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# EXPRESSED YEAST-CELL PLASMA (BUCHNER'S "ZYMASE").

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*Introduction.*—In 1897 a communication was published by Professor E. Buchner* in which he described a method by means of which he claimed to have isolated for the first time the active alcoholic ferment from the yeast-cell and to have demonstrated its action upon fermentable sugars. Since then Buchner, mainly in conjunction with Rapp, has from time to time given an account of his further investigations in this direction, and these investigations are still in progress.

* 'Berichte d. deutsch. Chem. Ges.,' 1897, p. 117. *Vide* also succeeding papers, 1897—1900, *ibid.*

These further investigations, Buchner considers, are confirmatory the conclusion drawn by him from his original experiments, viz., the activity of the yeast-cell as an alcoholic ferment depends upon action of a soluble enzyme of an albuminoid character elaborated the living cell. To this soluble ferment Buchner applies name "Zymase."

The subject presented so many phases not only of special but of general biological interest that we were led to pursue its invest tion. We considered this the more necessary since Buchner's exp ments were carried out entirely with bottom-fermentation yeasts, it appeared of interest to ascertain whether top-fermentation ye (as used in English brewing) give parallel results. At the outset carefully adhered to Buchner's method of expressing the cell plasn but owing to the tediousness of the process we were led, after m attempts, to adopt the following arrangement for the extraction of cell plasma or juice.

*Method of Preparation of the Cell Plasma.*—The yeast as recei from the brewery is a thick, pasty, frothy mass, consisting of ye cells intermixed with more or less fermented wort. For the purp in view it is necessary to separate the yeast-cells from all adher matter which would by its presence influence the composition of expressed juices. The purification of the yeast is thus a necess preliminary operation, and is accomplished as follows:—

To the pasty mass of crude yeast is added an equal part of wa and the mixture stirred together. This suspension of yeast-cells then centrifugalised, whereby the contained cells are separated a thick creamy mass at the bottom of the containing vessel. The sup natant liquor is decanted and the mass of cells again stirred int suspension in a fresh quantity of water. The mixture is again cen fugalised, and the process repeated until the last added water con away clear and colourless. The final product of this process is a fi mass of yeast-cells closely packed together, with a minimal quantity adherent water. It is necessary to remove even this quantity water if a natural juice is to be obtained. The pasty mass of yeast wrapped in a double thickness of "hydraulic chain cloth" and int duced into one of a series of shallow iron trays, so constructed that t pile can be strongly compressed in a hydraulic press and the express liquor run off. In this process, which is a modified form of filt pressing, the last adherent portions of water are removed from t yeast-cell, and the mass of yeast as removed from the cloths appears a perfectly dry white powder, consisting of yeast-cells with appro mately dry exteriors. The pressure necessary to produce this result from 70—100 atmospheres.

The disintegration of the yeast-cell is the next process. This accomplished by a mechanical contrivance which maintains the yea gether with a proportion of added silver sand, in a condition of iolent agitation, in such a manner that in the rapidly succeeding utual impacts of yeast-cell and sand-particle the cell wall is ruptured d the contents expelled.*

If the dry mass of yeast and sand be watched while disintegrating will be seen to become rapidly pasty, and through successive stages viscidity it finally reaches a perfectly fluid condition. A micro-opical examination at the end of the process fails to discover any hole cells. During the continuance of this process, and in fact uring the whole time that elapses between the rupture of the first ll wall and the examination of the final product, the material is kept ol by means of a brine circulation. The brine is maintained at a mperature of $-5^{\circ}$ C. by means of expanding anhydrous ammonia. his suffices to keep the disintegrating mass at about $15^{\circ}$ C. If this ecaution is not adopted the temperature will rise to nearly boiling oint, owing to the mechanical production of heat by the impacts and iction of the disintegrating mass.

It now remains to separate the escaped intracellular juices from the spended cell walls. This is accomplished by a repetition of the me process by which the adherent water was removed from the iginal yeast. To reduce the mass to a consistency capable of being ealt with by the press, kieselguhr is added (Buchner uses this sub-ance together with sand for grinding). The addition of this substance so serves as a filtering material, and allows the expression from the ughy mass—as from a sponge—of a perfectly clear opalescent pro-uct in which no suspended particles can be discovered. A pressure from 200—300 atmospheres is requisite to express the contained uid. Such are the main outlines of the method which has been lopted in the preparation of the juice on which the observations that llow were made.

It may be useful to give figures representing the method as it perates in practice on an averagely successful preparation.

From 100 grammes of dried and pressed yeast will be obtained om 30—35 c.c. of expressed juice. The weight of sand employed r grinding will be 100 grammes, and the weight of kieselguhr ecessary to reduce the ground mass to a suitable consistency for essing will be about 80 grammes. The specific gravity is usually om 1050—1060, and the time necessary to completely disintegrate e above quantity of dried yeast is usually $3\frac{1}{2}$ hours.

The physical properties of the juice correspond closely with those scribed as characteristic by Buchner. The contained proteolytic

* The precise details of this process, which has been successfully employed for e disintegration of micro-organisms, internal organs, glands and muscle fibres, ill form the subject of a separate paper.

enzyme was of a very active character, and produced a rapid digestio of the proteid constituents of the juice.

