CLXIII. THE EFFECT OF FERMENTATION ON THE WATER-SOLUBLE VITAMIN CONTENT OF WORT.

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In a previous communication [1924] the writer has shown that there is much less water-soluble B vitamin in beer than in the corresponding amount of malt used in its manufacture. In this connection it should be stated that Harden and Zilva [1924] in their investigation of the subject, report the beer samples tested by them to be free from water-soluble B vitamin. It was indicated that further experiments were in progress to determine if possible where the main loss occurred in this process. The prevalent idea appears to be that beer is indebted to the yeast cell for its content of this factor. The correctness of this theory is dependent on the answer to the question whether yeast does or does not synthesise the water-soluble B factor during the fermentation of the wort. The results obtained by Eijkman and his co-workers [1922] showed that during the process of fermentation of wort by the yeast cell the water-soluble factor passes from the wort into the yeast cell. The quantitative experiments described below corroborate their findings and in addition show that there is no actual synthesis of this factor during the process.

The experiments were as follows:

To 1200 g. of coarsely ground malt were added 3 litres of water previously heated to 150° F., and the mixture was maintained at this temperature for two hours. At the end of this period the wort was filtered through glass wool and the grains repeatedly washed with water at 150° F. till another 3 litres had been used. The filtrates were mixed, this wort having a gravity of about 1057 and a volume of roughly 5 litres. This was then brought to the boil and then autoclaved for 20 minutes at 125° C. The precipitate which had formed during this last process was removed by filtration through sterile glass wool from the dark coloured wort which was equally divided into two sterile 3 litre flasks. Each flask thus contained roughly 2½ litres of wort. For the fermentation a small amount of top yeast was obtained from a local brewery, plated out on wort-gelatin and colonies morphologically resembling *S. cerevisiae* were sub-cultured into sterile wort of similar gravity to the above, and after vigorous growth, again sub-cultured and grown in 15 cc. lots of sterile wort in test-tubes. After growing for three days at a temperature of 69° F., each tube yielded about 1 g. of moist yeast, on centrifuging down the liquid for ten minutes

at 3000 R.P.M. Two of these tubes of fermented wort were taken, the contents of one added to those of one of the 3 litre flasks, and fermentation allowed to proceed at 69° F. for three days, after which time the gravity had dropped to 1012–1014. The contents of the other tube were just brought to the boil and then added to the second 2500 cc. of sterile wort to compensate.

When active fermentation had ceased in the first flask the beer was cleared of yeast cells by centrifuging. The weight of moist yeast obtained was 50–55 g. Thus about a fifty-fold multiplication had occurred. The volume of the beer was about 2200 cc. The yeast was suspended in water and alcohol added to make the strength of the latter 20 %, when the total volume of the yeast suspension was 440 cc. Thus 440 cc. of this are equivalent to 2200 cc. of beer. The yeast suspension was kept in the ice-box and always well shaken just before use. The beer was brought to the boil and again autoclaved. The other $2\frac{1}{2}$ litres of wort were subjected to the same process to compensate for any loss of vitamin through heating. Each of the 3 litre flasks was fitted with sterile tubes and covered delivery pipettes for syphoning over the liquid. The sterility of the beer and wort was thus maintained.

The precipitate was suspended in aqueous alcohol (4: 1), and the volume made up 440 cc. This was also kept in the ice-box.

The four test substances were then examined for their content of water-soluble growth-promoting factor on rats quantitatively, by adding to the basal diet (see previous paper), which was deficient in water-soluble B vitamin, quantities of 10 cc. of wort, 10 cc. of beer, 0·5 cc. of yeast suspension and 1 cc. of precipitate respectively; these amounts being increased to 15 cc., 15 cc., 0·75 cc. and 1·5 cc. respectively.

The result illustrated in Fig. 1, with rats of the same family, has been obtained with five other sets.

Fig. 2 shows the result of an experiment to test whether there was any actual synthesis of the water-soluble growth promoting factor by the yeast cell growing under the above conditions. In this case the yeast cells were not centrifuged down after fermentation, but the flask contents just brought to the boil and then rapidly cooled. 10 cc. increasing to 15 cc. of wort and an equal volume of the beer-yeast-mixture were then fed to rats with the results shown. It should be mentioned that the wort was similarly brought to the boil and cooled, in order to compensate.

A consideration of the above results shows, first, that the yeast has abstracted the growth promoting factor almost quantitatively from the wort. The presence of vitamin B in the beers of commerce as described in the author's previous paper is probably due to the fact that wort in the vat is much more heavily seeded with yeast for fermentation. Since it has been shown that within certain limits the number of yeast cells at the end of the fermentation process is independent of the number used for seeding, the multiplication will vary inversely with the quantity used in seeding. I am informed that about one pound of yeast is used per barrel and at the end of fermentation this has

increased to six pounds—a much smaller multiplication than was obtained in the experiments described above, in which the increase was fiftyfold.

