

the 100% division passes through the point at which the horizontal line corresponding to the asymptote cuts the ordinate of the curve. The oblique lines then cut the ordinate of the curve at a series of points. From the curve, read off the time corresponding to each of these points, add up the times, and divide by the time corresponding to the oblique line for 85.4%. Call the result S. The value of  $n (=1/p)$  is found by consulting Table I.

TABLE I.

n	S	n	S
1.0	3.083	2.2	1.631
1.2	2.603	2.4	1.518
1.4	2.335	2.6	1.431
1.6	2.097	2.8	1.357
1.8	1.908	3.0	1.300
2.0	1.753		

In this way a time-dilution curve can be analyzed in less than a minute, and, if the curve is of the usual form, the value of  $n$  is obtained with an error no greater than  $\pm 0.05$ , for values not shown in the table can be found by interpolation. When large numbers of time-dilution curves are obtained in the course of an investigation, a rapid method of analysis is a great convenience, although it is often necessary to analyze the curves more carefully later on.

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## Effect of Heat on Vitamin G Potency of Desiccated Yeast.

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In the course of their investigations on the nutritive requirements of the chick Elvehjem, Kline, Keenan and Hart<sup>1</sup> found that about half of the vitamin G potency of desiccated yeast was destroyed by heating at 100°C. for 6 days. With the hope of securing evidence of the supposed duality of vitamin G, as postulated by Sure<sup>2</sup> and others, we have studied the effect of heat on both the growth-promoting and dermatitis-preventing activities of dried yeast. After

<sup>1</sup> Elvehjem, C. A., Kline, O. L., Keenan, J. A., and Hart, E. B., *J. Biol. Chem.*, 1932, **99**, 309.

<sup>2</sup> Sure, B., Smith, M. E., and Kik, M. C., *Science*, 1931, **73**, 242.

preliminary desiccation the yeast was heated in an electric oven at either 105°C. or 150°C. for various lengths of time. The resulting products were fed, at a level of 1.5 gm. per mouse per week, to 3-weeks-old mice maintained on an otherwise G-deficient diet. The basal ration was prepared by mixing 200 gm. of vitamin-free casein, 265 gm. of cornstarch, 205 gm. of sucrose, 200 gm. of lard and 40 gm. of McCollum's salt mixture. Each animal received apart from the basal diet 2 drops of a tested cod liver oil and an ample amount of a vitamin B preparation. The latter was made from wheat germ by extraction with 93% alcohol, distillation of the extract *in vacuo*, and separation of the potent aqueous residue from an inert oil.

The vitamin G activity of the yeast preparations, as measured by the growth response of the mice, is recorded in Fig. 1. No decrease

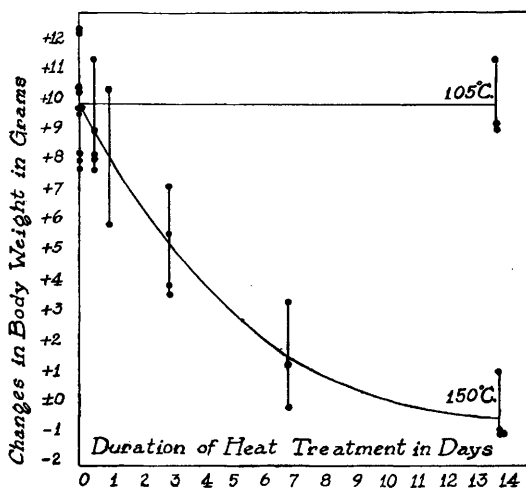


FIG. 1.—The Inactivation of Vitamin G by Heat.

Dots represent growth of mice in 6 weeks plotted against the length of time at which the desiccated yeast has been heated at either 105°C. or 150°C. The curves are drawn through the average value of each group.

in the vitamin G potency of yeast heated at 105° was observed. Further experiments with mice fed varying amounts of dried yeast, heated at 105° for 2 weeks, would be necessary to reveal whether a slight inactivation occurs. From the limited data available, however, it may be concluded that the destruction of vitamin G under these conditions is certainly not extensive. Moreover, Block and Farquhar,<sup>3</sup> who used the rat as their experimental animal, have recently reported that they observed no decrease in the growth-pro-

<sup>3</sup> Block, R. J., and Farquhar, L. R., *J. Biol. Chem.*, 1933, **103**, 643.

moting activity of yeast that had been heated at 100° for 2 or even 4 weeks.

Our data show further that the growth-promoting activity of yeast is slowly lost as a result of prolonged heating at 150°. There is a little vitamin G remaining even after one week of such treatment, but the vitamin is entirely destroyed in 2 weeks. Each of the 4 mice receiving the yeast that had been heated at 150° for 2 weeks developed typical skin lesions, as described by Bing and Mendel,<sup>4</sup> and all were dead in 7 weeks. At this time the mice in the other groups were still alive and their skins were in good condition. It appeared that the mice which had received sufficient vitamin to permit some growth had likewise received enough of the anti-dermatitis factor to meet their requirements, and the experiment was therefore discontinued.

As a guide to further experiments the slow rate of destruction of vitamin G at 150° is of special significance. If inactivation at the much lower temperature of 100° should occur, one could hardly expect to demonstrate it by biological assay except after unusually long periods of heat treatment.

From the results described herein it is concluded that the heating of previously desiccated yeast at 105°C. for 2 weeks probably does not affect its vitamin G content. Heating the yeast at 150°C. for the same length of time brings about a complete loss of activity; the resulting product neither permits growth nor prevents dermatitis on a vitamin G deficient diet. If there should prove to be separate factors for growth and dermatitis prevention in so-called vitamin G preparations they would thus appear to be destroyed at identical rates by heating in the dry condition.

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<sup>4</sup> Bing, F. C., and Mendel, L. B., *J. Nutrition*, 1929, **2**, 49.