It occasionally happens that great reluctance is displayed by th juice in leaving the kieselguhr sponge when under pressure. Thi has most frequently happened with very new yeasts—that is, yeas skimmed from the fermentation vats and used directly for the pre paration of the juice. There is some evidence to lead us to suppos that this difficulty is correlated with a similar difficulty which is me with when attempting to prepare as near the living condition a possible an intracellular juice of an organ or tissue. For instance, liver removed from a dog at the moment of death and at once disin tegrated will yield no juice on pressing, even if the pressure be raise to a thousand atmospheres or more; whereas a liver not so fresh wil yield its juice without difficulty. That kieselguhr has the power o arresting the passage of certain albuminous bodies can easily b demonstrated. Thus we have found that egg globulins are almos entirely retained in a kieselguhr sponge, and even albumin and serun proteids are retained to a certain extent. It therefore is suggeste that the juice that was used in the following work was in every cas far removed in nature from the condition in which it existed wher alive within the yeast-cell; but on the other hand it is much neare the living condition than that obtained by Buchner, owing to the fac that he employed water to extract his juice, and water as will be shown has a decided action on the juices we obtained.

We are therefore placed in the difficult position that those conditions in which the juice is nearest its living condition are just those when it cannot be obtained by the convenient method of pressing. Under such circumstances resort must be had to centrifugalising; but the process is tedious in the extreme, and by the time it is completed in all probability the juice has altered in composition. We hope soon to be in a position to overcome this difficulty.

*Properties of the Cell Plasma.*—In the course of our experiments we employed yeast from five different breweries, which we will designate as A, B, C, D, and E. The first three (A, B, and C) were breweries in the London district, D was in the south of England, and E was one of the very few bottom-fermentation breweries in this country. The greater number of our experiments were made with yeasts from A and B, the yeasts from C, D, and E being only used in one or two instances.

From the outset we found that in practically all cases the juice obtained from the yeasts freely evolved gas, both when standing alone and with the addition of sugar. Our results in the latter respect were fully equal to, and in some instances surpassed, those of Buchner.

We were, however, early confronted with the fact that the *auto-* or *self-fermentation* of the juice gave rise to a considerable volume of gas, a olume which in many cases exceeded that given by the same amount f juice to which sugar had been added. This auto-fermentation pparently escaped the observation of Buchner, who only incidentally efers to it in one of his papers, and who does not appear to have nade any correction for the gas evolved from the juice itself in any f his experimental results. The extent to which this fermentation ccurs may be seen from the subsequent tables (Tables I and II); in ne experiment, for instance, 100 c.c. of the fresh juice gave no less han 2·98 grammes or 1500 c.c. of carbon dioxide. This spontaneous volution of gas takes place even when the juice is kept at a temperaure sufficiently low to maintain it in a solid condition. In all probbility the gas which Buchner mentions as being evolved on heating Zymase" is due to this cause.

In our earlier experiments we determined the carbon dioxide evolved rom the juice alone, or from its admixture with sugar, by measuring he volume of saturated salt solution which was displaced by the gas; ut later we adopted a modification of Hart's double titration method, n which the carbon dioxide was absorbed by sodium hydroxide olution, and the amount determined by double titration.

In the experiments on the relationship of the carbon dioxide and lcohol formed, we absorbed the carbon dioxide evolved in 33 per ent. potassium hydroxide solution contained in Mohr's potash bulbs. The alcohol formed in these experiments was estimated by distillation nd determination of the specific gravity of the distillate, the weight f absolute alcohol corresponding to the gravity of the distillate being hen found by reference to spirit tables.

Control experiments were made in all cases—that is to say, when we were determining the amount of carbon dioxide or of alcohol, formed by any juice from sugar, a corresponding quantity of the juice was placed under identical conditions, but without the addition of any sugar, and the amount of gas evolved or of alcohol formed was carefully determined by the same methods as those used in the experiments in the presence of sugar. We employed antiseptics to inhibit the possible action of yeast-cells or other micro-organisms, the nature of the antiseptics used depending on the object of the experiment. The antiseptics principally employed were sodium arsenite, toluol, and thymol, all at the rate of 1 per cent.

In our earlier experiments we employed 40 per cent. of cane-sugar, this being the concentration which Buchner first considered the most favourable; but we subsequently reduced this to 10 per cent., as we found that greater action was obtained with the lesser concentration. In fact, the larger amount of sugar appeared to exercise a retarding influence on the activity of the juice.

*Nature of Results obtained.*—In Table I we give the results of some of our experiments, in which the gas evolved was measured by the displacement of salt solution. The volume of gas evolved is express in this and the following tables on 100 c.c. of juice, although t quantity actually used was, as a rule, either 25 to 40 c.c.