Secondly it is evident that there has been no synthesis of the factor by the yeast cell during the process, but rather a small fraction of the factor originally present in the wort has disappeared. It would be rash to conclude from these experiments that the yeast during this process has actually used up some of the growth-promoting factor since the difference is small, but the results suggest this.

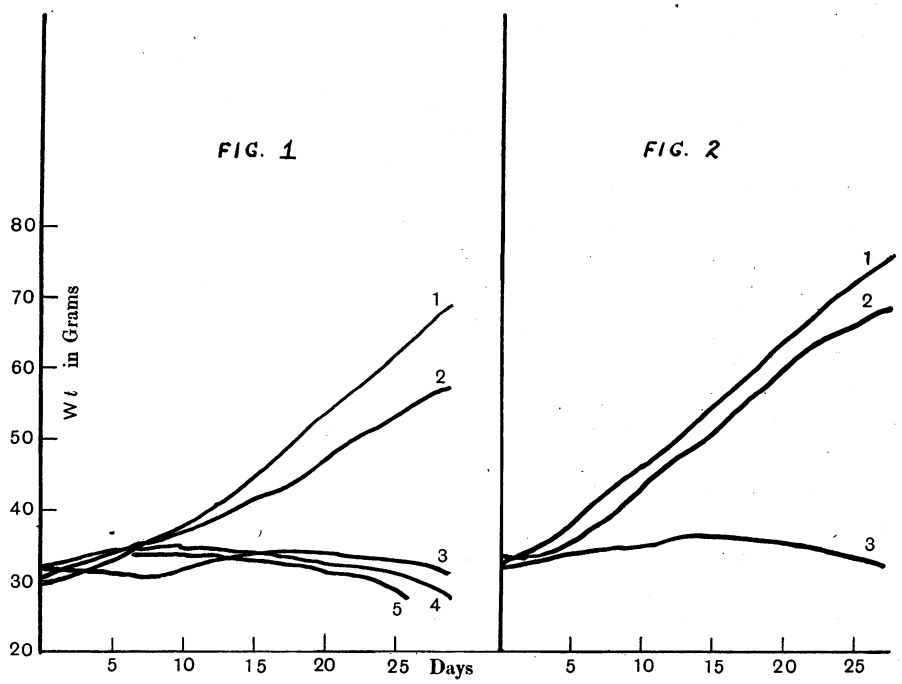

Fig. 1. Curve 1, 10–15 cc. of wort added to the basal diet of this rat. Curve 2, 0·5–0·75 cc. of yeast suspension added to the basal diet. Curve 3, 10–15 cc. of beer added to the basal diet. Curve 4, rat on basal diet only. Curve 5, 1–1·5 cc. of the precipitate suspension added to the basal diet. All these rats were females.

Fig. 2. Curve 1, 10–15 cc. of wort added to the basal diet of this rat. Curve 2, 10–15 cc. of the beer yeast suspension added to the basal diet. Curve 3, rat on basal diet only. The three rats were males.

Thirdly, the experiments described above, and others in which larger quantities of the precipitate were used, show that the precipitate from the first autoclaving did not carry down any measurable quantity of the factor.

The relationships of the water-soluble B vitamin to the yeast cell have long been the subject of experimental investigation—and controversy. Some of the aspects of the problem have been discussed by Heller [1922] and others. The difficulties have been increased rather than diminished by the work of Funk and Dubin [1921] corroborated by Heaton [1922], and more recently by Deas [1924]. From the work of these authors there can no longer be any

doubt that bios is distinct from vitamin B. Whether vitamin B is essential for the growth and multiplication of the yeast cells the above results do not prove, but suggest it. Clark [1922] showed that bios is rapidly and completely taken up from wort when the latter is shaken up with yeast, even when a large amount of yeast is used with no multiplication taking place. Eijkman showed that under similar conditions the antineuritic factor is not removed. This contrast has been pointed out, not so much with the idea of drawing another distinction between bios and the water-soluble B factor, as suggesting the possibility that vitamin B is taken up by the yeast cell during its growth and multiplication.

SUMMARY.

1. When the yeast cell is allowed to grow in beer wort—its optimum medium—it abstracts almost quantitatively the water-soluble growth-promoting factor from the medium, when grown under the above conditions.

2. The organism does not synthesise any of this factor during this process, but if anything actually uses up some of that which it takes up from the wort.

3. The main loss of vitamin B in the preparation of beer is thus accounted for.

Finally it may be added that the question of the loss of the growth-promoting factor during the malt-wort transition is at present being investigated.

In conclusion I would express my indebtedness to Professor Edward Mellanby for his help throughout the work. The expenses of this research have been defrayed by the True Temperance Research Committee.

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