The supposed Alcoholic Enzyme in Yeast.

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EARLY in the present year a paper was published by Dr. E. Buchner (1), in which he stated that he had succeeded in extracting from Yeast a liquid possessing the power of setting up the alcoholic fermentation in solution of cane-sugar. His method was the following:—One kilogram of Yeast was dried by pressure until it formed a friable powder. When so dried it was mixed with an equal weight of fine quartz sand and with 250 grams of a fine infusorial earth (Kieselguhr), and the whole carefully ground in a mortar. Water was added to the fine powder, now become pasty owing to the breaking up of the Yeast-cells, 100 cc. being used to the kilogram of Yeast. The mixture was then wrapped in a cloth and gradually submitted to strong pressure in a hydraulic press, the pressure being worked up to 400–500 atmospheres to the square inch. The resulting liquid measured about 300 cc. The cake, when removed from the press, was ground up again, and the powder extracted with a further 100 cc. of water. This was again subjected to the action of the press, and a further 150 cc. of liquid was obtained. Each kilogram of Yeast thus furnished about 450 cc. of expressed liquid, of

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which only 150 cc. represented added water, and the remainder was extracted from the living Yeast-cells.

This turbid liquid was then mixed with four grams of the same Kieselguhr and well shaken. On filtering it through paper a clear yellow liquid was obtained, having an aroma like that of active Yeast.

This liquid was the material in which Buchner claimed that the long-sought alcohol-producing enzyme was present. It had a specific gravity of 1·0416 at 17° C.

In its properties it closely resembled solutions of the other enzymes already known to physiologists. It lost its power of inducing fermentation on being heated, but parted with it at a much lower temperature than most enzymes, viz. at 45–50° C., its proteids being coagulated at the same temperature. The coagulum was very bulky, the extract being extremely rich in proteid matter. The coagulation was preceded by the evolution of carbon dioxide.

When this Yeast-extract was mixed with a 37% solution of cane-sugar, a regular evolution of carbon dioxide commenced, and proceeded with an energy which varied according to the temperature to which the liquid was exposed; it was active even so low as 0° C., but became more obvious at the temperature of the laboratory, and still more energetic at 40° C. The extract was found to work upon the same sugars as the Yeast itself, and to refuse to attack those on which the cells had no action.

The activity noticed was not interfered with by chloroform or other antiseptics, nor was it abolished by filtering the extract under pressure through a porcelain filter. There was thus a strong presumption at least that no living Yeast-cells were present in the extract, and that whatever happened was due to something extracted from the cells by the enormous pressure employed in the process.

In a subsequent paper (2) Buchner admits that this active principle is not contained in all Yeasts, the so-called 'German Yeast' being free from it. He further states that the active liquid can only be preserved for a single day at the ordinary

temperature of the laboratory or for two days at 0° C. The inactive liquid which gave a bulky coagulum at 45°–50° C. when gradually heated, while in possession of its powers, seems to lose the power of forming the coagulum as its fermentative property disappears. Buchner suggests that the supposed enzyme is proteid in character, and is digested rapidly in the liquid, with the other proteids it contains, under the influence of peptic enzymes extracted from the cells.

Buchner says further that if the Yeast be very carefully dried, it can be heated for six hours to a temperature of 100° C. without destroying the power of the Yeast to induce fermentation. This temperature is sufficient to kill the cells, but, according to the author, is not high enough to destroy the enzyme they contain. Hence a fermentation can be obtained similar to that set up by his Yeast-extract.

The appearance of these researches excited a good deal of interest, especially among physiologists and chemists, the more so as the Yeast-plant has always been the chief support of the vitalistic theory of fermentation put forward by Pasteur. It is only natural that researches have been undertaken by many workers with the view of confirming or disproving Buchner's statements. I have for several months been at work upon the subject, and have embodied my experiences in the present paper.