It will be noticed that in nearly every instance more gas w obtained from the auto-fermentation of the juice than from t fermentation in the presence of cane-sugar. This was usually t case with the juice from the yeasts of A, C, and D breweries; ho far it is due to the distinctive character of the yeasts or to the hig sugar concentration we are at present not prepared to say. Anoth point to be noted is the great variation in the activity of the jui from different samples of yeast. We noticed this throughout tl whole of our experiments, but we are unable to correlate it with an of the physical properties of the juice, such as gravity, &c. It wi also be seen that by far the greater part of the action is at an en after twenty-four hours, there being either no further increase in tl amount of gas evolved, or comparatively little. This we also foun common to the majority of our experiments, as will be seen fro subsequent tables.

Table I.—Volume of Carbon Dioxide evolved by Cell Juice fro different Yeasts, with and without the addition of Cane-sugar.

| Source of yeast. | Age of yeast from collection. | Carbon dioxide from 100 c.c. of juice. | | | |
|---|---|---|---|---|---|
| | | After 24 hours. | | After 48 hours. | |
| | | Alone. | With 40 per cent. sugar. | Alone. | With 40 per cent. sugar. |
| | | c.c. | c.c. | c.c. | c.c. |
| A* | Fresh | 520 | 280 | 600 | 472 |
| A* | 1 day | 240 | 808 | — | — |
| A* | 2 days | 133 | 164 | — | — |
| A* | Fresh | 308 | 186 | 308 | 324 |
| A* | Fresh | 400 | 228 | 400 | 340 |
| A* | 1 day | 285 | 270 | 285 | 320 |
| A* | 1 day | 162 | 84 | 162 | 150 |
| A† | 2 days | 990 | 234 | 990 | 290 |
| A† | Fresh | 90 | 170 | — | — |
| A† | 3 days | 760 | 560 | 788 | 592 |
| A‡† | Fresh | 900 | 2165 | — | — |
| A* | 5 days | 280 | 220 | — | — |
| C* | 1 day | 550 | 180 | — | — |
| C† | 1 day | 540 | 100 | — | — |
| C* | 3 days | 160 | 200 | — | — |
| A* | 7 days | 120 | 200 | — | — |
| A* | 1 day | 65 | 90 | — | — |

* Toluol used as antiseptic. † Sodium arsenite used as antiseptic.
‡ In this experiment 10 per cent. cane-sugar was employed.

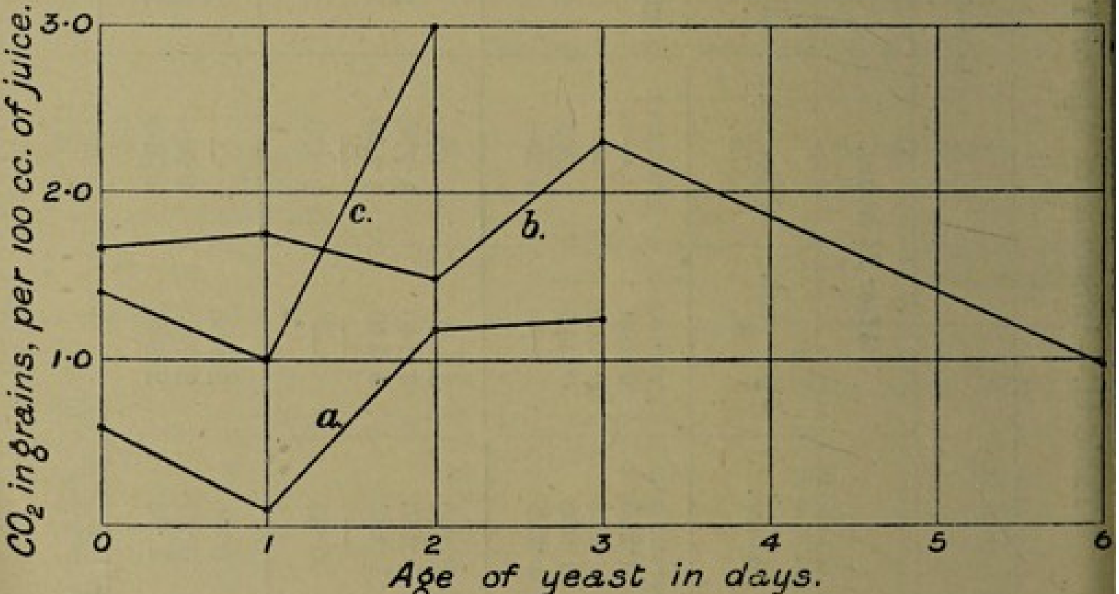
Table II.—Influence of Age of Yeast on Activity of Juice.

