the two populations this indicates increased total volume of the cells with or without cytologically more dense cells. The total volume of the cells from the population grown in the heavy water is greater than that from the control population in distilled water (table 1), when they are centrifuged into calibrated volume tubes. This difference increases with the age of the populations. As the mean cell size is not very different, less close packing occurred in centrifuging the yeast obtained in the heavy water. The rela-

tion between the several criteria of yeast growth will be given elsewhere.[3]

The dry weight of the yeast is greater when the cells are grown in water containing the heavier isotope of hydrogen and this difference also increases with the age of the populations (table 1). The ash content of the yeast grown in this dilute heavy water is less (9.5 per cent) than that of the control yeast (11.3 per cent) showing that the difference in the dry weight is due to non-mineral storage material. The glycogen content of the yeast from the heavy water cultures was nearly twice that of the controls but their nitrogen content was only 69 per cent of that of the control yeast populations. The increase of glycogen and loss of nitrogen is characteristic of old populations of yeast and is also observed in the ageing or ripening of commercial yeast.

The greatest effect on the yeast is shown by the nephelometric method to occur at the two times when the rate of population growth is greatest for the two cycles of growth which occur when this yeast is grown in the Williams' medium at 28°C. At these two times the cells are smaller and have a proportionately greater surface for the exchange of nutrient material with respect to their volume (fig. 1, *A*, *B*). The effect at this time leads to an earlier reorganization within the cells making those grown in the heavy water appear to be aged with respect to the controls. This ageing, or cellular reorganization, normally occurs as the culture medium becomes less favorable, from the exhaustion of food and the accumulation of the toxic excretion products of the cells. Hence, the effect of the water containing deuterium is unfavorable from the standpoint of a maintained constant environment. On the other hand, if the criterion of growth is bulk or weight, the dilute heavy water may be said to stimulate the yeast growth.

A single experiment made with two dilutions of the heavy water indicated that the optimum effect occurred with about half of the concentration of the heavy water used in my experiments (i.e., one part in four thousand). Further dilution gave less difference as shown by the nephelometric criterion plotted on figure 1, *C*.

[3] An abstract appears in *Anat. Rec.*, 1933, **57** (4) Suppl. 36 and the details will be published in the near future.

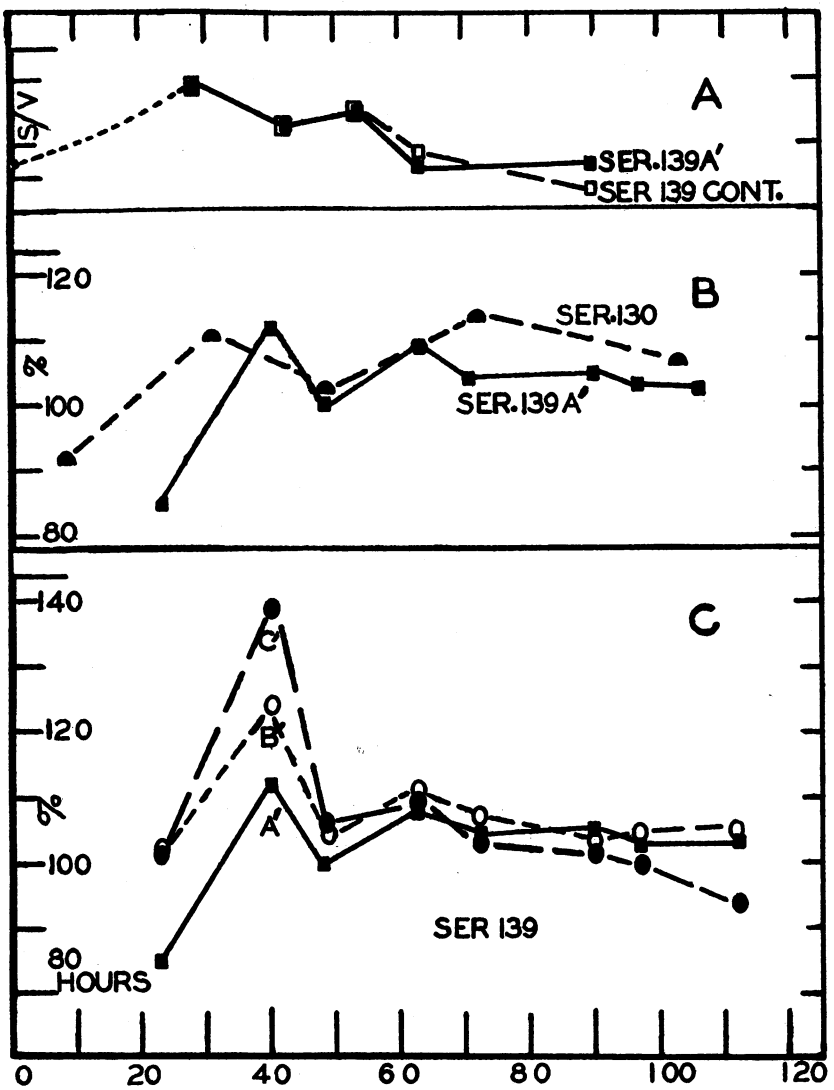

FIG. 1. *A*, the relation of the surface to the volume of the average yeast cell during growth. (Ser. 139 *A'* in heavy water, 139 cont. the control in distilled water). *B*, the growth of yeast in heavy water as a percentage of the growth of the control cultures in distilled water for two independent experiments. *C*, the growth in heavy water, *A'*; in 1 part heavy water to three parts distilled water, *B'*; and in 1 part heavy water to 1 part distilled water, *C'*.

Pascu (1934) has found that the fermentation of glucose by yeast is much less in concentrated heavy water (100 per cent and 60 per cent deuterium oxide), but Macht and Davis (1934) report no difference with a concentration of heavy water about the same as used in my experiments. Barnes and Larson (1933) have reported a 10 per cent decreased liberation of carbon dioxide by zymin preparations from sugar in solution in the same strength heavy water as that used here.

A greater effect is observed on the first cycle of growth when the energy for growth and maintenance appears to come mainly from the fermentation of the sugar in the medium. The effect during the second growth cycle is less and the energy then comes mainly from an oxidative process. The effects occur when the ratio of surface to the volume of the cells is greatest. Thus, the presence of the heavy isotope of hydrogen may alter the permeability of the cell or may have more effect on the enzymes of the cell. The result is increased bulk of yeast and earlier ageing of the cells. While a change in the bound water of the cell colloids is not excluded it seems to be a less satisfactory explanation than an effect on the cell enzymes (Barnes and Larson, 1933). The results of the experiments are marked, but the stimulation observed is much less than that known to occur with yeast growth stimulants such as thallium (Richards, 1932b), or some of the "bios" preparations (Williams and Truesdale, 1931).

Until more heavy water becomes available it will not be possible to determine the optimum concentration, the effects on fermentation and respiration, and the extent to which the yeast may be adapted to greater concentrations of the heavy isotope of hydrogen. The amount of protium which may be replaced by deuterium in the cell must be determined under carefully controlled conditions. Until this information becomes available from critical, quantitative study it is premature to speculate further on the nature of the effects observed in these experiments or to generalize on the rôle which the newly discovered isotope of hydrogen may play in living matter.

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