On reading Buchner's account of his experiments it appears that he lays great stress on the evolution of carbon dioxide from the mixtures of his extract with the various solutions of sugar which he used. To him such an evolution of gas was evidence of fermentation, and his deduction was that an enzyme was at work in his liquids. There are, however, two other points to which attention may fairly be called in conducting the experiments. The sugar-solution should gradually become of a less specific gravity as the fermentation proceeds; conditions being constant, this diminution of specific gravity should be regular as well as progressive. Also there should be a gradual and continuous formation of alcohol in the liquid as the fermentation proceeds. If

a true fermentation is set up, not only should these three phenomena be capable of separate demonstration, but there should be a definite quantitative relation between them.

I have carried out several series of experiments with different Yeasts, using in the first place the ordinary high fermentation Yeast used by our local brewers at Cambridge. I have made one series on a sample of low fermentation Yeast kindly supplied me by my friend Mr. Armstrong, of the Tottenham Lager Brewery, London.

I followed Buchner's method of preparation of the extract as closely as I could. In the process of grinding up the Yeast-cells with Kieselguhr and fine sand, I examined each instalment in the mortar with a $\frac{1}{6}$-inch objective, and kept on the grinding till about 75 to 80% of the Yeast-cells were ruptured. My extracts differed from his in quantity, never measuring so much as his figures led me to expect. In physical peculiarities there was a very close correspondence between us, my preparations coagulating at the stated temperature, and possessing the proper colour and smell.

On mixing the extract and the sterilized sugar-solution, the latter being sometimes solution of cane or grape sugar, and sometimes a wort obtained from the brewery, I have always failed to observe the copious evolution of gas which Buchner speaks of. I carried out the experiments in freshly sterilized Ehrlenmeyer flasks fitted with a mercury-manometer. Instead of a rise of the mercury in the distal limb, soon after the experiment was started there was almost uniformly a rise in the proximal limb, indicating an absorption and not an evolution of gas. In no case did I ever get a measurable rise of the mercury in the distal limb of the manometer unless the liquid contained some Yeast-cells that had been imported into the flask. This happened occasionally, even filtration through porcelain and the addition of chloroform sometimes failing to prevent it.

Not being able to get the copious evolution of CO_2, I turned my attention to the specific gravity of the fermenting solution. It seemed possible that a small quantity of sugar might be

undergoing decomposition, but that the mercury-manometer was not sufficiently delicate to give evidence of it.

In the bulk of my experiments I relied on the plan of taking the weight of a specific-gravity-bottle full of the fermenting liquor at the same temperature at approximately regular intervals. The same course of action could generally be observed, and I will therefore only quote two typical observations.

The first of these extended over a period of about ten days, the fermenting liquid being a solution of cane-sugar (40°/₀) mixed with an equal volume of the Yeast-extract. This was filtered twice through porcelain before being experimented with, and the flask was allowed to stand in an incubator at 33° C. The weighings were taken in a sp.-gr.-bottle of 25 cc. capacity. The successive weights are given in the following table:—

Date.	Time of day.	Hours of fermentation.	Weight in gms.	Loss.	Loss per hour.
May 5	12 noon	—	40·7445	—	—
„ 6	11 a.m.	23	40·7385	·006	·00026
„ 7	5 p.m.	30	40·732	·0065	·00021
„ 8	5 p.m.	24	40·726	·006	·00025
„ 10	11 a.m.	42	40·723	·003	·000075
„ 11	11 a.m.	24	40·722	·001	·000041
„ 12	5 p.m.	30	40·715	·007	·00023
„ 15	5 p.m.	72	40·702	·013	·00018

During the first three days the course of action resembled that of a weak enzyme, and appeared to confirm Buchner's views. After six days the activity had fallen to one-sixth the original amount, which again would not be surprising. During the next few days, however, it went up again to the original amount, which was quite contrary to one's experience of the action of enzymes.

In the second experiment I wish to quote, the extract was mixed with its own volume of 40°/₀ solution of glucose and digested in an incubator as before. The 25 cc. weighed 40·0915 gms., and after thirty hours' digestion it fell to 40·0905, showing a loss of ·0003 gm. per hour. After this time it gained in weight, and the mercury in the proximal

limb of the manometer rose slightly, pointing to an absorption of some constituent of the air in the flask.