| Source of yeast. | Age of yeast. | Gas evolved per 100 c.c. of juice. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | After 24 hours. | | | | After 48 hours. | | | |
| | | Alone. | 5 per cent. C.S. | 10 per cent. C.S. | 15 per cent. C.S. | Alone. | 5 per cent. C.S. | 10 per cent C.S. | 15 per cent. C.S. |
| | | grammes. | grammes. | grammes. | grammes. | grammes. | grammes. | grammes. | grammes. |
| A. | Fresh..... | 0·215 | 0·145 | — | — | 0·615 | 0·325 | — | — |
| | 1 day..... | 0·105 | 0·015 | — | — | 0·120 | 0·200 | — | — |
| | 2 days.... | 0·930 | 0·445 | 0·395 | 0·140 | 1·205 | 0·965 | 0·855 | 0·570 |
| | 3 days.... | 1·145 | — | 0·280 | — | 1·285 | — | 0·950 | — |
| B. | Fresh..... | 1·480 | 3·030 | 3·870 | — | 1·705 | 3·550 | 5·060 | — |
| | 1 day..... | 1·450 | 3·140 | 3·770 | 3·630 | 1·770 | 3·590 | 4·600 | 4·050 |
| | 2 days.... | 1·480 | 3·540 | 4·410 | 3·880 | 1·480 | 3·920 | 5·300 | 3·970 |
| | 3 days.... | — | — | — | — | 2·310 | 2·480 | 6·600 | 3·300 |
| | 6 days.... | 0·83 | — | 1·370 | 3·230 | 0·970 | — | 1·950 | 3·720 |
| B. | Fresh..... | 0·90 | 2·800 | — | 3·720 | 1·420 | 2·930 | — | 4·450 |
| | 1 day..... | 0·97 | 2·040 | 4·040 | 3·590 | 1.230 | 2·930 | 4·440 | 4·170 |
| | 2 days.... | 1·32 | 2·980 | 3·390 | 3·150 | 2·980 | 3·290 | 3·700 | 3·000 |

*Influence of Age of Yeast on Activity of Juice.*—In Table II we give some of our results on the influence of the age of the yeast, *i.e.*, the time which elapsed between the time of collection of the yeast in the brewery, and that of pressing and grinding, on the activity of the cell plasma obtained. The same table shows to some extent the influence of sugar concentration on the amount of gas evolved.

It will be seen that the results are very variable, but that the general tendency is for the activity of the juice to increase to a certain point with the age of the yeast, the maximum being reached about the 3rd or 4th day from collection. After the maximum is reached there is a very rapid decline in the activity of the juice. The variation in the amount of auto-fermentation is not so great, but the tendency of this is to follow the same direction. The results are shown diagrammatically in figs. 1 and 2, the former showing the auto-fermentation and the latter the fermentation in presence of sugar, both when the gas evolved from the auto-fermentation is included and when it is deducted from the total amount.

FIG. 1.—Showing the influence of age of yeast on auto-fermentation after 48 hours.

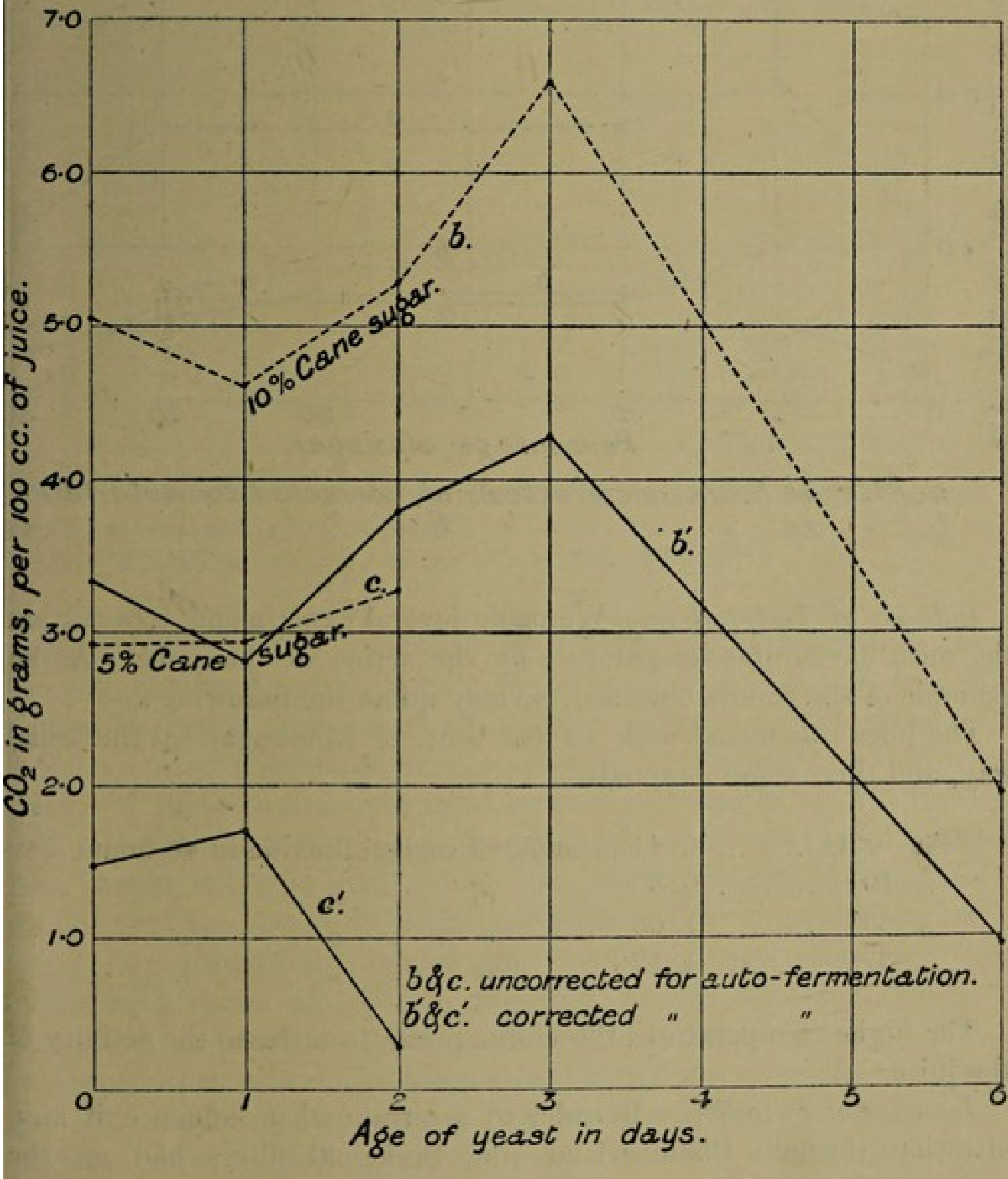



This increase up to a certain point of the activity of the juice with the age of the yeast is the reverse of that which takes place with bottom-fermentation yeasts, as described by Buchner and other Continental observers.

*Influence of Storage on Activity of Juice.*—When the expressed juice is kept even at or below freezing point, its power both of auto-fermentation and of decomposing sugar rapidly diminishes.

*Influence of Amount of Sugar present.*—We carried out a series of experiments to determine the most favourable concentration of sugar; the results show that the smaller amounts—5 to 10 per cent.—give the most favourable results, whilst the larger quantities sensibly retard the action, *i.e.*, less gas is obtained from the juice plus sugar than from the juice alone. This probably explains to some extent the results we

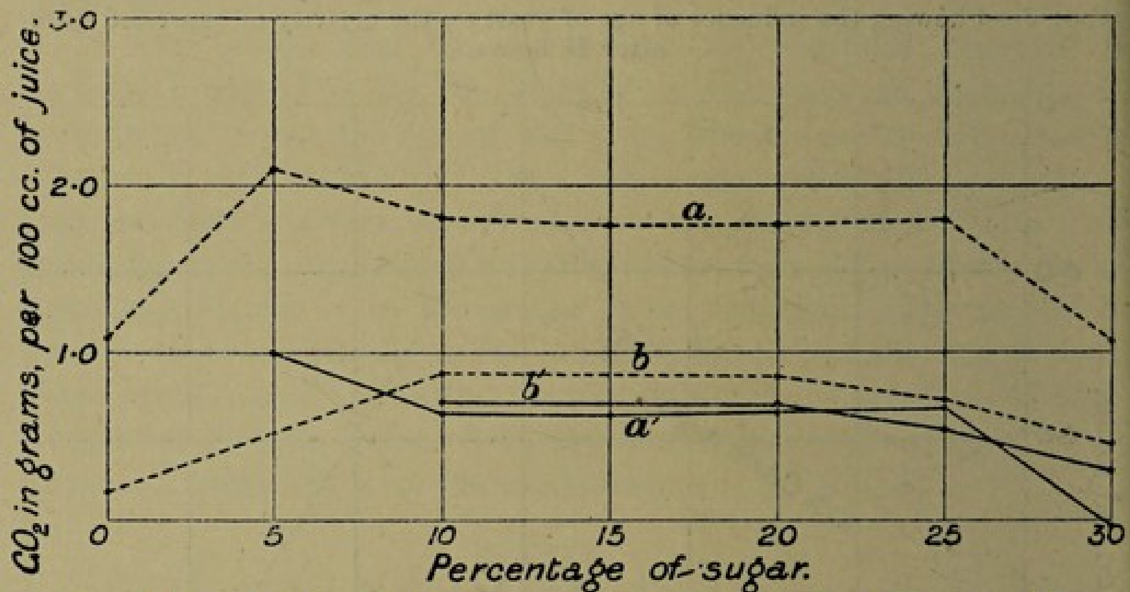
FIG. 2.—Showing the influence of age of yeast on the activity of expressed juice after 48 hours.




obtained in our earlier experiments in which 40 per cent. of sugar was employed. Some of the results are shown diagrammatically in fig. 3.

*Influence of different Sugars.*—In order to determine if the nature of the sugar employed had any influence on the amount of gas evolved, we carried out a series of experiments with cane-sugar, dextrose, maltose, and levulose at different concentrations, using the same sample of juice for each set of experiments. The results, as a whole, show that more carbon dioxide is given off from cane-sugar than from either of the other sugars.

Fig. 3.—Showing the influence of different concentrations of sugar on gas evolved.




a, After 48 hours (uncorrected); a′, corrected for auto-ferm.
b, " 24 " " ; b′, " " "

*Influence of Temperature.*—We made several experiments to ascertain the most favourable temperature for the action of the juice. As an example of the results obtained, we may quote the following:—

The juice was mixed with 10 per cent. of cane-sugar in the usual way, and there were obtained—

| | | |
|---|---|---|
| At 0° C.... ..... | 0·41 | gramme of carbon dioxide in 48 hours. |
| „ 10 ......... | 0·83 | „ „ „ |
| „ 25 ......... | 1·05 | „ „ „ |
| „ 37 ......... | 1·17 | „ „ „ |

The higher temperatures therefore appear to increase the activity of the juice.