Dr. Blackman was kind enough to measure for me, by means of his very delicate apparatus (described by him in the Phil. Trans. (3)), the amount of CO_2 that was being evolved by the fermenting liquid per hour in both of these experiments. The quantity varied very slightly from time to time, did not show a diminution *pari passu* with the diminution of the weight of the sugar-solution, and did not amount to more than ·01 cc. per hour, which is not more than one-tenth of the quantity which would have been produced had the loss of weight of the sugar-solution been caused by the splitting of a corresponding amount of sugar into CO_2 and alcohol.

At the end of the experiment I examined the digestions for alcohol in the usual way, by neutralizing, distilling off two-thirds of the liquid, adding distilled water to make up the original bulk, and taking the specific gravity. After the ten days of the first experiment quoted, the liquid contained ·2% of alcohol.

I may quote further a series of experiments I made to ascertain if alcohol was produced during the fermentations, and whether, if so, the quantity increased in any proportion to the duration of the digestion.

I mixed 50 cc. of a freshly prepared Yeast-extract with 100 cc. of a 40% solution of glucose, and divided it into five equal parts, A, B, C, D, and E, which were set in an incubator at 29° C. in flasks fitted with gauges or manometers. Prior to starting the digestion A was boiled to serve as a control. It gave the usual bulky coagulum at 45° to 50° C. The experiments were started on May 28, and the weight of a specific-gravity-bottle filled with the liquid was 27·350 gms. One flask, C, was examined at intervals of twenty-four hours, and the weights obtained were the following :—

May 31, 27·338 gms.
June 1, 27·340 „
„ 2, 27·338 „

The contents of this flask were then examined for alcohol, and found to contain ·2%.

B was allowed to digest for three days longer. It then contained the same proportion of alcohol. When boiled it gave hardly any coagulum, the proteids being apparently digested, as Buchner says they were in his experiments.

E was examined after four more days. It contained almost exactly the same percentage of alcohol.

Two days later, the control flask A was similarly examined, and found to contain the same amount of spirit.

There had thus been during the whole time no additional amount of alcohol formed.

From a consideration of the whole series of my experiments I can see no satisfactory evidence in favour of the existence of Buchner's enzyme, at any rate in the Yeasts in use in English brewing. The diminution in specific gravity in the digesting fluid in the quoted series of experiments appeared to me at first to point in that direction, but had the enzyme been present I think I should have found this diminution more regular, and I should have found at least ten times the CO_2 evolved. The amount of alcohol formed would also have corresponded to the weight of sugar lost. The extraction of the Yeast has in all my experiments led to the presence of a measurable quantity of alcohol in the digestions at the outset. The Yeast-extract which I obtained from the Tottenham Yeast contained ·4% of spirit. In no case did I find evidence that this initial amount of alcohol was increased.

My own results receive confirmation from some researches published quite recently by Will (4), and by Lindner (5), Delbruck, and others, who have also failed to extract the enzyme by the method detailed by Buchner.

I do not think that Buchner's statement that the activity of the enzyme is correlated with the presence of coagulable proteid in the Yeast-extract is correct. Certainly I found many extracts containing quite a considerable amount of coagulable proteid to be entirely without enzyme-action when digested with saccharine solutions.

I made some experiments to ascertain whether I could confirm Buchner's statement that the Yeast-cells will, when dry, retain their activity after being heated for several hours to 100° C. I carefully dried the Yeast by exposure to air, after pressing out the Yeast-liquor in a screw-press. When quite mealy to the fingers, I placed a quantity in a beaker *in vacuo* over strong sulphuric acid, and kept it there three days. I then treated half of it to 100° C. for six hours, according to Buchner's directions. On digesting the two quantities with beer-wort at 23° C., there was a copious fermentation at once set up by the unheated sample, but the heated one had lost all power of inducing it.

For the present, therefore, I must contend, in opposition to Buchner, that at any rate our English Yeasts do not contain any alcohol-producing enzyme.

Bibliography.

(1) Buchner: Ber. d. d. chem. Gesell., 1897, 30, 117–124.
(2) „ „ „ „ „ 30, 1110–1113.
(3) Blackman: Phil. Trans., 1896.
(4) Will: Zeits. f. d. Ges. Brauw., 1897, p. 363.
(5) Lindner: Wochenschr. f. Brau., 1897, p. 364.