*Influence of Filtration.*—In order to ascertain what influence, if any, filtration through Chamberland and Berkefeld filters had on the activity of the juice, we carried out a series of experiments with different juices, carefully testing their gas-producing activity before and after filtration. Thymol was used as an antiseptic in each case The results are given in Table III, and it will be seen that filtration decreases to a considerable extent, but without entirely destroying, both the auto-fermentation and the action of the juice on sugar. This decrease in gas-evolving power is accompanied by a very considerable ll in gravity of the juice. These experiments agree with those of uchner on the same point.

Table III.—Influence of Filtration on the Activity of the Juice.

| Source of yeast. | Age of yeast. | Gravity before filtration. | Gravity after filtration. | Gas evolved from 100 c.c. of juice. | | | |
|---|---|---|---|---|---|---|---|
| | | | | Before filtration. | | After filtration. | |
| | | | | Alone. | With 10 per cent. C.S. | Alone. | With 10 per cent. C.S. |
| | days. | | | gramme. | gramme. | gramme. | gramme. |
| B * | 3 | — | — | — | 0·67 | — | 0·31 |
| B | 2 | — | — | 0·43 | 0·65 | 0·00 | 0·10 |
| B | 2 | — | — | 0·18 | 1·56 | 0·25 | 0·84 |
| B † | 2 | — | — | 1·23 | 0·83 | 0·11 | 0·43 |
| B | 2 | 1055 | 1018 | 0·57 | 0·65 | 0·24 | 0·23 |
| B | 3 | 1045 | 1030 | 0·25 | 1·55 | 0·24 | 1·17 |

* In this experiment the filtration was through a Chamberland filter; in the maining experiments a Berkefeld filter was used.

† After 72 hours in this case; all the others after 48 hours.

*Influence of Dilution.*—In considering the nature of the action of the uice and of the agent to which the evolution of gas was due, it ppeared important to ascertain the effect of dilution on the action of he juice. All experiments were conducted by adding the weighed uantity of sugar to the juice itself, so that no water at all was introuced. If the action were a purely enzymic one, dilution to a limited xtent should not appreciably affect the result; whereas if the action ere due to other causes, it might be influenced to a greater or less xtent. We accordingly carried out a series of determinations on ilution with water alone, with physiological salt solution (0·75 per ent. sodium chloride), and with water in the presence of cane-sugar. he experiments with sugar were made in two ways: in the one, the ugar was added to the juice in the usual way (10 per cent.), and ater was then added to bring about the desired dilution, the atio of the sugar to juice being therefore kept constant; in the ther, the dilution was made with a 10 per cent. solution of sugar, so hat the ratio of sugar to the total volume was maintained throughout. he results obtained are set out in Table IV. An examination of the esults will at once show that the auto-fermentation of the juice is reatly influenced by dilution both with water and with salt solution. The addition of an equal volume of water sensibly retards the action

Table IV.—Influence of Dilution on Activity of Juice.

| Source of yeast. | Age of yeast. | Gas evolved from 100 c.c. of juice. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | After 24 hours. | | | | After 48 hours. | | | |
| | | Alone. | Diluted 1 : 1. | Diluted 1 : 2. | Diluted 1 : 3. | Alone. | Diluted 1 : 1. | Diluted 1 : 2. | Diluted 1 : 3. |
| (a) With water alone. | | | | | | | | | |
| | | grammes. | grammes. | gramme. | gramme. | grammes. | grammes. | gramme. | gramme. |
| A........ | 1 day..... | 1·49 | 1·25 | 0·02 | 0·00 | 1·71 | 1·32 | 0·05 | 0·00 |
| B........ | 5 days.... | 0·83 | — | 0·54 | — | 0·97 | — | 0·58 | — |
| B........ | 5 „ .... | 1·05 | — | 0·00 | — | 1·37 | — | 0·09 | — |
| B........ | 6 „ .... | 1·22 | 0·99 | 0·31 | 0·07 | — | — | — | — |
| (b) With 0·75 per cent. salt solution. | | | | | | | | | |
| A........ | Fresh..... | 0·90 | 0·05 | 0·00 | 0·00 | — | — | — | — |
| B........ | 5 days.... | 0·83 | — | 0·10 | — | 0·97 | — | 0·17 | — |
| B........ | 5 „ .... | 1·05 | — | 0·00 | — | 1·37 | — | 0·08 | — |
| (c) With water in presence of 10 per cent. cane-sugar on juice. | | | | | | | | | |
| B........ | 6 days.... | 1·30 | 0·10 | 0·10 | 0·05 | 1·30 | 0·10 | 0·10 | 0·05 |
| B........ | 6 „ .... | 1·66 | 0·23 | 0·10 | 0·08 | — | — | — | — |
| (d) With water in presence of 10 per cent. cane-sugar on total volume. | | | | | | | | | |
| B........ | 5 days.... | 1·77 | 0·67 | 0·47 | 0·55 | — | — | — | — |

in some cases, *dilution with a double volume practically stops the ion of gas.* With salt solution, the action is still more marked.

the presence of sugar the retarding action is still distinctly ent, especially when the concentration of the sugar decreases dilution. In this case the effect of dilution is fully as marked as e case of water alone or of salt solution. When the strength of ugar solution is maintained constant, the retardation is still conable, but not so great as in the other cases.

is paralysing effect of dilution on the activity of the juice is so ary to the behaviour of enzymes in general under similar condi-, that in our opinion it forms a grave objection to the acceptance uchner's enzyme theory. Since the above experiments were , we find that Wroblewski* has conducted dilution experiments like results.

connection with the question of the influence of dilution on ne action, it may be mentioned that when a sample of six-day was diluted to 1 in 1000 with cane-sugar solution, 50·5 per cent. e cane-sugar was found to be inverted, whilst with another juice, days old, a dilution of 1 in 100 showed an inversion of 79·5 per of the cane-sugar present. This offers a great contrast to the of dilution of the juice on the production of carbon dioxide.

*tio of Carbon Dioxide to Alcohol.*—In connection with the question her we had to do with a true alcoholic fermentation, it became rtant to determine if carbon dioxide and alcohol were produced e proportions ordinarily found, and if the amount of sugar which peared during the experiment bore any relation to the alcohol carbon dioxide. We carried out a large number of experiments a view to elucidate these points, and the results of some of the riments are shown in Table V. In experiments 1 to 5, the ol and carbonic acid estimations were made on the same fermenta- but in experiments 6 to 15 we carried out duplicate fermenta-, under identical conditions with the same juice, for the two rminations. We did this in order to ensure greater accuracy in alcohol estimation, since the escaping gas could be washed by ge through a little water, which was subsequently added to the llation flask. When we were estimating both products from the experiment this was not possible.

will be noticed from the table that the juice as it comes from the s always contains a considerable amount of alcohol, and we found xamination that this agrees fairly closely with the amount of ol contained in the yeast, even after the thorough washing and sing to which it had been subjected in the preliminary treatment. hen corrections are made for the amounts of alcohol and of carbon ide formed during the auto-fermentation of the juice, the ratio

* 'Centralbl. f. Physiol.,' 1899, p. 284.

Table V.—Showing Relation of Alcohol and Carbon Dioxide formed in Fermentations in the Cell-juice.

The results are expressed on 100 c.c. of Cell-juice.

| No. of expt. | Age of yeast. | Original alcohol. | Auto-fermentation. | | | Fermentation with 10 per cent sugar. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ formed. | Alcohol formed. | Increase of alcohol. | $CO_2$ formed. | Sugar fermented. | Increase of $CO_2$. | Alcohol formed. | Sugar fermented. | Increase of alcohol. |
| | days | grammes | grammes | grammes | grammes | grammes | grammes | grammes | grammes | grammes | grammes |
| 1 | 1 | 1·85 | 1·38 | 2·90 | 1·05 | 1·64 | 5·838 | 0·26 | 4·75 | 5·838 | 1·85 |
| 2 | 2 | 3·90 | 1·28 | 7·90 | 4·00 | 0·97 | 4·292 | none | 7·90 | 4·292 | none |
| 3 | 3 | 3·25 | 0·65 | 3·25 | none | 2·03 | 6·101 | 1·37 | 6·10 | 6·101 | 2·85 |
| 4 | 4 | 4·20 | 1·89 | 5·95 | 1·75 | 2·45 | — | 0·56 | 6·55 | 4·615 | 0·60 |
| 5 | 6 | 4·20 | 0·95 | 3·15 | none | 1·16 | 1·720 | 0·21 | 3·40 | — | 0·25 |
| 6 | 3 | 3·70 | 1·11 | 4·30 | 0·60 | 1·22 | 2·525 | 0·11 | 5·25 | 2·812 | 0·95 |
| 7 | 5 | 3·95 | 1·20 | 4·80 | 0·85 | 1·21 | 2·181 | 0·01 | 5·25 | 2·830 | 0·45 |
| 8 | 7 | 3·80 | 0·64 | 3·80 | none | 1·09 | 4·115 | 0·45 | 4·25 | 1·758 | 0·65 |
| 9 | 7 | 4·45 | 0·51 | 5·05 | 0·60 | 1·16 | 7·413 | 0·65 | 5·70 | 4·390 | 0·65 |
| 10 | 3 | 4·12 | 1·49 | 4·52 | 0·40 | 1·50 | 3·070 | 0·01 | 4·85 | 2·107 | 0·32 |
| 11 | 5 | 2·90 | 0·40 | 3·00 | 0·10 | 0·59 | 1·553 | 0·19 | 2·75 | 1·086 | none |
| 12 | 2 | 2·80 | 0·73 | 3·75 | 0·95 | 0·91 | 1·340 | 0·18 | 3·90 | 4·150 | 0·15 |
| 13 | 1 | 2·12 | 0·23 | 1·75 | 0·63 | 0·18 | none | none | 1·85 | none | 0·10 |
| 14 | 3 | 3·15 | 0·37 | 3·12 | none | 2·27 | 5·041 | 1·90 | 5·55 | 6·413 | 2·43 |
| 15 | 3 | 4·17 | 0·56 | 4·25 | 0·08 | 3·66 | 8·331 | 3·10 | 7·50 | 9·080 | 3·25 |

Experiments 1 to 7 and 12 to 15 were made with dextrose. Experiments 8 to 11 were made with cane-sugar. Thymol was used in all the experiments.

een the residual alcohol and the carbon dioxide is very variable, only in cases in which a very active juice is employed does the ratio oximate to that found by Pasteur.* With a weak juice there appears e little or no connection between the two, the amount of alcohol ied being, as a rule, greater than the carbon dioxide. The small ıtities of alcohol to be determined may be thought to be account- for the discrepancy between the two products, but the following nple of a determination carried out in duplicate shows that our hods were capable of considerable accuracy.

| | I. Gramme. | II. Gramme. |
|---|---|---|
| Carbon dioxide evolved ......... | 0·327 | 0·337 |
| Alcohol formed .................... | 0·95 | 0·95 |
| Sugar fermented .................. | 1·167 | 1·158 |

ne very remarkable fact comes out in all the above experiments, ıely, that the amount of sugar which disappears is greatly *in excess* hat actually fermented, as deduced either from the alcohol or from carbon dioxide formed. The closer the relationship, however, veen the two products, the less is the excess of sugar which dis- ears.

t occurred to us that there might be some constituent of the juice ch interfered with the correct determination of the sugar, but we this to the test and found that when we added sugar to the juice, then killed its action by heat before any fermentation could take ce, the whole of the sugar could be accounted for by Pavy's method letermination.

Ve also submitted the residual product after fermentation had en place to hydrolysis with very dilute acid with a view to break any hydrolysible compound which might have been formed between constituents of the juice and the excess sugar which had disap- ıred, but without any result: the reducing power before and after atment remained the same. The sugar had therefore apparently appeared as such, and had not simply been rendered unrecognisable ordinary tests.

We are at present only able to chronicle this most interesting fact, at the present stage of our work it would be premature to make y theoretical deductions; but in connection with this remarkable appearance, we venture to throw out the following suggestion:— ıring the life of the yeast, sugar is consumed by the organism with resulting production of carbon dioxide and alcohol. Considered detail, this process probably occurs in two stages—(1) a building up d incorporation of the sugar molecules into the actively living proto-

* Cane-sugar yields 51·11 per cent. of alcohol and 49·42 per cent. $CO_2$.
Dextrose " 48·55 " " " 46·95 " "

plasm (anabolism); and (2) a breaking down of this complex ma into simpler products, of which carbon dioxide and alcohol ar constant and principal constituents (katabolism). May it not be after the expression of the cell-juice from the cell the same seri actions continues to take place, at least for so long a time as rapidly changing and unstable cell-juice remains in a condition proximately identical with that in which it existed in the living If this hypothesis be admitted, then the varying activities of the are at least partly explicable, for if we designate by $\chi$ the hypothe protoplasmic constituent of the cell with which the sugar comb then we may imagine the processes which take place in the expre cell-juice (in which we assume $\chi$ to continue to exist) to be somev as follows:—

(*a*) In the case of auto-fermentation the $\chi$-sugar combination, l up during the life of the cell, continues to decompose, after the pression of the juice, yielding carbon dioxide and alcohol.

(*b*) In the case of the disappearing sugar, the formation of $\chi$-sugar combination continues to a certain point, depending on activity of the juice, but the decomposition of this combination co to an end before the whole of the sugar has been liberated in the fo of carbon dioxide and alcohol. In the case of a very active juice may imagine this process to continue until practically the whole of combination has been decomposed. In the case of a weak juice, building-up process takes place more rapidly than the breaking-do process, and, consequently, when the activity of $\chi$ ceases, there rema an excess of sugar in the form of the $\chi$-sugar combination.

We are continuing our investigations with the yeast-cell plasma, a shall hope to communicate our further results to the Society in d course. In the meantime it may be convenient to briefly summar the results we have already obtained, which so far appear to be leadi us in the direction not of an enzyme explanation of the process, b rather of a theory which refers the phenomenon to the vital activi of the yeast-cell protoplasm.

(1.) The top-yeast of English breweries yields, by suitable tre ment, a cell-juice which possesses the transient power decomposing sugar into alcohol and carbonic acid.

(2.) The amount of gas formed by an active juice is as great as, even greater than, that found by E. Buchner.

(3.) The cell-juice as prepared by us undergoes a very considerab auto-fermentation, in some instances exceeding that give by a mixture of the same juice and cane-sugar.

(4.) A moderate dilution (1 : 2) with water or physiological sa solution practically stops all fermentative activity.

(5.) Only with a very active cell-juice does the ratio between th alcohol and carbon dioxide formed approximate to that found in ordinary alcoholic fermentation.

(6.) When the cell-juice is allowed to act on sugar—either cane-sugar or dextrose—the quantity of sugar which disappears is considerably in excess of that which can be accounted for by the production of carbon dioxide and alcohol.

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